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Iodine status and thyroid function among lactating women in Saharawi refugee camps, Algeria

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Preface

The present study on iodine status and thyroid function among lactating women in the Saharawi refugee camps was done as a part of the Master's degree in Food, Nutrition and Health at Akershus University College (AUC). The study was conducted in collaboration with researchers from AUC and the non-governmental organisation Norwegian Church Aid (NCA), who provided information and financial support. Without their support, this project would not have been possible.

Several people have contributed to this thesis. Big thanks go to my supervisor Sigrun Henjum for advice and encouragement along the way. Thanks to Ingrid Elisabeth Barikmo for the opportunity to do field work in the Saharawi refugee camps and for support during the whole period.

Thanks to my fellow master student Inger Aakre for good cooperation and unforgettable time during the field work. I would like to thank each of the field workers: Ahmed Labed Belid, Salamu Ali, Abderrahàman Mohamed Lehabib Merras, Najat Bah, Najwa Mohamed Cheikh, Monina Mohmed Bazied, Nadjana Sid Abed Allah and Bashir Abas, who did a tremendous effort in translating questionnaires, collect data and drive back and forth to places.

Thanks to Eirik Kirkerud from NCA for sharing information and experiences from the refugee camps, bio-engineer Ellen Raael from AUC for useful information on the procedures for blood sample collection, the library at AUC for answering my questions and not least to my family and friends for being there for me during this process.

At last, a huge thanks to the Saharawi health director, Alien Abdullah, for all the help and support during our stay in the refugee camps and to the participants for their cooperation. It has been an immense experience and a great learning profit to be able to do field work and learn about the Saharawi people, their culture and the situation in the camps. I hope this study will benefit the people living in the camps.

Oslo, May 2011 – Navnit Kaur Grewal

Abstract

BACKGROUND: Insufficient iodine intake as well as excess iodine intake may cause thyroid diseases. Endemic goitre and high urinary iodine concentration (UIC), probably caused by excess iodine, has been reported among Saharawi refugees. To what extent long-term excess iodine intake have influenced the thyroid function of the refugees is unknown.

OBJECTIVE: The main objective was to assess iodine status and thyroid function among lactating women in the Saharawi refugee camps.

METHOD: A baseline for a cohort study was performed among 111 lactating Saharawi women (18-50 years) living in the Algerian desert. Samples of casual urine, breast milk, public drinking water, goat - and camel milk were collected for determination of iodine concentrations. Dietary iodine intake through intake of water and milk was registered using 24-h recall. Thyroid function was assessed through serum levels of thyroid stimulating hormone (TSH), thyroglobulin (Tg), tyroxin (T_4), thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb). In selected samples triiodothyronine (T_3) and thyrotropin receptor antibody (TRAb) were determined.

RESULTS: Median UIC was 350 µg/L. The breast milk iodine concentration (BMIC) showed a median concentration of 479 µg/L. Median iodine concentrations in public drinking water, goat - and camel milk was 102, 952 and 2020 µg/L, respectively. The median dietary intake of iodine among the women was 407 µg/day. Thyroid function abnormalities were found in 23.4 % of the women: 12.6 % had subclinical hypothyroidism, 5.4 % autoimmune thyroiditis, 5.4 % subclinical hyperthyroidism, 3.6 % clinical hypothyroidism and 0.9 % clinical hyperthyroidism. Further, 17.1 % of the women had elevated serum Tg levels and positive TgAb was detected in 14.4 %. The age distribution was higher among women with thyroid abnormalities ($p = 0.01$). Dietary iodine intake correlated well with UIC ($r_s = 0.24$, $p = 0.01$) and BMIC ($r_s = 0.47$, $p < 0.001$). The prevalence of thyroid function abnormalities increased with higher UIC, although no significant correlation between UIC and TSH or UIC and Tg was found.

CONCLUSION: The lactating women had high levels of iodine in urine and breast milk, probably caused by excessive dietary iodine intake with public drinking water as a major contributor and animal milk presumably increasing the iodine intake when consumed. The high prevalence of thyroid abnormalities indicates that the long-term excess iodine intake might have influenced the thyroid function negatively.

Table of contents

List of tables

List of figures

List of pictures

List of abbreviations

AUC	Akershus University College
BMI	Body Mass Index (kg/m^2)
DIT	Diodotyrosine
ECHO	European Community Humanitarian Office
FAO	Food and Agriculture Organization of the United Nations
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine Deficiency Disorders
IIH	Iodine Induced Hyperthyroidism
IRLI	International Resource Laboratories for Iodine
MIT	Monoiodotyrosin
n	number
NCA	Norwegian Church Aid
NGO	Non-governmental organisation
NIFES	National Institute of Nutrition and Seafood Research
NIRU	Nutritional Intervention Research Unit
REK	Regional Committees for Medical and Health Research Ethics
SMH	Saharawi Ministry of Health
SPSS	Statistical Package for the Social Sciences
T_3	Triiodothyronine
T_4	Tyroxin
Tg	Thyroglobulin
TPO	Thyroid peroxidase
TRH	Thyrotropin Releasing Hormone
TSH	Thyroid Stimulating Hormone (thyrotropin)
UIC	Urinary Iodine Concentration (µg/L)
UN	United Nations
UNHCR	United Nations High Commissioner of Refugees
UNICEF	United Nations Children's Fund
WFP	World Food Program
WHO	World Health Organization
µg	Microgram (mcg)

1 INTRODUCTION

It is well documented and accepted that iodine deficiency is associated with the development of thyroid function abnormalities (Hetzel & Dunn, 1989). However, the effect of iodine on the thyroid gland is complex and the relationship between iodine intake and the risk of developing thyroid disease is U-shaped, where both low and high intake are associated with an increased risk of thyroid disease (Laurberg, Bulow Pedersen, Knudsen, Ovesen & Andersen, 2001). Thirty-four countries have reported to have more than adequate median urinary iodine (de Benoist, McLean, Andersson & Rogers, 2008). Some of these countries even exceed 300 µg/L, an urinary iodine concentration which is categorised excessive for school-aged children by the World Health Organization (WHO) (WHO, 2007). The high levels of iodine may increase the risk of developing thyroid abnormalities, such as thyroiditis, hypothyroidism, hyperthyroidism and goitre (Pennington, 1990). The most vulnerable groups are especially under-privileged people, pregnant and lactating women and further preschool children (WHO, 2007).

Today, approximately 165 000 refugees from Western Sahara live in the refugee camps near Tindouf in Algeria (UNHCR, 2002). Previous studies have revealed some of the nutritional problems among the refugees living in the camps (UNHCR, 2002, 2005), including high prevalence of goitre. A cross-sectional study conducted in 2007 found that a total of 11 % of the school-aged children and 18 % of the women who participated had goitre by palpation. Furthermore, the ultrasound showed that 56 % of the children and 22 % of the women had enlarged thyroid volume (SMH/NCA/AUC, 2008). Goitre usually occurs due to iodine deficiency. The contrary has been seen in the Saharawi population, where 79 % had a urinary iodine concentration above 300 µg/L (SMH/NCA/AUC, 2008).

The study of high iodine intake and its health effects is still unclear. To what extent the long-term excess iodine intake has influenced the thyroid function of the refugees is still unknown. Thus, the aim of this thesis was to investigate this relationship.

1.1 Context and delimitation

The work of this thesis included a field work period in the Saharawi refugee camps. Another master student and I were to plan and undertake this nutritional study together looking at different aspects of the data collected. Planning of the data collection was done together with our respective supervisors.

Lactating women and infants from 0-7 months were the study group. The nutritional study included taking urine samples from mother and infant and further blood- and breast milk sample from the mother. Water and milk samples were collected. In addition four questionnaires were developed before the field work, which included background information, dietary iodine intake, breastfeeding practices and anthropometrical measurements.

This master thesis focuses on lactating women, including urinary iodine, breast milk iodine, dietary iodine intake and thyroid function of the women. Thus, iodine status of the infants and breastfeeding practices were not included in this thesis. Pictures from the refugee camps used in this thesis are taken by me with permission.

2 OBJECTIVES

Main objective for this thesis

To assess iodine status and thyroid function among lactating women in the Saharawi refugee in Algeria, through assessing the urinary iodine, breast milk iodine, dietary iodine intake and blood constituents.

Specific objectives for this thesis

1. To describe the urinary iodine concentration
2. To describe the breast milk iodine concentration
3. To describe the iodine concentration in public drinking water and animal milk
4. To describe the dietary iodine intake
5. To assess thyroid function through blood constituents
6. To assess association between the urinary iodine concentration and dietary iodine intake
7. To assess association between the urinary iodine concentration and thyroid function
8. To assess iodine status and thyroid function in the low - and high iodine area in the refugee camps

In order to achieve the main objective, specific objectives were studied. All objectives were explored at the group level. However, previous studies along with the present study revealed high concentration of iodine in the public drinking water consumed by the refugees and the water is considered to be the main dietary iodine source among the refugees (Pezzino et al., 2008; SMH/NCA/AUC, 2008). Among the four Sahrawi refugee camps, there has been found significant higher iodine concentration in the two camps named Auserd and Aaiun compared to Smara and Dajla (SMH/NCA/AUC, 2008). Therefore, this thesis tried to investigate the impact of iodine in public drinking water on the urinary iodine, breast milk iodine, dietary iodine intake and thyroid function (objective 1-5) among the women by dividing the group into the "low iodine area" (which refers to women living in Smara and Dajla) and the "high iodine area" (which refers to women living in Auserd and Aaiun) (objective 8). The association between the different indicators (objective 6 and 7) were only assessed at the group level.

3 THEORETICAL BACKGROUND

Iodine (I) is a non-metallic element belonging to the halogen family of the periodic table. It occurs in a variety of chemical forms, such as iodide (I^-), iodate (IO_3^-) and elemental iodine (I_2). There is a natural cycle of iodine in nature between the ocean, the atmosphere, rainfall and runoff of rainfall into streams and rivers. Most of the iodine resides in the ocean (Semba & Delange, 2008). In human nutrition, iodine is generally absorbed as iodide (Sharp, 2011).

3.1 Iodine in human nutrition

Iodine is an essential micronutrient of human nutrition, as it is an integral part of the thyroid hormones. Only few food groups are found to contain significant amounts of iodine. The sources may vary between countries, but the main foods with natural high iodine content are fish and seafood (Rasmussen et al., 2009). A study conducted by Julshamn et al. (2001) in Norway reported that the level of iodine content in fish fillets varied widely, both within a species and between species. Lean fish species like cod, haddock and saithe were reported to have a mean iodine concentration twice as high as in fatty fish species, such as farmed salmon, herring and mackerel (Dahl & Meltzer, 2009; Julshamn, Dahl & Eckhoff, 2001).

The iodine content in soil and drinking water is highly dependent on its geological origin, which is why the iodine content in animal meat, crops and water varies appreciably within different geological localities (Rasmussen et al., 2009). In Denmark alone, variations of iodine content in drinking water was observed from less than 2 µg/L to more than 30 µg/L, with a small town reaching 100 µg/L (Rasmussen, Larsen & Ovesen, 2000). Furthermore, in some countries iodine is given to dairy cattle along with mineral supplements, and for countries consuming high amounts of milk and dairy products, this may be the most important source of iodine. Iodine added in food processing and iodine fortifications, such as iodised salt, are still the most important sources internationally. However, even in countries with iodine fortification, intake of other iodine-rich foods can have a significant impact on the total iodine intake (Rasmussen et al., 2009).

3.1.1 Iodine requirements

Iodine cannot be stored in the body for long periods and therefore a daily supply is required (Vandepas, 2006). Table 3-1 shows the iodine intake recommended by the WHO[1] and the Institute of Medicine US Food and Nutrition Board (IOM). The recommendations by the WHO are based on the intake estimated to cover the needs of "nearly all" healthy individuals in the specified life stage (WHO, 2007). For adolescents and adults they recommended a daily iodine intake of 150 µg. During pregnancy and lactation there is an increased requirement of iodine. This is due to at least three factors; an increased requirement of thyroxin (T_4) to maintain a normal metabolism in the mother, transfer of T_4 and iodide from mother to foetus and an increased loss of iodide through the kidney (Delange, 2004). Pregnant and lactating women are therefore recommended a daily iodine intake of 250 µg (WHO, 2007). The IOM operate with slightly other values, which are defined as the average daily intake sufficient to meet the iodine requirement of 97-98 % of healthy individuals in a life stage (IOM, 2001).

Table 3-1 Recommended daily intake (RDI) of iodine (µg/day) by age or population group

WHO		IOM	
Age or population group	RDI (µg/day)	Age or population group	RDI (µg/day)
Children 0-59 months	90	Infants 0-12 months	110-130
Children 6-12 yr	120	Children 1-8 yr	90
		Children 9-13 yr	120
Adults > 12 yr	150	Adults ≥ 14 yr	150
Pregnancy	250	Pregnancy	220
Lactation	250	Lactation	290

Sources: Institute of Medicine US Food and Nutrition Board [IOM] (2001); WHO (2007).

Tolerable upper level intakes of iodine

While a physiological amount of iodine is required for insuring a normal thyroid function, a large excess of iodine can be harmful as well. It has not been easy to define the upper threshold limit of iodine intake, as it is affected by the level of iodine before exposure to iodine excess. Consequently, both basal status of iodine intake and age will determine the upper limit of iodine intake (WHO, 2004a).

Although the upper limit of iodine intake among lactating women has not yet been defined by the WHO, the WHO has suggested that a daily intake above 500 µg should not be exceeded

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[1] These recommendations are also used by UNICEF and ICCIDD.

by pregnant and lactating women, as it would not provide any additional benefit for health. Levels above may on the contrary be associated with impaired thyroid function (Andersson, de Benoist, Delange & Zupan, 2007). According to the European Commission's Scientific Committee on Food (SCF), iodine intake of 600 µg/day in adults, including pregnant and lactating women is considered to be acceptable (SCF, 2002), while the IOM has concluded that intakes up to 1100 µg/day in adults and pregnant women is acceptable due to the lack of evidence for adverse health effects at exposures significantly higher than this (IOM, 2001).

3.2 Iodine and thyroid metabolism

Iodine is crucial in the synthesis of thyroid hormones thyroxin (T_4) and triiodothyronine (T_3), which are vital regulators of the metabolic rate, physical - and mental development in humans (Sharp, 2011).

Most iodine ingested, is reduced in the gut and absorbed almost completely in the proximal small intestine as iodide. Some iodine-containing compounds, such as thyroid hormones, are absorbed intact. In the circulation, iodide is removed by the thyroid gland and the kidney. With an adequate iodine supply, approximately 10 % of absorbed iodine is taken up by the thyroid. However, this fraction can exceed to 80 % in the presence of chronic iodine deficiency (Zimmermann, 2009). Other tissues may concentrate iodine, such as the salivary glands, choroid plexus and the lactating mammary gland. These tissues employ a similar mechanism as the thyroid for the uptake of iodide (Dorea, 2002; Sharp, 2011). The metabolism of molecular iodine may differ somewhat from that of iodide, with less uptake by the thyroid and more to fatty tissues, including the mammary gland (Dunn, 2006). With the exception of the lactating breast, the other tissues are considered minor pathways of uncertain significance (IOM, 2001).

The human body contains about 15-20 mg of iodine. From this, around 70-80 % is found in the thyroid gland (Fisher & Oddie, 1969; Semba & Delange, 2008). The iodide is selectively concentrated in amounts required for adequate thyroid hormone synthesis, while the remaining iodine is removed from the blood by the kidney and excreted in urine (Sharp, 2011).

3.2.1 Thyroid hormone synthesis

The human thyroid gland, which is located in the lower part of the neck wrapped around the front of the trachea, is composed of many spherical structures called follicles. Each of these

follicles contains a thick, gel-like substance called colloid. This space is filled with a large glycoprotein called thyroglobulin (Tg), which contains the thyroid hormones T_4 and T_3 (Vander, Sherman & Luciano, 2001).
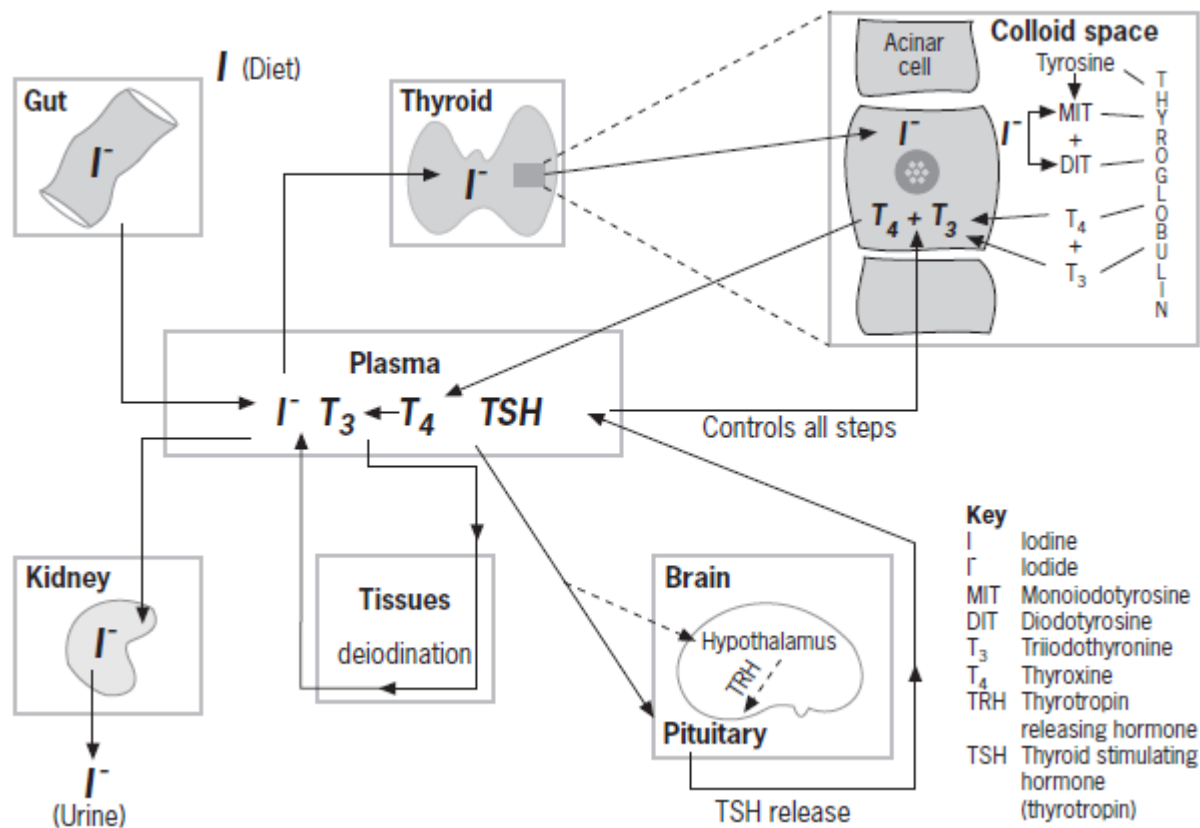
Figure 3-1 Thyroid hormone production and regulation (WHO, 2004a).

Circulating iodide is taken up by a sodium iodide transporter (NIS) and transported actively from the circulation into the thyroid cell (figure 3-1). From there, it migrates to the cells colloidal space, where it is oxidised to iodine (I_2) by a thyroid peroxidase enzyme (TPO). The iodine reacts with tyrosine within the peptide chains of Tg and form monoiodotyrosine (MIT) and diiodotyrosine (DIT). The molecule then undergoes a coupling reaction that forms T_4 and T_3. Thyroglobulin, still containing the hormones, is stored in the follicular lumen until needed. Endosomal and lysosomal protease digests Tg and release the hormones into the circulation. In the circulation, T_4 and T_3 rapidly attach to several binding proteins synthesised in the liver, which migrates to target tissues. Here, T_4 deiodinates to the metabolically active form T_3. The iodine of T_4 returns to serum iodine and the cycle of iodine follow again or excrete in the urine. Iodine remaining as DIT and MIT in Tg is recycled within the thyroid. This is an important mechanism for iodine conservation. (Dunn, 2006; Vanderpas, 2006).

3.2.2 Regulation of thyroid hormone synthesis

There are several factors modulating hormone synthesis and release. The major regulator of thyroid function is thyroid stimulating hormone (TSH), also known as thyrotropin. This protein hormone is secreted by the pituitary in response to circulating levels of thyroid hormone. The TSH secretion increases when circulating thyroid hormone decreases. Several sites within the thyrocyte are affected by TSH, the principal actions being to increase thyroidal uptake of iodine and to break down Tg in order to release thyroid hormone into the circulation. The secretion of TSH is in turn regulated by thyrotropin-releasing hormone (TRH), which is secreted by the hypothalamus and responsive both to thyroid hormone and to TSH feedback (Dunn, 2006).

3.2.3 Action of thyroid hormones

Most of the body cells are affected by the action of thyroid hormones. Some of the major functions of thyroid hormones are the maintenance of the metabolic rate, cellular metabolism, physical - and mental development (Sharp, 2011).

Metabolism

Thyroid hormones regulate the basal energy economy of the body by regulating the basal metabolic rate and have major effects on the metabolism of proteins, carbohydrates and lipids. As a result the hormones set basal rate of body heat production and of oxygen consumed by the body, also known as the calorigenic action of the thyroid hormones. A wide variety of physiological activities, including heart rate, respiration and oxygen consumption are affected by this action. Much of the regulation thyroid hormones are involved in occurs through their influence on gene expression, however the thyroid hormones may influence energy metabolism through direct or indirect regulation of mitochondrial activities (Goglia, Moreno & Lanni, 1999; Semba & Delange, 2008).

Growth and development

Thyroid hormones are essential for normal body growth as the synthesis of growth hormone (GH) partly is regulated by thyroid hormones and physiological concentrations of circulating thyroid hormones appears to be necessary to maintain normal pituitary GH secretion (Giustina & Wehrenberg, 1995). They play a major role for postembryonic or perinatal growth and development, including functional differentiation of bone. Thus, thyroid dysfunction may lead to severe disturbances in bone metabolism (Klaushofer et al., 1995).

Theoretical background

Brain development

Thyroid hormones play an important role in the development of the brain and the central nervous system during foetal and neonatal life (Chan & Kilby, 2000; Delange, 2000). With an insufficient supply of thyroid hormones during prenatal and postnatal period of differentiation and maturation of the brain, mental retardation occurs.

3.3 Iodine deficiency

It is estimated that 2 billion people in the general population have insufficient iodine intake (de Benoist et al., 2008). Iodine deficiency has many adverse health effects, which collectively are termed iodine deficiency disorders (IDD). These disorders results from inadequate production of thyroid hormones due to insufficient amount of iodine. The main consequences of the IDD are goitre, cretinism, damaged reproduction, increased foetal and infant mortality and socioeconomic deprivation (Dunn, 2003; Zimmermann, Jooste & Pandav, 2008).

Goitre

Goitre is one of the classic signs of iodine deficiency, and can take place at all ages, including in newborn (Zimmermann et al., 2008). It begins as an adaption to iodine deficiency. When iodine intake is low, secretion of TSH increases in order to maximize uptake of available iodine. As the thyroid continues to secrete inadequate amounts of hormone, more TSH is released by the pituitary, with all its various effects including thyroid enlargement. Goitre is initially characterised by diffuse homogenous enlargement, so-called "simple-goitre", reflecting generalised hyperplasia, but with continued iodine deficiency or an increased demand of thyroid hormone, such as in pregnancy and adolescence, the cycle may begin anew with increased TSH and further thyroid enlargement. Over time the hyperplasia becomes more focal and nodules are often developed, resulting in multinodular goitre. The nodules may be adenomas, cysts, collections of colloid or at times follicular cancer (Dunn, 2003).

The prevalence of goitre is higher in women than in men. It is not due to larger thyroids or that it is not a continuum of larger thyroid volumes among women, but rather that a larger proportion of women develop growth of the thyroid when exposed to iodine deficiency. In areas with severely iodine-deficiency goitre prevalence has a peak early in life, while in areas with mild iodine-deficiency the peak appears around middle age or later (Knudsen et al., 2002).

Cretinism

Cretinism is caused by severe iodine deficiency in utero and is considered to be the most severe effect of iodine deficiency. It is characterised by the combination of mental retardation, along with varying degrees of short stature, deaf-mutism and motor rigidity, or less commonly by severe hypothyroidism (Hetzel, 1994; Zimmermann et al. 2008). The two phenotypes are described as neurologic cretinism and myxedematous or hypothyroid cretinism, where the former is attributed to the lack of sufficient thyroid hormone in the critical period in foetal development, while the latter is thought to come from sustained hypothyroidism in late foetal life and early postnatal life (Dunn, 2003). Both types of cretinism may occur together or separately and are often associated to areas with severe iodine deficiency sufficiently to cause goitre in 30 % or more of the population (Hetzel, 1994).

IDD through lifespan

Damaged reproduction is the most important disorder induced by iodine deficiency (Dunn & Delange, 2001). During pregnancy, insufficient iodine impairs the development of the brain and severe iodine deficiency increase the risk of stillbirths, abortions and congenital abnormalities of the foetus, further with an increased risk of infant mortality and cretinism (Zimmermann et al., 2008). According to a meta-analysis conducted by Bleichrodt and Born (1994), children living in iodine deficient areas showed a reduced intelligence quotient (IQ) of 13.5 below children from an iodine sufficient area (Bleichrodt & Born, 1994). In adults, a further consequence of iodine deficiency is impaired socioeconomic development, as they are less educable, make poorer employees and are less productive than their iodine-sufficient peers (Dunn, 2003).

Effect of goitrogens and other micronutrients

Generally, IDD are primarily caused by insufficient iodine intake, but substances known as goitrogens can interfere with synthesis and utilisation of thyroid hormones (Engel & Lamm, 2003; Gaitan, 1990). Exposure to goitrogens, such as thiocyanates can occur through the ingestion of staple food, such as cassava, maize and sweet potato, and this may aggravate the effects of iodine deficiency by inhibiting the trapping of iodide by the thyroid gland. Flavonoids, generally found in millet, sorghums, beans and groundnuts, may inhibit the thyroid peroxidase, the peripheral metabolism of thyroid hormones and further the action of TSH (Gaitan, 1990).

Coexisting deficiencies of iodine, iron, selenium, vitamin A and zinc can impair thyroid function, as these elements are essential for normal thyroid hormone metabolism (Zimmermann 2006; Zimmermann & Kohrle, 2002). While iron deficiency impairs the thyroid hormone synthesis by reducing activity of heme-dependent thyroid peroxidase and limit the effectiveness of iodine intervention programs (Zimmermann, Adou, Torresani, Zeder & Hurrell, 2000), combined selenium and iodine deficiency may lead to myxedematous cretinism (Zimmermann & Kohrle, 2002).

3.3.1 Prevention of IDD

Endemic goitre and cretinism has been recognised as public health problems for centuries and iodine deficiency is considered to be the most common cause of preventable mental retardation. In 1851 it was hypothesised that iodine deficiency was the cause of goitre. However, little attention was paid to iodine deficiency in public health programs until the period of 1970-90 when controlled studies showed that iodine supplementation not only eliminated, but also improved cognitive function in the remaining populations. Today, most countries integrate the control of iodine deficiency as a part of the national nutrition strategies (Zimmermann, 2008a).

Several interventions of proven high efficacy, such as iodised oil capsules (IOC) and universal salt iodisation (USI)[2] have been used to eliminate iodine deficiency (UNICEF, 2008). However, the goal to virtually eliminate iodine deficiency within 2000 set by the World Summit for Children in 1990 (UNICEF, 2007) with the support of the United Nations (UN) has not yet been reached. Some progress has been made, but efforts need to be accelerated to eliminate this debilitating health issue. Not only iodine deficiency, but excessive intakes of iodine should be monitored as well, as this is an issue of concern in some localities, including the Saharawi refugee camps.

3.4 Iodine excess

Based on an analysis of urinary iodine in school-aged children from 1993-2003 conducted by Andersson et al. (2005), it was shown that 29 countries had a median urinary iodine which was more than adequate (> 200 µg/L). Among these, five countries had an excessive iodine intake shown by median urinary iodine exceeding 300 µg/L (Andersson, Takkouche, Egli,

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[2] USI is defined as when all salt for human and animal consumption is iodised to the internationally agreed recommended levels (WHO, 2007).

Allen & de Benoist, 2005)[3]. More recently, national representative data on urinary iodine in school-aged children collected between 1997 and 2006 was published. These estimates were used alongside 2003 estimates for the remaining countries without new data to generate updated global and regional estimates of iodine nutrition. Here, iodine intake was more than adequate, or even excessive, in 34 countries (> 200 µg/L), which is an increase from 29 in 2003 (de Benoist et al., 2008)[4]. Data were however insufficient to estimate the global prevalence in pregnant women. As described in the previous section, iodine deficiency is associated with the development of thyroid function abnormalities. Similarly, iodine excess can also impair thyroid function, as the effect of iodine on the thyroid is U-shaped (Clark, 1990; Laurberg et al., 2009; Seal, Creeke, Gnat, Abdalla & Mirghani, 2006).

3.4.1 Sources of excessive intake of iodine

Excess intake of iodine may occur through consumption of foods such as seaweed, milk, bread, salt and natural iodine-rich drinking water (Pennington, 1990, Roti & Uberti, 2001, Zhao, Chen & Maberly, 1998). Various drugs and food preservatives contain large amounts of iodine. Dietary supplements can contain iodine, considered to be the physiological daily requirement (Roti & Utiger, 2001). Radioactive substances used in certain X-ray procedures (Pennington, 1990) and water filtration systems (Pearce et al., 2002) may also contribute to excess intake of iodine.

3.4.2 Consequences of excess iodine

The effect of iodine on thyroid function is highly variable and depends on the health of the thyroid gland (Dunn, 1998; Lee, Bradley, Dwyer & Lee, 1999). While some individuals tolerate large excess without side effects, others may respond adversely to levels close to recommended intakes (table 3-1). During pregnancy and lactation, high levels of iodine absorbed by the mother, particularly from iodine-rich food, drinking water, medicines or drugs, can harm the health of the infants (Sun & Yang, 2009). In a normal healthy thyroid, most adults can tolerate iodine levels up to 600-1100 µg per day (Eastman & Zimmermann, 2009). However, the levels are much lower among those who have been exposed to iodine deficiency for a prolonged period, those with thyroid disorders and those who are sensitive to iodine (Eastman & Zimmermann, 2009; Pennington 1990). Healthy adults exposed to short-

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[3] Data based on national studies on UIC in 126 of the 192 WHO Member States. Data is missing for the remaining 66 WHO Member States (Andersson et al., 2005).

[4] Data based on national studies on UIC in 130 of the 193 WHO Member States. New national representative data were available for 41 countries, which are used alongside 89 country estimates produced in 2003 (of which 53 are national representative). Data were missing for the remaining 63 WHO Member States (de Benoist et al., 2008).

term high levels of iodine intakes may have mild inhibitory effects on thyroid function, while consequences of prolonged exposure to high intakes of iodine, particularly in children, are less clear and more research is needed (Zimmermann, Ito, Hess, Fujieda & Molinari, 2005). The possible consequences of iodine excess are thyroiditis, hypothyroidism, goitre, hyperthyroidism, sensitivity reactions and acute responses (Pennington, 1990).

Iodine-induced thyroiditis

Iodine supplementation may lead to the induction of autoimmune thyroiditis and excessive iodine intake may precipitate spontaneous thyroiditis in strains genetically predisposed for this. The mechanisms possibly involved in iodine-induced thyroiditis include damage to the thyroid and cell injury by free radicals and triggering of autoimmune reactivity by increasing the immunogenicity of Tg (Eastman & Zimmermann, 2009). The incident of thyroiditis is usually higher in iodine replete populations rather than iodine deficient populations and iodine has been incriminated as partially responsible for the increased incidents following the introduction of iodised salt (Pennington, 1990). This was for instance observed in Turkey, where iodine supplementation was used to eliminate iodine deficiency in Eastern Black Sea Region (Emral, Bastemir, Erdogan & Gullu, 2006). The supplementation resulted in the elimination of iodine deficiency, but was accompanied by an increase in the prevalence of autoimmune thyroiditis and thyroid dysfunction (Emral et al., 2006).

Iodine-induced hyperthyroidism

Iodine prophylaxis has eliminated endemic goitre in many countries, but its main complication has been iodine-induced hyperthyroidism (IIH) (Wiersinga & Braverman, 2003). A multicenter study conducted in seven African countries showed that the occurrence of IIH after the introduction of iodised salt was due to poor monitoring of the quality of the iodised salt and of the iodine intake in the population. Countries which recently (less than 2 years) had been introduced to iodised salt were more exposed than others (Delange, de Benoist & Alnwick, 1999).

The biological basis of IIH most often appears to be mutational events in the thyroid cells, which leads to autonomy of function. When the mass of cells becomes sufficient and the supply of iodine is increased, thyrotoxicosis may occur. These changes can occur in localised foci in thyroid gland or in the process of nodule formation. Only some nodules keep their capacity to store iodine, become autonomous and result in hyperthyroidism after iodine supplementation. Elderly are principally affected to the risks of IIH and the incidence reverts

to normal or even below normal after one to ten years of iodine supplementation (Eastman & Zimmermann, 2009; Stanbury et al., 1998). In iodine-sufficient areas, IIH has been reported in patients with and without previous thyroid disease (Roti & degli Uberti, 2001). By monitoring iodine exposure and measure its effects in humans, IIH can to some point be prevented (Dunn, Semigran & Delange, 1998).

Iodine-induced hypothyroidism

Despite the highly effective regulatory mechanism, iodine excess can induce hypothyroidism, with or without goitre (Wiersinga & Braverman, 2003). Iodine is efficiently handled by the thyroid gland when the availability of iodine becomes scarce, as well as when iodine is available in excessive quantities. The thyroid handles the latter situation by acutely inhibiting the organification of iodine, also known as the Wolff-Chaikoff effect. It prevents the thyroid from synthesising large quantities of thyroid hormones by rejecting the large quantities of iodide. The acute Wolff-Chaikoff effect last for a few days and then, through the so-called "escape" phenomenon, the organification of iodide resumes and the normal synthesis of T_4 and T_3 returns. However, in some normal individuals, newborn and foetuses, in patients with autoimmune thyroiditis, Graves' disease, patients previously treated with radioimmunoassay (RAI), surgery or antithyroid drugs, the escape from the inhibitory effect of large doses of iodide is not achieved and clinical or subclinical[5] hypothyroidism ensues (Markou, Georgopoulos, Kyriazopolou & Vagenakis, 2001). Patients with underlying or previous thyroid disease are particularly prone to iodine-induced hypothyroidism (Clark, Cavalieri, Moser & Ingbar, 1990).

Studies have demonstrated vulnerability of the thyroid gland to excess iodine exposure during prenatal and postnatal period. Maternal ingestion of iodine has caused neonatal hypothyroidism and goitre (Wiersinga & Braverman, 2003). In Korea, dietary iodine intake in lactating women was reported to be high, causing subclinical hypothyroidism in their preterm infants due to excessive iodine intake from breast milk with a median breast milk iodine concentration of 2529, 1153 and 822 µg/L at the first, third and sixth weeks, respectively (Chung, Shin, Yang, Choi & Kim, 2009).

While rare events of hypothyroidism was found in healthy adults residing in Hokkaido, Japan, who consumed large quantities of iodine-rich seaweed (approximately 200 mg/day) (Suzuki,

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[5] Mild hypo- or hyperthyroidism is often referred to as subclinical hypo - or hyperthyroidism (Dayan & Panicker, 2009). It is a state where serum TSH is above or below the upper and lower reference limit for the assay, while free T_4 is within the reference interval of the assay (Karmisholt, Andersen & Laurberg, 2011).

Theoretical background

Higuchi, Sawa, Ohtaki & Hornichi, 1965), the incidence of hypothyroidism was detected in 12.1 % of Japanese subjects with a morning urinary iodine excretion of ≥ 75 μmol/L, in contrast to 2.3 % in subjects with a lower urinary iodine excretion (Konno, Makita, Yuri, Iizuka & Kawasaki, 1994). Furthermore, both Tajiri et al. (1986) and Mizukami et al. (1993) found results indicating the existence of a reversible type of hypothyroidism sensitive to iodine restriction characterised by relatively minor changes in lymphocytic thyroiditis. This suggests that attention should be given to this type of hypothyroidism, as thyroid function may revert to normal with iodine restriction alone (Mizukami et al., 1993; Tajiri, Higashi, Morita, Umeda & Sato, 1986).

Iodine-induced goitre

The pathophysiology of endemic goitre caused by excessive iodine intake is not well defined, but may involve a damaged thyroid parenchyma (Wolff, 1969). A study conducted by Boyages et al. (1989) found major autoantibodies of thyroid growth-stimulating immunoglobulins (TGI) (60 %) in children with goitres caused by iodine excess, while none of the healthy children were positive for TGI. By this they concluded that autoimmune growth factors such as TGI may play a primary role in the pathogenesis of thyroid growth in this condition (Boyages et al., 1989). More recently, Zimmermann et al. (2005) conducted a study on 6 to 12 years old children supporting previous studies indicating that increased thyroid size associated with high iodine intake may be due to autoimmune-mediated lymphoid infiltration of the thyroid, inhibition of thyroid hormone release that increases serum TSH and thyroid stimulation, or both (Zimmermann, et al., 2005).

High prevalence of thyroid dysfunction and goitre due to chronic iodine excess from water filters was observed in a survey of 102 Pearce Corps volunteers in Niger, West Africa (Pearce et al., 2002). During the prolonged excess iodine exposure, it was found a marked increase in serum total iodine concentration, and the prevalence of goitre, elevated serum TSH and elevated serum thyroid peroxidise antibody (TPOAb) values was increased. All abnormalities decreased after removal of iodine excess from the drinking water, with a decrease in goitre prevalence from 44 % of the subjects during excess iodine ingestion to 30 % after removal of excess iodine (Pearce et al., 2002). In the Saharawi refugee camps in Algeria, endemic goitre was also found to be due to excessive iodine intake. Drinking water and milk were the main dietary sources with high levels of iodine content (Henjum et al., 2010).

4 SUBJECTS AND METHODS

4.1 Study area

Since 1976, a large number of Saharawi refugees have moved from Western Sahara toward the Algerian desert near Tindouf. Western Sahara stretches from the southern border of Morocco to Mauritania and reaches inlands from its coastline to the borders of Algeria and Mauritania. The Saharawi people are the native inhabitants. Western Sahara was a Spanish colony from 1884, until Morocco invaded the country when Spain withdrew in 1975. The Moroccan and Mauritanian forces encountered resistance from the Saharawi movement, known as the Polisario, but the Moroccan troops had already seized control over the northern, and rapidly the southern zone. In 1991, UN negotiated a ceasefire and the mission was to organise a referendum that would allow Saharawi people to vote. This has still not taken place (Human Rights Watch [HRW], 2008).

Figure 4-1 Localisation of the four Saharawi refugee camps (Source: UNHCR, 2006).

The refugees live in the camps named Smara, Auserd, Dajla and Aaiun, around Tindouf (figure 4-1). Due to harsh geographical and climate conditions, the refugees are a vulnerable group dependent on food aid from the abroad. Until 1985, the main assistance was provided by the Government of Algeria, through the Algerian Red Crescent. After the request of the Government, United Nation High Commissioner of Refugees (UNHCR) and World Food Program (WFP) have assisted in meeting the basic food and non-food needs of the refugees together with the European Community Humanitarian Office (ECHO) and different non-governmental organisations (UNHCR/WFP, 2007). However, the refugees still remain vulnerable.

4.2 Study design and subjects

A baseline for a cohort study was carried out during October and December 2010 in the Saharawi refugee camps. In the cohort, women and their infants will be followed over time to monitor their health outcomes. The next follow up is set out to be two years after the baseline.

Among the refugees living in the camps, lactating women with infants between 0-7 months was the target group chosen for this study. The WHO recommends the inclusion of pregnant and lactating women and further young children in iodine studies as they are the most vulnerable groups of iodine deficiency. Fertile women are vulnerable as iodine deficiency during pregnancy may reduce production of thyroid hormones and cause damages on the foetus (WHO, 2004b, 2007). The WHO have further proposed specific iodine studies concerning these groups, as more research in this field is needed. Some of the proposals are to investigate the relationship between high intakes of iodine and thyroid disease and function, investigate breast milk iodine concentration (BMIC) and identify the best indicators to assess iodine nutrition and monitor the impact to control iodine deficiency and excess (Andersson et al., 2007).

4.2.1 Sample size

The study was conducted on a representative sample of lactating women from the four refugee camps. To estimate the sample size, the prevalence of goitre, which was 18 % among the women between the ages of 15-45 from the study conducted in 2007, was used (SMH/NCA/AUC, 2008). The required sample size was calculated using Epi Info Statcalc. With a 95 % confidence interval and by accepting a 5 % margin of error, it was estimated that the sample size should consist of 100 women. A 10 % of dropout throughout the cohort was considered and it was decided to include 110 women.

4.2.2 Selection procedure

It was not possible to access name list of the population in the refugee camps for the random selection of women. Therefore, women were collected to participate through the dispensaries[6] in the dairas in each of the four camps. A daira is a district with its own dispensary and each of the four camps consisted of 6-7 dairas[7]. It was desired to include women from all dairas. To estimate the number of participants needed from each camp and daira, a list of beneficiaries[8] was used. It was assumed that an equal percentage of lactating women lived in each camp and daira and the sample size of 110 women were divided according to the number of beneficiaries. This gave an equal division of women from the low iodine area (Smara and Dajla, n = 56) and women from the high iodine area (Auserd and Aaiun, n = 55) (appendix 4).

When arriving to a camp, the Saharawi health director informed the director of the hospital in the camp and the director of the dispensary in each daira about the purpose of the study. The inclusion criteria for the participating women were that they had infants between 0-7 months and were breast feeding. The director from each dispensary was asked to find women from their registry or women who they knew matched the criteria and ask if they wanted to participate.

The selection procedure was performed a day before the household visits in each daira. When arriving to the daira, women were brought to the dispensary and were informed about the study and the consent letter by a member of the research team. Those who wanted to participate and fit the criteria received an identification number (ID number) and their names and location was written down on a list together with the ID number. Blood samples were taken at the dispensary and the women received equipment and information about the arrival of the field workers to their households. The next morning a health worker from the dispensary who participated during the selection procedure helped find the location of the women on the list.

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[6] A local health administration which provides the most basic primary healthcare services to the community in the daira.

[7] Although 27 de Febrero is considered to be a separate camp in the Saharawi refugee camps, it is included as a daira of Smara in the present study due to the small population size compared to the other camps. It further uses the same public drinking water source as Smara.

[8] Beneficiaries are the number of people in the Saharawi refugee camps who need assistance. People studying abroad, in military service or living in the liberated area are not included. It is considered that more people live in the camps, but these are the numbers of vulnerable agreed upon by the UN and Polisario. The list does not include name or any information about the refugees.

Dropout and final selection

In total, 150 women were asked to participate (table 4-1). Among these, women who did not want to participate, did not have infants between 0-7 months or were not breast feeding, were excluded and gave a total dropout rate of 26.0 %. The final selection consisted of 111 lactating women.

Table 4-1 Cause of exclusion and final selection of women, Saharawi refugee camps 2010

Sample	n	%
Asked to participate	150	100
Excluded women		
Refusal	6	4.0
Infants above 7 months	16	10.7
Not breast feeding	17	11.3
Total exclusion	39	26.0
Final selection	111	74.0

4.2.3 Ethics and Informed Consent

The study was approved by the Regional Committees for Medical and Health Research Ethics (ref.: 2010/2513) and conducted with the permission from the Saharawi health authorities.

During the study, when the team arrived to the dispensaries to recruit participants, women were first explained the purpose of the study using an information sheet, which was translated into Spanish and Arabic. It was emphasised that it was voluntary to participate in the study and that they could withdraw at any time. Further, it was made clear that refusal to participate would have no negative impact on their entitlement to food aid or other services. The women decided for themselves and their infants. If agreement was given, written consent for sample collection, questionnaires and anthropometric measurements was obtained. The English version of the informed consent letter used in the study is given in appendix 1.

As this study is set out to be a cohort, it was necessary to collect name and location of the women and their infants to be able to locate them for the next follow-up. However, these records were only known by the research team and are confidential. By using these lists with name and ID number it was possible to find back to women who had not been able to give samples when the field workers arrived at their household and the field workers could go back Since the study was a request from the Saharawi health authorities, most of the women were positive to participate. No reward was given. However, women were promised feedback of the test results from their samples and anthropometric measurements and help if a serious

health condition would be detected.

4.3 Preparations in field

During the first two weeks, preparations for field work were made and a pilot study conducted.

Training of field workers

A meeting was held at the Saharawi Ministry of Health's office in Rabouni[9]. The meeting included the whole team working within the project and was organised to explain and discuss the purpose and schedule of the project. The team consisted of eight local fieldworkers, 4 men and 4 women, all recruited by the Saharawi health director. Among these, two were nutritionists[10], two bio-engineers, one medical doctor, two translators and one driver. Besides Hasanía and Arabic, all of them spoke Spanish fluently and the translators further spoke English.

The two local nutritionists, together with the Norwegian research team, were responsible for the field work in the households, which included collecting samples of urine, breast milk, public drinking water and milk, collect questionnaires and perform anthropometrical measurements. The questionnaires consisted of background information, breast feeding practices and 24-h and 7 days recall on dietary iodine intake. A one week of theoretical and practical training was given, with a translator present. Overall, the training went well, as the local nutritionists were familiar with conducting similar studies. During the training, some changes in the questionnaires were made after discussions with the nutritionists.

The bio-engineers were present to take blood samples in the evening of recruitment at the dispensary. Both had experience of taking blood samples and only needed a short update of the equipment which was used. The doctor was available as a medical contact.

Translators were primary available to improve communication, reduce misunderstandings and translate questionnaires into Spanish. During the training, one of the translators was further trained to inform participating women of the study and how to use the equipment, which were

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[9] Rabouni is located near the four refugee camps and is the place where the Saharawi president and different ministries are located. The UN organisations have their offices in Rabouni and various NGO's are also located here. Although each of the refugee camps has their own local hospital, the central hospital is located in Rabouni.
[10] Local health workers who previously have been educated and trained in nutrition through a nutritional project conducted by NCA.

distributed the day of recruitment.

Pilot study

After preparations and training of fieldworkers, a pilot study was conducted in a daira called "27. de Febrero". Two lactating women with infants under 7 months were asked to participate. Blood samples were taken by the bio-engineer. When arriving to the household of the women, the nutritionists were responsible for collecting urine and breast milk samples, ask the questionnaires and perform anthropometrical measurements on one woman each. Even though the fieldwork was planned to be conducted in two teams, both teams went together to each household during the pilot. This was done so both nutritionists could watch each other, minimize differences in the method of asking questions, make corrections and adjustments according to the experiences from the pilot. Overall, the pilot went well and only minor changes were made.

Practical performance

Approximately one week was set to conduct field work in each camp (appendix 5). After recruiting, taking blood samples and handing out equipment to women participating, two teams went to the households of the women the next day. The team leader was responsible for organising the team and each team consisted of at least one from the Norwegian research team, which was present to observe and double check the registrations done, before leaving a household. Response that was forgotten to be filled out was completed if possible. Lack of answers or obvious errors discovered in retrospect was set as "missing". Both teams had a car with a driver responsible for transport of team members and equipment. Communication between the field workers and the participating women took place in the Saharawi refugees' mother language, Hasanía.

4.4 Assessment of iodine status and thyroid function

Different methods are used to assess iodine status in populations. However, the methods generally recommended are UIC, thyroid size by neck inspection and palpation and/or thyroid ultrasonography, serum thyroid stimulating hormone (serum TSH) and serum thyroglobulin (serum Tg). These indicators are complementary. While UIC show a more recent iodine intake, Tg shows an intermediate response, whereas changes in goitre rate reflects more long-term iodine nutrition (Zimmermann, 2008b).

In the present study, UIC, BMIC, iodine concentration in public drinking water and animal milk, dietary iodine intake and blood constituents were assessed, along with background questionnaires and anthropometrical measurements. These indicators, shown in table 4-2, are further described in this chapter.

Table 4-2 Methods, variables and number of lactating women or samples used in this study

Age (years)	Variables	Number of women	Number of samples
18-50	Urinary iodine concentration	111	-
18-50	Breast milk iodine concentration	110	-
-	Iodine concentration in public drinking water	-	24
-	Iodine concentration in goat milk	-	13
-	Iodine concentration in camel milk	-	34
18-50	Dietary iodine intake	111	-
18-50	Blood constituents	111	-
18-50	Background questionnaire	111	-
18-50	Anthropometrical measurements	111	-

4.4.1 Urinary iodine

Urinary iodine concentration is currently the key biochemical indicator in epidemiological assessment of iodine status and may assess iodine excess as well as deficiency (Dunn, 1993; ICCIDD, 2000). It is a good marker of recent iodine intake, as approximately 90 % of iodine absorbed in the body eventually excretes in the urine (Vanderpas, 2006). This method is feasible as urine samples are easy to collect in small beakers, which are transferred to tubes and tightly sealed with screw caps. Usually, no more than 0.5-1 ml of urine is required, although it depends on the method used. The samples do not require refrigerator, preservatives or immediate determination. Although they can be kept in the laboratory for months, it is preferable to store the samples in refrigerator to avoid unpleasant odour. Further,

attention to avoid contamination with iodine at all stages is required and evaporation should be avoided, as it may increase the concentration (Bruijns & Smit, 2003; WHO, 2007).

The use of UIC to assess iodine status has its limitation, as iodine excretion in individuals can vary somewhat from day to day, or even within a given day, depending on the degree of hydration (ICCIDD, 2000; Rasmussen, Ovesen & Christiansen, 1999). Therefore, a single urine sample from an individual may be misleading in the interpretation of an individual's iodine status. However, this variation tends to even out among populations, if a sufficient number of specimens are collected (WHO, 2007; Zimmermann, 2008b). A 24 hour sample is preferred for the measurement of urinary iodine, but is difficult and impractical to obtain under field conditions (Hetzel & Dunn, 1989). Instead casual samples have been proposed. This method reflects a 24 hour collection, if taken in adequate numbers within a population (Dunn, 1993). According to a study conducted by Andersen et al. (2008), it has been estimated that 100-500 casual urine samples are required from each group or sub group to assess the population iodine excretion (Andersen, Karmisholt, Pedersen & Laurberg, 2008). Still, limitation can occur if the distribution of a population is heterogeneous and reliability is reduced if there are wide differences in diet or socioeconomic background (ICCIDD, 2000). It can further depend on the urinary volume produced by the group (Laurberg et al., 2007).

Analysis of urinary iodine

Most of the methods used to analyse urinary iodine depend on the role of iodide as catalyst in the reduction of ceric ion (Ce^{4+}) to cerous ion (Ce^{3+}) coupled to the oxidation of arsenite (As^{3+}) to arsen (As^{5+}). This reaction is known as the Sandell-Kolthoff reaction (Dunn, 1993). The colour of the ceric ion is yellow, while the cerous ion is colourless. Thus, the course of the reaction can be followed by the disappearance of the yellow colour as the ceric ion is reduced. The colour disappearance is directly proportional to the amount of iodide catalysing it if other reactants are held stable and iodine levels down to several nanograms can be detected. This specificity and high sensitivity has made the Sandell-Kolthoff reaction the basic method for the detection of iodine in urine (Dunn, 1993). However, a digestion or ashing step is necessary to prepare urine samples by this method for the removal of interfering substances, such as nitrate and thiocyanite. Ammonium persulfate is considered to be one of the safe alternatives as the oxidizing agent to eliminate the interfering substances in the urine before the colorimetric measurement by the Sandell-Kolthoff reaction (Pino, Fang & Braverman, 1996).

Assessment of urinary iodine concentration in the refugee camps

Urine samples were collected using the casual sampling method. Women received urine beakers the day of recruitment and the beakers were collected from their household the day after. Information was given on how to use the beaker and women were told to only use the beaker to collect urine sample to avoid contamination. If needed, because of loss or contamination, the women received a new beaker. As urine samples from both women and infants were collected, ID number and a sign was marked on the beaker for women and field workers to clearly see who it belonged to.

Picture 4-1 Vacuette Urine System

Vacuette Urine System was used to collect urine samples (picture 4-1). Urine samples were collected in 100 ml sterile urine beakers of plastic with screw caps and integrated transfer device. Urine was aspirated from the urine beakers into self-priming 9.5 ml Vacuette vacuum urine tubes by the field workers and marked with ID number. The tubes were sealed to prevent evaporation. The vacuum tubes were stored in a freezer at -20°C during the field study, before they were transported to Norway and kept in a cold storage at -20°C at AUC. A week later they were transported by World Courier to the Nutritional Intervention Research Unit (NIRU) in Cape Town, South Africa, for analysis. Urinary iodine concentration was determined using the Sandell-Kolthoff reaction. Ammonium persulfate was added before analysis for the removal of any interfering substances. Urine was diluted 1:2, 1:5, 1:10 and 1:20 to determine the absolute iodine values of the samples.

Interpretation

The epidemiological criteria for assessing iodine nutrition based on UIC are presented in table 4-3. Children and non-pregnant women with median UIC between 100-199 µg/L are considered by the WHO to have optimal iodine nutrition. According to the values of UIC, the iodine intake and nutrition in these groups can be defined. This does however not apply for pregnant and lactating women or children less than two years old. To define adequate dietary iodine intake among lactating women, a median UIC of 100 µg/L can be used. However, no other categories of iodine intake are defined. The reason why lactating women have lower

median UIC than pregnant women, although they have the same requirements, is because of iodine excretion in breast milk (Andersson et al., 2007; WHO, 2007).

Table 4-3 Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations (UIC) in different population groups

Median UIC (µg/L)	Iodine intake	Iodine nutrition
School aged children		
< 20	Insufficient	Severe iodine deficiency
20-49	Insufficient	Moderate iodine deficiency
50-99	Insufficient	Mild iodine deficiency
100-199	Adequate	Optimal
200-299	Above requirements	Likely to provide adequate intake for pregnant/lactating women, but may pose a slight risk to the overall population
> 300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid diseases)
Pregnant women		
< 150	Insufficient	
150-249	Adequate	
250-499	More than adequate	
≥ 500	Excessive	
Lactating women		
< 100	Insufficient	
≥ 100	Adequate	
Children less than 2 years old		
< 100	Insufficient	
≥ 100	Adequate	

Source: (WHO, 2007).

4.4.2 Breast milk iodine

To determine iodine excretion in breast milk, women received Medela Harmony manual breast pumps along with the urine beakers. The bottle was marked with a black line indicating that they had to give a sample of 20 ml, although usually not more than 10-15 ml is required for analysis. They could choose if they wanted to collect milk either by manual expression or use the breast pumps they had received. When arriving to their households the next day, the breast milk samples were collected and bottled in 50 ml plastic tubes and ID number was

written on the bottle. The samples were stored in a freezer at -20 °C during the field study until transported to Norway.

The samples were transported by World Courier to the National Institute of Nutrition and Seafood Research (NIFES) in Bergen, Norway, for analysis. The determination of iodine in milk was carried out using inductively coupled plasma mass spectrometry (ICP-MS), as described by Dahl et al. (2003). A test sample of 1 gram milk was added to 5 ml deionised water and 1 ml tetra methyl ammonium hydroxide (TMAH) and extraction was carried out in a dry oven at 90°C for three hours. Samples were diluted with water and filtered before analysis.

4.4.3 Iodine concentration in drinking water and animal milk

As public drinking water, goat - and camel milk are the main dietary iodine sources in the refugee camps, samples were collected to estimate iodine concentrations, which further were used to analyse the dietary iodine intake among the women.

From each of the four refugee camps, 6 water samples were taken of the public drinking water, for analysis. The reason for the few samples from each camp was that iodine concentration in water has recently in 2007 (SMH/NCA/AUC, 2008) and 2009 (SMH/NCA/UC, 2010) been analysed and no changes in water supply has been made since then. Thus, the samples taken were to evaluate if the concentration has remained the same the past few years and to evaluate the dietary iodine intake among the women. The samples were bottled in 50 ml plastic tubes with screw caps. The screw cap ensured that the plastic tube was tightly sealed to avoid evaporation. Name of the camp where the sample was taken, was written on the tube and stored in room temperature during the field study. Analysis was performed at the NIRU in Cape Town, South Africa. The water samples were analysed by the Sandell-Kolthoff reaction in a similar way as the urine samples, described in section 4.4.1.

Women who consumed goat - or camel milk the last seven days, were asked to give a sample if possible. The milk samples were bottled in the same 50 ml plastic tubes as public drinking water. Milk samples were marked with ID-number, milk type and if the milk was from local animals in the camps or the liberated area[11]. Samples were stored in a freezer at -20°C during the field study, before transported to Norway. The samples were analysed at NIFES in

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[11] It is an area in Western-Sahara close to the Algerian border, which the Saharawi people have managed to liberate from Morocco. Thus, the name "the Liberated area".

Bergen, Norway, using the ICP-MS in a similar way as the breast milk samples described in section 4.4.2.

4.4.4 Dietary iodine intake

To assess dietary intake of a nutrient or a food chemical, food consumption data are combined with data on the concentration of the nutrient or chemical in foods. Depending on the nutrient or food chemical of concern, the resulting dietary intake of this is then compared with the relevant toxicological or nutritional reference value (Parmar, Miller & Burt, 1997).

There are different methods to estimate food consumption, for instance through food consumption surveys at an individual or household level or through food production statistics. Food consumption at the individual level usually provides the most precise estimates (WHO, 2008). Assessments may be undertaken for both current and past intakes. Current intake can be assessed using three main methods; 24-h dietary recall, estimated food records and weighed food records. These methods can be combined to improve accuracy and validation of the data collected (Gibson, 2005).

24-hour recall

A 24-h dietary recall, consist of estimates of types and amounts of foods and beverages consumed by an individual the previous day or 24 hour prior to the recall interview. Additional information, such as the source of the foods, time and place of consumption may also be provided. Usually, the interview is conducted in person by a trained interviewer, but telephone, Internet or self-administered intake may be performed (Gibson, 2005). The advantage of this method is its ability to estimate nutrient intake of population groups and compare nutrient intakes with specific dietary recommendations. However, the major limitation is that a 24-h recall seldom is representative of usual intake, and intake may be under- or overestimated (Gibson, 2005; WHO, 2008).

Assessment of dietary iodine intake in the refugee camps

In order to assess the women's iodine intake, a dietary questionnaire was developed prior to the field study (appendix 3). The 24-h recall was used, because urinary iodine excretion reflects iodine intake the last 24 hours (WHO, 2007). Seven days recall was used to give extended information about the dietary iodine intake. Because of limited time resources, it was decided to only ask about foods and beverages which contained iodine. As public drinking water and milk are the main dietary iodine sources in the camps with a significant content of iodine, intake of public drinking water and milk (goat- and camel milk) was estimated. Further, intake of Cadida[12] -, artificial - and other milk, along

Picture 4-2 A Saharawi woman showing the amount of water consumed the last 24 hours

with tea and soup was estimated as these items may contain a significant amount of water and are consumed regularly by the Saharawi refugees.

The fieldworker started by introducing the women to the procedure of the 24-h and 7 days recall, by first asking about the type of milk consumed the previous day from the time they woke up till the time they went to bed. If milk was consumed they were asked to show the amount, before continuing with water, tea and soup consumed the last 24 hours and 7 days. A digital kitchen scale measuring to the nearest gram was used to determine the amount. Women used their own bowls in which they drank milk, water and ate soup; further their own tea cups to measure the exact amounts of consumption, before pouring it over to the kitchen scale (picture 4-2). If they had consumed a mix of milk and water, they were asked to show the distribution of milk and water separately.

The amounts registered from the 24-h and 7 days recall was combined with the average iodine concentration in public drinking water in the six water samples from each camp and iodine concentration in goat - and camel milk in the samples given by women. For women with no available goat - or camel milk sample, the average iodine concentration in goat- and camel milk from the other samples was used to determine iodine concentration in the milk consumed by the women.

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[12] Processed cow's milk (1.6 % fat content).

4.4.5 Blood constituents

Two blood constituents, TSH and Tg, are recommended for the assessment of iodine nutrition (Zimmermann, 2008b). Both serve as indicators of thyroid function. In a population survey, dried whole blood spots on filter paper or serum samples can be used to measure TSH and Tg (WHO, 2007; Zimmermann, Moretti, Chaouki & Torresani, 2003). Both methods are well established. It is essential to use sterile equipment, either lancets for blood spot collection or needles and syringes for collecting whole blood from which the serum is separated, following the standard procedures for handling blood products or objects contaminated with blood (WHO, 2007).

Thyroid stimulating hormone

TSH levels in neonates are a valuable indicator of iodine nutrition in a population. However, in adults, the difference between individual TSH values in iodine-sufficient groups compared with groups with deficient or excessive iodine nutrition is not great and much overlap occurs. Thus, it may not be a practical marker of iodine nutrition in adults (WHO, 2007).

Serum TSH is however widely used in thyroidology as a sensitive marker for both hypothyroidism and hyperthyroidism (WHO, 2007). Often, TSH alone is used to screen for thyroid diseases. This is usually adequate; however it may miss some rare diagnoses, which is why free T_4 (FT_4) and in some cases free T_3 (FT_3), is measured for initial discovery and identification of thyroid disease. FT_3 assays are generally not as robust as FT_4, but may be useful in some clinical situations, particularly to identify hyperthyroidism (Dayan & Panicker, 2009). Table 4-4 provides a matrix for the interpretation of TSH, FT_4 and FT_3.

Table 4-4 General interpretation of thyroid function tests

	Raised free T_4 or T_3	Normal free T_4 and T_3	Low free T_4 or T_3
Raised TSH		Mild (subclinical) hypothyroidism	Primary hypothyroidism
Normal TSH	Pituitary hyperthyroidism Resistance to thyroid hormone Recent ingestion of T_4	Euthyroid	Pituitary hypothyroidism Nonthyroidal illness
Low TSH	Primary hyperthyroidism	Mild (subclinical) hyperthyroidism	

Source: Dayan & Panicker (2009).

Normal TSH, FT_4 and FT_3 indicates euthyroidism[13], raised TSH and low FT_4 or FT_3 suggests clinical hypothyroidism, and low TSH and raised FT_4 or FT_3 suggests clinical hyperthyroidism. Furthermore, abnormally raised TSH with normal FT_4 and FT_3 suggests subclinical hypothyroidism and low TSH with normal FT_4 and FT_3 suggests subclinical hyperthyroidism. The other combinations of thyroid function tests are rare (Dayan & Panicker, 2009).

Additional thyroid tests for thyroid antibodies, such as thyroid peroxidase antibody (TPOAb) and thyrotropin receptor antibody (TRAb), can be useful to confirm certain diagnosis and in some situations, predict clinical outcomes (Dayan & Panicker, 2009). Today, TPOAb is the most commonly used and the best biochemical analysis to detect autoimmune thyroid disease. TRAb is often used to diagnose autoimmune hyperthyroidism (Bjøro, 2002).

Thyroglobulin

Serum Tg is a sensitive marker of thyroid function and iodine status in epidemiological studies. It is the most abundant protein of the thyroid, by providing the matrix for thyroid hormone synthesis. Small amounts of Tg can be detected in the blood of all healthy individuals. When the thyroid is hyperplastic or injured, much larger amounts are released. (Knudsen et al., 2001; WHO, 2007, Zimmermann et al., 2006). In this setting, it reflects iodine nutrition over a period of months or years, which is in contrast to UIC, which assesses more recent iodine intake (WHO, 2007). Together with UIC, serum Tg is the most appropriate indicator of iodine status and thyroid function under conditions of increasing iodine supply, as serum Tg have close association to thyroid volume, thyroid nodularity and iodine excretion (Knudsen et al., 2001). Excess intake of iodine may on the other hand also inhibit thyroid function, by either the Wolff-Charkoff effect or inhibition of Tg proteolysis with reduction in hormone secretion (Bilek & Zamrazil, 2009). The various physiological and pathophysiological conditions due to iodine intake and concentrations of serum Tg are shown in table 4-5. Normal or sometimes increased serum Tg, depending on iodine intake, suggests eufunction of the thyroid gland. Decreased serum Tg suggests hypofunction, while increased serum Tg suggests hyperfunction of the thyroid gland.

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[13] Normal thyroid gland function.

Table 4-5 Influence of thyroid status and iodine intake on concentration of circulating thyroglobulin (Tg)

	Iodine intake	Serum Tg concentrations
Eufunction of thyroid gland	Deficiency	Increased
	Adequate	Normal
	Excess	Normal or increased
Hypofunction of thyroid gland	Deficiency	Decreased
	Adequate	Decreased
	Excess	Decreased
Hyperfunction of thyroid gland	Deficiency	Increased
	Adequate	Increased
	Excess	Increased

Source: Bilek & Zamrazil (2009).

The presence of circulating antibodies against Tg (TgAb) may however interfere with the determination of serum Tg using immunoassay kits. Thus, it is necessary to measure TgAb at the same time (Bilek & Zamrazil, 2009).

Assessment of blood constituents in the refugee camps

During the stay in each refugee camp, blood samples were taken of women concurrently with the recruitment. If consent was given, blood samples were taken at the dispensary by the bio-engineer in a separate room from the recruitment area (picture 4-3).

Blood samples were collected using BD Vacutainer Blood Collection System. The equipment used was sterile and the bio-engineer always wore gloves before taking

Picture 4-3 Blood sample taken of a woman at the dispensary in one of the refugee camps

the samples. Gel tubes were used to collect blood samples, so the gel could lie as a barrier between the serum and the blood cells after centrifugation. ID number was written on the gel tubes and 4 ml of blood, which gave 2 ml of serum after centrifugation, was collected from each woman. The Vacutainer gel tubes were turned 8-10 times to the sides, before they were left to rest for 30-120 minutes and transported to the local hospital in the camp to be centrifuged.

At the local hospital, the strength of the centrifuge was set between 1500 and 2000 G. A balanced amount of gel tubes were placed in the centrifuge and the samples were centrifuged for 10 minutes. By using a pipette, the serum was transferred over to a new tube which was sealed and labelled according to the ID number on the gel tube. A new pipette was used for each serum sample to avoid contamination. The serum was stored in a freezer at -20°C at the local hospital. After completion in each camp, serum samples were transported in cooler bags with frozen elements and placed at the central hospital in Rabouni at -20°C until transported to Norway, where they were stored in a cold storage of -20°C at AUC. The samples were transported in cooler bags with frozen elements to Oslo University Hospital by car, for analysis.

All serum samples were analysed for TSH, FT_4, TPOAb, Tg and TgAb. When necessary, some were further analysed for FT_3 and TRAb. Analysis of TSH, FT_4, Tg, TgAb, FT_3 and TRAb was performed at the Central Laboratory (MBK) and analysis of TPOAb was performed at the Department of Immunology and Transfusion Medicine at the Oslo University Hospital in Norway. Methods and reference levels used are shown in table 4-6.

Table 4-6 Methods and reference levels used for thyroid function tests

Thyroid function test	Method	Reference level[*]
TSH	Electrochemiluminescence Immunoassay (ECLIA)	0.2-3.4 mIU/L
FT_4	Electrochemiluminescence Immunoassay (ECLIA)	9.0-21.0 pmol/L
FT_3	Electrochemiluminescence Immunoassay (ECLIA)	2.7-6.3 pmol/L
TPOAb	Fluorescent Enzyme Immunoassay (FEIA)	< 34 IU/mL
TRAb	Electrochemiluminescence Immunoassay (ECLIA)	< 1.0 U/L
Tg	Immunofluorometric Assay (IFMA)	< 30 µg/L
TgAb	Fluorescent Resonance Energy Transfer (FRET)	< 50 kU/L

[*]Based on reference levels used by Oslo University Hospital in Norway (MBK, 2011). The levels may differ somewhat from reference levels used by other institutions.

Diagnostic criteria for thyroid diseases

All women with positive TPOAb (> 34 IU/mL) were diagnosed with autoimmune thyroiditis. The presence of TPOAb between 35 and 100 IU/mL may be found in healthy subjects, without thyroid disease and they were thus not included as affected by autoimmune thyroiditis. Clinical hypothyroidism was diagnosed in women with TSH above 4.0 mIU/L and

FT_4 below 9.0 pmol/L or in women with TSH levels above 10.0 mIU/L. Women with TSH levels between 4.0-10.0 mIU/L, but normal FT_4 were diagnosed with subclinical hypothyroidism. Clinical hyperthyroidism was determined in women with low or undetectable TSH ($\geq$ 0.05 mIU/L) and high FT_4. FT_3 and TRAb were measured in women with suppressed TSH, to determine if there was presence of subclinical, clinical or autoimmune hyperthyroidism. If FT_3 was above 10 pmol/L or TRAb above 1.0 U/L, along with suppressed TSH and/or increased FT_4, hyperthyroidism was diagnosed. Subclinical hyperthyroidism was diagnosed in women with decreased TSH > 0.2 mIU/L, but normal FT_4.

4.4.6 Additional questionnaires and measurements

Very little research has been conducted on the nutritional status during lactation and no reference data exist for this target group. However, body mass index (BMI)[14] may be a useful indicator of postpartum nutritional status, as poor maternal postpartum status, reflected in low BMI is associated with poor lactation performance and poor infant growth. The level of BMI below which there is a risk has not been reported, but based on the lower limit of BMI (<18.5 kg/m^2) suggested for thin adults, a cut-off for BMI of 20.3 kg/m^2 at one month postpartum for women 150 cm tall has been estimated. Throughout the first six months of lactation, BMI is expected to decline steadily and the value of 18.5 kg/m^2 as for non-pregnant non-lactating can be used as cut-off for identifying women at risk. Based on the recommendations for modest gestational weight gain by overweight and obese women, upper limit of BMI ($\geq$25 kg/m^2) recommended for non-pregnant non-lactating women would most likely apply to lactating women as well (WHO, 1995).

Background questionnaires and anthropometrical measurements in the refugee camps

Background questionnaire was developed prior to the study and information, such as age, marital status and education, was collected from each participating woman (appendix 2). Women were further measured for height and weight to calculate BMI. Weight was determined using Seca UNICEF electronic digital scale measuring to the nearest 0.1 kg. The weights were calibrated before the start and during the field work. Participating women were asked to take of shoes and as much clothes as possible. Due to cultural sensitivities, women were allowed to wear some clothing. Weight was written down, and according to the amount of clothes on the women the weight of the clothes was estimated and noted. Height was measured by using a height board, with a plate attached 90° at the bottom and an adjustable

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[14] BMI = weight (kg) / (height (m))^2

plate to place 90° at the top of the head. Standing on the height board, women were asked to take of shoes, stand and look straight ahead with buttocks, shoulders and the back of the head in to the height board. Height was noted to the nearest millimetre.

4.5 Data processing and statistical analysis

Data processing and statistical analysis were performed in SPSS version 18.0 (SPSS Inc., 2010) and Microsoft Office Excel 2007 (Microsoft Office Home and Student, 2007). Data were manually entered into SPSS and frequency analysis and analysis of minimum and maximum values for all variables were made to detect any wrong values. Data were examined for normal distribution using Kolmogorov-Smirnovs test. Data on UIC, BMIC, public drinking water, animal milk, dietary iodine intake and blood constituents did not adhere to normal distribution. They were presented as median and 25th - and 75th percentiles and proportion in percentage. Continuous variables were compared by the Mann-Whitney *U* test or Spearman's rank correlation. The Chi-Square test was used to compare proportions of categorical variables. Statistical significance was indicated by $p < 0.05$.

Background information such as age, height, weight and BMI were presented as mean ± SD. BMI was further categorised as underweight (<18.5 kg/m^2), normal weight (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obese (≥30.0 kg/m^2) (WHO, 2006). Variables like marital status, education, and number of childbirths and child deaths were presented as percentage of the total study population.

When analysing for the association between UIC and thyroid function, UIC was further categorised as low UIC (> 100 µg/L), adequate and above requirements UIC (100-299 µg/L) and excessive UIC (≥ 300 µg/L) according to the median UIC definitions for school-aged children (WHO, 2007).

5 RESULTS

5.1 Study population

Background characteristics of the women are presented in table 5-1. The mean (± SD) age was 31 (± 6) years and the mean (± SD) BMI was 26.9 (± 4.6) kg/m^2. Three percent of the women had BMI below 18.5 kg/m^2. Forty-one percent were overweight and 26 % obese. All women in the study were married. Most of them (94%) had some form of education, from less than 6th grade to higher education. Only six percent had higher education. Almost one fifth (19 %) of the women were working outside home. Fifty-three percent had lived in the refugee camps their entire life and 74 % percent had lived in the same refugee camp their entire life. Seventy-seven percent had more than one child and 16% reported at least one child death.

Table 5-1 Background characteristics of lactating women sampled (n=111), Saharawi refugee camps 2010

Characteristic	% (n)	Mean ± SD
Age (years)	-	31 ± 6
Height (m)	-	1.56 ± 0.05
Weight (kg)	-	65.9 ± 11.7
BMI (kg/m^2)*	-	26.9 ± 4.6
BMI < 18.5	3 (3)	-
BMI 18.5-24.9	30 (33)	-
BMI 25.0-29.9	41 (46)	-
BMI ≥ 30.0	26 (29)	-
Married	100 (111)	-
Education	94 (103)	-
Less than 6th grade	22 (24)	-
6th grade - Primary	5 (5)	-
7-9th grade - Secondary	45 (49)	-
10-12th grade - High school	16 (18)	-
Higher education	6 (7)	-
Work outside home	19 (21)	-
Lived in the refugee camps their entire life	53 (59)	-
Lived in the same refugee camp their entire life	74 (82)	-
More than one childbirth	77 (86)	-
At least one child death	16 (18)	-

*BMI calculations based on the WHO BMI classifications for adult underweight (< 18.5 kg/m^2), normal range (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2) and obesity (≥ 30.0 kg/m^2) (WHO, 2006). Note that three of the women less than two months postpartum are listed as normal range, but had BMI less than 20.3 kg/m^2 and can be considered to be underweight (WHO, 1995).

5.2 Urinary iodine concentration

The median UIC among the lactating women was 350 µg/L (range 64-1880 µg/L). Figure 5-1 shows the frequency distribution of the UIC. In total, 5 % had UIC below 100 µg/L, while 36 % had UIC between 100 and 299 µg/L. Thirty-one percent had UIC between 300 and 499 µg/L, 21 % with a concentration between 500 and 999 µg/L and 7 % of the women had UIC exceeding 1000 µg/L.
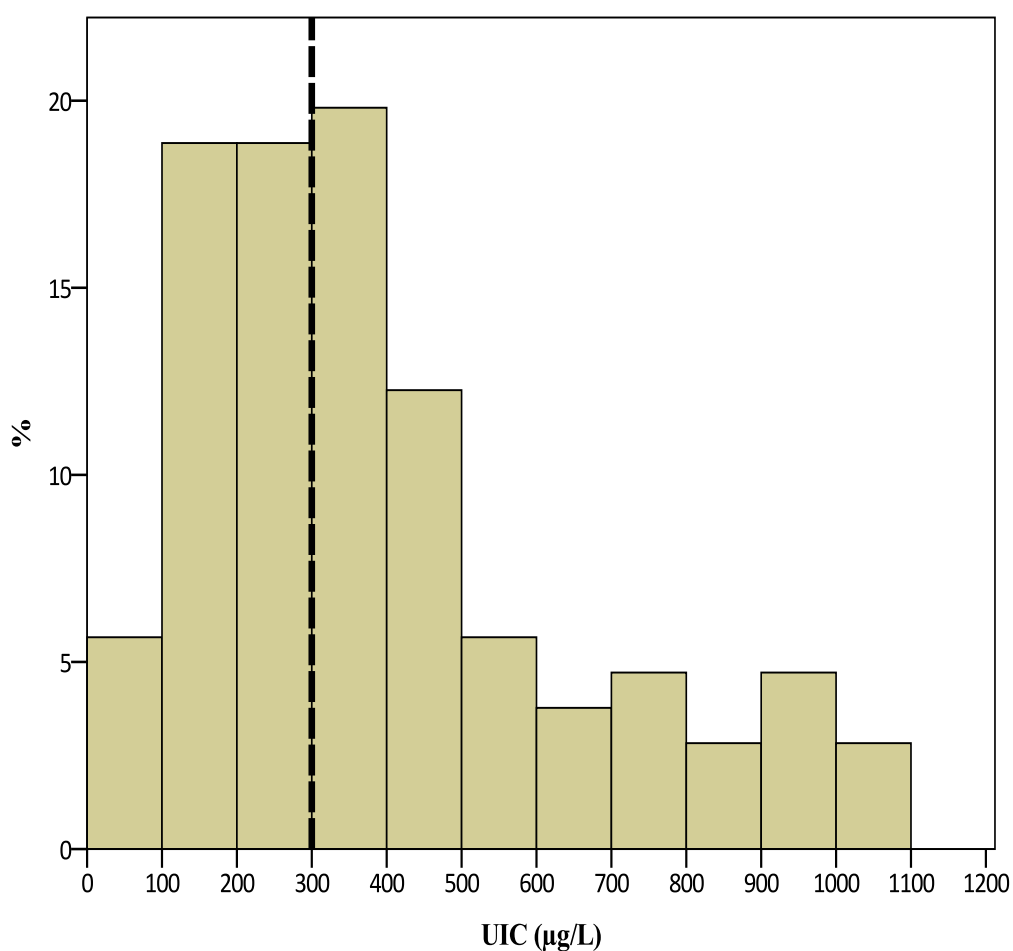
Figure 5-1 Frequency distribution of urinary iodine concentration (UIC, µg/L) in lactating women (n = 106), Saharawi refugee camps 2010. The dashed line indicates the cut-off limit for excessive UIC for school-aged children set by WHO. Five outliers (UIC > 1300 µg/L) are excluded.

5.3 Breast milk iodine concentration

Median BMIC among the women was 479 µg/L (range 132-1760 µg/L). None of the women had BMIC below 100 µg/L (figure 5-2). Eighteen percent had BMIC between 100 and 299 µg/L, 36 % had BMIC between 300 and 499 µg/L and 39 % had BMIC between 500 and 999 µg/L. Seven percent had BMIC exceeding 1000 µg/L. There was a weak, but significant correlation between UIC and BMIC (r_s = 0.20, p = 0.04) among the women (n = 110), and the iodine concentration was significant higher in BMIC than UIC (p < 0.001).
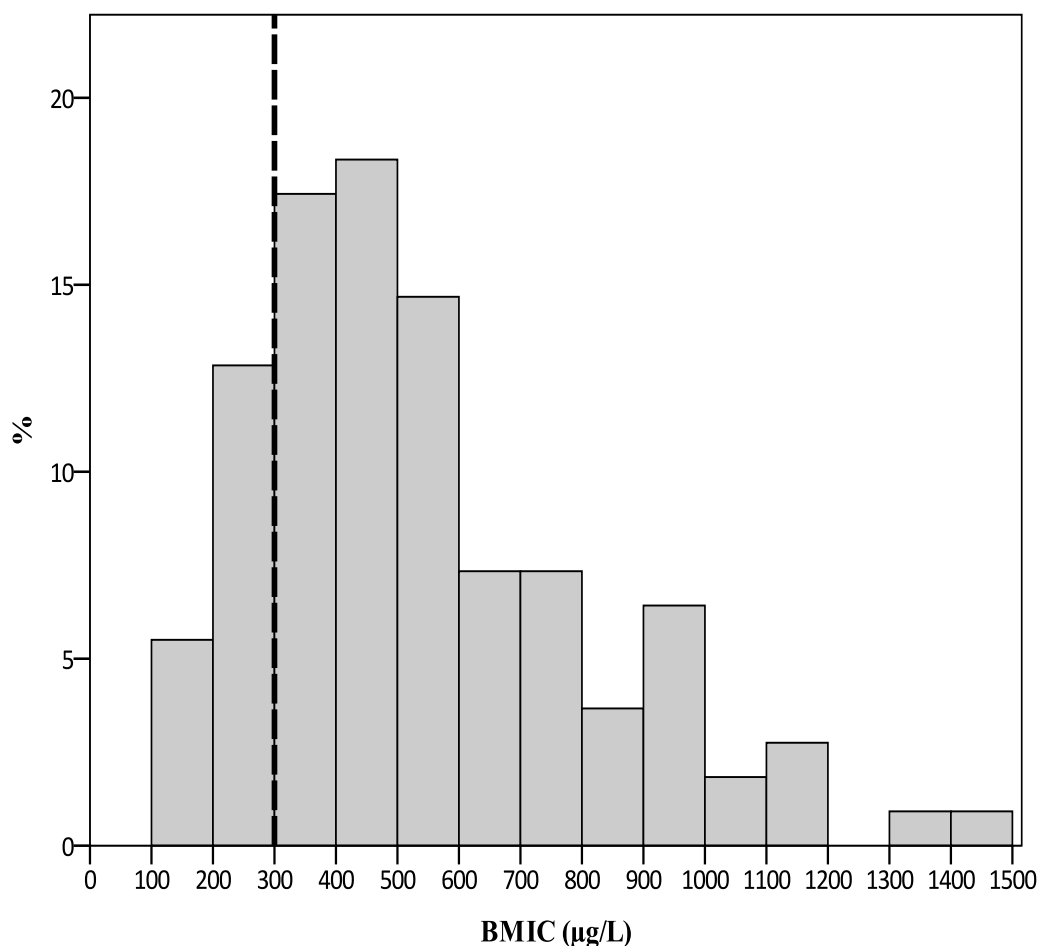
Figure 5-2 Frequency distribution of breast milk iodine concentration (BMIC, µg/L) in lactating women (n = 109), Saharawi refugee camps 2010. The dashed line indicates the cut-off limit for excessive UIC for school-aged children set by WHO .One outlier (BMIC = 1760 µg/L) is excluded. One breast milk sample was missing.

Total iodine concentration

The total iodine concentration (UIC and BMIC, µg/L) showed a median iodine concentration of 894 µg/L (range 320-2858 µg/L) among lactating women (n = 110). None of the women had a total iodine concentration below 300 µg/L. Twelve percent had a total iodine concentration between 300 and 499 µg/L, 48 % between 500-999 µg/L and 40 % had a total iodine concentration above 1000 µg/L.

5.4 Iodine concentration in drinking water and animal milk

Median iodine concentration in public drinking water (n = 24) was 102 µg/L (range 44-289 µg/L) (table 5-2). There were large variations in median iodine concentration in water between the camps, with a median iodine concentration of 90, 236, 76 and 267 µg/L in Smara, Auserd, Dajla and Aaiun, respectively. Iodine concentration in public drinking water in Auserd and Aaiun was significant higher than Smara and Dajla (p < 0.01). There were no significant differences between Auserd and Aaiun (p = 0.06) or between Smara and Dajla (p = 0.05).

Median iodine concentration in goat milk (n = 13) was 952 µg/L (range 323-3540 µg/L) and in camel milk (n = 34) 2020 µg/L (range 210-11100 µg/L) (table 5-2). There were great variations in the iodine concentration in the goat - and camel milk samples, with a difference of 3247 µg/L between the minimum and maximum range of iodine concentration in the goat milk samples (n = 13) and 10 890 µg/L between the minimum and maximum range of iodine concentration in the camel milk samples (n = 34). No significant differences were found between the iodine concentration in goat milk between the camps (n = 12) and the liberated area (n = 1) (p = 0.42) or in camel milk between the camps (n = 17) and the liberated area (n = 15) (p = 0.47).

Iodine concentration in goat milk and camel milk was significant higher than public drinking water (p < 0.001). Among the two milk types, camel milk had a significant higher iodine concentration than goat milk (p = 0.007).

Table 5-2 Iodine concentration (µg/L) in public drinking water (n=24), goat milk (n=13) and camel milk (n=34) in the Saharawi refugee camps 2010

Sources	n	Iodine concentration (µg/L)	
		Median	Range
Drinking water			
Smara	6	90[a]	74-105
Auserd	6	236[b]	118-256
Dajla	6	76[a]	44-82
Aaiun	6	267[b]	94-289
Total	24	102[c]	44-289
Goat milk			
From camps	12	955[f]	323-3570
From liberated area	1	836[f]	836-836
Total	13	952[d]	323-3570
Camel milk			
From camps	17	1960[g]	210-11100
From liberated area	15	2490[g]	598-6140
Unknown	2	4230	1990-6470
Total	34	2020[e]	210-11100

[a,b]Different letters indicating significant differences in iodine concentration in public drinking water between the camps ($p < 0.01$), Mann-Whitney *U*-test.
[c,d,e]Different letters indicating significant differences in iodine concentration in public drinking water, goat milk and camel milk ($p < 0.01$), Mann-Whitney *U*-test.
[f]Letter indicating no significant differences in iodine concentration in goat milk between the camps and the liberated area ($p > 0.05$), Mann-Whitney *U*-test.
[g]Letter indicating no significant differences in iodine concentration in camel milk between the camps and the liberated area ($p > 0.05$), Mann-Whitney *U-test.*

5.5 Dietary iodine intake

There were no significant differences between 24-h and 7 days dietary iodine intake registered among the women (p = 0.08). Thus, only 24-h dietary iodine intake is presented, as UIC reflects iodine intake the last 24 hours.

Based on the 24-h recall, the median dietary iodine intake was 407 µg/day (range 43-1991 µg/day). Twenty-six percent had dietary iodine intake below 250 µg/day and of these 5 % had an intake under 100 µg/day (figure 5-3). Thirty-four percent had an iodine intake within the range of 250-499 µg/day. A total of 30 % had a dietary iodine intake between 500 and 999 µg/day, while 11 % of the women had an iodine intake exceeding 1000 µg/day.
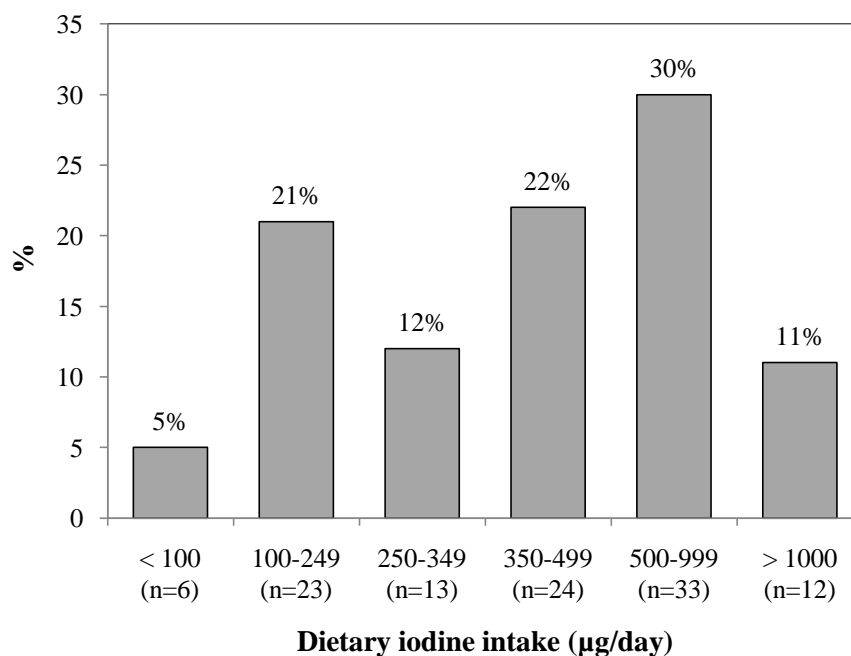
Figure 5-3 Frequency distribution of dietary iodine intake (µg/day) from 24 hour recall in lactating women (n=111), Saharawi refugee camps 2010.

Figure 5-4 shows the frequency distribution of women who consumed public drinking water, goat - and camel milk the last 24 hours. All women in the study (n = 111) consumed water the last 24 hours. Nineteen percent of the women (n =21) reported drinking goat milk, while half of the women (n = 56) drank camel milk the last 24 hours.

Of the total intake of water, goat - and camel milk among the women (n = 111) the last 24 hours, water contributed with approximately 65 %, goat milk with 6 % and camel milk with 30 % of the total dietary iodine intake.
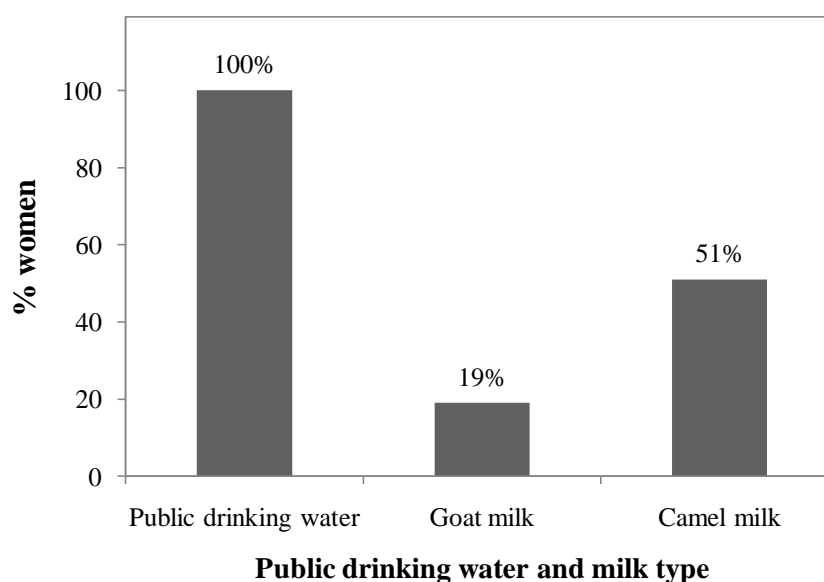
Figure 5-4 Frequency distribution of lactating women (n=111) who had consumed public drinking water, goat - and camel milk the last 24 hours, Saharawi refugee camps 2010.

5.6 Thyroid function

Thyroid function abnormalities are presented in table 5-3. A total of 23.4 % (n = 26) of the women had thyroid function abnormalities.

Autoimmune thyroiditis

Three subjects (2.7 %) had low positive TPOAb (35-100 IU/mL) and were not considered to have autoimmune thyroiditis. Autoimmune thyroiditis (TPOAb > 100 IU/mL) was found in

Results

5.4 % (n = 6) of the women, with one of the women being euthyroid[15] and five having autoimmune thyroiditis along with clinical or subclinical hypo-/hyperthyroidism.

Subclinical – and clinical hypothyroidism

Subclinical hypothyroidism was the most prevalent thyroid abnormality among the women and was found in 12.6 % (n = 13) of the women, with positive TPOAb occurring in one of the women. Clinical hypothyroidism was diagnosed in 3.6 % (n = 4) of with positive TPOAb in one of the women.

Subclinical – and clinical hyperthyroidism

Subclinical hyperthyroidism was found in 5.4 % (n = 6) of the women, with positive TPOAb detected in two of the women. One woman (0.9 %) had clinical hyperthyroidism with positive TPOAb.

Table 5-3 Thyroid function abnormalities among lactating women (n = 111), Saharawi refugee camps 2010

Thyroid function abnormalities	n	%
Autoimmune thyroiditis	6	5.4
Euthyroidism	1	0.9
Clinical hyperthyroidism	1	0.9
Clinical hypohyroidism	1	0.9
Subclinical hyperthyroidism	2	1.8
Subclinical hypothyroidism	1	0.9
Clinical hypothyroidism	3	2.7
Subclinical hyperthyroidism	4	3.6
Subclinical hypothyroidism	13	11.7
Total	32[*]	28.8

[*]Note that even though a total of 32 thyroid function abnormalities are described, one of the women with autoimmune thyroiditis was euthyroid and five had one of the other abnormalities, which give a total of 26 women (23.4 %) with thyroid function abnormalities

The results further revealed that 17.1 % of the women had increased serum Tg concentration ($\geq$ 50 µg/L). TgAb ($\geq$ 50 kU/L) was detected in 14.4 % of the women. The age was significant higher among women with thyroid function abnormalities compared to women with normal thyroid function (p = 0.01). For a more detailed description of the serum levels of TSH, FT_4, TPOAb, Tg, TgAb, FT_3 and TRAb, see appendix 6.

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[15] Normal thyroid gland function (TSH levels within the normal range) although TPOAb is detected.

5.7 Association between UIC and dietary iodine intake

There was a weak, but significant correlation between 24-h dietary iodine intake and UIC, r_s = 0.24 (p = 0.01) among the lactating women (n = 111) (figure 5-5). The 24-h dietary iodine intake correlated stronger with BMIC, r_s = 0.47 (p < 0.001) (figure 5-6). The total iodine concentration (UIC and BMIC) showed a significant correlation, with the 24-h dietary iodine intake, r_s = 0.51 (p < 0.001) among the women (n =110).
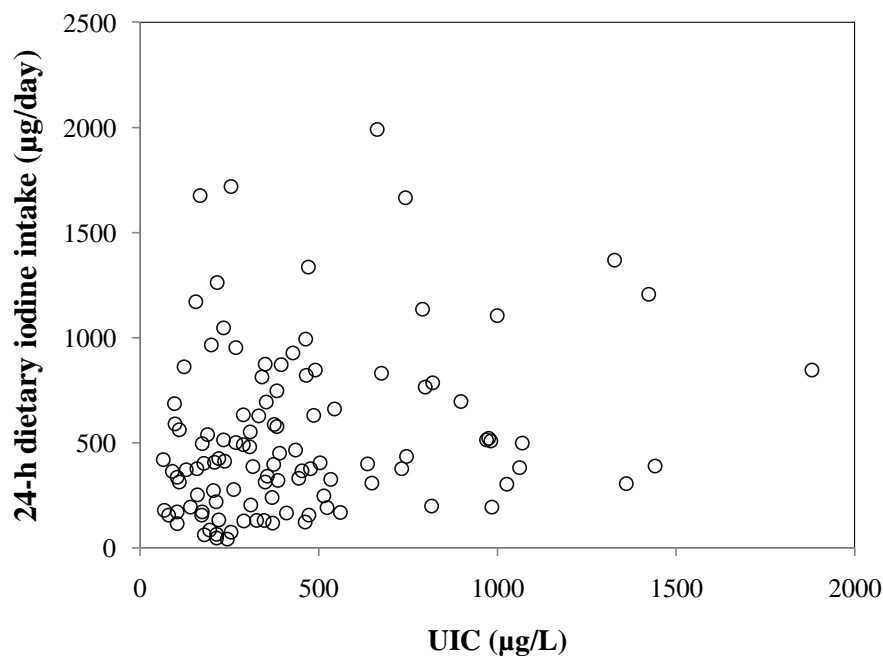
Figure 5-5 Association between urinary iodine concentration (UIC, µg/L) and 24-h dietary iodine intake (µg/day) among lactating women (n = 111), Saharawi refugee camps 2010.
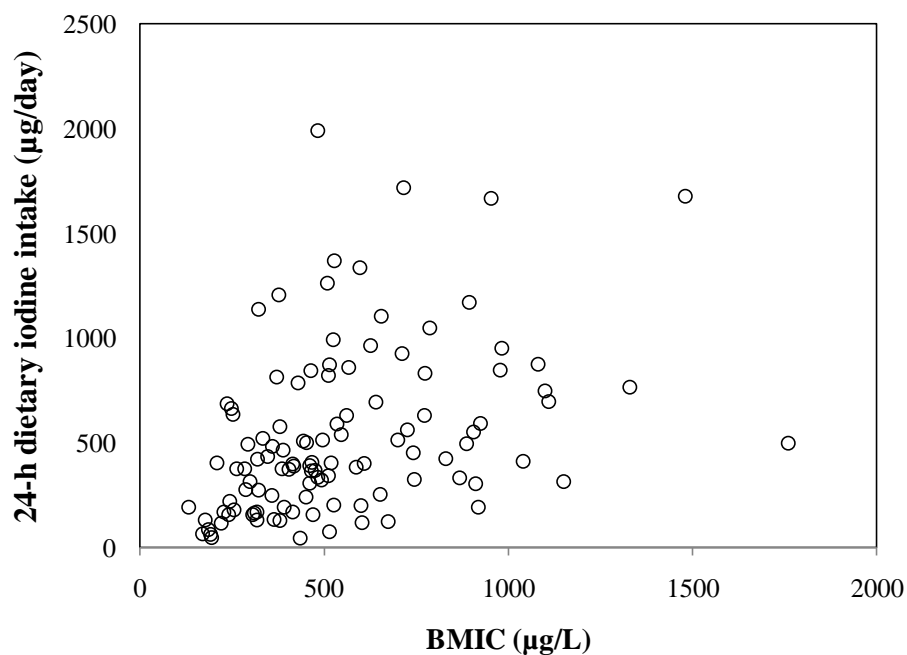
Figure 5-6 Association between breast milk iodine concentration (BMIC, µg/L) and 24-h dietary iodine intake (µg/day) among lactating women (n = 110), Saharawi refugee camps 2010. One breast milk sample was missing.

5.8 Association between UIC and thyroid function

There was no significant correlation between UIC and TSH among lactating women (n = 111), r_s = 0.10 (p = 0.32) or between total iodine concentration (UIC and BMIC) and TSH among lactating women (n = 110), r_s = 0.09 (p = 0.33). There was further no significant correlation between UIC and Tg among lactating women (n = 111), r_s = 0.07 (p = 0.47). The association between total iodine concentration (UIC and BMIC) and Tg was stronger than UIC alone among the women (n =110), r_s = 0.16 (p = 0.10), however no significant correlation was found.

Table 5-4 shows the thyroid function among lactating women with low (> 100 µg/L), adequate (100-299 µg/L) and excessive (≥ 300 µg/L) UIC, based on the recommendations for school-aged children. One of six (16.7 %) of the women with low UIC, 8 of 40 (20.0 %) of the women with adequate UIC and 16 of 65 (24.6 %) of the women with excessive UIC, had thyroid function abnormalities. One of the women with adequate UIC and three women with excessive UIC had autoimmune thyroiditis associated with another thyroid abnormality.

Table 5-4 Thyroid function among lactating women (n =111) with low, adequate and excessive urinary iodine concentration (UIC), Saharawi refugee camps 2010

Thyroid function	UIC (µg/L)[*]		
	> 100	100-299	≥ 300
	% (n)	% (n)	% (n)
Normal	83.3 (5)	80.0 (32)	75.4 (49)
Autoimmune thyroiditis	-	2.5 (1)	-
Autoimmune thyroiditis + 1[≠]	-	5.0 (2)	4.6 (3)
Clinical hypothyroidism	-	-	4.6 (3)
Subclinical hyperthyroidism	-	-	6.2 (4)
Subclinical hypothyroidism	16.7 (1)	12.5 (5)	9.2 (6)
Total	100 (6)	100 (40)	100 (65)

[*]Based on the WHO UIC classification for low (< 100 µg/L), adequate and above requirements (100-299 µg/L) and excessive (≥ 300 µg/L) UIC among school-aged children (WHO, 2007).
[≠]Autoimmune thyroiditis associated with another thyroid abnormality

5.9 Low and high iodine area

Table 5-5 shows median UIC, BMIC, total iodine concentration, iodine concentration in drinking water and dietary iodine intake in the low - and high iodine area. Median UIC among lactating women (n = 56) from low iodine area was 249 µg/L (range 64-1880 µg/L). Lactating women (n = 55) from the high iodine area had median UIC of 385 µg/L (range 98-1424 µg/L). UIC in women from the high iodine area was significant higher than median UIC in women from the low iodine area (p = 0.003).

Median BMIC among lactating women (n = 55) was 379 µg/L (range 132-978 µg/L) in the low iodine area, while the median BMIC was 608 µg/L (range 209-1760 µg/L) among women (n = 55) in the high iodine area. BMIC was significant higher in the high iodine area (p < 0.001)

The low iodine area had a median iodine concentration of 80 µg/L (range 44-105 µg/L) in public drinking water (n = 12) (table 5-5), while the median iodine concentration in public drinking water (n = 12) in the high iodine area was 254 µg/L (range 94-289 µg/L). Iodine concentration in public drinking water in the high iodine area was significant higher than the low iodine area (p < 0.001).

Results

Median dietary iodine intake was 305 µg/day (range 43-1991 µg/day) among lactating women (n = 56) in the low iodine area, with water contributing with 45 %, goat milk with 4 % and camel milk with 51 % of the total dietary iodine intake in this area. Median dietary iodine intake was 552 µg/day (range 119-1718 µg/day) among lactating women (n = 55) in the high iodine area, with water contributing with 76 %, goat milk with 6 % and camel milk with 17 % of the total iodine intake. The dietary iodine intake was significant higher among lactating women in the high iodine area than the low iodine area (p < 0.001) (table 5-5).

Table 5-5 Iodine concentrations in the low - and high iodine areas, Saharawi refugee camps 2010

Source	Unit	Low iodine area		High iodine area		p-value[1]
		n	Median $(P_{25}\text{-}P_{75}{}^{*})$	n	Median $(P_{25}\text{-}P_{75}{}^{*})$	
UIC	µg/L	56	249 (163-484)	55	385 (288-675)	0.003
BMIC	µg/L	55	379 (263-514)	55	608 (450-905)	< 0.001
Total iodine concentration	µg/L	55	675 (518-909)	55	1061 (872-1429)	< 0.001
Drinking water	µg/L	12	80 (73-92)	12	254 (230-269)	< 0.001
Dietary iodine intake	µg/day	56	305 (159-508)	55	552 (387-874)	< 0.001

*25th - and 75th percentiles
[1]Mann-Whitney *U*-test

Thyroid function in the low - and high iodine area

In the low iodine area, median TSH was 2.15 mIU/L, while in the high iodine area, median TSH was 2.3 mIU/L (p = 0.431). Median Tg was higher in the high iodine area (22.0 µg/L) compared to the low iodine area (14.0 µg/L). However no significant differences were found (p = 0.148).

Twenty-three percent of the women in the low iodine area had thyroid function abnormalities, while 22 % of the women in the high iodine area had thyroid function abnormalities. No significant differences were found between the low - and high iodine area (p = 1.0).

6 DISCUSSION

6.2 Methodological considerations

6.2.1 Study design and subjects

The baseline study had a cross sectional design. The advantage with this study design is that it is relatively easy to perform in a large number of subjects and is convenient for health surveillance purposes (Woodward, 2005). It can however not be used to draw causal inferences. This is due to two factors. Firstly, the direction of any potential causal relation remains unknown. Secondly, the findings may be spurious, as the estimates for associations are largely afflicted by confounding factors (Woodward, 2005).

Due to the political situation in the refugee camps, it was not possible to access name list of the population, which was the method first proposed for the random selection of women. If a name list had been available with information of women who had recently given birth, the women could have been randomly selected through the lists. Hence, a convenience sampling method was used. This method involves taking individuals into the study who happens to be available at the time of data collection and who consent to participate (Gibson, 2005). This may however lead to systematic biases. Bias can be defined as a condition that causes a result to depart from the true value in a consistent direction (Gibson, 2005). One of the principal biases is selection bias, which may arise when there is a systematic difference between the characteristics of the individuals selected for the study, and the characteristics of those who are not, which will make it impossible to generalise the results to the target population (Gibson, 2005). One selection bias occurring using the convenience sampling method may for instance be that those with higher levels of education may volunteer to participate and not be representative for the target population. As women living in the refugee camps were the study population, this does not seem to affect this study. Background information further revealed that only a few of the participating women had higher education. Furthermore, the subjects which were included had to fit the inclusion criteria. The first women to come to the dispensary in each daira who fit the inclusion criteria were included until the estimated number of women needed was obtained. It was difficult to find the estimated number of women needed in some of the dairas, because there were not enough women who fitted the inclusion criteria. As a result, women were sometimes selected from other dairas.

Because of a long travelling distance, women living with greater distance from the dispensary might have had more difficulties to participate. This may have given women living near the dispensary a greater chance to be chosen for the study. However, when the health workers at the dispensary knew about women who could participate, but were not able to come due to the distance, a car was sent to bring them to the dispensary.

Only 4 % refused to participate. The low refusal rate is probably due to the fact that the health director gave consent to the study and his judgement is highly respected, and the fact that women wanted to participate. During the recruitment it was discovered that not all women had health cards[16] for their infants. One criterion to participate could have been that the women had health cards. This would however have excluded a large proportion of the women, as many of the women did not obtain one. It was thus not used as criteria. As a result, the present study relied on the mothers' recall of the birth date of her child to evaluate if the women fitted the inclusion criteria. When it was discovered that the infants were above 7 months of age, the woman and the child were excluded. Women who were not breastfeeding were further excluded. The number of women required according to the sample size calculations was collected and the results from this study are thus considered to be representative for the lactating women living in the Saharawi refugee camps.

6.2.2 Urinary iodine concentration

Urinary iodine is the key biochemical marker in epidemiological assessment of iodine status and a good marker of recent iodine intake (WHO, 2007). Casual urine samples were collected from the women and this method has some limitations. Firstly, urinary iodine may vary from day to day and even within a day, which is why UIC only can be classified at a population and not an individual level, if a sufficient number of subjects are collected (Rasmussen et al., 1999). An individual can therefore not be classified as a high or low iodine consumer from a casual urine sample. It is desirable to measure a sufficient number of individuals from a population to obtain a reasonably narrow confidence interval (WHO, 2007). According to WHO (2007), 30 urine determinations from a defined sampling group are usually sufficient. Andersen et al. (2008), has more recently tried to estimate the number of urine samples needed to determine UIC in a population or an individual to describe the reliability of estimates and asses the precision of results of subgroup analysis. According to their findings approximately 125 casual urine samples (100 when using estimated 24 hour iodine

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[16] Health cards is a relatively new system used in the refugee camps and are given to newborns. The health cards should include birth date, height and weight of the child when born along with other information.

excretions) are needed to estimate the iodine level in a population with 95 % confidence within a precision range of ± 10 % and 500 casual urine samples within a precision range of ± 5 % (Andersen et al., 2008). In the present study, 111 casual urine samples were collected. Using the estimates of Andersen et al. (2008), median UIC among lactating women in the refugee camps will most likely with 95 % confidence lie between 350 µg/L ± 10 %, corresponding to 315-385 µg/L, which indicates that the true median iodine excretion lies within this range. However, this study represents a subgroup of the population, respectively lactating women, living in the Saharawi refugee camps which is a limited geographical area. Furthermore, the sample size calculations in this study are based on the number of women needed for the results to be representative with a precision range of ± 5 % for this group of the population. Thus, the median UIC among lactating women in the refugee camps will most likely with 95 % confidence be between 350 µg/L ± 5 %, corresponding to 333-368 µg/L.

Secondly, another limitation is that UIC responds to 24 hour urinary iodine excretion if the volume produced by the group is 1 litre per day, which it often is for school-aged children (Laurberg et al., 2007). For adolescents and adults however, the average urine volume is more likely 1.5 litres per 24 hour. Hence, urinary iodine excretion for this group could be 50 % higher, which suggests that an UIC of 100 µg/L corresponds to a urinary iodine excretion of 150 µg per 24 hour. Thus, urinary iodine excretion of groups of healthy adolescents and adults measured as µg per 24 hour is often equal to UIC measured as $\mu g L^{-1}$ x 1.5 (Laurberg et al., 2007). However, it can be discussed to what degree this can be applied on refugees living in the desert with high temperatures, high sweat production and probably low intake of fluid due to the low water quality. Some of the urine samples collected seemed to be concentrated and had a dark yellow colour, which might indicate that the urine volume produced is less than 1.5 litres per 24 hour. To improve the accuracy and interpretation of the results in this study, 24 hour urinary volume could be measured in a small group of lactating women to see how well UIC corresponded to urinary iodine excretion in lactating women. Furthermore, when evaluating iodine intake using urinary iodine excretion, it should be made correction for the amount of iodine excreted through other routes, mostly in faeces, which usually is 10 % of intake (Laurberg et al., 2007). For lactating women, iodine is also excreted in breast milk, which was measured and corrected for in this study.

6.2.3 Analysis of iodine concentrations

The laboratory in NIRU, South Africa, which was used to analyse both urine and water samples, is an internationally well recognised laboratory. It is one of the 12 laboratories

worldwide which was identified by the International Resource Laboratory for Iodine (IRLI), established by CDC[17], WHO, UNICEF, ICCIDD and MI[18], to serve as an effective resource for their region and accurately measure iodine in urine and salt. The Programme for Ensuring the Quality of Urinary Iodine Procedures (EQUIP) is a CDC standardized program designed to assist laboratories to monitor the degree of variability and bias in their assessment of urinary iodine and is widely used by the IRLI laboratories (WHO, 2007). The Sandell-Kolthoff reaction used to analyse iodine concentration in urine and water samples is well known, includes an initial digestion step to get rid of interfering substances and is the most applied method (Jooste & Strydom, 2010).

Animal milk samples along with breast milk samples can however not be analysed using the same method as the urine and water samples and were analysed at NIFES in Norway using the ICP-MS. This method is considered to be the most accurate method for analysis of iodine and is often used as a "gold-standard", as it to a high degree is accurate and precise in its estimates (Jooste & Strydom, 2010). The transport of all samples was done carefully in cooling bags with cooling elements and should further not have interfered with the iodine concentrations found. This gives reasons to believe that estimates of iodine concentrations in the different samples are reliable.

6.2.4 Registration of dietary iodine intake

Because of limited time resources, it was decided to only ask about specific foods which contained significant amounts of iodine. Only foods and beverages containing water and milk were registered. Water used to cook rice and spaghetti was excluded, as it would be difficult to estimate the amount consumed and these food items were not frequently consumed. Other dietary iodine sources in the camps were dairy products, salt, tuna fish and bread stabiliser, but none of these items are reported to be consumed frequently or contain substantially amounts of iodine compared to the levels found in drinking water and animal milk (Barikmo et al. 2011).

Normally, a single 24-h recall is not considered to be sufficient to reflect the usual intake of an individual (Gibson, 2005). However, the 24-h recall on the dietary iodine intake made it possible to compare dietary iodine intake with the UIC on a group level. As only public drinking water and animal milk was registered, the study could explore to what extent these

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[17] Center for Disease Control of the United States of America
[18] The Micronutrient Initiative

two dietary iodine sources correlated with UIC. The weight scales used to estimate the intake of water and milk were regularly checked and no differences in measurements between the two scales were found.

Certain factors require attention when evaluating the registered intakes. During the measurements of nutrient intakes, random and systematic errors may occur, of which some are discussed here. Recall biases may have occurred, as the women were asked to recall and weigh the amount of milk and water consumed the last 24 hours and 7 days. The women live in an area where most of the population are not familiar with recalling amounts of consumed foods. The consumption of food and beverages are usually done from shared serving dishes. For instance, a bowl with water circulates between family members from which all drink, which might indicate a lack of accuracy of the volume consumed by the women alone. However, according to Gibson (2005), a modification of the 24-h recall can be used to collect information on rural populations in developing countries. Gibson (2005) suggests that recall interviews should be conducted in the respondent's home whenever possible around familiar environment, as it may improve the recall of foods consumed. Furthermore, bowls and plates should be provided for the use on the recall to help the respondent to visualize the amount of food consumed (Gibson, 2005). This was taken into consideration during the recalls, which were performed in the women's households, where they showed the amount consumed from their own bowls.

Another source of inaccuracy can be interviewer biases. Interviewer biases may be random across days and subjects or systematic for a specific interviewer. They may further exist as an interaction between certain interviewers and certain respondents (Gibson, 2005). The 24-h recalls were performed in Hasanía by the local nutritionists. Some of the respondents were further familiar to the interviewers, especially those living in the same camp as them. Although they were trained how to ask questions, certain ways of posing the questions may have influenced the results and due to the language barriers it is difficult to distinguish variations in the way of asking. However, women showed the amount of consumption themselves and registrations were usually checked. Furthermore, the interviewers were trained together and the questions were standardised, which gives reasons to believe that the interviewer differences were minor.

As samples of public drinking water from the camps and animal milk from households were obtained, the amount consumed could directly be linked to the iodine concentrations found in

the samples. As some of the women did not have goat - or camel milk samples available, the median iodine concentrations from the other samples were used to estimate the iodine concentration from milk consumed by the remaining women. The results showed that iodine concentrations in the animal milk samples varied widely. The actual iodine concentration in the milk consumed by women without their own milk sample may vary from the median iodine concentration used. It should thus be taken into consideration that some under - or overestimates on the dietary intake may have occured. However, as this only applies for some of the women and as usually small amounts of milk were consumed, this will most likely even out on a group level. Hence, it might only have interfered with the results to a small degree.

Validity of the 24-hour recall

The quality of a method used to estimate food consumption can be expressed by its validity as to what extent the method measured what it was intended to measure (Frost Andersen, 2000). Some under - or overestimations may have been done due to the exclusion of some food items containing iodine and the use of median iodine concentration found in other milk samples than those specifically consumed by the women. Still, the estimated 24-h dietary iodine intake seemed to correlate well with the levels of iodine found in urine and breast milk. Errors will however always occur despite the methods used for dietary assessment (Frost Andersen, 2000). They have been taken into account and have been discussed above. The registrations are, despite these potential errors, considered to have been performed with the most achievable accuracy as possible within the context in which the study has been conducted.

6.2.5 Analysis of thyroid function

Whole dried blood spots on filter paper would have been the easiest way to collect blood samples, as only a finger prick of blood is needed. Also, it would probably have been easier to perform on the infants. However, only a few institutions can analyse dried blood spots and it was thus decided to collect serum samples instead. This is a well recognised method if the standard procedures are followed and this was done in the best possible way in the refugee camps. The samples were stored in the freezer and brought with cooling elements in cooling bags, but were thawed during transportation to Norway, because of long journey (two days). However, the blood constituents which were analysed are usually stable up to 1-2 weeks in room temperature (e-mail correspondence with the Oslo University Hospital, 19th of August, 2010) and should thus not have affected the results. The different analytical techniques which were used are well recognised and conducted with good quality assurance (MBK, 2011). Test

analysis and interpretations were further performed in collaboration with the Oslo University Hospital.

6.2.6 Data processing and statistical analysis

Although the sample size required to do the statistical analysis were obtained, lack of statistical findings may be due to few observations. Several different grouping variables were performed, still the same tendencies were observed. The treatment of the data was done carefully, and is hence considered to be reliable.

6.1 Results and findings

6.1.1 Iodine concentrations in urine and breast milk

Median UIC among lactating women in the present study was lower compared to the previous studies conducted in the Saharawi refugee camps (table 6-1). The minimum range was very similar to the studies conducted by UNHCR (2002) and SMH/NCA/AUC (2008), while the maximum range was twice as high in the previous studies. However, it has been demonstrated that the sodium iodide symporter is markedly up regulated in the mammary gland during lactation, which results in excretion of iodine in breast milk (Azizi & Smyth, 2009; Bazrafshan, 2005). The BMIC varies with dietary iodine intake and in a sufficient iodine area, the concentration of iodine in breast milk should be in the range of 100-150 µg/L (Azizi & Smyth, 2009). According to the results in the present study, median BMIC among lactating women was three to four times higher than this range (479 µg/L). The high excretion of iodine in breast milk can further explain the lower UIC among lactating women compared to other study groups from previous studies.

Table 6-1 Urinary iodine concentration (UIC) among Saharawi refugees from 2002-2010

Year	Study	Study group	Age (Y/M)[*]	Sex (M/F)[1]	n	UIC (µg/L) Median	UIC (µg/L) Range
2002	UNHCR	Adolescents	10-19 Y	M/F	122	1200	60-3900
2007	Henjum et al.	Children	6-14 Y	M/F	416	565	102-3594
2007	SMH/NCA/AUC	Women	15-45 Y	F	398	466	54-3640
2010	Present study	Infants	0-7 M	M/F	111	722	130-4363
2010	Present study	Lactating women	18-50 Y	F	111	350	64-1880

[*]Y = Years, M = Months
[1]M = Male, F = Female

Discussion

The median UIC of 350 µg/L in the present study indicates an adequate iodine intake among lactating women in the population according to the cut-off value set by the WHO ($\geq$ 100) (WHO, 2007). Iodine concentrations found in urine and breast milk together showed an increase in the total iodine concentration among the women. The median iodine concentration increased from 350 µg/L to 894 µg/L, which is more than 50 % increase from UIC alone and confirm that the lactating breast is a significant pathway for iodine concentration. It is difficult to interpret if the iodine intake exceeds the recommended intake, as no cut-off value for excessive UIC among lactating women exists. However, based on the cut-off value of median UIC of 300 µg/L, which is excessive for school-aged children (WHO, 2007), both median UIC of 350 µg/L and median total iodine concentration of 894 µg/L among lactating women indicates excessive iodine intake. The median total iodine concentration of 894 µg/L among the women even exceeds the cut-off value of median UIC of 500 µg/L, which is excessive for pregnant women, who have the same iodine intake recommendation of 250 µg/day as lactating women (WHO, 2007) and further confirms the high iodine intake among the women. The high iodine concentrations found in the present study are in accordance to previous studies conducted among the refugees in the Saharawi camps presented in table 6-1.

6.1.2 Iodine content in drinking water and animal milk

Iodine content in drinking water

There has not yet been established a guideline value for the iodine in drinking water due to lack of relevant data on the effects of excess iodine (WHO, 2003). However, median iodine level in the drinking water (102 µg/L) in the present study was considerably higher than the usual iodine level of < 15 µg/L in drinking water, as described by the SCF (SCF, 2002). Smara and Dajla (referred to as the low iodine area) had a significant lower iodine concentration in the water compared to Auserd and Aaiun (referred to as the high iodine area). Still, both of the areas had much higher iodine concentration than 15 µg/L. All of the drinking water samples had an iodine concentration above 15 µg/L and only one of the samples had an iodine concentration below 50 µg/L, which usually is the iodine concentration found in sea water (SCF, 2002). Adults are usually recommended to consume 2 litre of water per day and with the high levels of iodine found in some of the water samples, this amount alone exceeds the recommended daily iodine intake. As shown by the median iodine concentration in the drinking water in Smara, Auserd, Dajla and Aaiun, the consumption of 2 litre of water per day will correspond to an iodine intake of 180, 472, 152 and 534 µg/day, respectively. Iodine

from drinking water will then give the refugees in Auserd and Aaiun an iodine intake almost twice as high as the recommended intake of 250 μg/day.

What are the reasons for the high levels of iodine in the drinking water and the differences between the refugee camps?

The Sahara desert, where the refugees reside, is a former area of sea and the iodine content in food and water usually depends on the iodine content in the adjacent soil (Rasmussen et al., 2009). Thus, the location of the refugee camps, in the mineral-rich desert, might have led to the high iodine concentrations in the local drinking water.

Table 6-2 Iodine concentration in water and milk in the Saharawi refugee camps 1998-2010

Year	Study	Iodine concentration (μg/L)					
		Drinking water		Goat milk		Camel milk	
		n	Median (range)	n	Median (range)	n	Median (range)
1998	Pezzino et al.	3	259-724-934[*]	-	-	-	-
2007	SMH/NCA/AUC	95	108 (55-545)	16	370 (70-13071)	3	5563 (540-11980)
2009	SMH/NCA/AUC	18	160 (36-376)	147	994 (101-9323)	18	2471 (357-7799)
2010	Present study	24	102 (44-289)	13	952 (323-3570)	34	2020 (210-11100)

[*]Iodine concentration in drinking water from Dajla, Rabouni and Aaiun, respectively (Pezzino et al., 1998).

Comparing the present results with results from previous studies in the camps shows a decrease in the iodine concentration in drinking water (table 6-2). This is probably due to changes in water supply during the past years. A study conducted by Pezzino et al. (1998) revealed that the drinking water from Rabouni (supplying Smara and Auserd) and the drinking water from Aaiun (supplying Aaiun and Auserd), had an iodine concentration of 724 and 934 μg/L, respectively. This was much higher than the iodine concentration in drinking water in Dajla, which is a camp 150 km from the others. Here, the iodine concentration in drinking water was 259 μg/L (Pezzino et al., 1998). Over the past years, the refugee camps have received new water sources. Aaiun and Auserd received water from deep wells (about 100 m) in 2002-2003, Dajla got a new water source in 2004, and the water in Smara has been treated by reverse osmosis since 2005. The osmotic plant used in Smara has shown to work

efficiently giving the refugees acceptable drinking water (Barikmo, Henjum, Dahl, Oshaug & Torheim, 2011). Although the iodine concentrations have decreased in all of the camps during the past years due to changes in water sources, the present study along with the previous studies, described in table 6-2, reveal that the iodine concentration in the drinking water is still high. Especially in Auserd and Aaiun, the deep wells supplying these two areas with drinking water seem to still contain great amounts of iodine.

Iodine content in milk

The iodine concentration was significant higher in camel milk than goat milk and both had significant higher iodine concentration than public drinking water. The study conducted by SMH/NCA/AUC (2010) in 2009 and the present study shows an increase in the median iodine concentration in goat milk and an decrease in the median iodine concentration in camel milk from the study conducted in 2007 (table 6-2). This may be due to differences in the number of animal milk samples collected in the different studies, as there in the present study, along with the previous studies is shown to be wide variations in iodine concentrations between the samples. The reason for the wide variations of iodine concentration in the animal milk may be due to different sources of water provided to the animals. According to Barikmo et al. (2011), some animals in the Saharawi refugee camps were given water from old family wells, which no longer were used by humans. In this case, the iodine concentration in goat milk exceeded 13 000 µg/L (Barikmo et al., 2011). However, high iodine concentrations were also found in animal milk where the animal was given the same water as consumed by humans, for instance 540 µg/L in a camel consuming water with an iodine concentration of 70 µg/L. This indicates that iodine in animal milk is concentrated beyond the levels of iodine in water, leading to high levels of iodine in animal milk, thus aggravating the concentration of iodine consumed by humans (Barikmo et al. 2011).

The high iodine concentrations in animal milk may also be explained by high iodine concentration in fodder consumed by the animals. In the study by SMH/NCA/AUC (2010) conducted in 2009, participants were asked for what type of fodder was given to the animals. Goats were usually given household food and cartoon and camels hay and greens. The garbage and cartons contained low amounts of iodine. There is however concern for other toxins, such as dioxin, polychlorinated biphenyls (PCB), polyvinylchloride and heavy metals such as lead and mercury (SMH/NCA/AUC, 2008), which should be analysed. The iodine content was higher in animals fed with fresh greens than hay and may be explained by the

evaporation of iodine in the drying process. The high iodine content in the fresh greens from the refugee area may however be explained by the high iodine content in the soil where the refugees reside, as described previously. The higher iodine concentrations found in camel milk than goat milk can be due to the fact that a greater proportion of camels are fed with greens than goats. Another possibility is a higher ability in camels to concentrate substances in milk, which can influence the results for nutrients and toxins (SMH/NCA/AUC, 2010).

In the study conducted in the refugee camps in 2007, women were asked about household livestock and milk samples were taken to investigate the impact of household livestock on the prevalence of goitre (Henjum et al., 2010). The study revealed a positive association between enlarged thyroid volume and the presence of livestock in the household (Henjum et al., 2010). This may suggest that women with their own household livestock may be more exposed to thyroid function abnormalities, as they might have a greater consumption of animal milk. However, women in the present study were not asked if they had their own livestock. It is thus difficult to conclude to what degree household livestock may have contributed to the development of thyroid function abnormalities due to a more frequent consumption of excess iodine through animal milk.

6.1.3 Dietary iodine intake

Lactating women are recommended an iodine intake of 250 µg/day (WHO, 2007). The dietary iodine intake in the present study, which is based on the estimated 24 hour dietary intake of water and milk revealed a median iodine intake of 407 µg/day, which suggests that the women have more than adequate intake of iodine. Forty-one percent of the women exceeded an iodine intake of 500 µg/day, which according to WHO should not be exceeded by lactating women as no further health benefit can be expected (Andersson et al., 2007). Thirty percent of the women had an iodine intake above 600 µg/day, which is the upper level set by SCF (SCF, 2002) and 10 % of the women even exceeded 1100 µg/day which is the upper level set by IOM (IOM, 2001). The dietary iodine intake confirms the findings from the UIC and BMIC, which suggests that the women have consumed adequate and even excessive levels of iodine.

The 7 days recall which was performed in the present study revealed that the amount of iodine consumed through water and milk the last seven days was almost the same. This might indicate that the women had been consuming high levels of iodine for a long period of time and not only the last 24 hours. Public drinking water was consumed every day, while the intake of goat - and camel milk varied from day to day and was sometimes only consumed

one day a week. When women were asked if they consumed milk the last 24 hours, it was usually only small amounts of milk which was mixed with water, to make the water taste better. Frequently, what they reported as consumption of goat - and camel milk, contained greater amounts of water than milk. This suggests that the water is the main dietary iodine source and that the consumption of milk might further increase the iodine intake.

6.1.4 Thyroid function

When the prevalence of thyroid disorders is less than 5 % in a population, the disorders are usually due to genetically conditions (WHO, 2007). In the present study however, 23.4 % of the women had thyroid abnormalities, over one fifth of the women, which is a high prevalence and signal the presence of a public health problem.

It has been discussed to what degree pregnancy and lactation can have an impact on the incidence of thyroid dysfunction due to hormonal changes in this period. However, reports have concluded that maternal thyroid status is relatively stable during gestation unless iodine supplies are limited (Liberman, Pino, Fang, Braverman & Emerson, 1998). Hence, pregnancy itself does not usually affect serum TSH concentrations. Thyroid dysfunction occurring during the postpartum period has however earlier been reported (Bjøro, 2002; Nicholson, Robinson, Smallridge, Ladenson & Powe, 2006). Postpartum thyroid dysfunction (PPTD) is an autoimmune disorder that may occur during the first year postpartum and has been shown to occur in 1 of 12 women in the general population worldwide, 1 of 17 women in the United States and is 5.7 times more likely to occur in women with positive TPOAb (Nicholson et al., 2006). It is usually a mild condition occurring weeks to months postpartum. It begins as an initial hyperthyroidism and then develops into hypothyroidism before it turns to normal. The thyroid function should be monitored in the first months of postpartum and especially in subjects with positive TPOAb, to see if it turns back to normal (Bjøro, 2002).

The prevalence of thyroid abnormalities is usually more prevalent among women than men and increases with age. Women over 40 years may thus be more exposed than others (Bjøro, 2002). However the prevalence of abnormalities in the present study is still high. Although the age among women with thyroid abnormalities were significant higher than women without, only a few of the women with abnormalities were over 40 years old. Eight of the 26 women were between 20-29 years, 13 between 30-39 years, 4 between 40-49 years and one woman reaching 50. This might indicate that other environmental influences, such as iodine, may have played a role in the development of thyroid abnormalities among the women.

Autoimmune thyroiditis

In this study, autoimmune thyroiditis was found in 5.4 % of the women determined by TPOAb levels above 100 IU/mL. Furthermore, 14.4 % had positive TgAb (> 50 kU/L). Chronic autoimmune thyroiditis is usually seen in all subjects with high concentrations of TPOAb and TgAb in the serum. Iodine intake has been suggested as the most important among the environmental factors which contributes to the development of thyroid autoimmunity (Prummel, Strieder & Wiersinga, 2004). There are relatively few comparative studies with different iodine intake in the analysis of thyroid antibodies. A comparative study conducted on elderly subjects (66-70 years), both males and females, in Iceland with stable high iodine intake (median UIC around 300 µg/L, n = 100) and Jutland in Denmark with long-standing low iodine intake (median UIC about 40-60 µg/L, n = 423) showed a prevalence of various thyroid abnormalities (Laurberg et al., 1998). The prevalence of both TPOAb and TgAb were nearly twice as high in Jutland, compared to Island with the highest iodine intake (Laurberg et al., 1998). This was somewhat unexpected as autoimmune thyroid diseases are more common in iodine sufficient areas, than iodine deficient areas (Lind et al., 1998). However, the susceptibility clearly increases with age and this can be a factor triggering the prevalence among this age group along with iodine intake. The lymphocytic infiltrations of the thyroid in humans may be more common after an increase in iodine intake, which is in accordance to the previous findings by Laurberg et al. (1998), showing that Graves' disease manifested at a considerably younger age in Iceland than Jutland (Laurberg et al., 1998). Subjects above 13 years (n = 3761) living in three different regions in China with mild iodine deficiency (median UIC 84 µg/L), more than adequate iodine intake (median UIC 243 µg/L) and excessive iodine intake (median UIC 651 µg/L) showed an increase in the prevalence of autoantibodies with increasing iodine intake (Teng et al., 2006). TPOAb was found in 9.2, 9.8 and 10.5 % and TgAb in 9.0, 9.0 and 9.4 %, respectively (Teng et al., 2006). Although no significant differences were detected, the results suggested that more than adequate and excessive iodine intake could trigger the incidence and prevalence of autoimmune thyroiditis in humans (Teng et al., 2006), which is further supported by Camargo et al. (2008).

Subclinical - and clinical hypothyroidism

In a review, Lind et al. (1998) have found that 5 in 1000 (0.5 %) have clinical and 15 in 1000 (1.5 %) have subclinical hypothyroidism in an iodine sufficient area (Lind et al., 1998). The prevalence was higher in the present study, with 4 in 111 (3.6 %) with clinical

Discussion

hypothyroidism and 14 in 111 (12.6 %) with subclinical hypothyroidism. Iodine-induced hypothyroidism usually occurs when individuals are not able to escape from the inhibitory effect of large doses of iodide (Markou et al., 2001). Studies from other countries have confirmed an increase in hypothyroidism in areas exposed to excessive nutritional iodine (Camargo et al., 2008; Teng et al., 2006). Five years of excessive iodine intake in the population living in Sao Paulo, Brazil, may have increased the prevalence of autoimmune thyroiditis and hypothyroidism in subjects genetically predisposed for thyroid autoimmune diseases (Camargo et al., 2008). Among 678 women, autoimmune thyroiditis associated with hypothyroidism was detected in 39 women (5.75 %) and subclinical hypothyroidism in 18 women (2.65 %) (Camargo et al., 2008). In China, hypothyroidism was detected in 0.3, 0.9 and 2.0 % and subclinical hypothyroidism in 0.9, 2.9 and 6.1 % in the mildly deficient, more than adequate and excessive areas (Teng et al., 2006). The higher prevalence of hypothyroidism among subjects with an excessive iodine intake is further supported by Laurberg et al. (1998). In his study, the prevalence of subclinical hypothyroidism was four times higher in Iceland where the iodine intake was high, compared to Jutland, with a lower iodine intake (Laurberg et al., 1998).

Subclinical hypothyroidism is common in populations with high iodine intake. An increased risk of cardiovascular morbidity and mortality is shown in subjects with TSH above, but not below 10 mIU/L with no increase in total mortality (Karmisholt et al., 2011). However, subclinical hypothyroidism should be monitored as it can progress to hypothyroidism or regress to normal thyroid function. No evidence-based recommendations exists, but Karmisholt et al. (2011), have made a list of recommendations for monitoring untreated subclinical hypothyroidism, which suggests that this condition should be followed up on if persistent.

Subclinical - and clinical hyperthyroidism

The prevalence of clinical hyperthyroidism is 2 in 1000 (0.2 %) and the prevalence of subclinical hyperthyroidism is 6 in 1000 (0.6 %) in an iodine sufficient area (Lind et al., 1998). In the present study clinical hypothyroidism was detected in 1 in 111 (0.9 %) and subclinical hyperthyroidism in 6 in 111 (5.4 %), which is higher than the prevalence described in the iodine sufficient area. Iodine-induced hyperthyroidism has been reported to occur when there is a sudden increase in iodine supplementation (Stansbury et al., 1998). The Saharawi refugees have been exposed to high levels of iodine for a long period and background

information on the women in the present study did not show any sudden movement between the camps or outside the camps. To what degree the long-term excessive iodine intake in the refugee camps have increased the prevalence of clinical - and subclinical hyperthyroidism is difficult to conclude. According to Laurberg et al. (1998), hyperfunction of the thyroid gland was more prevalent among the subjects in the low iodine area in Jutland compared to Iceland. In China, clinical hyperthyroidism was found in 1.6, 2.0 and 1.2 % of the subjects and subclinical hyperthyroidism in 3.7, 3.9 and 1.1 % of the subjects in the mildly deficient, more than adequate and excessive iodine areas (Teng et al., 2006). Based on these findings Yang et al. (2007) concluded that chronic iodine excess did not apparently increase the risk of autoimmune hyperthyroidism or influence the incidence and outcome of subclinical hyperthyroidism (Yang et al., 2007). Camargo et al. (2008) found similar prevalence as Teng et al. (2006) in Sao Paulo, Brazil, with 1.77 % of the women having clinical hyperthyroidism and 2.06 % having subclinical hyperthyroidism, but could not reach to a conclusion if there was a relationship between excess iodine intake and hyperthyroidism (Camargo et al., 2008).

6.1.5 Association between the different indicators

UIC and dietary iodine intake

The results showed weak, but significant correlation between UIC and dietary iodine intake and the correlation was stronger when iodine excretion in breast milk was included. As only intake of water and milk was reported, the results indicate that the women in the Saharawi refugee camps are exposed to large amounts of iodine through water and milk consumption.

UIC and thyroid function

There were an increasing number of thyroid abnormalities with higher iodine concentrations in urine in the women. Still, no significant correlations between increasing UIC and serum TSH or serum Tg levels were found. Even when correcting for BMIC, the same tendencies were found and no significant correlation was detected.

Looking at the types of thyroid abnormalities shows that the one subject with low UIC had subclinical hypothyroidism and the seven women with adequate to more than adequate UIC had autoimmune thyroiditis or subclinical hypothyroidism. Clinical hypothyroidism and subclinical hyperthyroidism were only found in women with excessive UIC (defined as $\geq$ 300 µg/L) along with autoimmune thyroiditis and subclinical hypothyroidism. This suggests that women with higher UIC may be more prone for the development of thyroid abnormalities.

Discussion

Low and high iodine area

The present results revealed similar tendencies as the previous studies by UNHCR (2002) and SMH/NCA/AUC (2008), with a significant higher UIC and dietary iodine intake in the high iodine area compared to the low iodine area and suggests that public drinking water is a significant iodine source and has a great impact on the UIC and BMIC levels among the lactating women.

There were however not found any significant differences in the prevalence of thyroid abnormalities or TSH levels between the two areas. The concentration of Tg is usually increased in areas with excess iodine (Bilek & Zamrazil, 2009). Similar tendencies were observed in the present study. The median Tg was higher among the women living in the high iodine area compared to the low iodine area, although no significant differences were found. Background information was checked to see if the women from the low iodine area had recently lived in other camps from the high iodine area, but only one woman was found to have moved the past two years. However, looking at the median UIC, BMIC and total iodine concentration in the low iodine area shows that the women are exposed to higher levels of iodine than recommended by the WHO in this area too. Furthermore, the amount of milk consumed by women in the different areas may have had an impact on the iodine concentrations found in the women. All women in the present study live in the Saharawi refugee camps which are areas where excessive amounts of iodine have been observed for several years and this makes all subjects susceptible for developing thyroid function abnormalities.

Concluding remarks

As discussed in this thesis, thyroid dysfunction has been reported due to iodine excess. It is known that environmental influences may play a major role in the prevalence of thyroid abnormalities in different regions and geographical locations. High intake of iodine is a risk factor triggering the prevalence and this gives reasons to believe that the long-term excessive intakes of iodine among the refugees living in the Algerian desert may have influenced the thyroid function in the wrong direction. Dunn et al. (1998), Pearce et al. (2002) and others have suggested that iodine-induced abnormalities are not permanent and may decrease after the removal of iodine excess. Further studies are however required to determine to what extent the excess iodine have resulted in negative health consequences for the Saharawi refugees.

76

7 CONCLUSION AND FUTURE WORK

The present study confirms previous findings of excessive intakes of iodine among the Saharawi refugees, shown through the high urinary iodine - and breast milk iodine concentrations. The iodine concentrations in urine and breast milk correlated well with the dietary iodine intake. The women seem to be exposed to high levels of iodine through the consumption of iodine-rich drinking water, and the intake is presumably increased by the consumption of animal milk. The prevalence of thyroid abnormalities among the refugees is high. Although no significant associations between urinary iodine and serum levels of TSH and Tg were found, the high prevalence indicate that the long-term excessive iodine intake may have influenced the thyroid function negatively.

The present study suggests that there is a need for screening of thyroid function, monitoring of iodine intake and reduce the intake into recommended levels. In the Saharawi refugee camps, the public drinking water is shown to have a significant impact on the levels of iodine found in the refugees, which suggests that the water should be continued to be purified, so all refugees have access to acceptable drinking water. The purified water should further be given to the animals to decrease the levels of iodine found in the animal milk consumed by the refugees.

To improve the reliability of this study, some suggestions have been made for the future work. Although the goitre rate has been measured among the refugees in previous studies, it was desired to measure the goitre rate in the present study. However, no one was available to make these measurements. The women were not asked if they previously had been diagnosed with goitre, which might have improved the understanding of the development of thyroid abnormalities.

From the study start, we planned to take blood samples of the infants, to investigate the impact of a high iodine intake through breast milk on their thyroid function. However, it was not possible to collect sufficient amount of blood needed for analysis and no medical doctor who was trained to take blood samples of the infants, was available. In the future, it is desirable to take blood samples of the infants.

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Appendix 1

Ministrio de Salud Publica

República Árabe Saharaui Democrática

Consent form to participate in the iodine study among Saharawi refugees.

We are doing research on why the Saharawi population has a problem with goitre. This study is a part of the Ministry of Health's work for examine and reducing this problem. If you decide to participate you and your child will be asked to contribute with:

- a sample of urine
- a blood sample
- a sample of breast milk
- measure your height and weight
- ask about your breast feeding practises
- ask you questions about your background
- ask you questions about your water and milk intake
- ask you about your water sources and milk sources
- small samples of some of the animal milk you drink and the water, if necessary

You and your family are randomly selected. We are asking mothers with children from 0 to 7 months of age to participate in the study. You will be anonymous in the way that no names will be on the form where your answers are filled in. An ID number will be made up for the survey. A separated list with the id number and name will be kept by the doctor in case we find something in the tests that need treatment. We want to follow up with further examinations in the years to come, to map the iodine situation for your children. We therefore ask you if it is accepted that we contact you again for further samples. The first follow-up study will be in two years and the ID list will be used to find back to you.

If you and your children take part in this project you help the Ministry of Health to map the iodine situation among lactating women and the children. You also contribute to get necessary help to solve the problem for the Saharawi people. Taking part in this project is entirely up to you, and no one will hold it against you or your children if you decide not to participate. If you take part, you may stop at any time without any negative consequences.

The study is collaboration between the Saharawi Ministry of Health, Norwegian Church Aid and Akershus University College. The contact person for the study is Dr. Abderrahàman, Ministry of Health and Nutritionist Ingrid Barikmo, Norwegian Church Aid.

---***--------------***---

I agree to take part and also let my child take part in this iodine study. I know that we have to give blood, urine and breast milk samples and answer questions and that we can stop at any time.

_____   _____
Signature   Date

Akershus University College

NORWEGIAN CHURCH AID

Appendix 2

97 = Forgot to ask
98 = Not answered
99 = Do not know

Iodine survey in the Saharawi camps November-December 2010

Background questionnaire to the women

1. **Date**.... .... □□.□□.□□

2. **Id number woman** .... □□□-□

3. **Id number child** .... □□□-□

4. **Camp** . ... □□
   1=27. February, 2=Smara, 3=Auserd, 4=Dajla, 5=Aaiun

5. **Daira** ..(write)_____ □□
   (Daira according to a list)

6. **Barrio** (write)_____ □□
   (Barrio according to a list)

7. **Name of the interviewer**_____

8. **Time of the interview**: .... a) start □□-□□ .... b) stop □□-□□

Ask the following questions to the mother:

9. How old are you? .... □□ years

10. Marital status:.. .... □□
    0=Not married, 1=Married, 2=Divorced, 3=Widowed

11. Have you lived in different Saharawi refugee camps?... .... □□
    0=No, 1=Yes

If YES

   11.1.a) Which camp_____**11.1.b)** Period_____

   11.2.a) Which camp_____**11.2.b)** Period_____

   11.3.a) Which camp_____**11.3.b)** Period_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

12. Have you lived in other areas outside the Saharawi refugee camps?............................☐☐
  0=No, 1=Yes

If YES

    12.1.a) Which area_____**12.1.b)** Period_____

    12.2.a) Which area_____**12.2.b)** Period_____

    12.3.a) Which area_____**12.3.b)** Period_____

13. How long education do you have ....☐☐
  0=None, 1=Less than 6th grade, 2= 6th grade, 3=7 to 9th grade, 4=10 to 12th grade, 5=Higher education

14. Have you attended any courses in the refugee camps? .. ..☐☐
  0=No, 1=Yes

If YES

    14.1.a) Which_____**14.1.b)** How long_____

    14.2.a) Which_____**14.2.b)** How long_____

15. What language do you speak, read or write? (mark with X)

       15.1.a) Hasanía .... **15.1.b)** speak ☐

       15.2.a) Arabic.................. **15.2.b)** speak ☐..........**15.2.c)** read ☐.....**15.2.d)** write ☐

       15.3.a) Spanish .... **15.3.b)** speak ☐..........**15.3.c)** read ☐.....**15.3.d)** write ☐

       15.4.a) Other (which?) .... **15.4.b)** speak ☐..........**15.4.c)** read ☐.....**15.4.d)** write ☐

16. Are you working outside the home at the moment? ....☐☐
  0=No, 1=Yes

 If YES

    16.1 with what?_____

17. How many people live in this household here and now (count those at 12october school in the area but not those that are abroad or other places in Algeria)?..................................☐☐

18. Gender of Head of household ....☐☐
  0=Man, 1=Woman

19. Age of Head of household.. ....☐☐ years

20. How many children do you have?

    20.1.a) alive ....☐☐ **20.1.b)** dead ....☐☐

21. How many children are under 5 years?........................ ....☐☐

Appendix 3

97 = Forgot to ask
98 = Not answered
99 = Do not know

Iodine survey in the Saharawi camps November – December 2010

24 hour and 7 days recall on milk and water intake among women

1. Date.... .... □□.□□.□□

2. Id number woman .... □□□-□

3. Id number child .... □□□-□

4. Camp . ... □□
  1=27.February, 2=Smara, 3= Auserd, 4=Dajla, 5=Aaiun

5. Daira (write)_____ □□
  (Daira according to a list)

6. Barrio (write)_____ □□

  (Barrio according to a list)

7. Name of the interviewer_____

8. Time of the interview: .... a) start □□-□□ .... b) stop □□-□□

Information to the mothers (read): In this questionnaire, we will ask you about your consumption of milk, water, tea and soup during the last 7 days. Yesterday is the last 24 hours, which refer to food and drink consumed from the time you woke up yesterday until the time you woke up today. We will start with milk and continue with the other foods and drinks.

97 = Forgot to ask
98 = Not answered
99 = Do not know

MILK

9. Did you drink any of the following milk types the last 7 days? (Ask all milk types)

9.1 Goat milk.. ....☐☐

       0=No, 1=Yes

9.2 Camel milk ....☐☐

       0=No, 1=Yes

9.3 Cadida milk ....☐☐

       0=No, 1=Yes

9.4 Powder milk ....☐☐

       0=No, 1=Yes

9.5 Other milk ....☐☐

       0=No, 1=Yes

If NO on all the questions above, go to Question 30.

If YES on any of the questions above, continue with the questions according to the milk type they have consumed (10-29).

97 = Forgot to ask
98 = Not answered
99 = Do not know

Goat milk

10. How was the distribution of milk and water?............... ☐☐☐☐ g milk   ☐☐☐☐ g water

11. How much goat milk did you drink in total per day each of the last 7 days?

    a) Yesterday (Day 1) ....☐☐☐☐ g

    b) Day before yesterday (Day 2) ....☐☐☐☐ g

    c) Day 3 ....☐☐☐☐ g

    d) Day 4...☐☐☐☐ g

    e) Day 5 ....☐☐☐☐ g

    f) Day 6.. ....☐☐☐☐ g

    g) Day 7 ....☐☐☐☐ g

  12. Where did you get the goat milk? ....☐☐

    *1=From my own household, 2=I bought it/got it (**Note**:Use the day that is coming first on the list of days)*

 If you bought it/got it,

    12.1 Where did you buy/get the goat milk? (write)_____

13. Is this the amount of goat milk you usually drink in a week?:....................................☐☐

    0=No, 1=Yes

If NO,

    13.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

Camel milk

14. How was the distribution of milk and water?............... ☐☐☐☐ g milk   ☐☐☐☐ g water

15. How much camel milk did you drink in total per day each of the last 7 days?

    a) Yesterday (Day 1) ....☐☐☐☐ g

    b) Day before yesterday (Day 2) ....☐☐☐☐ g

    c) Day 3 ....☐☐☐☐ g

    d) Day 4.. ....☐☐☐☐ g

    e) Day 5 ....☐☐☐☐ g

    f) Day 6... ....☐☐☐☐ g

    g) Day 7 ....☐☐☐☐ g

16. Where did you get the camel milk? .... ☐☐

    *1=From my own household, 2=I bought it/got it (**Note**:Use the day that is coming first on the list of days)*

 If you bought it/got it,

    16.1 Where did you buy/get the camel milk? (write)_____

17. Is this the amount of camel milk you usually drink in a week?.................................. ☐☐

    0=No, 1=Yes

If NO,

    17.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

Cadida milk

18. How was the distribution of milk and water?............... ☐☐☐☐ g milk   ☐☐☐☐ g water

19. How much Cadida milk did you drink in total per day each of the last 7 days?

    a) Yesterday (Day 1) ....☐☐☐☐ g

    b) Day before yesterday (Day 2)................................ ....☐☐☐☐ g

    c) Day 3 ....☐☐☐☐ g

    d) Day 4.. ....☐☐☐☐ g

    e) Day 5 ....☐☐☐☐ g

    f) Day 6.. ....☐☐☐☐ g

    g) Day 7 ....☐☐☐☐ g

20. Where did you get the Cadida milk? ....☐☐

    *1=From my own household, 2=I bought it/got it (**Note**:Use the day that is coming first on the list of days)*

If you bought it/got it,

    20.1 Where did you buy/get the Cadida milk? (write)_____

21. Is this the amount of Cadida milk you usually drink in a week? ....☐☐

    0=No, 1=Yes

If NO,

    21.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

Powder milk

22. How was the distribution of milk and water?............... ☐☐☐☐ g milk ☐☐☐☐ g water

23. How much powder milk did you drink in total per day each of the last 7 days?

    a) Yesterday (Day 1) ....☐☐☐☐ g

    b) Day before yesterday (Day 2) ....☐☐☐☐ g

    c) Day 3 ....☐☐☐☐ g

    d) Day 4... ....☐☐☐☐ g

    e) Day 5 ....☐☐☐☐ g

    f) Day 6... ....☐☐☐☐ g

    g) Day 7 ....☐☐☐☐ g

24. Where did you get the powder milk? ....☐☐

    *1=From my own household, 2=I bought it/got it (**Note**:Use the day that is coming first on the list of days)*

If you bought it/got it,

    24.1 Where did you buy/get the powder milk? (write)_____

25. Is this the amount of powder milk you usually drink in a week? ....☐☐
    0=No, 1=Yes

If NO,

    25.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

Other milk

26. How was the distribution of milk and water?............... ☐☐☐☐ g milk   ☐☐☐☐ g water

27. How much other milk did you drink in total per day each of the last 7 days?

    a) Yesterday (Day 1) .... ☐☐☐☐ g

    b) Day before yesterday (Day 2) .... ☐☐☐☐ g

    c) Day 3 .... ☐☐☐☐ g

    d) Day 4... .... ☐☐☐☐ g

    e) Day 5 .... ☐☐☐☐ g

    f) Day 6.. .... ☐☐☐☐ g

    g) Day 7 .... ☐☐☐☐ g

28. Where did you get the other milk? ....☐☐

    *1=From my own household, 2=I bought it/got it (**Note**:Use the day that is coming first on the list of days)*

If you bought it/got it,

    28.1 Where did you buy/get the powder milk? (write)_____

29. Is this the amount of other milk you usually drink in a week? ....☐☐
    0=No, 1=Yes

If NO,

    29.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

WATER

30. Did you drink water the last 7 days? .... ☐☐

  0=No, 1=Yes

 If NO, go to Question 34.

 If YES, continue with the questions below.

31. How much water did you drink in total per day each of the last 7 days?

  a) Yesterday (Day 1) .... ☐☐☐☐ g

  b) Day before yesterday (Day 2) .... ☐☐☐☐ g

  c) Day 3 .... ☐☐☐☐ g

  d) Day 4 .... ☐☐☐☐ g

  e) Day 5 .... ☐☐☐☐ g

  f) Day 6 .... ☐☐☐☐ g

  g) Day 7 .... ☐☐☐☐ g

32. From where did you get the drinking water .... ☐☐

  *1= Public water, 2= Water from own well, 3= Sweet water, 4= Bottled water, 4= Other water (**Note**: Use the day that is coming first on the list of days)*

33. Is this the amount of water you usually drink in a week? .... ☐☐
  0=No, 1=Yes

If NO,

  33.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

TEA

34. Did you drink tea the last 7 days? ....☐☐

   0=No, 1=Yes

If NO, go to Question 38

If YES, continue with the questions below.

35. How much tea did you drink in total per day each of the last 7 days?

   a) Yesterday (Day 1) ....☐☐☐☐ g

   b) Day before yesterday (Day 2) ....☐☐☐☐ g

   c) Day 3 ....☐☐☐☐ g

   d) Day 4 ....☐☐☐☐ g

   e) Day 5 ....☐☐☐☐ g

   f) Day 6 ....☐☐☐☐ g

   g) Day 7 ....☐☐☐☐ g

36. From where did you get the tea water ....☐☐

   *1= Public water, 2= Water from own well, 3= Sweet water, 4= Bottled water, 4= Other water (**Note**: Use the day that is coming first on the list of days)*

37. Is this the amount of tea you usually drink in a week?.. ....☐☐
   0=No, 1=Yes

If NO,

   37.1 Why not? (write)_____

97 = Forgot to ask
98 = Not answered
99 = Do not know

SOUP

38. Did you eat soup the last 7 days? ....☐☐

0=No, 1=Yes

If YES, continue with the questions below.

39. How much soup did you eat in total per day each of the last 7 days?

    a) Yesterday (Day 1) ....☐☐☐☐ g

    b) Day before yesterday (Day 2)...................................... ....☐☐☐☐ g

    c) Day 3 ....☐☐☐☐ g

    d) Day 4.. ....☐☐☐☐ g

    e) Day 5 ....☐☐☐☐ g

    f) Day 6.. ....☐☐☐☐ g

    g) Day 7 ....☐☐☐☐ g

40. Is this the amount of soup you usually eat in a week?... ....☐☐

0=No, 1=Yes

If NO,

    40.1 Why not? (write)_____

Thank you for your time and for answering our questionnaire ☺

List of participants from each area, camp and daira

Area	Camp	Daira	Beneficiaries[1]	Estimated participants[2]	Participants included[3]
LOW IODINE AREA	SMARA	B. Lehlu	4150	4	4
		Mahbes	4594	4	2
		Farsia	5779	5	6
		Ejdeiria	5675	5	4
		Hauza	5291	5	6
		Tifariti	4881	4	4
		Mheiriz	4998	4	5
		27. de Feb	9050	8	10
		Subtotal	44418	39	41
	DAJLA	B.Enzaran	2395	2	2
		A. El Beida	1948	2	2
		G. El Fula	2647	2	2
		Bjudur	2544	2	2
		Umdreiga	3000	3	3
		El Argub	2047	2	2
		Jreifa	2549	2	2
		Subtotal	17130	15	15
HIGH IODINE AREA	AUSERD	Aguenit	3986	4	6
		Tichla	4298	4	2
		La Guera	5350	5	3
		B. Ganduz	4548	4	5
		Miyex	4750	4	7
		Zug	4348	4	2
		Subtotal	27280	25	25
	AAIUN	Amgala	6000	5	2
		Dchera	5349	5	7
		Daora	6148	5	5
		Hagunia	5690	5	6
		Buchraa	6198	6	4
		Guelta	6047	5	6
		Subtotal	35432	31	30
TOTAL			**124260**	**110**	**111**

[1] Beneficiaries are the number of people in the Saharawi refugee camps who need assistance. People studying abroad, in military service or living in the liberated area are not included. It is considered that more people live in the camps, but these are the numbers of vulnerable agreed upon by the UN and Polisario

[2] Numbers of participants which were estimated to be included in the study, based on the number of beneficiaries in each camp and daira

[3] Number of participants from each camp and daira included in the study

Appendix 5

Time schedule for preparations and field work, Saharawi refugee camps 19.10-07.12, Algeria 2010

	Sat 23/10 *Rabouni*	Sun 24/10 *Auserd*	Mon 25/10 *Auserd*	Tue 26/10 *Rabouni*	Wed 27/10 *Rabouni*	Thur 28/10 *Rabouni*	Fri 29/10 *Rabouni*
9 am-14 pm	Meeting	Training (theory)	Training (practice)	Translation of Q	Training (practice)	-	-
14 pm-16 pm	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch
16 pm-18 pm	-	Training (theory)	Training (practice)	Translation of Q	Training (practice)	-	-
	Sat 30/10 *27. De Feb*	Sun 31/10 *27. De Feb*	Mon 01/11 *27. De Feb*	Tue 02/11 *Smara*	Wed 03/11 *Smara*	Thur 04/11 *Smara*	Fri 05/11 *Rabouni*
9 am-14 pm	Pilot	Household visit	Household visit	-	Household visit	Household visit	-
14 pm-16 pm	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch
16 pm-18 pm	Recruitment	Household visit Recruitment	Household visit Recruitment	Household visit Recruitment	Household visit Recruitment	Household visit	-
	Sat 06/11 *Smara*	Sun 07/11 *Smara*	Mon 08/11 *Auserd*	Tue 09/11 *Auserd*	Wed 10/11 *Auserd*	Thur 11/11 *Auserd*	Fri 12/11 *Rabouni*
9 am-14 pm	-	Household visit	-	-	Household visit	Household visit	-
14 pm-16 pm	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch
16 pm-18 pm	Recruitment	Household visit	Recruitment	Household visit Recruitment	Household visit Recruitment	Household visit	-
	Sat 13/11 *Rabouni*	Sun 14/11 *Rabouni*	Mon 15/11 *Rabouni*	Tue 16/11 *Rabouni*	Wed 17/11 *Rabouni*	Thur 18/11 *Rabouni*	Fri 19/11 *Rabouni*
	-	-	-	-	-	-	-
	Sat 20/11 *Dajla*	Sun 21/11 *Dajla*	Mon 22/11 *Dajla*	Tue 23/11 *Dajla*	Wed 24/11 *Rabouni*	Thur 25/11 *Rabouni*	Fri 26/11 *Rabouni*
9 am-14 pm	-	Household visit	Household visit	Household visit	-	-	-
14 pm-16 pm	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch
16 pm-18 pm	Recruitment	Household visit Recruitment	Household visit	-	-	-	-
	Sat 27/11 *Aaiun*	Sun 28/11 *Aaiun*	Mon 29/11 *Aaiun*	Tue 30/11 *Aaiun*	Wed 01/12 *Aaiun*	Thur 02/11 *Aaiun*	Fri 03/11 *Rabouni*
9 am-14 pm	-	Household visit	Household visit	-	Household visit	Household visit	-
14 pm-16 pm	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch	Lunch
16 pm-18 pm	Recruitment	Household visit Recruitment	Household visit	Recruitment	Household visit Recruitment	Household visit	-

Appendix 6

Blood constituents - Test results and interpretation

ID	TSH 0.2-3.4 mIU/L[*]	FT$_4$ 9.0-21.0 pmol/L[*]	TPOAb < 34 IU/mL[*]	Tg < 30 µg/L[*]	TgAb < 50 kU/L[*]	FT$_3$ 2.7-6.3 pmol/L[*]	TRAb < 1.0 U/L[*]	Thyroid abnormalities
-	1.2	13	<34	18	17			
-	5.4	14	<34	14	14			Subclinical hypothyroidism
-	1.1	13	<34	8.6	19			
-	2.5	12	<34	25	15			
-	3.8	13	<34	20	10			
-	3.2	11	<34	12	16			
-	14	9.6	1231	0.27	32			Autoimmune thyroiditis and subclinical hypothyroidism
-	0.90	10	<34	4.7	16			
-	1.6	14	<34	16	14			
-	2.2	13	<34	23	48			
-	0.15	17	332	197	43	7.2	<1.0	Autoimmune thyroiditis and subclinical hyperthyroidism
-	3.2	14	<34	16	20			
-	1.7	10.0	<34	360	20			
-	8.6	6.5	1357	281	999			Autoimmune thyroiditis and hypothyroidism
-	1.6	11	<34	25	21			
-	1.8	11	<34	7.3	29			
-	1.5	13	<34	25	18			
-	1.6	15	<34	34	19			
-	1.6	14	<34	57	26			
-	1.3	11	<34	6.8	21			
-	2.1	11	<34	8.9	15			
-	2.2	14	<34	39	15			

[*] Based on reference levels used by Oslo University Hospital in Norway (MBK, 2011).

Blood constituents - Test results and interpretation

ID	TSH 0.2-3.4 mIU/L[*]	FT$_4$ 9.0-21.0 pmol/L[*]	TPOAb < 34 IU/mL[*]	Tg < 30 µg/L[*]	TgAb < 50 kU/L[*]	FT$_3$ 2.7-6.3 pmol/L[*]	TRAb < 1.0 U/L[*]	Thyroid abnormalities
-	2.0	12	252	24	8.247			Autoimmune thyroiditis (euthyroid)
-	3.8	12	<34	25	19			
-	1.7	13	<34	6.8	37			
-	2.2	11	<34	12	11			
-	68	5.7	45	6.0	201522			Hypothyroidism
-	2.1	13	<34	6.8	27			
-	3.5	11	<34	2.3	16			
-	2.0	10.0	<34	2.8	24			
-	2.5	12	<34	23	8.359			
-	1.7	13	<34	7.2	19			
-	2.5	13	<34	14	19			
-	0.95	12	<34	51	274			
-	1.6	13	<34	30	8.168			
-	19	9.7	<34	60	16			Hypothyroidism
-	2.4	15	<34	9.7	18			
-	1.5	10	<34	18	8.679			
-	1.4	15	<34	9.9	10			
-	13	10	49	95	16			Hypothyroidism
-	2.2	14	<34	283	13			
-	4.7	12	<34	12	32			Subclinical hypothyroidism
-	2.4	13	<34	21	82			
-	4.9	14	<34	23	4.764			Subclinical hypothyroidism

[*] Based on reference levels used by Oslo University Hospital in Norway (MBK, 2011).

Blood constituents - Test results and interpretation

ID	TSH 0.2-3.4 mIU/L	FT$_4$ 9.0-21.0 pmol/L	TPOAb < 34 IU/mL	Tg < 30 µg/L	TgAb < 50 kU/L	FT$_3$ 2.7-6.3 pmol/L	TRAb < 1.0 U/L	Thyroid abnormalities
-	2.3	11	<34	34	12			
-	2.5	13	<34	30	12			
-	2.7	12	<34	50	16			
-	2.9	11	<34	10	6.178			
-	3.1	13	<34	30	25			
-	0.010	18	109	30	244	7.0	<1.0	Autoimmune thyroiditis and subclinical hyperthyroidism
-	2.4	11	<34	30	2.885			
-	1.2	12	<34	7.2	3.966			
-	0.020	25	<34	0.15	84	8.50	<1.0	Subclinical hyperthyroidism
-	1.0	16	<34	11	27			
-	2.9	12	<34	17	15			
-	3.4	12	<34	7.3	56			
-	6.1	11	<34	22	9.583			Subclinical hypothyroidism
-	1.5	14	<34	1.3	11			
-	5.0	15	<34	34	15			Subclinical hypothyroidism
-	3.1	12	<34	14	22			
-	5.4	9.3	<34	64	55			Subclinical hypothyroidism
-	2.2	9.2	<34	62	4.309			
-	2.5	16	<34	6.4	52			
-	3.0	13	<34	34	14			
-	1.1	13	<34	2.5	13			
-	2.3	11	<34	23	13			

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Blood constituents - Test results and interpretation

ID	TSH 0.2-3.4 mIU/L[*]	FT$_4$ 9.0-21.0 pmol/L[*]	TPOAb < 34 IU/mL[*]	Tg < 30 µg/L[*]	TgAb < 50 kU/L[*]	FT$_3$ 2.7-6.3 pmol/L[*]	TRAb < 1.0 U/L[*]	Thyroid abnormalities
-	1.7	10	<34	6.9	6.32			
-	1.6	10	<34	11	21			
-	0.98	13	<34	15	14			
-	2.6	9.1	<34	12	19			
-	2.9	11	<34	11	36			
-	4.0	14	<34	6.4	13			Subclinical hypothyroidism
-	4.8	11	<34	11	9.777			Subclinical hypothyroidism
-	1.6	12	<34	4.8	71			
-	6.2	12	<34	1.3	46			Subclinical hypothyroidism
-	3.0	11	<34	10.0	3.931			
-	0.84	13	<34	6.0	7.719			
-	2.2	12	<34	7.2	2.951			
-	4.0	12	<34	27	74			Subclinical hypothyroidism
-	2.5	13	<34	23	18			
-	0.020	41	1465	120	54	13.20	<1.0	Autoimmune hyperthyroidism
-	2.3	10	<34	22	6.375			
-	0.70	15	<34	25	11			
-	2.2	11	<34	9.6	15			
-	3.2	12	<34	19	15			
-	8.0	9.5	<34	82	7.735			Subclinical hypothyroidism
-	0.010	18	<34	8.2	71	7.40	<1.0	Subclinical hyperthyroidism
-	3.8	13	<34	26	1.505			

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Blood constituents - Test results and interpretation

ID	TSH 0.2-3.4 mIU/L[*]	FT$_4$ 9.0-21.0 pmol/L[*]	TPOAb < 34 IU/mL[*]	Tg < 30 µg/L[*]	TgAb < 50 kU/L[*]	FT$_3$ 2.7-6.3 pmol/L[*]	TRAb < 1.0 U/L[*]	Thyroid abnormalities
-	0.79	11	<34	3.6	12			
-	8.9	12	<34	76	17			Subclinical hypothyroidism
-	0.030	19	45	17	163	7.30	<1.0	Subclinical hyperthyroidism
-	1.5	12	<34	8.9	30			
-	1.2	14	<34	4.4	32			
-	0.74	17	<34	18	17			
-	1.5	15	<34	26	25			
-	1.3	12	-	23	6.995			
-	4.7	13	<34	30	10			Subclinical hypothyroidism
-	2.5	12	<34	56	13			
-	2.0	14	<34	9.4	11			
-	2.1	11	<34	14	17			
-	1.2	13	<34	133	189			
-	2.6	14	<34	36	29			
-	3.4	8.9	<34	13	5.431			
-	1.1	13	<34	11	30			
-	2.6	12	<34	39	23			
-	0.35	12	<34	97	19			
-	0.42	15	-	9.0	95			
-	0.070	16	<34	131	12	6.10	1.10	Subclinical hyperthyroidism
-	1.5	12	<34	32	9.809			
-	0.73	11	<34	3.7	47			
-	1.9	11	<34	73	29			

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