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Aspects of feeding patterns in the first two years of life in Iranian infants

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"Never dieth that one, whose heart is alive with love
our durable impression is recorded in the world"

"Hafez, Iranian poet"

"To my mother with warm hug and to my late father with respect"

"To my husband and my lovely daughters"

ABSTRACT

Background: The unique way of providing infants with a perfect nutrition necessary for good growth and development is breastfeeding. Exclusive breastfeeding for the first 6 months is recommended by the World Health Organization. Maternal intake of long chain polyunsaturated fatty acids (LCPUFA), especially omega-3 fatty acids (FAs), during pregnancy and lactation influence the content in breast milk. Docosahexaenoic acid (DHA) is important for infant growth and the development of brain and vision. For vitamins A and D, Iranian authorities recommend supplements for infants from15 days to 2 years of age.

Aim: To investigate the nutritional situation for Iranian infants by describing the current situation regarding breastfeeding prevalence, promotion and support. Furthermore, to investigate the nutritional content of FAs in colostrum related to mother's diet as well as status for vitamin A and D in infancy.

Methods: Cross-sectional analytic methods have been combined in this thesis to examine the quality of the feeding patterns. Study I and II are based on questionnaire and interview data from mothers of 63 071 infants up to 2 years of age in all 30 provinces of Iran. The data of breastfeeding rates were collected in 2005-2006 (Sep 15th – Jan 15th) by trained health workers in the Integrated Monitoring Evaluation System (IMES) in the Family Health Office of the Ministry of Health. A translated version of a survey, used to assess the current breastfeeding situation in Europe, was also used in study I. Study II used the questionnaire data from IMES to pin point important determinants for early discontinuation of breastfeeding. In study III, breast milk was collected early after delivery from 120 mothers, 60 in each province of Guilan and Kermanshah (coastal area and inland, respectively) during July to September 2008. The mothers were interviewed regarding dietary intake using a food frequency questionnaire. The FA composition of colostrum was measured with gas chromatography. Study IV investigated 7112 infants 15-23 months of age who attended Health Care Centers during early summer of 2001. The sampling method was unequal clusters with unequal household sizes. Vitamins A and D were analyzed with high performance liquid chromatography in an accredited laboratory.

Findings: The policy questionnaire showed that 466 hospitals were accredited as Baby Friendly Hospitals, covering more than 80% of the births in Iran in 2006. On a national level, 89% and 57% of children were breastfed at one and two years of age, respectively. Exclusive breastfeeding rates at 4 and 6 months of age were 57% and 28%. The most common reason for stopping exclusive breastfeeding before six months was, according to the mothers, the physician's recommendation. After 6 months, the mothers stated the major reason to be insufficient breast milk. Mothers in coastal areas had significantly higher intake of fish or seafood during pregnancy than the mothers in inland areas. High fish and seafood intake was associated with significantly higher DHA concentration and lower arachidonic acid/DHA ratio. The mean (SD) concentrations of vitamin A and D were 2.09, (0.83) μmol/L and 61.3 (31.4) nmol/L, respectively. About 1.2% of the infants had plasma levels indicating deficient or insufficient vitamin A. Deficient and insufficient levels of vitamin D were found in 2.8% and 32.9% of infants, respectively.

Conclusions: The general breastfeeding situation, vitamin A status in Iranian infants, and LCPUFA status in colostrum in women were good in comparison with several countries. However, the exclusive breastfeeding prevalence was lower than previously reported. Physicians and other health professionals have an important role in supporting mothers to breastfeed. There is a need for further investigation in regards to the adequacy and compliance of vitamin D supplementation to infants.

LIST OF PUBLICATIONS

I. Olang B, Farivar K, Heidarzadeh A, Strandvik B and Yngve A. *Breastfeeding in Iran: prevalence, duration and current recommendations* International Breastfeeding Journal 2009, 4:8 doi10.1186/1746-4358-4-8

II. Olang B, Heidarzadeh A, Strandvik B and Yngve A. *Physician's role; important for support of breastfeeding* (Submitted)

III. Olang B, Haji Faraji M, Ali MA, Hellstrand S, Palesh M, Azadnyia E, Kamali Z, Strandvik B and Yngve A. *Long chain polyunsaturated n-3 fatty acids differ significantly in colostrum in relation to fish intake during pregnancy* (Submitted)

IV. Olang B, Naghavi M, Bastani D, Strandvik B and Yngve A. *Optimal vitamin A and suboptimal vitamin D status are common in Iranian infants* Acta Pædiatrica IN PRESS

CONTENTS

LIST OF ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha-linolenic acid
BFHI	Baby Friendly Hospital Initiative
BFHs	Baby Friendly Hospitals
DHA	Docosahexaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FFQ	Food frequency questionnaire
GC	Gas chromatography
HPLC	High performance liquid chromatography
IMES	Integrated Monitoring Evaluation System Survey
LA	Linoleic acid
LCPUFA	Long chain polyunsaturated fatty acids
MOH	Ministry of Health
NFNC	National Food and Nutrition Council
NNFTRI	National Nutrition and Food Technology Research Institute
BFPS	Breastfeeding Promotion Society
UFMS	University/Faculty of Medical Sciences
25(OH)D	25-hydroxy vitamin D

DEFINITIONS

The definitions used in this thesis are consistent with the WHO terminology [1-4].

Exclusive breastfeeding means only breast milk and no other liquids or solids except for those containing vitamins or medicines to the baby. *Partial breastfeeding* includes some breastfeeds, and some artificial feeds, either milk or cereal, or other food. *Any breastfeeding* includes partial and exclusive breastfeeding

Discontinuation is defined as complete cessation of breastfeeding between 7-24 months of age. *Early discontinuation of breastfeeding* means complete cessation of breastfeeding before 6 months of age.

Colostrum is defined as the yellowish, sticky breast milk produced for 3 days after birth.

Malnutrition essentially means not enough as well as too much food, or the wrong types of food. Clinically, malnutrition is characterized by inadequate or excess intake of energy, macronutrients and micronutrients such as vitamins, and sometimes related to the frequent disease and disorders.

1 INTRODUCTION

1.1 THE IMPORTANCE OF BREASTFEEDING

The unique way of providing infants with a perfect nutrition necessary for good growth and development is breastfeeding [5]. Breastfeeding is also a necessary and important part of the reproductive process with important implications for the health of mothers [6]. According to a population-based review of evidence, exclusive breastfeeding, in the first six months of age, is the most advantageous way of feeding infants [7], which is also recommended by the World Health Organization (WHO). Since breast milk is no longer enough after this age to meet the nutritional needs of the infants, they need to be fed by complementary foods with continued breastfeeding up to 2 years of age [5, 6]. Complementary feeding which very often covers the period from 6 to 18-24 months, a vulnerable period of life for infants, is a transition period from exclusive breastfeeding to family foods. It is the time when malnutrition starts in many infants, contributing significantly to the high prevalence of malnutrition in children less than five years of age worldwide [6]. The WHO estimated that 2 out of 5 children are stunted in low-income countries [8-10]. Fetal and postnatal nutrition also have long-term effects on the risk for development of diseases later in life [11].

1.2 SUPPORT OF BREASTFEEDING PRACTICES

Cessation of breastfeeding is linked to an increased risk of acute and chronic diseases in childhood [12]. Low rates and early cessation of breastfeeding have important adverse effects on health, social and economic implication for women, children, the community and environment, and results in greater expenditure on national health care provision and may increase inequalities in health.

The WHO concludes that exclusive breastfeeding, defined as giving breast milk without any food or liquid until 6 months of age, confers benefits to mothers and infants [2]. Globally, less than 40% of infants are exclusively breastfed until six months of age [13]. Factors positively influencing the extent of breastfeeding have been shown to be mothers' socio-demographic and psychosocial background such as high education

and a supportive family [14]. Other important factors are the routines in health care and the messages conveyed by health professionals and physicians [15].

1.3   SUPPLEMENTARY FEEDING AND NUTRITIONAL STATUS

Exclusive breastfeeding increases survival and optimized growth of low birth weight infants [16]. Breast milk alone after six months may not meet the infant's growing requirements of some micronutrients, especially iron.

In several countries, vitamins A and D are provided as supplements to babies during breastfeeding, since breast milk alone may not meet the infant's requirements of vitamin A and D [17, 18]. Vitamins A and D are fat-soluble compounds and, in Iran as well as in most countries, the recommended intake for infants from 25 days up to two years of age is 450 μg/day and 10 μg/day, respectively [19].

Vitamin A is important for vision, protein synthesis and cell differentiation, as well as for growth and vitamin D is important for mineralization of bone growth and metabolism [20]. Vitamin D deficiency may occur in exclusively breastfed infants who are not sufficiently exposed to sunlight, i.e. confined indoors during daylight hours or covered by clothing while outdoors. Living in urban centers, where high rise buildings and pollution can block sunlight, provides another risk. A darker skin pigmentation and use of sun screen lotions can further lead to an increased need for vitamin D supplementation [11, 21]

1.4   DIET DURING LACTATION AND NUTRITIONAL COMPONENTS OF BREAST MILK

In the last 20 years, several studies have examined the maternal intake of fish, seafood and omega (n)-3 long chain polyunsaturated fatty acids (LCPUFA) during pregnancy and lactation in relation to the impact on infant's health outcome [22, 23]. Maternal health during pregnancy as well as fetal and child health have the potential to be improved by LCPUFA [24]. To reach the requirement of essential nutrients, it is important that a high quality diet is available for the mother, infant and child [25].

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Mothers should be advised to eat a balanced varied diet. Some fatty acids (FAs) are necessary to get from diet, since they cannot be synthesized in the human body, these essential FAs belong to the n-3 and the n-6 series, having a carbon-carbon double bond in the n-3 or the n-6 position (Figure 1.1) [24, 26]. Usually, enough n-6 is supplied by different vegetable oils as linoleic acid (LA), which is transformed to the other LCPUFA of this series. More importantly, the arachidonic acid (AA) is necessary for normal growth and also for the nerve system and brain development.
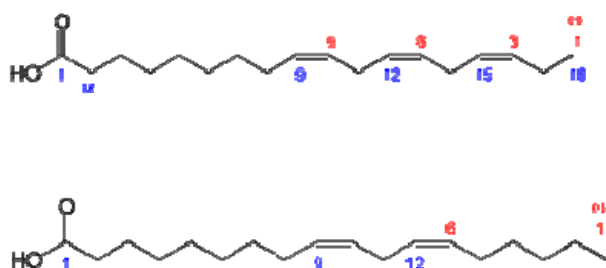
Figure 1.1 The molecular formula of n-3 and n-6 fatty acids

Important n-3 fatty acids in nutrition are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The human body cannot synthesize the n-3 ALA, but can synthesize all the other necessary n-3 fatty acids from ALA but EPA and especially DHA are synthesized in very low amounts. Therefore, ALA obtained from food need to be complemented by fish or seafood rich in EPA and DHA, since both fatty acids are important for normal development and especially DHA that accretes in the nervous system in high rate during the perinatal period [27]. Essential FAs deficiency has an anti inflammatory effect [28].

DHA thus is very important for the baby. Fats in the traditional diet in the countries of the Mediterranean basin are typically rich in olive oil, but the high consumption of vegetables, and to some extent, fish and seafood result in a balanced intake of n-3 and n-6 fatty acids.

Fat in breast milk is present as triglycerides and a small part as phospholipids. Long chain polyunsaturated fatty acids are necessary for growth and neural development

[26]. There are three main sources for LCPUFA; fish, fish oil and egg yolk (depending on chicken feed) [26].

Trans fatty acids [29], originating from highly processed foods interfere with the elongation of n-3 fatty acids, whereas olive oil increases the incorporation of n-3 fatty acids in the cell membranes [30]. There is a rising interest in a balanced and varied diet not only preconceptionally but also during pregnancy and in the breastfeeding period in order to reduce fetal, neonatal, and maternal risks, since the DHA concentration in the mothers' plasma determines the concentration in the breast milk [31].

The n-6 to n-3 ratio is important to maintain cardiovascular health [20]. Both n-3 and n-6 fatty acids are essential. N-3 and n-6 compete for the same metabolic enzymes, thus the n-6 to n-3 ratio will significantly influence the ratio of the ensuing hormones, and will alter the body's metabolic function. In Paleolithic time the ratio of n-6 to n-3 was probably about 1:1-2:1, and the present general recommendation is about 4:1-5:1 but more than 10:1 is commonly reported [20].

Maternal supplementation with a proper balance of n-3 and 6 fatty acids during pregnancy and lactation provides more DHA to the infant [32]. Human milk is the sole source of nutrition for the breastfed infant and if maternal diet is appropriate, it provides all of the fatty acids needed [33] for the infant's growth and development, including fatty acids for synthesis of membrane lipids and adipose tissue [29].

In settings where micronutrient deficiency in infants under six months of age is of concern, improved maternal intake during pregnancy and lactation, instead of premature introduction of complementary foods, is the most effective and less risky way of preventing deficiencies [34]. Maternal diet enrichment with DHA will mitigate the reduction of this FA in breast milk which normally occurs during prolonged breast feeding [26].

1.5 IRAN

Iran is located in Western Asia (Middle East) [35] and has a population of 72 million. It covers 1.648 million km^2. It is the 18th largest country in the world and the fourth in

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Asia. The country has 30 provinces, 336 districts, 1012 cities and 64 000 villages [36, 37]. Iran is a middle income country [38] with a gross national income of 3998 purchasing power parities (ppp) US dollars per capita in 2007, with under 6% of the population living on less than two dollars a day [39].

In 2006, Iran's human development index (HID) value was 0.777, the gender related development index value was also 0.777 and the gender empowerment measure value was 0.345 [40].

Education and employment

The adults' literacy rate which was 65% in 1991 improved and reached 84% in 2006 [40] while it was higher in women. Gender parity index (women to men) in primary, secondary and tertiary level education enrolment was 0.97, 0.75 and 0.84 in 1991, whereas in 2005, it was 1.22, 0.94 and 1.09, respectively [41]. However, female literacy as a percentage of male literacy rate is 88% [40].

The other improvement is increasing the employment rate in women. In 1991, while the employment to population ratio for men and women were 73% and 18%, respectively, it increased to 67% and 33% in 2005 [41]. The unemployment rate among women, however, is still high, especially among those with high education. The unemployment rate for youth (15-24 years old) women and men was 32% and 20%, respectively in 2005 [41].

Population

Iran experienced a remarkable increase in population within 15 years i.e. the population reached from 33 million in 1975 to 66 million in 1990 [42]. The urbanization rate has been increasing over the past decades. It was 54% in 1976 and increased to 68% in 2006. Total fertility rate and annual growth rate were 2.1 and 1.2 in 2004, respectively [36].

1.5.1 The health care system in Iran

During the last three decades, Iran has made remarkable progress in the health sector. The extensive system of a health care network, with its base in the communities and with easy access to basic preventive and curative services at its heart, ensured provision

primary health care to the vast majority of population. At present, 95% of the rural population enjoys health care services through the health houses staffed by community health workers so called Behvars. The system greatly improved many of the main health indicators, especially in maternal and child health, and family planning (Table 1) [43].

Table 1 Health indicators in Iran

Indicator	Year	Number
Life expectancy at birth (years)	1970-1975	55.3
	2000-2005	70.3
Infant mortality rate (per 1000 live births)	1970	122
	2001	35
Under-5 years mortality rate (per 1000 live births)	1970	191
	2001	42
Maternal mortality ratio (per 100 000 live births)	1988	90
	2001	37
Total fertility rate (per woman)	1970-1975	6.4
	2000-2005	2.3
One-year-olds immunized against tuberculosis (%)	1988	88
	2001	93
One-year-olds immunized against measles (%)	1988	83
	2001	96

The primary health network of Iran (Figure 1.2) is an integrated and stratified health care system.
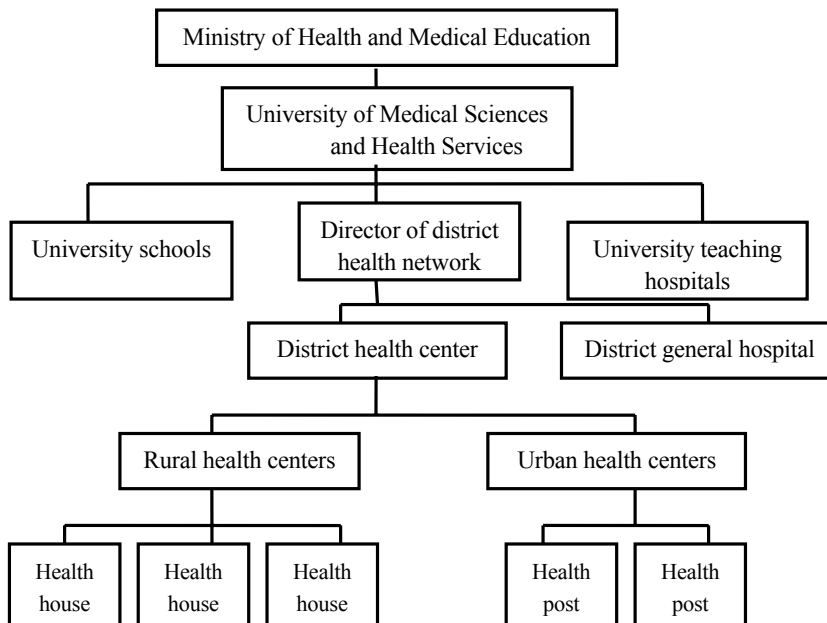
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│ University schools │  │ Director of district │  │ University teaching │
│               │  │   health network    │  │     hospitals       │
└───────────────┘  └──────────────────┘  └──────────────────┘
                    ┌─────────────────┐  ┌──────────────────────┐
                    │ District health center │  │ District general hospital │
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          ┌──────────────────┐      ┌──────────────────┐
          │ Rural health centers │      │ Urban health centers │
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Figure 1.2 Health care networks in Iran

Health houses in rural areas, and health centers and health posts in urban areas are the first points of contact between the community members and primary health care system. The health houses and health posts are supported by rural or urban health centers. The rural and urban health centers are administrated by district health centers, one in each district or more than one in a few large districts [43]. As a result of prioritization of rural areas and effective delivery of primary health care, health outcomes in rural areas of the country are now almost equal to those in urban areas, with indicators for infant and maternal mortality nearly identical [44].

The University or Faculty of Medical Sciences (UFMS), at least one in each province, plays an important role both in medical education and in the provision of health services. The vice of the universities are the executive directors of the provincial health services and in charge of all district health centers and hospitals. They, in turn, report to the Ministry of Health (MOH) in Tehran [43].

When a new programme is developed, health workers at the provincial level attend an initial meeting at the MOH about programme goals, strategies, and activities, followed by regular updating workshops [45].

Each level of the primary health care system then educates the next level under them in the organization [46].

Health posts usually only have a family health unit, staffed mainly by midwifes and family health technicians. There are environmental health technicians in some and a general physician in a few of them. The staff work at the family planning, prenatal and postnatal health care, child health care and immunization. [47].

The urban centers are also staffed by administrative personnel and at least one general physician. Some of the centers, based on population density, cover one or more health posts. The main functions of the urban health centers, in addition to what is done at the health posts, are the provision of services for referred cases, and guidance and administration of the affiliated health posts [47].

1.5.2 Maternal and newborn health

Currently, about 97% of all births take place with skilled attendants, resulting in a considerable decrease in maternal mortality rate, e.g. from 90 to 38 per 100 000 live births in 1995 and 2005, respectively [48-50]. The mortality rate for infants was 30 per 1000 live births in 2008, and it was, for the population less than 5 years of age, 34 per 1000 in 2006 [49, 50].

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1.5.3 Situation of breastfeeding in Iran

The National Committee of Breastfeeding Promotion was established at the MOH in 1991 by the Minister of Health at that time, Professor Alireza Marandi [51]. The major strategy of the committee was to promote breastfeeding for preventing morbidity and mortality in infants [52] and improve their health. Breastfeeding could save seven million children less than one year from death worldwide [53]. Iran as a member of WHO has especially been active in the promotion of breastfeeding and has had a strong political management for support of breastfeeding. Iran voted in favor of the International Code of Marketing Substitutes [51].

Policy strategy in healthy children in 2004 in Iran [51]:
  1. Decrease of mortality rate for children less than one year of age (male & female)
  2. Decrease of mortality rate for children less than five years of age (male & female)
  3. Decrease of nutritional growth abnormalities in children
  4. Increase percentage of breast feeding to two years
  5. Increase percentage of exclusive breastfeeding in infants up to 6 months
  6. Increase percentage of complementary feeding in 6-9 months children
  7. Prescription of 1cc Vitamin A and D (drop or syrup) to neonate, 15 days after birth and change Vitamins A and D to Multivitamin drop after two months of age (controversial)
  8. Prescription of Iron supplements 12.5 mg/day from 6 months to one year and continuing to 2 years of age. The MOH provides vitamin and iron supplements up to one year of age free of charge
  9. Increase the number of BFHs
  10. Decrease mental and physical growth abnormality

1.5.4 Fish and fishery area in Iran

The Caspian Sea is about 422000 km^2 in area with 6397 km of coastline, of the latter, more than 900 km is in Iran [54]. The Iran's fishery area is shown in figure 1.3 [55].
The most important fish species in the Caspian Sea are Caspian kutum (R.frisii kutum), Mullet (L. aurata and L. saliens) and Pikeperch (S. lucioperca) [54]. Their total PUFA

content were 23%, 22% and 20%, respectively [56]. The highest content per g fat is found in kutum (32.7±9.1g/100 g).

1.5.5   Fat and fatty acid consumption in Iranian nutrition

Iran is undergoing a socioeconomical and a nutritional transition [57]. Both under nutrition and nutrient deficiency diseases (anemia, iodine deficiency and some mineral and vitamin deficiencies), as well as overnutrition in the form of obesity, diabetes mellitus, cancers, and cardiovascular diseases are present in the population [57]. The dietary energy intake of fat, carbohydrate and protein in Iran are 25%, 65% and 10%, respectively [58].

The National Food and Nutrition Council (NFNC) of the Ministry of Agriculture in Iran reported that the mean fat intake per capita was 66 grams per day [58]. That is equal to 22% of the daily energy intake. They also reported saturated FA, MUFA and PUFA constitute 13%, 7% and 1.5% of total energy intake, respectively [59].
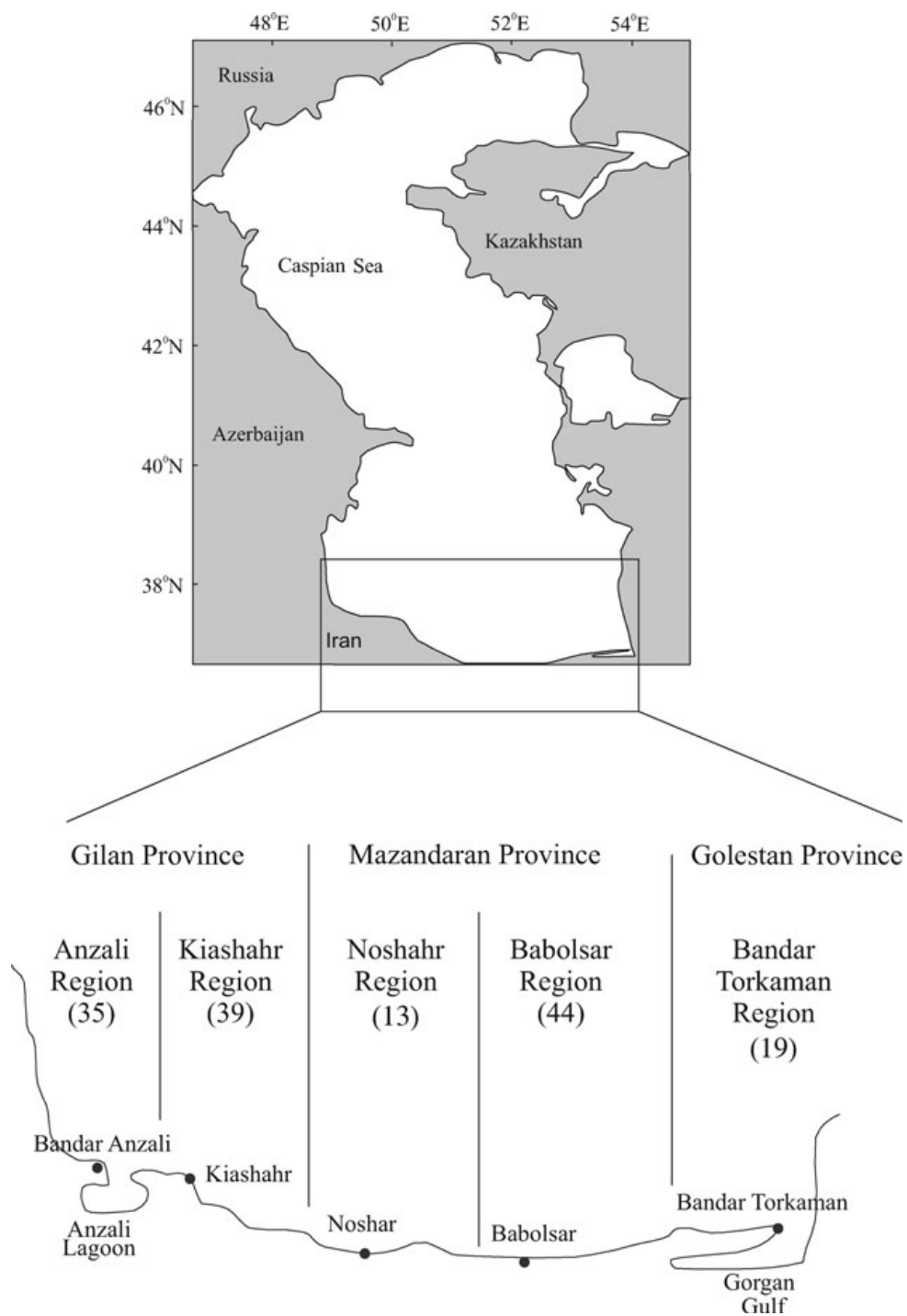
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Figure 1.3 Caspian Sea fishery area in Iran
Source [54]

1.6 RATIONALE FOR THE STUDY

Iran belongs to the Eastern Mediterranean Region of the WHO [60]. In this region nutrition policy is mainly concerned with efforts to decrease malnutrition. A systematic review of nutritional status in this region [60] showed that malnutrition has decreased during the last two decades. The diagnosis of malnutrition was in 55% of cases coexisting with a pediatric disease [51].

Many studies have been conducted on breastfeeding in Iran during the last twenty years but the study in this thesis comprised the first national study in MOH regarding breastfeeding prevalence at national level. Based on the Iranian Demographic Health Survey (DHS) information from the year 2000, the rate of infants being exclusively breastfed at the age of six months was 44% [51].

Previous research from Iran [60-62] showed that interventions can reduce severe malnutrition to moderate and mild. According to the MOH of Iran, in 1999, mild to moderate malnutrition was most prevalent in children between 6 months and one year of age. Micronutrient malnutrition (i.e. iron, zinc) was stated as one of the most important problems limiting economic progress in Iran in 2000. One of the nutritional problems in Iran is a low fish consumption in the diet. The average annual consumption of sea foods in the world is 18 kilograms per capita; it is on average 6.7 kilograms per capita and year in Iran [51].

2 OBJECTIVES

2.1 GENERAL OBJECTIVE

To investigate nutritional status in Iranian infants by:

> ➤ Describing the current situation regarding breastfeeding prevalence, promotion and support and the nutritional content of colostrum related to mother's diet as well as nutritional status regarding some important nutrients in early infancy

2.2 SPECIFIC OBJECTIVES

1. To describe the current situation regarding breastfeeding rates as well as breastfeeding promotion and support in Iran

2. To investigate regional difference in fish intake during pregnancy and whether that is related to the fatty acid concentration in breast milk

3. To assess the nutritional status regarding vitamins A and D in Iranian infants

3 METHODS

3.1 STUDY SETTING

Study I and II were conducted in Family Health Office of the MOH (2006). Study III was performed in the National Nutrition and Food Technology Research Institute (NNFTRI), Tehran; Moutazedi Hospital, Kermanshah; and Alzahra Hospital, Rasht, in 2008. Study IV was conducted in 2001 in the Family Health Office and the Office for Nutrition Promotion of the MOH in Iran (Figure 3.1).

3.2 OVERALL STUDY DESIGN

Cross sectional analytic methods have been combined in this thesis to examine the quality of feeding patterns for Iranian infants. In study I, we aimed to clarify the situation in 2006 regarding breastfeeding rates as well as breastfeeding promotion and support. Furthermore, we surveyed the implementation of breastfeeding policies in Iran. In study II, we aimed to clarify the most prevalent causes of cessation of partial breastfeeding as well as the reasons for the short duration of exclusive breastfeeding, compared to the WHO recommendation. In study III, we aimed to estimate essential fatty acids and LCPUFA concentrations in breast milk in two districts (Kermanshah and Guilan) of Iran, which differ in regard to dietary fish intake in 2008. In study IV, we investigated the status of vitamins A and D among infants aged 15 to 24 months in Iran. Study IV is based on a survey performed in Iran in 2001, covering all the different provinces as well as urban and rural characteristics.

Figure 3.1 Map of Iran

The summary of the methods used in this study is shown in Table 3.1.

Table 3.1 Summary of methods

Title of study	Design	Data collection	Year of data collection
I: Breastfeeding in Iran: prevalence, duration and current recommendations	Cross-sectional	Policy & IMES questionnaires	IMES, Sep-Jan, 2005-2006 Policy questionnaire, February, 2007
II: Physician's role; important for support of breastfeeding	Cross-sectional	IMES questionnaire	IMES, Sep-Jan, 2005-2006
III: Long chain polyunsaturated n-3 fatty acids differ significantly in colostrum in relation to fish intake during pregnancy	Cross-sectional	Food frequency questionnaire, structured interview and GC for fatty acid concentrations in breast milk	July-September, 2008
IV: Optimal vitamin A and suboptimal vitamin D status are common in Iranian infants	Cross-sectional	Questionnaire and HPLC for serum concentrations of vitamins A & D	National survey, May-June, 2001

3.3 THE INTEGRATED MONITORING EVALUATION SYSTEM SURVEY (IMES)

Breastfeeding rates and their discontinuation were studied (studies I and II), using the Integrated Monitoring Evaluation System Survey (IMES), which collects data regarding the content and consequences of provision and delivery health services for promoting health in the country. The study population was obtained with regard to the general objective of IMES and on the basis of data required for Family and Population Health Office of the MOH in 2005-2006. The study population was based on data from IMES collected as follows: all mothers who had given birth at hospitals or other health facilities, all mothers who had taken their infants for the first vaccination at one of the

rural or urban centers, all infants less than 2 years of age who lived in urban or rural areas and had been referred to Health Care Center or Health House to receive health care, vaccination or treatment for any disease.

The data were gathered in urban and rural areas by health workers who were trained in the specifics of collecting health data including the definition of exclusive breastfeeding in the previous 24 hours. IMES data collection was performed in all rural areas through direct visits by trained interviewers who were employees of the MOH at UFMS and were sufficiently familiar with concepts and guidelines of provision of healthcare as well as asking the IMES questions through a questionnaire. The questionnaire was validated in a pilot study in late 2004 [51]. It was in Persian, the feeding patterns' questions were translated to English. Questions about infant feeding patterns at ages 4, 6 and less than 24 month were included. Questions also covered the name of province, district and health center, number of household, mother's age and education, birth date, infant's age, weight, length and head circumference, breastfeeding exclusively, breastfeeding duration and any breastfeeding. The data was collected from 15 September 2005 to 15 January 2006.

Sampling method and sample size (IMES)
The MOH used a multistage sampling method with three kinds of sampling:
1. Random cluster sampling with non-equal clusters with equal sample size. In each district 20 clusters of households were identified by random sampling from a list of electricity subscribers (common way of identifying households in Iran). Subsequently, two children were selected from each cluster to enter the study.

2. Sampling in the urban areas was made through inviting clients who came to Health Centers for vaccination (convenience sampling).

3. Random systematic sampling was used to sample infants in rural areas. The sampling frame was the list of child care users which covered more than 99% of the infants in rural areas.

3.4   STUDY I

For exclusive breastfeeding rate data from IMES, only those infants who were between 1 day and 6 months of age were included. However, for general breastfeeding data, all infants were included (Table 3.2).

Table 3.2 IMES sample size, 2005-2006

Age, months	Total number	Urban/rural %	Proportion distribution	Cumulative %
0-4	2186	62/38	4.4	4.4
>4-6	6248	61/39	10.8	15.2
>6-9	15833	56/44	20.5	35.7
>9-12	5290	54/46	9.3	45
>12-18	17159	58/42	28	73
>18-24	16355	56/44	27	100
Total	63 071	57/43	100	100

The policy questionnaire

In order to assess the current situation in regard to breastfeeding protection, promotion and support, a questionnaire was used during the period February to May 2007. This was a Persian translation of a questionnaire to authorities, constructed and used previously to assess the breastfeeding situation in Europe. The questionnaire was sent to the central committee of breastfeeding at the MOH, where it was completed based on provincial and national data in 2006.

The questionnaire was used to investigate how the following topics were implemented in Iran as a whole and in its provinces:
1. Breastfeeding policy and/or action plans
2. Leadership for breastfeeding promotion
3. Training of health care staff

4. Baby Friendly Hospital Initiative implementation (BFHI) [63]

5. Endorsement of the WHO International Code of Marketing for breast milk substitutes [64]

6. Legislation for working mothers

7. Community outreach, including mother support

8. Information, education, communication

9. Monitoring of breastfeeding duration and exclusiveness

10. Activities directed towards disadvantaged groups

3.5 STUDY II

The data collection was performed by interviewing mothers (IMES) during September 15th, 2005 to January 15th, 2006, using a preprinted questionnaire format by health workers. In this paper we used only the data about early discontinuation of exclusive breastfeeding and any breastfeeding, use of breast milk substitutes or other drinks in bottle and the use of pacifier.

In the analysis of early discontinuation of breastfeeding, only those infants were included, who were between 1 day and 6 months of age at the time of investigation (n=8434). For any breastfeeding, infants between 7-24 month of age (n=52637) were studied (Table 3.2). Breastfeeding was reported as no breastfeeding or any breastfeeding and in the 0-6 months also as exclusive breastfeeding. Regarding the reasons for discontinuation given from the mothers, we divided these into two categories, the reason for discontinuation of exclusive breastfeeding before six months of age and the reason for discontinuation of any breastfeeding after six months of age. Breast milk substitutes or other drinks in bottles (sweetened water, tea, juice, etc) were registered as well as the use of pacifier.

Data analysis (study I & II)
IMES data were analyzed using STATA version 8.0 and Survey Analysis commands by using each medical university as separate strata and each data collection area as primary sampling unit (PSU) and proportion of sampled person to population of under 2 years of age infants as weight. Breastfeeding rates were calculated with 95% confidence interval (CI). The rates of discontinuation in different age groups were

calculated with 95% confidence interval (CI). Pearson regression was used to correlate the use of pacifier to breastfeeding duration.

3.6   STUDY III

This study was a collaboration between the NNFTRI in Tehran, Iran; Unit for Public Health Nutrition at Karolinska Institutet in Stockholm, Sweden; and the Faculty of Health, Nutrition and Management at Akershus University College, Norway. The fatty acid composition of breast milk at delivery was examined in a random sample and the dietary intake of fish and sea food was assessed by use of a structured interview using a food frequency questionnaire developed by NNFTRI (FFQ).

Two regions of Iran were chosen, the Guilan province near and the Kermanshah province far from the Caspian Sea. One hundred and twenty mothers, 60 in each province, were randomly selected in two public provincial hospitals (Alzahra Hospital in Rasht, capital of Guilan and Moutazedi Hospital in Kermanshah, capital of Kermanshah) during July to September 2008. Anthropometry characteristics of the mothers were collected through interview and those for infants were copied from neonatal identification card (ID). The anthropometric characteristics of the mothers (self-assessed) and infants (measured) are given in Table 3.3.

Table 3.3 Data of pregnant mothers and their infants in the provinces of Kermanshah and Guilan. Values for each province represent mean ± standard deviation

	Kermanshah	Guilan
Mothers:	n=60	n=60
age (y)	26.6±6.7	26.2±6.4
weight before pregnancy (kg)	59.6±11.6	63.7±13.9
weight gain during pregnancy (kg)	9.9±4.7	12.2±5.5
Babies:	n=58*	n=62**
gestation age (w)	38.9±1.4	38.4±1.3**
birth weight (g)	3173±423	3115±489**
Birth length (cm)	49.7±2	49.6±1.5**
birth head circumference (cm)	34.9±1.6	34.4±1.6**

Anthropometric data missing on two infants from Kermanshah
**data including two sets of twins in the Guilan province*

Breast milk sampling and fatty acid analysis

When the mothers were in the post partum ward, 8 to 72 hours after delivery and after first feeding to infant, 2 to 5 ml of colostrum were collected by hand into tubes, kept in an ice box and sent within half an hour to the provincial health centre for freezing at -20 °C until analysis. When all samples were gathered, they were sent to the laboratory at NNFTRI in Tehran in a cold chain vehicle. The milk samples were completely homogenized by Vortex (Heidolph Vortex Shaker REAX 1. 2400 rpm. 220V. 30W Germany) for 30 sec. Fat was extracted from five hundred microlitres of the homogenized milk, hydrolyzed and the FAs derivatized to fatty acid methyl ester (FAME) for analysis by capillary gas chromatography (GC-CP3800, Varian, USA). The fatty acids were identified by comparing the retention times of FAME with a standard FAME mixture (SUPELCO 37 Comp. FAME mix, (595, North Harrison Road, Bellefonte, Pennsylvania (PA) 16823-0048, USA). These analyses were performed at the NNFTRI in Tehran. All samples were analysed in duplicate. The results of FA analysis were expressed in GC area percent.

Dietary measurements

An FFQ (in Persian) containing 17 items was used to estimate the fish and dietary fat consumed during pregnancy. Dietary measurements as well as anthropometric measures of the mother were completed by the author of this thesis at an interview of the participants, 8 to 72 hours after delivery. The frequency response options for the dietary intake were arranged in five categories from "none", "not regularly", "once a week", "and twice a week" and "more than twice a week". In the analysis of fish or seafood intake these five categories were reduced to three, named "non/not regularly", "once a week" and "twice a week or more".

Data Analysis

Data analysis was performed with Excel and Statistical Package for the Social Sciences software 17.0 for Windows (SPSS Inc, Chicago, IL, USA. Standard t-test was used to compare the mean differences of total FAs in breast milk and weight gain during pregnancy between the mothers in the two provinces. The DHA concentration of total FAs in breast milk and the AA/DHA ratio were compared with the frequency of eating fish or seafood during pregnancy by using Kruskal-Wallis test and Mann Whitney´s U-test. Differences were considered as statistically significant at $p < 0.05$.

3.7 STUDY IV

This study is based on a population-based survey in Iran, performed in 2001.

Data collection

Iran can be divided into 11 regions with 28 provinces (Figure 3.1), based on ethnographic, demographic, epidemiologic and socioeconomic factors. The study population was households in all provinces [36] (Table 3.4), including infants from 15 months to 2 years of age, who came to the Health Care Centers during the period May 25th to June 2nd 2001 for the second dose of measles vaccine or for general health control. This sampling frame has been considered the best way to choose a random sample of this age group [65].

There were 880 clusters of households from all 11 regions (Table 3.4), 504 urban and 376 rural clusters, randomly selected throughout the country. In each cluster, 10 infants were chosen, a total of 8800 infants (4400 infants for measuring vitamin A and vitamin D, respectively). The health care staff at the selected health centers informed mothers about the study. Those mothers who agreed to participate also completed a questionnaire. Only healthy infants were included. Venous blood was collected randomized for measuring vitamin A and vitamin D. The infants were in mother's lap during the blood sampling. Ultimately, vitamin A and vitamin D samples were collected from 3099 and 4013 infants, respectively. The samples were sent to district accredited laboratory for centrifugation, the sera coded and frozen at -20 °C until analysis. All samples were analyzed in an accredited laboratory [66] by high performance liquid chromatography (HPLC) in Tehran, transported there by a cold chain vehicle. Vitamin A was analysed as retinol and vitamin D as 25-hydroxy vitamin D (25(OH)D).

Reference values of vitamins A and D

Reference values of vitamin A and D were obtained in the literature, as indicated bellow [17, 19]:

Plasma retinol concentration:
    Optimal >1.4 µmol/L
    Suboptimal > 0.7 but <1.4 µmol/L
    Insufficient: 0.35-0.7 µmol/L
    Deficiency <0.35 µmol/L

Plasma 25(OH)D concentration:
    Optimal >75 nmol/L
    Suboptimal: 50-75 nmol/L
    Insufficient: 25-50 nmol/L
    Deficiency <25 nmol/L

Data analysis

The data were analysed using STATA version 9 and SPSS version 17. Mean and standard deviation (SD) are given. Comparison between groups was made by independent sample test (t-test). Significance levels were set at P-value < 0.05.

Table 3.2 Number of infants investigated in the 11 regions of Iran, 2001

Region	Provinces	Vitamin A N	Vitamin D N
1	Guilan, Mazandaran	353	374
2	E.Azarbaijan, W.Azarbajjane, Ardebil	334	347
3	Golestan, N. Khorasan	275	332
4	Semnan, Khorasan Razavi	244	422
5	Sistanbalochestan, S. Khorasan	272	365
6	Yazd, Isfahan, Chahalmahal & N. Kerman	299	319
7	Boshehr, Hormozgan, S. Khuzestan	279	352
8	Tehran	299	393
9	Zanjan, Ghazvin, Ghom, Markazi	337	389
10	Ilam, Kurdistan, Kermanshah, Lorestan, Hamedan, N. Khuzestan	294	411
11	Fars, South of Kerman, Kohgiloyeh BoyerAhmad	113	309
Total	All	3099	4013

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4 ETHICAL CONSIDERATIONS

Approval for the national studies (I, II &IV) was obtained from the Medical Ethics Committee of the Ministry of Health in Iran. Informed consent was obtained from mothers before the interview (study I & II). Informed consent was obtained from all mothers in study IV. Approval to conduct these analyses (I, II & IV) was obtained from the Medical Ethics Committee at the MOH in Iran in February 2007. Study III was approved by the Medical Ethics Committee of the Kermanshah University of Medical Sciences in 2008. Approval for conducting the analyses of the results of all studies for this thesis was obtained from Regionala Etikprövningsnämnden in Stockholm in 2009.

5 FINDINGS

5.1 STUDY I

Breastfeeding in Iran: prevalence, duration and current recommendations

5.1.1 Breastfeeding rates (IMES)

At a national level, a mean of 89% (95%CI:79.3-96.1) and 57% (95%CI:33.1-91.8) of infants were breastfed in the age groups 12-15 months and 20-23 months, respectively (Figure 5.1.1).
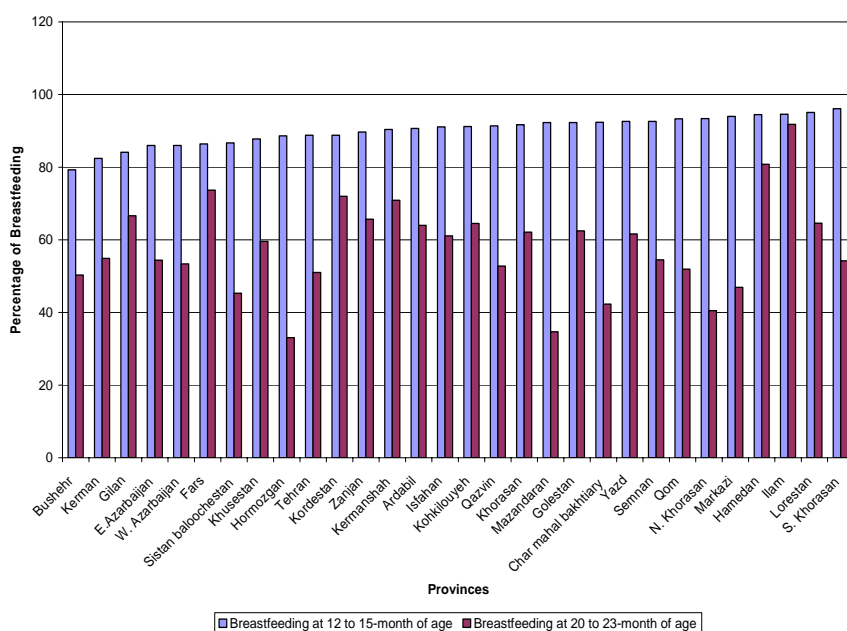

Figure 5.1.1 Breastfeeding rates at 12 to 15 and 20 to 23 months of age per province, IMES, 2005-2006

The exclusive breastfeeding rates were 57% (95%CI:38.8-79.6) at 4 months and 28% (95%CI:15.4-52.7) at 6 months. (Figure 5.1.2)
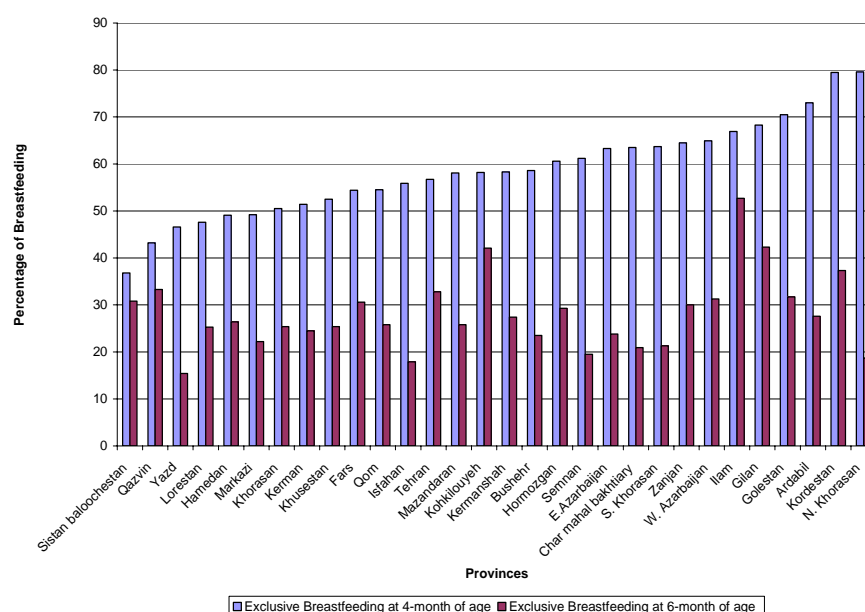
Figure 5.1.2 Exclusive breastfeeding rates at 4 and 6 months of age per province, IMES, 2005-2006

The exclusive breastfeeding rates in rural areas were 58% (95%CI: 15.9-87.6) and 29% (95%CI: 5.3-53.8) at 4 and 6 months of age respectively, and corresponding figures in urban areas were 56% (95%CI: 40.3-81) and 27% (95%CI: 12.7- 41.1).

5.1.2 Breastfeeding policy

The policy questionnaire showed that out of the 566 hospitals across the country 466 hospitals were accredited as Baby Friendly Hospitals, covering more than 80% of the births in 2006. The legislation for working mothers met the International Labour Organization standards that cover women with formal employment. The MOH and Breastfeeding Promotion Society [67] (BFPS) were responsible for producing booklets, pamphlets, breastfeeding journal, CD, workshops and websites.

Baby Friendly Hospital Initiative (BFHI)

Each province had a BFHI coordinator in the provincial health center who reports to the Deputy of Treatment Affairs in each UFMS. The proportion of births covered by BFHs was more than 80 percent. All BFHs are evaluated annually by the Breastfeeding Office at the MOH. Eighty two percent (466/566) of hospitals across the country were accredited as BFHs in 2006. The number of BFHs in each province varies from 3 to 72. The number of hospitals is related to the population in each district or province. The number of BFHs in relation to the population varied from 0.28 to 1.31 per 100,000 inhabitants.

Training

Policy questionnaire showed that a national board set standards and certified pre-service education at the MOH. The 18-hour UNICEF/WHO course on promotion of breastfeeding and BFHs had been introduced by the MOH. The 40-hour WHO/UNICEF course on breastfeeding counseling had been introduced by the MOH in all UFMS. Most of these courses were endorsed and lead to credits. The training covers nurses, midwives, general physicians, pediatricians and obstetricians.

5.2   STUDY II

Physician's role; important for support of breastfeeding

The mean age for early discontinuation of breastfeeding (before six months of age) was 3.2 months of age, ranging from 2.4 to 3.8 months of age. Early discontinuation of breastfeeding was identified in only 5.3% of the mothers. The main reason for early discontinuation of *exclusive* breastfeeding during the first 6 months of life given by the mothers was that discontinuation was advised by the physician (54%).

The mean age of discontinuation of any breastfeeding between 7 to 24 months of age was 13.8 months, ranging from 12.1 to 16.3 months of age. The percentage of discontinuation of breastfeeding before and after six months of age is shown in Figure 5.2.1.
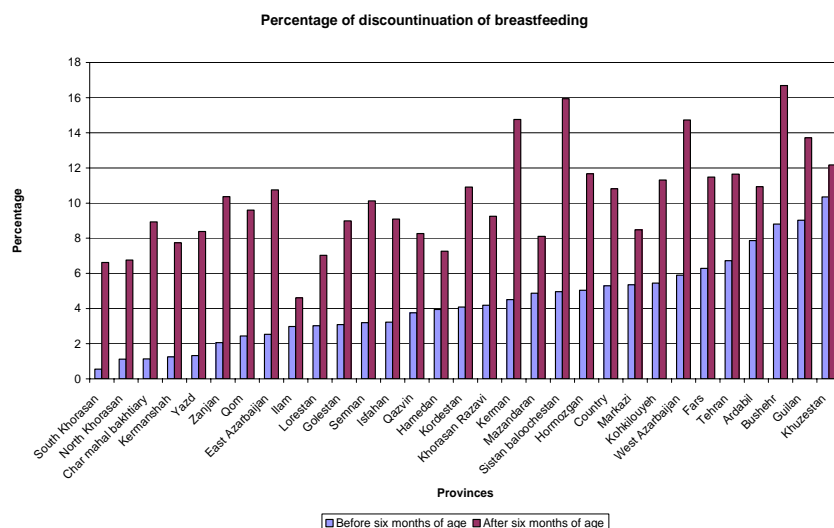
Percentage of discountinuation of breastfeeding

Figure 5.2.1 Percentages of discontinuation breastfeeding before and after six months of age, per province, IMES 2005-2006

The reason given for discontinuation of any breastfeeding from 7 to 24 months of age was most often insufficient amount of breast milk (false or true) (45%). Breast refusal (27%) was as common as stating no specific reason (25%). Return to work outside home was seldom (3%) stated as a reason for discontinuation of breastfeeding.

5.3 STUDY III

Long chain polyunsaturated n-3 fatty acids differ significantly in colostrum in relation to fish intake during pregnancy

5.3.1 Characteristics of the two study populations

One hundred and twenty subjects were included in the two provinces. The mean (SD) age was 26.6 (6.7) years in Kermanshah and 26.2 (6.4) in Guilan, ranging 14-40 years and 15-43 years, respectively. Weight before pregnancy was not available in 12 cases (4 in Kermanshah and 8 in Guilan). The mean weight before pregnancy (SD) did not differ between the groups, (59.6 (11.6) kg in Kermanshah and 63.7 (13.9) kg in

Guilan). There were no differences in birth weight, length and head circumference of the babies between 58 infants in Kermanshah and 62 infants in Guilan.

5.3.2 Dietary data

The result of the FFQ showed that the subjects in Guilan had a significantly higher intake of fish or seafood during pregnancy compared to the mothers in Kermanshah (p<0.001).

Consumption of saturated fat from other animal sources during pregnancy was significantly higher in Kermanshah (p = 0.001) and the intake of olives and olive oil was significantly higher in Guilan (p<0.001). There were no differences in the intake of other vegetable oils (such as sunflower and corn oil) between the two provinces.

5.3.3 Fatty acid composition of breast milk at delivery

Table 5.3.1 shows the concentration of the major fatty acids in breast milk in the two districts. Breast milk samples from the mothers in the two districts were similar regarding the EFA, LA and ALA. However, the total n-3 PUFA and DHA in breast milk were significantly higher in the Guilan compared to the Kermanshah district (p = 0.039 and p<0.001, respectively). Total n-6 PUFA and AA concentrations did not differ between the two groups. The ratios of total n-6/n-3 fatty acids as well as AA/DHA in breast milk of the mothers from Guilan were significantly lower (p = 0.013 and p = 0.002, respectively) than those found in mother's milk from Kermanshah. Mead acid (ETA; 20:3n-9) was not identified.

The concentration of DHA was higher in breast milk after premature than after term delivery, being 0.76 (0.26) vs. 0.56 (0.27) (p=0.03), but that of AA concentration showed no significant differences. The AA/DHA ratio was lower after premature delivery 1.91 (0.44) vs. 2.94 (2.10) than after term delivery (p=0.016).

Table 5.3.1 The concentration of major fatty acids in colostrum (weight %) of mothers in Kermanshah and Guilan provinces of Iran. Mean±SEM

Fatty acids	Kermanshah (n=60)	Guilan (n=60)	p-value
$\sum$ SFA	39.4±0.8	40.7±0.77	0.16
$\sum$ MUFA	32.9±0.41	31.6±0.4	0.14
C18:2ω6 (LA)	16.30±0.33	16.03±0.38	0.60
C18:3ω3 (ALA)	0.81±0.03	0.81±0.03	0.97
C20:4ω6 (AA)	1.40±0.04	1.32±0.04	0.28
C20:5ω3 (EPA)	1.31±0.06	1.35±0.06	0.60
C22:5ω3 (DPA)	0.35±0.01	0.40±0.02	0.10
C22:6ω3 (DHA)	0.50±0.02	0.66±0.03	0.001
$\sum$ ω3FA	3.04±0.10	3.35±0.11	0.04
$\sum$ ω6FA	17.7±0.36	17.4±0.41	0.53
$\sum$ PUFA	20.8±0.41	20.8±0.42	0.95
AA/DHA	3.40±0.31	2.28±0.14	0.001
$\sum$ ω6/$\sum$ω3	6.10±0.20	5.44±0.17	0.01

Analysis of breast milk from term deliveries showed significant correlation between fish and seafood intake and a higher DHA level (p = 0.048) and lower AA/DHA ratio (p = 0.022). This difference persisted to be significant when all milk samples were included.

The youngest mothers (14-23 years) had the highest concentration of PUFA in breast milk (p = 0.01), mainly due to high concentrations of n-6 fatty acids (p = 0.03). There was no significant correlation between age groups and fish or seafood intake.

5.4   STUDY IV

Optimal Vitamin A and suboptimal vitamin D status are common in Iranian infants

5.4.1   Vitamin A

The mean (SD) of vitamin A was 2.09 (0.83) μmol/L. Optimal and suboptimal concentrations of vitamin A were found in 88.4% and 10.4% of the infants (>1.4 μmol/L, and > 0.7 but <1.4, respectively). Only 1.2% of the infants had deficient or insufficient concentrations of vitamin A (< 0.35 μmol/L or > 0.35 - < 0.7). Vitamin A

deficiency was identified in 9 out of 11 regions, altogether only in 20 out of the 3099 infants (0.7%). There was no difference in vitamin A concentrations by gender or between urban and rural areas. The mean vitamin A concentration was higher in region 11, and the lowest mean value was in region 5 which has a generally lower socio-economic status (p= 0.001).

5.4.2 Vitamin D

 The mean (SD) of vitamin D concentration was 61.3 (31.4) nmol/L. The percentage of infants with vitamin D deficient levels, i.e. serum concentration <25 nmol/L, was 2.8%. Insufficient concentration, 25-50 nmol/L, was found in 32.9%. Suboptimal and optimal concentrations of vitamin D were found in 44.1% and 20.2%, representing 50-75 nmol/L and >75 nmol/L, respectively. Deficient levels of vitamin D were found in all regions. There was no difference in vitamin D concentration between urban and rural areas, but there was a significant difference by gender (p=0.006), girls having lower concentrations than boys.

6 DISCUSSION

6.1 FINDINGS

The most important finding in this study was that at national level, the prevalence of breastfeeding was high, 89% and 57% of infants were breastfed at one and two-years of age, respectively. However, exclusive breastfeeding rates at 4 and 6 months of age at national level was unexpectedly low, averaging 56.8% and 27.7%, respectively, and was higher in rural than in urban areas. In view of the facts that, out of the 566 hospitals across the country, 466 hospitals were accredited as Baby Friendly Hospitals, covering more than 80% of the births in 2006, a higher breastfeeding prevalence would have been expected. A national board set standards and certified pre-service education at the MOH. The results suggest that confidence was lacking that the breast milk was satisfying nutrition for the infants, or that not support enough was available for the mothers during the first 6 months after delivery.

The analysis of early breast milk showed generally high concentrations of LCPUFA in Iranian mothers compared to most published data from other countries. Mothers in Guilan had significantly higher intake of fish or seafood during pregnancy than the mothers in Kermanshah ($p<0.01$), and this was associated to significantly higher DHA concentration ($p = 0.048$) and lower AA/DHA ratio ($p = 0.022$). There were no significant differences in the total polyunsaturated fatty acid status and in linoleic and α-linolenic acid concentrations between the two provinces. The total n-6 fatty acids and AA did not differ, reflecting the high and similar intake of vegetable oils in the two provinces. Consumption of saturated fat from other animal sources was statistically higher in Kermanshah ($p = 0.001$) and olive or olive oil intake was significantly higher in Guilan ($p<0.001$), reflecting a more healthier dietary intake in Guilan. If this was associated to socio-economic factors could not be evaluated since such information was missing.

The mean (SD) concentration of vitamin A in the infants was good and deficiency was rare (0.7%). On the other hand, the mean (SD) concentration of vitamin D was suboptimal but still deficiency was rare (2.8%). The lower vitamin D concentrations in girls than boys would be a matter of concern in view of the probable multiple functions and importance of this vitamin ($p = 0.006$).

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Three of the studies (I, II& IV) were based on analysis of national data on feeding patterns and status of the important fat-soluble vitamins, which is the first overview of these patterns in Iran. Still there is concern about the short duration of exclusive breastfeeding and about the compliance to use vitamin A and D supplements. The general observation that infant feeding practices rarely correspond to current global recommendations is of major public health nutrition policy concern and raises questions whether compliance with recommendations is feasible in any population [68].

The WHO recommends routine use of iron and folic acid supplementation to women during pregnancy as a part of antenatal care [69, 70]. In Iran, some guidelines exist for supplementation and diet during pregnancy, such as iron and folic acid supplementation. There are however no guidelines regarding the intake of n-3 and n-6 fatty acids during pregnancy. We demonstrated, in study III, the usefulness of n-3 FA taken from fish and sea-food. Study III showed that there were no significant differences between the total PUFA and EFA status (linoleic and α-linolenic acid) concentrations in Iranian mothers' breast milk. Total n-3 fatty acids and DHA in breast milk were significantly higher in coastland compared to inland. In the WHO recommendation regarding caring practices [71], health professionals are responsible to support initiation of breastfeeding during the first hour of life, introducing exclusive breastfeeding and breastfeeding on demand during the first six months of age and not use bottles, teats or pacifiers. However, study I and II showed that about 72% of Iranian infants were not exclusive breastfed to the age of six months and that around 15% of infants less than one year used pacifier.

6.1.1 Breastfeeding promotion and support (study I & IV)

The any breastfeeding situation in Iran is good, as shown in this study based on nationally representative data, in comparison with several countries (study I). However, the exclusive breastfeeding prevalence was low and had maybe demonstrated a decrease compared to a report from MOH in 2000, when the exclusive breastfeeding rate was 44% at 6 months [51]. Since that study was not based on national data and the variation between different provinces in our study was high, we can at least summarize

that the prevalence of exclusive breastfeeding had not increased, thus the results were far from meeting the WHO recommendation.

In study II was also shown that the mean age of the infant for discontinuation of breastfeeding varied greatly between the provinces of Iran, but total discontinuation of any breastfeeding was seldom reported before two years of age. Despite this high prevalence of any breastfeeding, exclusive breastfeeding at 6 months of age was only reported in 28%, ranging from 15.4% to 52.7% depending on province (study I). This figure is not very different from that reported from other countries, like Turkey, Kuwait and Czech Republic (22.4%, 26.1% and 23%, respectively) [72-74] but lower than reported from Brazil, 44% [75], and some European countries, like Austria and Sweden (34% in 2003) [73]. Other countries have lower percentage of exclusive breastfeeding at 6 months than Iran, such as Spain reporting 23% and the Netherlands 17% [73]. In a study from 2001, the Australian National Health Survey reported that less than 50% of the Australian infants were receiving any breastfeeding at six months of age [76], and in US in 2004 the percentage of any breastfeeding was 74% at 12 months of age but exclusive breastfeeding rate at six months of age was only11% [77].

The breastfeeding rate was higher in rural than urban areas, as also shown by others [78], which might be due to tradition. The high rate of any breastfeeding also in urban areas might be related to the fact that most of the workplaces, schools, and universities have day care very close to mothers' workplace so that they can continue to breastfeed their infant. The right to take breastfeeding breaks at work is valid for 24 months.

Large provinces have UMS and small provinces have a Medical Faculty. There was a difference in the number of BFHs in the different provinces, which could be due to a difference in the number of hospitals in each Medical University or Medical Faculty. Useful approaches of the BFHs and Health Centers help mothers to train positioning [63], screening for adequate milk production at the regular visits to health professionals and consultation by trained health workers. BFHs covered more than 80% of deliveries across the country in 2006 and had applied the WHO [79] breastfeeding definition. In Iran a range of variables appear to influence breastfeeding, such as a positively integrated national program in health centers for the promotion of breastfeeding, developing the BHFs ("Ten steps to successful breastfeeding") [13], emphasizing the

rooming-in program, supporting programs for women who work, and other cultural issues [80, 81]. However, doctors' recommendations were the major reason to discontinuation of breastfeeding, despite the fact that the physiological and psychological beneficial effects of breastfeeding would be most acknowledged by pediatricians. Similar influences by the doctors have been reported previously [74]. Public health physicians are suggested to be more trained to encourage mothers to breastfeed [15]. One study showed that in a group of primary care physicians only 26% did not encourage exclusive breastfeeding [82]. One controlled Iranian study in 1999 illustrated that health professionals played a positive and significant role to educate breastfeeding practices to mothers [80]. The same study showed that the breastfeeding education and support of mothers during the first 4 months of lactation increased the rate of exclusive breastfeeding by 8 times.

The high prevalence of any breastfeeding might be associated with the health care system in Iran. Previous studies have illustrated that Iran has a national program in education of health profession and health workers [83, 84]. These programs are updated by WHO [85, 86] and followed in some countries [87]. Continuing Medical Education (CME) in Iran was implemented in 1991. CME is currently an important part of the Iranian physician's professional development. According to the literature [88, 89], there is a gap between the actual learning needs of participants and education provided. Traditional CME in Iran often uses text-based methods for planning rather than interactive training methods [90]. The main weakness of the present CME is that certifications are given to participants without any assessing or quality assurance of performance [90]. A national survey of CME in Iran showed that CME was not so effective in attaining the desired outcomes [91]. In Iran, physicians give advice in health centers and in private sector. We don't know exactly who recommended the mothers, in public sector or private sector, but health policy has more authority in governmental health system than in the private system [92].

The BFPS [67] supports mothers through a web site and telephone support service. There is no special organization for mother to mother support. Also the support of the family has been stressed by others [93] and was shown in another study [74] that also the family had a role in recommending mothers to discontinue breastfeeding at an early stage. In Iran, the couples who want to get married receive pre-marriage consultation.

But for delivery support, grandmothers and other relatives have a more important role than fathers.

Paid maternity leave in Iran was increased from 16 to 24 weeks in late 2006. After six months, reduction of work by one hour per day is optional, in order to promote breastfeeding up to 24 months of age. It is positive compared with some other countries [73]. However, for mothers without insurance, working outside governmental institutions and informal work, there is no paid maternity leave or right to breastfeeding breaks. Return to work constituted only 3% of reason for discontinuation of breastfeeding (study II) in contrast to other studies [94-96].

Study II showed that correlation between use of pacifier and continuation of exclusive and any breastfeeding was significantly negative, which has been shown also in another study [94].

6.1.2 Fat intake in relation to breast milk content (Study III)

For pregnant women, it is necessary to follow a healthy diet unless she has a specific health problem. For pregnant women it is required to have a variety in food that includes dairy products and also several fruits and vegetables. In study III, an FFQ containing 17 items that only covered the fish and dietary fat consumed during pregnancy was used. We therefore can not evaluate the general intake of the mothers, which can contribute, especially amount of vegetables for the intake of ALA, but also the kind of meat, since games have higher ALA than domestic animals in modern settings. Despite these limitations we found an association between mother's fatty acid status and the fish intake, confirming the importance of fish and seafood for the n-3 status. The PUFA status is also depending on vegetable oil intake, especially the n-6 fatty acid LA varies a lot in different oils [97, 98]. The fat consumption in Iranian consists to a great deal of vegetable oil, nuts (special walnut), fatty meat, animal fat, soy bean and cheese [99]. Those vegetable oils, nuts and soy bean have a high content of LCPUFA [98].

MUFA are found in olives and olive oil, canola oil, peanut oil and nuts. Olive or olive oil intake was significantly higher in coastland. There were no differences in the intake of other vegetable oils (sunflower and corn oil) during pregnancy between the two regions.

It would be of interest to follow these groups of infants for the further development. Breastfeeding, associated with higher intake of the infants of LCPUFA than formula fed infants, have been found to have lower incidence of different diseases later in life [100] and also supplementation of mothers or newborns with n-3 LCPUFA have been improving later mental and social development. The marked differences between Guilan and Kermanshah in content of n-3 fatty acids would make such follow up of interest.

6.1.3 The status of vitamins A and D in infants (study IV)

Study IV showed that deficient levels of vitamin A and vitamin D were rare during infancy. This possibly indicates that the recommendations of supplementation were generally followed. The concentration of vitamin A in breast milk is highly dependent on maternal diet and nutritional status [101]. In mothers with good nutritional status the amount of vitamin A in breast milk is adequate to meet the requirements for infants during the first 6 months of age, but in deficient mothers the amount of vitamin A concentration in breast milk will be suboptimal [101]. To treat the deficiency, maternal and/or infant supplementation are needed [101]. The adequacy of providing vitamin A as supplement is currently contested. Another study from 2001 on pregnant Iranian women showed that 25% of mothers had deficient and insufficient level of vitamin A [102]. One study [103] suggests that vitamin A supplementation during infancy should be supported, and findings from our studies (IV) [102] support this suggestion.

The concentration of vitamin A in breast milk is dependent on mother's vitamin A in plasma which normally decreases during prolonged lactation [104]. Sparks et al. [105] showed that mothers who had low vitamin A status during the last trimester of pregnancy tend to give birth to babies with low concentrations of vitamin A. Moreover, infants born with low reserves of vitamin A are vulnerable to vitamin A deficiency [106].

The concentration of vitamin D in breast milk is considered insufficient to meet infant requirements [101]. The concentration of vitamin D in infants is dependent on sun exposure or vitamin D supplement. During exclusive breastfeeding, vitamin D concentration in infants will decrease if it is not provided through exogenous sources [101]. Study IV showed that a third of the infants had insufficient levels of vitamin D. Low levels of vitamin D may be linked to other diseases in future, including cancer (breast and colon), high blood pressure and obesity [107]. In children, a vitamin D deficiency can cause rickets [17], a disease that results in soft, weak bones. Prevention of vitamin D deficiency would influence infants' and children's health outcome [19].

The vitamin D supplement that is distributed by MOH throughout the country consists of calciferol combined with vitamin A in droplet form. Twenty-five drops per day provides 1500 and 400 IU (450 µg/day and 10 µg/day) vitamins A and D, respectively. Supplementation starts fifteen days after birth and should continue until 2 years of life, according to the recommendation.

We have no way of knowing whether the parents gave their infants the supplement, so it is difficult to estimate an ideal dosage level. The sufficient vitamin A status suggests that supplementation has been given. Our study suggests that the recommendation is insufficient regarding the vitamin D supplementation.

On the other hand, in a study [108] about the percentage of use of supplements among Iranian infants in early 2006, we demonstrated that 85% of infants between 6 to 9 months of age were given the supplement (vitamins and iron). We have no data on vitamin levels at ages below one year and how large percentage of infants after one year are given supplementation. In a prospective Iranian study from 2007 [109], 75 breastfed infants were followed until 6 months of age. Three groups were given 200 IU or 400 IU per day or five drops of 50000 IU vitamin D3 every two months. The infants' blood samples were analyzed at six months of age to assess 25(OH) D levels. No insufficiency was found in any of the three groups. Suboptimal levels were 63%, 23% and 3% in group 1, 2 and 3, respectively, and correspondingly optimal levels in 37%, 69% and 77%. This would indicate that the current low dosage of vitamin D supplementation in infants might not be sufficient.

Low dosage of vitamin D in infants could be related to insufficient status of vitamin D in mothers [110]. Gender difference in vitamin D status is a concern in many countries, including Iran [111]. The risk of a lower concentration of vitamin D by age is increased by covering clothing [111], especially in countries with long seasonal dark periods or in heavily polluted areas. In region 11, located in the south of Iran, the weather in most seasons is sunny and warm and maybe children expose more of their skin. Region 1, located in the north of Iran, with more rainy days, cold weather and high humidity had more infants with low vitamin D. In region 5, low socioeconomic status (SES) and in region 8 (Tehran) the huge size of the city with a mixed population and more pollution might explain the low vitamin D status.

6.2 METHODOLOGICAL CONSIDERATION

6.2.1 Validity and reliability

Study I, II and IV use a method covering all the country and would be representative for the population nationwide. Furthermore, they demonstrate the same proportions of urban and rural population as known in Iran. The questionnaire used was validated according to a pilot project in IMES conducted in 2004 [51]. Results from this pilot project were used for calculating sample size.

Study III is in comparison a small study but represents two different provinces, where the result supports the assumption of different feeding pattern. However, it can not be excluded that contribution also by ethnicity [112] or genetics [113] might have influenced the results. Only a larger study could evaluate that.

The analytical methods, the GC for fatty acid analyses and the HPLC method for analyzing vitamin A and D, were performed in accredited laboratories. The HPLC method was confirmed by the WHO collaborate laboratory [66] in MOH.

6.2.2 Sampling bias and limitation

Study I, II and IV were based on large population from many Health Centers, where the data had been collected by many different people but all had the same instructions and education. The great number of cases might compensate for this.

In study II, we only have the mothers' view, which might be a limitation. On the other hand, it is the experience of the mothers which influence their decision about breastfeeding.

Study III had a small sample size and the unusually high levels of LCPUFA might partly been influenced by co-eluation, which could not be checked for. The absence of a common fatty acid in the colostrums, eicosatrienoic acid (20:3n-9, Mead acid) might be an indicator of this. On the other hand, low Mead acid can be expected when PUFA and LCPUFA are high. It would also have been valuable to know more about confounding factors regarding maternal habits and socio-economy. However, some influence would have been in opposite direction to our finding, e.g. smoking would lower DHA concentrations.

Study IV did not include any individual data of supplementation, which would have been of value to interpret the results in the context of supplementation or mother's diet, as well as the influence of complementary food.
Study IV was also conducted in a population based study, at this time the country was divided into 11 regions, based on a previous study, aiming at achieving a more socio-economically representative sample [114]. Migration of population between the regions made it impossible to pinpoint in which region 263 individuals were located, why the urban/rural division of individuals was slightly compromised. However, this number is representing only a small proportion of the entire sample for the vitamin D analysis (4013 individuals).

6.3 SOME IMPLICATIONS FOR POLICY AND RESEARCH

Based on our findings, policy interventions to promote and support a prolonged exclusive breastfeeding would be an appropriate development.

Such interventions should include educational programs designed especially for physicians (CME). There is a high need to change or improve the current content of this program. It was concluded that changing the CME program elements, mainly in the training and assessment methods, could be necessary to improve the effectiveness of progress [115, 116].

In the ten steps to successful breastfeeding in BFHIs national policy [117], further attention to steps two and three is recommended (2. Train all health care staff in skills necessary to implement this policy and 3. Inform all pregnant women about the benefits and management of breastfeeding). Also more research at district and provincial level is needed in order to fully comprehend the effects of the BFHI and the ten steps.

For having a more complete knowledge regarding nutritional status and feeding patterns, like complementary food, 7-24 months of age infants [118], more research is recommended.

For having a clear view of vitamin D concentration in mother's and infant's blood, new studies are recommended. Further studies are required to show whether increasing the dosage of vitamin D in supplement will result in higher concentration of vitamin D in blood.

6.4 CONCLUSIONS

Detailed information regarding the present infant feeding situation is needed to design and implement improvement programmes to achieve the national goals for infant feeding in Iran. There is a gap between the international (WHO) recommendation on breastfeeding (fully endorsed by the Iranian MOH) and the present results of the status of breastfeeding. The breastfeeding situation in Iran is good in comparison with several countries. However, the exclusive breastfeeding prevalence may have shown a reduction at 6 months and our results were far from meeting the WHO recommendation.

The cause of early discontinuation of exclusive breastfeeding during infancy was mainly physicians' recommendation and/or insufficient breast milk. Support for women needs considerable improvement in regards to the protection, promotion and support of breastfeeding. Physicians and other health professionals have an important role to play in encouraging and supporting mothers to breastfeed according to the recommendations until the prevalence of exclusive breastfeeding might further increase up to 6 months.

The LCPUFA status in colostrum in Iranian mothers was good compared with literature and DHA was higher and the ratio AA/DHA was significantly lower in mothers with high fish intake. These results suggest that fish or seafood should be recommended for consumption at least once a week by pregnant and lactating mothers. The high n-6 intake in the younger mothers might be of concern.

In comparison with developed and other low- and middle-income countries vitamin A deficient levels were rare in Iranian infants. Also vitamin D deficient levels were rare, but 33% had insufficient values. The concentrations of vitamin A were satisfactory, which might be due to the supplementary therapy, which is supported by the MOH. The concentrations of vitamin D showed that vitamin D supplementation probably is at too low dosage level or that the compliance is low. For a complete coverage of the problem, there is a need for an interventional study considering compliance, the adequacy of dosage and duration of vitamin D supplementation. The gender difference in vitamin D concentration is of concern and should be monitored.

7 ACKNOWLEDGEMENTS

8 REFERENCES

1. World Health Organization: **Indicators for assessing infant and young child feeding practices. conclusions of a consensus meeting held 6-8 November 2007 in Washington D.C., USA;** Available from: http://www.who.int/nutrition/publications/iycf_indicators_for_peer_review.pdf. Accessed July 14, 2008.

2. World Health Organization: **Infant Feeding Recommendation;** Available from**:** http://www.who.int/nut/. Accessed October 31, 2010.

3. World Health Organization: **Consultation on the Programmatic Aspects of the Management of Moderate Malnutrition, 2010;** Available from: http://www.who.int/nutrition/topics/moderatemalnutrition_consultation_programmaticaspects_MM/en/index.html**.** Accessed Dec. 2010.

4. World Health Organization: **Indicators for Assessing Breastfeeding Practice. Reprinted report of an informal meeting, June 11-12**. Geneva, Switzerland; 1991.

5. World Health Organization: **Global Strategy on Infant and Young Child Feeding. *55th World Health Assembly***. Geneva 2002.

6. World Health Organization/United Nations Children's Health Organization Fund: **Breastfeeding counselling: a training course**. 1993.

7. Yngve A, Sjostrom M: **Breastfeeding in countries of the European Union and EFTA: current and proposed recommendations, rationale, prevalence, duration and trends**. *Public Health Nutr* 2001, **4**(2B):631-645.

8. Claeson M, Gillespie D, Mshinda H, Troedsson H, Victora CG, Bellagio Study Group on Child S: **Knowledge into action for child survival**. *Lancet* 2003, **362**(9380):323-327.

9. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B: **Developmental potential in the first 5 years for children in developing countries**. *Lancet* 2007, **369**(9555):60-70.

10. Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E, Carter JA: **Child development: risk factors for adverse outcomes in developing countries**. *Lancet* 2007, **369**(9556):145-157.

11. European Commission, Karolinska Institute Department of Biosciences at Novum, Institute for Child Health IRCCS Burlo Garofolo Italy, WHO Collaborating Centre for Maternal and Child Health: **Infant and young child feeding: standard recommendation for the European Union**. 2006.

12. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, Lau J: **Breastfeeding and maternal and infant health outcomes in developed countries**. *Evid Rep Technol Assess (Full Rep)* 2007(153):1-186.

13. World Health Organization: **10 facts on breastfeeding;** Available from: http://www.who.int/features/factfiles/breastfeeding/en/index.html. Accessed Dec. 2010.

14. Yngve A, Sjostrom M: **Breastfeeding determinants and a suggested framework for action in Europe**. *Public Health Nutr* 2001, **4**(2B):729-739.

15. Labarere J, Gelbert-Baudino N, Ayral AS, Duc C, Berchotteau M, Bouchon N, Schelstraete C, Vittoz JP, Francois P, Pons JC: **Efficacy of breastfeeding support provided by trained clinicians during an early, routine, preventive visit: a prospective, randomized, open trial of 226 mother-infant pairs**. *Pediatrics* 2005, **115**(2):e139-146.

16. Agrasada GV, Gustafsson J, Kylberg E, Ewald U: **Postnatal peer counselling on exclusive breastfeeding of low-birthweight infants: a randomized, controlled trial**. *Acta Paediatr* 2005, **94**(8):1109-1115.

17. Casey CF, Slawson DC, Neal LR: **Vitamin D supplementation in infants, children, and adolescents**. *Am Fam Physician* 2010, **81**:745-748.

18. Vesel L, Bahl R, Martines J, Penny M, Bhandari N, Kirkwood BR, Group WHOI-lVASS: **Use of new World Health Organization child growth standards to assess how infant malnutrition relates to breastfeeding and mortality**. *Bull World Health Organ* 2010, **88**:39-48.

19. Nordic Nutrition Recommendations: *Integrating nutrition and physical activity*. Copenhagen: Nordic Council of Ministers; 2004.

20. Whitney EN, Rolfes SR: *Understanding nutrition*. Belmont, CA: Thomson/Wadsworth; 2008.

21. Pelletier DL, Frongillo EA: **Changes in child survival are strongly associated with changes in malnutrition in developing countries**. *J Nutr* 2003, **133**(1):107-119.

22. Cetin I, Koletzko B: **Long-chain omega-3 fatty acid supply in pregnancy and lactation**. *Curr Opin Clin Nutr Metab Care* 2008, **11**(3):297-302.

23. Innis SM: **Dietary omega 3 fatty acids and the developing brain**. *Brain Res* 2008, **1237**:35-43.

24. Horvath A, Koletzko B, Szajewska H: **Effect of supplementation of women in high-risk pregnancies with long-chain polyunsaturated fatty acids on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials**. *Br J Nutr* 2007, **98**(2):253-259.

25. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, Dudenhausen JW, Dupont C, Forsyth S *et al*: **The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations**. *J Perinat Med* 2008, **36**(1):5-14.

26. Schanler RJ: *Breastfeeding 2001, part 1: the evidence for breastfeeding*; 2001.

48

27. Hamosh M, Henderson TR, Kemper MA, Orr NM, Gil A, Hamosh P: **Long-chain polyunsaturated fatty acids (LC-PUFA) during early development: contribution of milk LC-PUFA to accretion rates varies among organs**. *Adv Exp Med Biol* 2001, **501**:397-401.

28. Lefkowith JB, Evers AS, Elliott WJ, Needleman P: **Essential fatty acid deficiency: a new look at an old problem**. *Prostaglandins Leukot Med* 1986, **23**(2-3):123-127.

29. Friesen R, Innis SM: **Trans fatty acids in human milk in Canada declined with the introduction of trans fat food labeling**. *J Nutr* 2006, **136**(10):2558-2561.

30. Crawford M, Galli C, Visioli F, Renaud S, Simopoulos AP, Spector AA: **Role of plant-derived omega-3 fatty acids in human nutrition**. *Ann Nutr Metab* 2000, **44**(5-6):263-265.

31. Krauss-Etschmann S, Shadid R, Campoy C, Hoster E, Demmelmair H, Jimenez M, Gil A, Rivero M, Veszpremi B, Decsi T *et al*: **Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial**. *Am J Clin Nutr* 2007, **85**(5):1392-1400.

32. Helland IB, Saugstad OD, Saarem K, Van Houwelingen AC, Nylander G, Drevon CA: **Supplementation of n-3 fatty acids during pregnancy and lactation reduces maternal plasma lipid levels and provides DHA to the infants**. *J Matern Fetal Neonatal Med* 2006, **19**(7):397-406.

33. Brenna JT, Lapillonne A: **Background paper on fat and fatty acid requirements during pregnancy and lactation**. *Ann Nutr Metab* 2009, **55**(1-3):97-122.

34. Popkin BM: **The shift in stages of the nutrition transition in the developing world differs from past experiences!** *Public Health Nutr* 2002, **5**(1A):205-214.

35. Wikipedia - the free encyclopedia: **Iran,** Available from: http://en.wikipedia.org/wiki/Iran.

36. Statistical Center of Iran: **Evaluation of population census data through demographic methods;** Available from: http://www.sci.org.ir/portal/faces/public/sci_en. Accessed Nov 2010.

37. World Health Organization-Regional Office For the Eastern Meaditerranean: **Country Profile: about IR of Iran.** [cited 2008 12 Oct]; Available from: http://www.emro.who.int/iran/countryprofile.htm.

38. The World Bank: **Country classification** [cited 2010 July]; Available from: http://web.worldbank.org/WBSITE/EXTERNAL/COUNTRIES/MENAEXT/I

RANEXTN/0,,menuPK:312962~pagePK:141159~piPK:141110~theSitePK:31
2943,00.html.

39.   United Nations: **National accounts estimates of main aggregates-United Nations Statistics Division- Per capita GNI at current prices- US Dollars.** [cited 2010 Dec]; Available from: http://www.un.org.ir

40.   United Nations: **United Nations Development Programme. 2008 statistical update, Iran, the human development index-going beyond income.** [cited 8 2009]; Available from: http://hdrstats.undp.org/2008/countries/country_fact_sheets/cty_fs_IRN.html.

41.   United Nations- Department of Economic and Social Affirs: **Millennium development goal indicators,** [cited 2010 Dec]; Available from: http:/mdgs.un.org/unsd/mdg/Data.aspx**.** Accessed June, 2010.

42.   United Nations- Department of Economic & Social Affairs: **World population prospects:The 2006 revision, population database,** [cited 2010 Dec]; Available from: http:/esa.un.org/unpp/index.asp?panel=2. Accessed July, 2010.

43.   World Health Organization: **Country cooperation strategy for WHO and the Islamic Republic of Iran, 2005-2009. Cairo: Regional Office for the Eastern Mediterranean**. 2006.

44.   The World Bank: **Country classification. Contry brief-Iran;** Available from: http://web.worldbank.org/WBSITE/EXTERNAL/COUNTRIES/MENAEXT/I RANEXTN/0,,contentMDK:20146413~menuPK:312964~pagePK:141137~piP K:141127~theSitePK:312943,00.html.

45.   World Health Organization: **Country Cooperation Strategy for World Health Organization and Islamic Republic of Iran**. 2004.

46.   Samavat A, Modell B: **Iranian national thalassaemia screening programme**. *BMJ* 2004, **329**(7475):1134-1137.

47.   World Health Organization- Regional Office for the Eastern Mediterranean: **Background information on the health system in the Islamic Republic of** Iran; Available from: http://www.emro.who.int/iran/Media/PDF/HealthSystems.pdf. 2009.

48.   Azemikhah A, Amirkhani M, Jalilvand P, Emami Afshar N, Radpooyan L, Changizi N: **National Maternal Mortality Surveillance System in Iran**. *Iranian J Publ Health* 2009, **38**:90-92.

49.   Uniten Nations Children's Fund (UNICEF): **Iran, Islamic Republic of, Statistics;** Available from: http://www.unicef.org/infobycountry/iran_statistics.html.

50.   World Health Organization: **Global Health Observatory;** Available from: http://apps.who.int/ghodata/?vid=10600.

51.   Ministry of Health: **Family and Population Health Office, Tehran, Iran**. 2006.

52.   Barzegar MA, Djazayery A: **Evaluation of rural primary health care services in Iran: report on vital statistics in West Azarbaijan**. *Am J Public Health* 1981, **71**(7):739-742.

53.   **Policy statement: organizational principles to guide and define the child health care system and/or improve the health of all children**. *Pediatrics* 2004, **113**(5 Suppl):1545-1547.

54.   Abdolmaleki S, Psuty I: **The effects of stock enhancement of pikeperch (*Sander lucioperca*) in Iranian coastal waters of the Caspian Sea**. *ICES Journal of Marine Science* 2007, **64**:973-980.

55.   Iranian Fisheries Organization: **Iranian Fisheries Organization; Available from:** http://en.ifro.ir/portal.aspx.

56.   Hedayatifard M, Jamali Z: **Evaluation of Omega-3 Fatty Acids Composition in Caspian Sea Pike Perch (Sander lucioperca)**. *Int J Agri Biol,* 2008, **10**(2):235-237.

57.   Meester F, Watson RR, Djazayery A, Jazayery S: *Wild-Type Food in Health Promotion and Disease Prevention [electronic resource] : The Columbus Concept*. Totowa, NJ: Humana Press Inc.; 2008.

58.   Djazayery A: **Effects of total energy and individual nutrient intakes on energy balance**. *World Rev Nutr Diet* 1987, **52**:143-208.

59.   Kimiagar SM, Ghaffarpour M, Houshiar-Rad A, Hormozdyari H, Zellipour L: **Food consumption pattern in the Islamic Republic of Iran and its relation to coronary heart disease**. *Eastern Mediterranean Health Journal* 1998, **4**(3):539-547.

60.   Djazayery A: **Regional overview of maternal and child malnutrition: trends, interventions and outcomes**. *East Mediterr Health J* 2004, **10**(6):731-736.

61.   Sheikholeslam R, Kimiagar M, Siasi F, Abdollahi Z, Jazayeri A, Keyghobadi K, Ghaffarpoor M, Noroozi F, Kalantari M, Minaei N *et al*: **Multidisciplinary intervention for reducing malnutrition among children in the Islamic Republic of Iran**. *East Mediterr Health J* 2004, **10**(6):844-852.

62.   Malekafzali H, Abdollahi Z, Mafi A, Naghavi M: **Community-based nutritional intervention for reducing malnutrition among children under 5 years of age in the Islamic Republic of Iran**. *East Mediterr Health J* 2000, **6**(2-3):238-245.

63.   Labbok MH: **Breastfeeding and Baby-Friendly Hospital Initiative: more important and with more evidence than ever**. *J Pediatr (Rio J)* 2007, **83**(2):99-101.

64. World Health Organization: **International Code of Marketing of Breast Milk Substitutes**. Geneva; 1981.

65. The Ministry of Health. Nutritional Promotion Office: *Researsh on status of micronutrient deficiency in Iran (Persian)*; 2001.

66. World Health Organization: **Reference Laboratories of Iran, Ministry of Health, EMRO, WHO Collaborating Centre for Quality Control and Clinical Chemistry;** Available from: http://apps.who.int/whocc/List.aspx?cc_code=IRA. 2010.

67. BFPS: **The Breastfeeding Promotion soceity. [cited 2010 Dec];** Available from: http://www.shiremadar.com/index.php?option=com_content&view=article&id=257&Itemid=176.

68. Sellen DW: **Comparison of infant feeding patterns reported for nonindustrial populations with current recommendations**. *J Nutr* 2001, **131**(10):2707-2715.

69. World Health Organization: **Multiple-micronutrient supplementation for women during pregnancy,** [cited 2010 Dec]; Available from: http://apps.who.int/rhl/pregnancy_childbirth/antenatal_care/nutrition/plcom2/en/index.html.

70. World Health Organization/UNICEF: **Planning Guide for national implementation of the Global Strategy for Infantand Young Child Feeding,** 2007; Available from: http://whqlibdoc.who.int/publications/2007/9789241595193_eng.pdf.

71. World Health Organization: **Nutrition Landscape Information System (NLIS), Country Profile Indicators, Interpretation Guide, [cited 2011 Jan];** Available from: http://whqlibdoc.who.int/publications/2010/9789241599955_eng.pdf.

72. Al-Awadi F A, Amine E k: **Recent trends in infant feeding patterns and weaning practices in Kuwait**. *Eastern Mediterranean Health Journal* 1997, **3**(3).

73. Cattaneo A, Yngve A, Koletzko B, Guzman LR: **Protection, promotion and support of breast-feeding in Europe: current situation**. *Public Health Nutr* 2005, **8**(1):39-46.

74. Yesildal N, Aytar G, Kocabay K, Mayda AS, Dagli SC, Bahcebasi T: **Breastfeeding practices in Duzce, Turkey**. *J Hum Lact* 2008, **24**(4):393-400.

75. do Nascimento MB, Reis MA, Franco SC, Issler H, Ferraro AA, Grisi SJ: **Exclusive breastfeeding in southern Brazil: prevalence and associated factors**. *Breastfeed Med* 2010, **5**(2):79-85.

76. Donath SM, Amir LH: **Breastfeeding and the introduction of solids in Australian infants: data from the 2001 National Health Survey**. *Aust N Z J Public Health* 2005, **29**(2):171-175.

77. (CDC) CfDCaP: **Breastfeeding trends and updated national health objectives for exclusive breastfeeding--United States, birth years 2000-2004**. *MMWR Morb Mortal Wkly Rep* 2007, **56**(30):760-763.

78. Qiu L, Zhao Y, Binns CW, Lee AH, Xie X: **Initiation of breastfeeding and prevalence of exclusive breastfeeding at hospital discharge in urban, suburban and rural areas of Zhejiang China**. *Int Breastfeed J* 2009, **4**:1.

79. World Health Organization: **Baby-Friendly Hospital Initiative. Revised, Updated and Expanded for Integrated Care;** Available from: http://whqlibdoc.who.int/publications/2009/9789241594967_eng.pdf.

80. Froozani MD, Permehzadeh K, Motlagh AR, Golestan B: **Effect of breastfeeding education on the feeding pattern and health of infants in their first 4 months in the Islamic Republic of Iran**. *Bull World Health Organ* 1999, **77**(5):381-385.

81. Zareai M, O'Brien ML, Fallon AB: **Creating a breastfeeding culture: a comparison of breastfeeding practises in Australia and Iran**. *Breastfeed Rev* 2007, **15**(2):15-20.

82. Leavitt G, Martínez S, Ortiz N, García L: **Knowledge About Breastfeeding Among a Group of Primary Care Physicians and Residents in Puerto Rico**. *Journal of Community Health* 2009, **34**(1):1-5.

83. WHO/UNICEF: **World Health Organization / United Nations Children's Health Organization Fund Breastfeeding counselling: a training course**. 1993.

84. World Health Organization: **Breastfeeding, management and promotion in a Baby Friendly Hospital: an 18-course maternity staff.** New York; 1993.

85. World Health Organization: **Baby-Friendly Hosoital Initiative: Breastfeeding Promotion and Support in A Baby Friendly Hospital, A 20-Hour Course for Maternity Staff;** Available from: http://whqlibdoc.who.int/publications/2009/9789241594981_eng.pdf.

86. World Health Organization: **Infant and Young Child Feeding Counselling: An Integrated Course;** Available from: http://whqlibdoc.who.int/publications/2006/9789241594745_eng.pdf. 2006.

87. Zakarija-Grkovic I, Burmaz T: **Effectiveness of the UNICEF/WHO 20-hour course in improving health professionals' knowledge, practices, and attitudes to breastfeeding: before/after study of 5 maternity facilities in Croatia**. *Croat Med J* 2010, **51**(5):396-405.

88. Grant J: **Learning needs assessment: assessing the need**. *BMJ* 2002, **324**(7330):156-159.

89. Norman GR, Shannon SI, Marrin ML: **The need for needs assessment in continuing medical education**. *BMJ* 2004, **328**(7446):999-1001.

90. Mohammadzadeh H: **Outcome-based continuing Medical Education,** PhD Thesis. Karolinska Institutet, Public Health Sciences; 2009.

91. Amini A, Kargarmaher M, Hatami F, Salami H: **Determining CME needs of general physicians in East Azerbijan province**. *Iranian Journal of Medical Education* 2002, **1**(8):12-30.

92. Palesh M, Tishelman C, Fredrikson S, Jamshidi H, Tomson G, Emami A: **"We noticed that suddenly the country has become full of MRI". Policy makers' views on diffusion and use of health technologies in Iran**. *Health Res Policy Syst* 2010, **8**:9.

93. Ekstrom A, Widstrom AM, Nissen E: **Breastfeeding support from partners and grandmothers: perceptions of Swedish women**. *Birth (Berkeley, Calif* 2003, **30**(4):261-266.

94. Scott JA, Binns CW, Oddy WH, Graham KI: **Predictors of breastfeeding duration: evidence from a cohort study**. *Pediatrics* 2006, **117**(4):e646-655.

95. Dennis CL: **Breastfeeding initiation and duration: a 1990-2000 literature review**. *J Obstet Gynecol Neonatal Nurs* 2002, **31**(1):12-32.

96. Froozani MD, Zavoshi R, Azordeghan F: **Duration and causes of cessation of breast feeding in working mothers in Ghazvin, Iran**. *Med J Islam Repub Iran* 1992, **6**(2):93-95.

97. Williams CM, Burdge G: **Long-chain n-3 PUFA: plant v. marine sources**. *Proc Nutr Soc* 2006, **65**(1):42-50.

98. ERNA: **Information Fact Sheets. Polyunsaturated fatty acids (PUFA), [cited 2010 Dec]; Available from:** http://www.erna.org/UserFiles/PUFA.pdf.

99. Food and Agriculture Organization: **Nutrition country profiles.** [database on the Internet] 2010. Available from: http://www.fao.org/ag/AGN/nutrition/IRN_en.stm. 2010.

100. Agostoni C, Riva E, Scaglioni S, Marangoni F, Radaelli G, Giovannini M: **Dietary fats and cholesterol in italian infants and children**. *Am J Clin Nutr* 2000, **72**(5 Suppl):1384S-1391S.

101. World Health Organization: **Nutrient adequacy of exclusive breastfeeding for the term infant during the first six months of life information; Available from:** http://whqlibdoc.who.int/publications/9241562110.pdf. 2002.

102. Neshati R: **Vitamin A status during pregnancy: A cross sectional study on pregnant women in Iran,** Master Program Public Health, Karolinska Institutet 2010.

103. Beaton GH, Ghassemi H: **Supplementary feeding programs for young children in developing countries**. *Am J Clin Nutr* 1982, **35**(4 Suppl):863-916.

104. Schanler RJ: *Breast feeding 2001, part II: the management of breastfeeding*; 2001.

105. Sparks JW: **Human intrauterine growth and nutrient accretion**. *Semin Perinatol* 1984, **8**(2):74-93.

106. Thurnham DI, Northrop-Clewes CA: **Optimal nutrition: vitamin A and the carotenoids**. *Proc Nutr Soc* 1999, **58**(2):449-457.

107. Gilbert-Diamond D, Baylin A, Mora-Plazas M, Marin C, Arsenault JE, Hughes MD, Willett WC, Villamor E: **Vitamin D deficiency and anthropometric indicators of adiposity in school-age children: a prospective study**. *Am J Clin Nutr* 2010, **92**(6):1446-1451.

108. Jyothi A.J S: **Begining of Complementary Feeding in Infants Over Six Months of Age in Iran** Master Program Public Health. Stockholm, Karolinska Institutet; 2010.

109. Shakiba M, Sadr S, Nefei Z, Mozaffari-Khosravi H, Lotfi MH, Bemanian MH: **Combination of bolus dose vitamin D with routine vaccination in infants: a randomised trial**. *Singapore Med J* 2010, **51**(5):440-445.

110. Lips P: **Worldwide status of vitamin D nutrition**. *J Steroid Biochem Mol Biol* 2010, **121**(1-2):297-300.

111. Arabi A, El Rassi R, El-Hajj Fuleihan G: **Hypovitaminosis D in developing countries-prevalence, risk factors and outcomes**. *Nat Rev Endocrinol* 2010, **6**(10):550-561.

112. van Eijsden M, Hornstra G, van der Wal MF, Bonsel GJ: **Ethnic differences in early pregnancy maternal n-3 and n-6 fatty acid concentrations: an explorative analysis**. *Br J Nutr* 2009, **101**(12):1761-1768.

113. Koletzko B, Lattka E, Zeilinger S, Illig T, Steer C: **Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children**. *Am J Clin Nutr* 2010.

114. Ministry of Health. Nutritional Promotion Office: *Researsh on status of micronutrient deficiency in Iran (Persian)*; 2001.

115. Shirazi M, Zeinaloo AA, Sabouri Kashani A, Alaeddini F: **Assessing the gap between current and desirable needs in TUMS* CME Unit: participants' viewpoints**. *Journal of Iranian Medical Education* 2004, **5**(1):17-22.

116. Westphal MF, Taddei JA, Venancio SI, Bogus CM: **Breast-feeding training for health professionals and resultant institutional changes**. *Bull World Health Organ* 1995, **73**(4):461-468.

117. **Family and Population Health Office, the Ministry of Health IR of Iran, Tehran, Iran, Baby Friendly Hospital Initiative (in Persian), 2006**.

118. World Health Organization: **Strengthening action to improve feeding of infants and young children 6-23 months of age in nutrition and child health programmes**. 2010.

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International Breastfeeding Journal

Research

Breastfeeding in Iran: prevalence, duration and current recommendations

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Abstract

Background: The need to promote breastfeeding is unquestionable for the health and development of infants. The aim of this study was to investigate prevalence, duration and promotion of breastfeeding status in Iran with respect to the Baby Friendly Hospital, government actions and activities by the Breastfeeding Promotion Society including comparison with European countries.

Methods: This retrospective study is based on data from 63,071 infants less than 24 months of age in all the 30 urban and rural provinces of Iran. The data of breastfeeding rates were collected in 20052006 by trained health workers in the Integrated Monitoring Evaluation System in the Family Health Office of the Ministry of Health to evaluate its subordinate offices. A translated version of a questionnaire, used to assess the current breastfeeding situation in Europe, was used.

Results: At a national level, 90% and 57% of infants were breastfed at one and two-years of age, respectively. Exclusive breastfeeding rates at 4 and 6 months of age at national level averaged 56.8% and 27.7%. Exclusive breastfeeding rates at 4 and 6 months of age in rural areas were 58% and 29%, and in urban areas 56% and 27%, respectively. The policy questionnaire showed that out of the 566 hospitals across the country 466 hospitals were accredited as Baby Friendly Hospitals, covering more than 80% of the births in 2006. A national board set standards and certified pre-service education at the Ministry of Health. Iran officially adopted the WHO International Code of Marketing of Breast Milk Substitutes in 1991. The legislation for working mothers met the International Labour Organization standards that cover women with formal employment. The Ministry of Health and Breastfeeding Promotion Society were responsible for producing booklets, pamphlets, breastfeeding journal, CD, workshops and websites. Monitoring of breastfeeding rates was performed every four years and funded by the Ministry of Health within the budgets assigned to the health care system.

Conclusion: In comparison to many European Union countries, Iran showed a favorable situation in terms of breastfeeding rates and promotion of breastfeeding. Iran still needs to increase the rate of exclusive breastfeeding during the first six months.

Background

The need to promote and support breastfeeding is unquestionable for the health and development of infants. It represents a public health priority everywhere, as confirmed by the Global Strategy on Infant Young Child Feeding, unanimously approved by the 55th World Health Assembly (WHA) in 2003 [1].

Breastfeeding provides all essential nutrients for the first 6 months of life. Breastfeeding plays an important role in ensuring food security for a large proportion of babies in the world, where food security is defined as having enough food to maintain a healthy and productive life today and in the future [2]. Breast milk contains the long chain polyunsaturated fatty acids which are especially important for the development of the brain and the nervous system [3]. Breastfeeding is also associated with a decreased risk for many early-life diseases [4].

Low rates and early cessation of breastfeeding have important adverse effects on health, social and economic implications for women, children, the community and environment, and results in greater expenditure on national health care provision [5].

Health in Iran

Iran is an Asian country with 30 provinces spread over an area of about 1,648,000 km^2 and has about 70 million inhabitants of whom 62% are living in urban and 38% in rural areas. According to UNICEF statistics in 2008 [6], the total fertility rate was 2 births per woman in 2006. The infant mortality rate was 30 per 1000 live births and the mortality rate in the population less than 5 years of age was 34 per 1000 in 2006 [6]. It seems that child mortality is going down over time and compared with other countries in the East Mediterranean region Iran had a good position [7].

The average annual growth rate of the urban population was 4.9% during 19701990 and 2.5% during 19902006. The gross national income per capita was US$3,000 in 2006. There were 850 active hospitals in Iran of which 566 had delivery and neonatal wards. In the urban areas 500 Health Centers provide health care and in the rural areas this is provided by 18,000 Health Houses. Iran has 41 Universities or Faculty of Medical Sciences (UFMS), all of them under jurisdiction of the Ministry of Health (MOH).

The capital province Tehran, has 12 million inhabitants and is divided into three geographic regions, covered by three different Universities of Medical Sciences (UMS) (Tehran 1 = Iran UMS, Tehran 2 = Tehran UMS and Tehran 3 = Shahid Beheshti UMS).

The breastfeeding situation in Iran

The National Committee of Breastfeeding Promotion was established at the MOH in 1991 by the Minister of Health at that time, Professor Alireza Marandi [8]. The major strategy of the committee was to promote breastfeeding for preventing morbidity and mortality in infants and to progress their health.

The mission of the Children's Health Office in the MOH was reviewed in 2004 [9]. It included 10 objectives: to decrease growth abnormalities in children due to malnutrition, decrease mortality rates in both neonates and infants, decrease mortality rate in children under 5 years, decrease mental and physical growth abnormality, increase the rate and duration of breastfeeding within the first two years of life, increase the percentage of exclusive breastfeeding in infants up to 6 months, increase the percentage of complementary feeding in 69 months children, prescribe vitamin A and D and iron supplements and increase the number of Baby Friendly Hospitals (BFHs). Exclusive breastfeeding is officially recommended in Iran until six months of age [9], with the addition of vitamin A and D from two weeks after birth.

Each district in a province had a Health Network in which there was a breastfeeding committee. All committees at district level were supervised by the main breastfeeding committee at the UFMS, located in the capital city of the province (Figure 1). Child health was monitored through regular visits at Health Centers and Health House visits from the first week of life until 24th month of age. Child health monitoring was continued until 72 months after birth. At these visits the child was weighed, length and head circumference measured and the general health status assessed. The vaccination programme was followed. Iran also has a private health sector but vaccination is performed by the MOH in more than 95% of children.

The exclusive breastfeeding rates up to 6 months in Middle East-North African (MENA) region in the period of 2000 to 2006 were 28% [6]. In the MENA region, exclusive breastfeeding rates in Pakistan, Iraq, Saudi Arabia, Egypt and Iran were 16%, 25%, 31%, 38% and 44%, respectively [6].

Based on Demographic Health Survey (DHS) statistics in 2000 about 90% of the infants in Iran received any breastfeeding, while exclusive breastfeeding at 6 months was less than 45%. The exclusive breastfeeding rate at 6 months was about 44% in 2000 and decreased to 27% in 2004 [9].

This study intends to clarify the current situation (2006) regarding breastfeeding rates as well as breastfeeding pro-
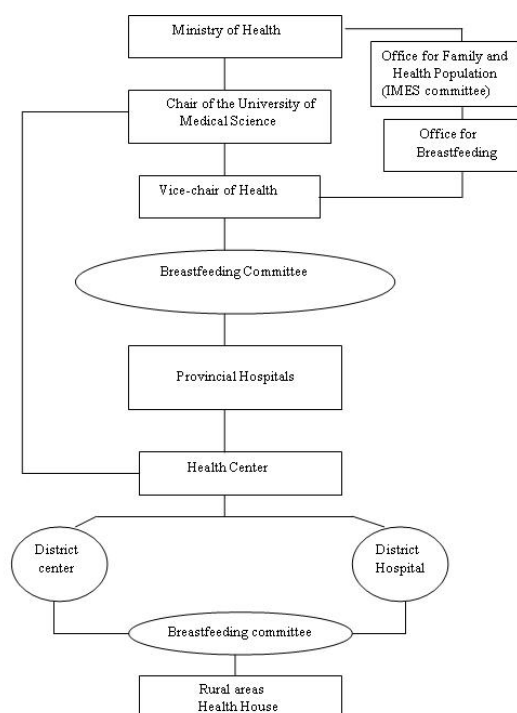
Figure 1
Health System Network and breastfeeding promotion organizational structure in Iran.

motion and support in Iran, as a first measure to design appropriate measures to stop the downwards trend in breastfeeding. This paper also reports on a survey of the implementation of breastfeeding policies in Iran.

Methods

Exclusive breastfeeding is defined as providing only breast milk and no other liquids or solids except for those containing vitamins, minerals or medicines to the baby from birth [10,11]. Exclusive breastfeeding is considered superior at least until an infant is six months of age [10,12].

Breastfeeding rates were studied, using the Integrated Monitoring Evaluation System Survey (IMES) which collects data regarding the content and consequences of provision and delivery health services for promoting health in the country. The study population was obtained with regard to the general objective of IMES and on the basis of

data required for Family and Population Health Office of the MOH in 20052006. The study population was based on data from IMES collected as follows: all mothers who had given birth at hospitals or other health facilities, all mothers who had taken their infants for the first vaccination at one of the rural or urban centers, all infants less than 2 years of age who lived in urban or rural areas and had been referred to Healthcare Center or Health House to receive healthcare, vaccination or treatment for any disease. It means that for exclusive breastfeeding rate data, only those infants were included who were between 1 day and 6 months of age. However, for general breastfeeding data, all infants were included (Table 1).

The data were gathered in urban and rural areas by health workers who were trained in the specifics of collecting health data including the definition of exclusive breastfeeding in the previous 24 hours. IMES data collection was

Table 1: Number and percentage distribution of the study population; data from Integrated Monitoring Evaluation System

Age, months	Total number	Urban/rural %	Proportion distribution	Cumulative %
04	2186	62/38	4.4	4.4
> 46	6248	61/39	10.8	15.2
> 69	15833	56/44	20.5	35.7
> 912	5290	54/46	9.3	45
> 1218	17159	58/42	28	73
> 1824	16355	56/44	27	100
Total	63 071	57/43	100	100

performed in all rural areas through direct visits by trained interviewers who were employees of the MOH at UFMS and were sufficiently familiar with concepts and guidelines of provision of healthcare as well as asking the IMES questions through a questionnaire (see below). The questionnaire was validated in a pilot study in late 2004. Answers to questions about feeding patterns in infants at 4, 6 and less than 24 month of age were made by mothers and were not the reviewers' opinion. Questions were about the name of province, district and health center, number of household, mother's age and education, birth date, infant's age, weight, length and head circumference, breastfeeding exclusively and any breastfeeding. The data were collected from 15 September 2005 to 15 January 2006. In this paper only breastfeeding data are reported.

Sampling method and sample size (IMES)
We used a multistage sampling method with three kinds of sampling:

1. Random cluster sampling with non-equal clusters. In each district we identified 20 clusters by random sampling from electrical power counters number list. Then two children were selected from each cluster to enter the study.

2. Sampling in the urban areas was from clients who came to Health Centers for vaccination (convenience sampling).

3. Random systematic sampling was used to sample infants in rural areas. The sampling frame was the list of childcare users which covered more than 99% of the infants in rural area.

The policy questionnaire
In order to assess the current situation in regard to breastfeeding protection, promotion and support, a questionnaire (available from the first author upon request) was used during February to May 2007. This was a translation of one used in Europe [5,13]. The questionnaire was sent to the central committee of breastfeeding at the MOH, where it was completed based on national data in 2006.

The questionnaire was used to investigate how the following topics were implemented in Iran as a whole and in its provinces:

1. Breastfeeding policy and/or action plans

2. Leadership for breastfeeding promotion

3. Training of health care staff

4. Baby Friendly Hospital Initiative (BFHI) [14]

5. Endorsement of the WHO International Code of Marketing for breast milk substitutes [15]

6. Legislation for working mothers

7. Community outreach, including mother support

8. Information, education, communication

9. Monitoring of breastfeeding duration and exclusiveness

10. Activities directed towards disadvantaged groups

Statistical analysis
IMES data were analyzed using STATA version 8.0 and Survey Analysis commands by using each medical university as a separate strata and each data collection area as primary sampling unit (PSU) and proportion of sampled person to population of under 2 years of age infants as weight. Breastfeeding rates were calculated with 95% confidence interval (CI).

Ethical approval
Approval to conduct this analysis was obtained from the ethics committee at the MOH in Iran in February 2007.

Results
The number, age and the distribution of participants in the IMES are shown in Table 1. The proportions between

urban and rural provinces are shown, indicating that the study population was representative for Iran.

Breastfeeding rates (IMES)

At a national level, a mean of 90% (95%CI: 79.3, 96.1) and 57% (95%CI: 33.1, 91.8) of infants had been breast-fed between 12 to 15 months and 20 to 23 months, respectively (Additional file 1; Table S1). The exclusive breastfeeding rates were 57% (95%CI: 38.8, 79.6) at 4 months and 28% (95%CI: 15.4, 52.7) at 6 months. The differences in exclusive breastfeeding rates at 4 and 6 months between regions are shown in Additional file 1; Table S1.

The exclusive breastfeeding rates in rural areas were 58% (95%CI: 15.9, 87.6) and 29% (95%CI: 5.3, 53.8) at 4 and 6 months of age respectively, and corresponding figures in urban areas were 56% (95%CI: 40.3, 81) and 27% (95%CI: 12.7, 41.1) (Additional file 1; Table S1 and Figure 2).

The exclusive breastfeeding rates at 4 and 6 months of age and their confidence intervals are presented in Additional file 1; Table S1. The results vary from 30% to 94% at 4 months of age and 13% to 55% at 6 months of age. The regions with the lowest exclusive breastfeeding rate at 4 month of age were Sistanblochestan, Qazvin and Yazd with low, middle and high socioeconomic status (SES), respectively. The lowest exclusive breastfeeding rate at 6 month of age was seen in Yazd, Isfahan and North Khorasan with high SESs. The highest rates could be seen in North Khorasan (high SES), Kordestan (low SES) and Ardebil (low SES) at 4 month of age. The highest rate of exclusive breastfeeding at 6 month of age was in Ilam (low SES), Guilan (middle) and Kohkilouyeh (low SES) (additional file 1; Table S1). These results indicate that socioeconomic status is not the most important factor for mothers to breastfeed.

Policy, planning and management

All provinces and UFMS are supposed to follow the national policy which is regulated by the MOH (Figure 1). Four objectives have been adopted by universities, including implementing the Ten Steps for Successful Breastfeeding and helping mothers to initiate breastfeeding soon after birth [16], to breastfeed exclusively for 6 months and to continue breastfeeding up to 2 years of age.

A national plan including general objectives and recommended strategies has been developed by the MOH [17]. Action plans including specific objectives, targets and activities have been developed by health workers in all provinces. All UFMS have a coordinator who should report these activities to the national coordinator at the MOH on a regular basis. Members of the provincial committee are all chairs and vice chairs of UFMS, experts from medicine, nursing and midwifery schools, pediatric nutritionists and gynecologists/obstetricians as well as nutrition departments in each province. The deputy of health at each UFMS coordinates these committees.

Training
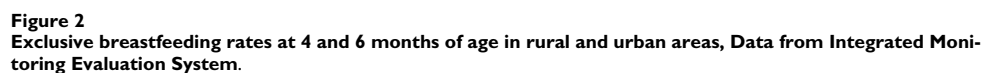
There is a national board that sets standards and certifies pre-service education at the MOH. The 18-hour UNICEF/WHO course on promotion of breastfeeding and BFH has been introduced by the MOH [18]. The 40-hour WHO/UNICEF course on breastfeeding counseling has been introduced by the MOH in all UFMS [19]. Most of these courses are endorsed and lead to credits. The training covers nurses, midwives, general physicians, pediatricians and obstetricians. Health workers have, in the majority of cases, completed the 18-hour course and are in the process to completing the longer course, since they need it to keep their positions. The course requirement in time is 36 hours for physicians and specialists.

Baby Friendly Hospital Initiative

Each province has a BFHI coordinator in the provincial health center who reports to the Deputy of Treatment Affairs in each UFMS. The proportion of births covered by BFHs is more than 80 percent. All BFHs are evaluated annually by the Breastfeeding Office at the MOH. Eighty two percent (466/566) of hospitals across the country were accredited as BFHs in 2006. Additional file 1; Table S1 shows the number of BFHs by province and UFMS. The number of BFHs in each province varies from 3 to 72. The number of hospitals is related to the population in each district or province. The number of BFHs in relation to the population varied from 0.28 to 1.31 per 100,000 inhabitants (Additional file 1; Table S1). The two provinces with lowest numbers of BFHs per 100,000 population were Qom and Sistanbalochestan, with 0.28 and 0.33, respectively. The two highest were in Yazd and Golestan with 1.31 and 1.05, respectively (Additional file 1; Table S1). The proportion of BFHs in relation to total number of hospitals in the different provinces varied between 0.5 and 1. The two lowest proportions of BFHs in relation to total number of hospitals were seen in Qom and Kermanshah with 0.5 and 0.56, respectively (Additional file 1; Table S1).

WHO International Code and subsequent relevant WHA resolutions

Iran officially ratified the WHO International Code of Marketing of Breast Milk Substitutes in 1991 [20] and also favored of the International Code of Marketing of Breast milk Substitutes and of subsequent relevant WHA resolutions. Iran adopted many provisions (e.g., no advertising of breast milk substitute to the public, no direct and indirect free samples or gifts to mothers or their relatives, no
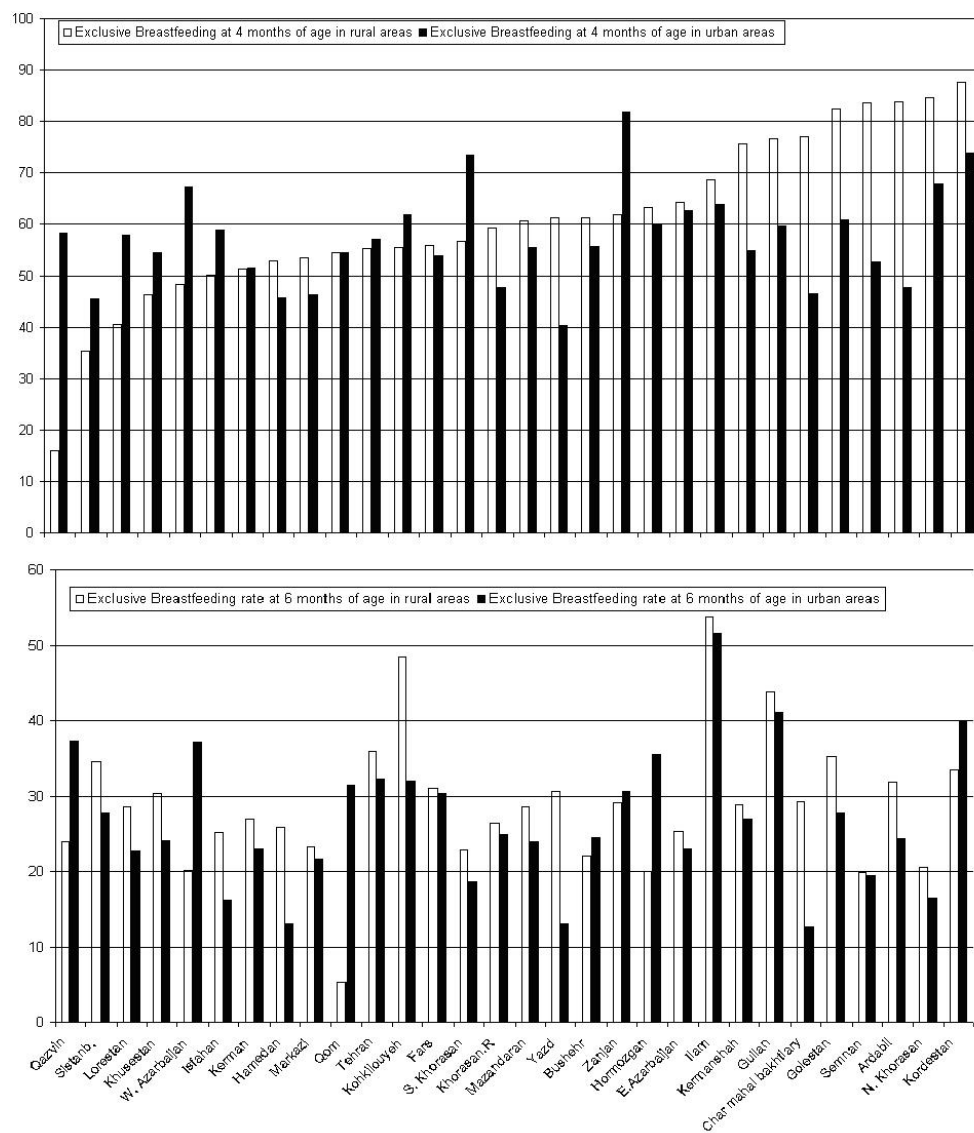
Figure 2
Exclusive breastfeeding rates at 4 and 6 months of age in rural and urban areas, Data from Integrated Monitoring Evaluation System.

gifts or personal samples to health workers and etc) of the Code also in its directive for the internal market of infants and follow on formulas.

Legislation for working mothers

The Maternity Protection act of the International Labor Organization (ILO) [21] sets standards for protecting and supporting breastfeeding in Iran only among governmental working mothers and working mothers who have insurance that is paid for by themselves or their employers. These standards include the provision of a minimum 24 weeks of paid maternity leave as well as the right to one paid breastfeeding break daily or reduction of work by one hour per day to promote longer duration of breastfeeding without loss of payment up to 24 months of age [22].

The ILO recommendations also state that maternity leave payments should be at least two-thirds of previous earnings. This is adhered to completely by government institutions in all provinces of Iran. There are day care centers and kindergartens next to most of the governmental offices. Maternity leave from work was extended from 16 to 24 weeks at the end of 2006. After six months, reduction of work by one hour per day is optional, in order to promote breastfeeding up to 24 months of age. It is important to note that for mothers without insurance, working outside governmental institutions, there is no paid maternity leave or right to breastfeeding breaks.

Community outreach, including voluntary mother to mother support

The Iranian Breastfeeding Promotion Society (BFPS) [23] is a non-governmental, non-political and non-profit organization, which was established in 1991. This society supports mothers through a web site and telephone support service. There is no special organization for mother to mother support [23].

Information, education, communication

The Breastfeeding Office at the MOH and BFPS (non-governmental) are responsible for producing pamphlets, booklets, leaflets, a breastfeeding journal, videos, CD, TV spots and web based information as well as workshops for staff. CD and videos are used for training mothers and physicians in BFHs. These materials are revised regularly by the National Committee of Breastfeeding. The World Breastfeeding Week activities are implemented in all provinces by the MOH and in Tehran is partially by UNICEF. There is also a governmental breastfeeding website [23].

Monitoring

Monitoring of breastfeeding rates is performed every four years by the Breastfeeding Office at the MOH within the budgets assigned to the health care system.

Disadvantaged groups

All of the women in Iran have the opportunity to use material for breastfeeding promotion from the MOH. There is a possibility for mothers without insurance to receive monetary support from Emdad Relief Committee, which is funded by public resources.

Discussion

Three important findings in our study were the identification of the rates of exclusive breastfeeding at 4 and 6 months of age, and the high proportion of accredited BFHs. The policy questionnaire showed that the Iranian authorities have made many measures to improve mother and infant care. Despite those efforts there were large variations in breastfeeding rates, which were not easily explained.

Large provinces have UMS and small provinces have Medical Faculty. The differences in number of BFHs were due to difference in the number of hospitals in each Medical University or Medical Faculty (e.g. Jahrom district has only one Medical Faculty and one hospital which is a BFH). BFHs covered more than 80% of deliveries across the country in 2006. BFHs had applied the WHO breastfeeding definition.

The highest exclusive breastfeeding rate at 6 month of age was shown in Ilam, which is a small province with low SES and a more rural population. It could be explained by old traditions that support breastfeeding in that area. The proportions of BFHs in relation to total number of hospitals and BFH to 100,000 population in Ilam were 0.71 and 0.91, respectively. The highest exclusive breastfeeding rate at 4 month of age was in North Khorasan with a high SES and high education but the exclusive breastfeeding rate at 6 month of age had dropped off. It could be explained by the return to work by mothers. The proportions of BFHs in relation to total number of hospitals and BFH to 100,000 population in North Khorasn were 1.0 and 0.61, respectively. The lowest exclusive breastfeeding rate at 4 month of age was shown in Sistanbalochestan but it was not the lowest exclusive breastfeeding rate at 6 month of age. Sistanbalochestan has low SES and more rural and low educated population. There is seasonal movement in this area. The proportions of BFHs in relation to total number of hospitals and BFH to 100,000 population in Sistanbalochestan were 0.88 and 0.33, respectively. The lowest exclusive breastfeeding rate at 6 month of age was illustrated in Yazd with high SES, high education and early return to work. The proportions of BFHs and BFH in relation to total number of hospitals to 100,000 population in Yazd were 0.92 and 1.31, respectively. Qom had the lowest proportions of BFHs in relation to total number of hospitals and BFH to 100,000 population despite the exclusive breastfeeding rates at 4

and 6 months of age were 54.4% and 25.8%, respectively. Also the urban population was higher than the rural population in Qom province and the SES was high. The regions with low rates of exclusive breastfeeding rate at 4 and 6 months are characterized by low to middle SES and also by return to work, while the North Khorasan and Guilan with high rates can be seen as affluent parts of the country, Ilam and Kordestan are regarded as culturally more traditional and with a more rural population.

Higher rates of exclusive breastfeeding in rural areas compared with those in urban areas indicated that this situation could be due to tradition rather than socioeconomic status or high education. A cross-sectional study in the north district of Iran (Babol) in 1998 showed that the prevalence of breastfeeding was 87% and 89% at 12 months in urban and rural areas and 18% and 53% at 24 months, respectively [24]. In our results the prevalence of breastfeeding was 95% at 12 months in urban and rural areas and 66.7% and 33% at 24 months, respectively. On the other hand in this area in our study there is no clear relationship between high and low socioeconomic status, high and low proportion of BFHs and BFH to 100 000 population and the breastfeeding rates, suggesting that the breastfeeding rate is determined by many interactive factors.

Lower rate of exclusive breastfeeding in working mothers demonstrate the need to educate mothers, but the extension of paid maternity leave might be more important [25]. Other factors may be of importance and more research is required for identifying explanatory factors.

The exclusive breastfeeding rate at 6 month of age was about 44% in 2000 and decreased to 27% in 2004. Our findings support the decreasing trend since in this study nearly 28% breastfed at 6 month of age. The Breastfeeding

Office became more active in late 2004, with support from the National Breastfeeding Committee. Iran had not had any International Board Certified Lactation Consultants (IBCLCs) until 2007 [26]. At the end of 2007, however, the first coordinator was appointed who was certified as an IBCLC. Furthermore paid maternity leave in Iran was increased from 16 to 24 weeks in 2007. Iran has a good family planning system; the growth rate of the population is under control and mothers are encouraged to work outside the home and have fewer children.

The status of BFH in March 2002 demonstrated that the number of Baby Friendly Hospitals in the world was 14,992, distributed over the eight regions [5]. The regions are West and Central Africa, Eastern and Southern Africa, Middle East and North Africa (MENA), East Asia and Pacific, South Asia, Americas and the Caribbean, Central and Eastern Europe and the Commonwealth of Independent States (CEE/CIS) and Industrialized Countries. Iran is located in the MENA region and had 376 BFHs in 2002. Thus Iran, as 1 out of 20 countries had 46% of the BFHs in this region. Iran was the first ranked country in terms of proportion of BFHs in the MENA region. Second and third ranked countries were Tunisia and Egypt, which had 17% and 15% of proportion of BFHs in MENA region, respectively. At the same time Saudi Arabia and United Arab Emirates had even fewer proportions of BFHs (0.25% and 0.5%, respectively). Despite the fact that Iran has a relatively good position from an international perspective; the MOH is planning to increase the number of BFHs. By comparison with some EU countries [5] Iran had an intermediate situation in regards to the number of BFHs (Table 2). Sweden had only a 34% exclusive breastfeeding rate at 6 months of age in 2000 despite 100% BFH indicating the complexity of the subject [5]. A recent review, regarding breastfeeding in Australia and Iran, showed that most Australian women discontinue breastfeeding before the

Table 2: BFH prevalence, percentage of births at BFH and exclusive breastfeeding (Excl. BF) prevalence in some EU countries [5] and Iran.

Country	Year	BFHs/total hospitals	% of birth at BFHs	Excl. BF at 4 mo. (%)	Excl. BF at 6 mo. (%)
Austria	1998	14/110	12	79	46
Germany	1998	18/1100	3	33	10
Italy	2000	7/700	1	47	13
Lithuania	2002	3/54	12	_	14
Portugal	1998	0/60	0	_	9
Sweden	2000	52/52	100	68	34
Iran	2006	466/566	≈80	57	28

recommended time [27]. The study attempted to identify variables influencing breastfeeding practices in Australia by comparing Australia with Iran, indicating comparatively high breastfeeding rate in Iran. Only approximately 4.5% of public and private hospitals in Australia [27] were BHF while the corresponding figure in Iran was 80%.

The breastfeeding tradition is well established in Iran, and the history of breastfeeding can be traced back to the fourth century in the Canon Medicine Text Book which was written by Avicenna [28]. This important textbook was extensively used in European medical schools for centuries after Avicenna's death. In the Canon of Medicine, a chapter was allocated to the care of the newborn infant dealing with hygiene, breastfeeding and raising the child.

Conclusion
The breastfeeding situation in Iran is good in comparison with several European countries and Australia. However, the exclusive breastfeeding prevalence had shown a downward trend at 4 and 6 months, which was confirmed in this new study. The results were far from meeting the WHO recommendation. The declining trend showed large big regional differences. Support for women needs considerable improvement in regards to the protection, promotion and support of breastfeeding, which would probably increase the national figures substantially.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
BO drafted the manuscript and coordinated the project between the MOH in Iran and Karolinska Institutet. KF was the head of Breastfeeding Office in the MOH and helped for preparing data collection in policy questionnaire. AB was responsible for IMES data and performed statistical analysis. BS helped to developing the study and writing the manuscript. AY participated in design of the study. All authors approved the final manuscript.

Additional material

Additional File 1
Table S1. Mean percentage of infants with exclusive breastfeeding (EXBF) and 95% confidence intervals (CI) at four and six months (m) of age and partial breastfeeding (BF) at one and two years of age in the different provinces in Iran.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1746-4358-4-8-S1.doc]

References
1. WHO: **Global Strategy on Infant and Young Child Feeding. 55th World Health Assembly.** *Genev* 2003.
2. *Nurturing the future: challenges to breastfeeding in the 21st century; 2327 September 2002, Arusha, Tanzania* Penang: World Alliance for Breastfeeding Action; 2004.
3. Innis SM: **Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids.** *J Pediatr* 2003, **143(4 Suppl):**S1-8.
4. **Breastfeeding trends and updated national health objectives for exclusive breastfeeding United States, birth years 20002004.** *MMWR Morb Mortal Wkly Rep* 2007, **56(30):**760-763.
5. Cattaneo A, Yngve A, Koletzko B, Guzman LR: **Protection, promotion and support of breast-feeding in Europe: current situation.** *Public Health Nutr* 2005, **8(1):**39-46.
6. *The state of the world's children. 2008, [Child survival]* New York:: Unicef; 2007.
7. **Child adolecent health** [http://www.who.int/child_adolescent_health/data/en/]
8. Marandi A, Afzali HM, Hossaini AF: **The reasons for early weaning among mothers in Teheran.** *Bull World Health Organ* 1993, **71(5):**561-569.
9. **The Ministry of Health IR of Iran, Breastfeeding office** [http://www.bfps.ir/(i5emlr45w1kdeg45tov0ysem)/Persian/Home.aspx]
10. Kramer MS, Kakuma R: *The optimal duration of exclusive breastfeeding [Elektronisk resurs] a systematic review* Geneva: Dept. of Nutrition for Health and Development, Dept. of Child and Adolescent Health and Development, World Health Organization; 2002.
11. Nelson WE, Behrman RE, Kliegman RM, Jenson HB: *Nelson textbook of pediatrics* Philadelphia: Saunders; 2004.
12. Khadivzadeh T, Parsai S: **Effect of exclusive breastfeeding and complementary feeding on infant growth and morbidity.** *East Mediterr Health J* 2004, **10(3):**289-294.
13. European Commission KIDoBaN, Unit for Preventive Nutrition, Institute for Child Health IRCCS Burlo Garofolo Italy, Unit for Health Services Research and International Health, WHO Collaborating Centre for Maternal and Child Health: **Infant and young child feeding: standard recommendation for the European Union.** 2006.
14. Labbok MH: **Breastfeeding and Baby-Friendly Hospital Initiative: more important and with more evidence than ever.** *J Pediatr (Rio J)* 2007, **83(2):**99-101.
15. WHO: **International Code of Marketing of Breast milk Substitutes, Geneva.** 1981.
16. Murray EK, Ricketts S, Dellaport J: **Hospital practices that increase breastfeeding duration: results from a population-based study.** *Birth* 2007, **34(3):**202-211.
17. Froozani MD, Permehzadeh K, Motlagh AR, Golestan B: **Effect of breastfeeding education on the feeding pattern and health of infants in their first 4 months in the Islamic Republic of Iran.** *Bull World Health Organ* 1999, **77(5):**381-385.
18. WHO: **New York: United Nations Children's Fund, Breastfeeding, management and promotion in a Baby Friendly Hospital: an 18-course maternity staff.** 1993.
19. WHO/UNICEF: **World Health Organization/United Nations Children's Health Organization Fund Breastfeeding counselling: a training course.** 1993.
20. Sokol E, Clark D, Aguayo VM: **Protecting breastfeeding in West and Central Africa: over 25 years of implementation of the International Code of Marketing of Breastmilk Substitutes.** *Food Nutr Bull* 2008, **29(3):**159-162.
21. ILO: **Maternity Protection Convention C183, Geneva: Internatinal Labour Organization.** 2000.

22. Humenick SS, Gwayi-Chore MO: **Leader or left behind: national and international policies related to breastfeeding.** *J Obstet Gynecol Neonatal Nurs* 2001, **30(5):**529-540.
23. BFPS: **Breastfeeding Promotion Society, Tehran, Iran.** 2007.
24. Hajian-Tilaki KO: **Factors associated with the pattern of breastfeeding in the north of Iran.** *Ann Hum Biol* 2005, **32(6):**702-713.
25. Yngve A, Sjostrom M: **Breastfeeding determinants and a suggested framework for action in Europe.** *Public Health Nutr* 2001, **4(2B):**729-739.
26. Good Mojab C: **An American IBCLC's observations on breastfeeding in Iran.** *J Hum Lact* 2008, **24(1):**13.
27. Zareai M, O'Brien ML, Fallon AB: **Creating a breastfeeding culture: a comparison of breastfeeding practises in Australia and Iran.** *Breastfeed Rev* 2007, **15(2):**15-20.
28. Modanlou HD: **Avicenna (AD 980 to 1037) and the care of the newborn infant and breastfeeding.** *J Perinatol* 2008, **28(1):**3-6.

II

Physician's role; important for support of breastfeeding

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Running title: Obstacles to breast-feeding

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Key words: Family, doctor advice, breast-feeding discontinuation, pacifier, bottle feeding

Abstract

We have previously shown that despite high prevalence of any breast feeding at 2 years of age in Iran, only 28% were exclusively breast-feeding at 6 months.

Objectives: To investigate causes of discontinuation of exclusive and any breast-feeding in mothers in Iran.

Design and setting: This retrospective study was based on questionnaires and interviews of 63 071 mothers of infants less than 24 months of age, divided in two populations before and after 6 months. The data were collected in 2005-2006 from all 30 provinces of Iran by trained health workers in the Integrated Monitoring Evaluation System in the Family Health Office of the Ministry of Health.

Results: Early total discontinuation of breast-feeding was found in 5.3% of infants before 6 months of age, at a mean of 3.2 months, more common in urban than rural areas. Reasons for discontinuation of exclusive breast-feeding (72%) were physicians' recommendation (54%) and insufficient breast milk (self-perceived or true, 28%).

Total discontinuation of breast-feeding between 7-24 months of age was identified in 11% of the infants at mean of 13.8 months. The most common reason for discontinuation of breast-feeding at this age was insufficient breast milk (self-perceived or true, 45%). Mothers' disease or medication (10%), infant's disease (6%), and return to work (3%) were uncommon causes. Use of pacifier was reported in 15%, and was negatively correlated to breast-feeding.

Conclusions: Physicians and other health professionals have an important role to play in encouraging and supporting mothers to breastfeed according to the recommendations.

Introduction

Breast-feeding is an ideal and unique way of providing nutrients for the healthy growth and development of infants [1]. Breast-feeding is providing ideal nourishment for the growth and development of infants and has a unique biological and emotional influence on the health of both mother and child [2, 3]. Cessation of breast-feeding is linked to an increased risk of acute and chronic diseases in childhood [4]. World Health Organization (WHO) concluded that exclusive breast-feeding, defined as giving breast milk without any food or liquid until 6 months of age, confers benefits to mothers and infants [5].

Factors positively influencing the extent of breast-feeding have been shown to be mothers' socio-demographic and psychosocial background such as high education in the mothers and a supportive family [6]. In contrast, in Iran, it was reported that higher maternal education had a negative influence as well as high income of the father [7, 8]. These factors were found to influence a large number of mothers to stop exclusive or any breast-feeding early and use formula or other breast milk substitutes [7].

We have previously shown that in 2006, only 57% of Iranian babies were exclusively breastfed at 4 and 28% at 6 months of age [9]. These figures were low in comparison with the WHO recommendation. In this context, it was surprising that the duration of any breastfeeding was long, and actually proved to be common at 2 years of age [9].

The current paper was written with the aim to identify causes for early discontinuation of breastfeeding, caused for early abandonment of exclusive breastfeeding as well as discontinuation between 7-24 months of age. This was done by getting access to more information from the Integrated Monitoring Evaluation System Survey (IMES), which collected the information constituting the basis for our previous report about the prevalence of breastfeeding in Iran [9]. In this paper we look at geographic differences, including urban/rural location, as well as reasons given by the mother.

Methods

Definitions

The definition of breast-feeding used was consistent with those given by WHO [5, 10]. *Exclusive breast-feeding* was defined as only breast milk given to the infants and no other liquids or solids except for those containing vitamins or medicines; *partial breast-feeding* included addition of some artificial feeds, either milk or cereal, or other food; *any breastfeeding* including partial and exclusive breastfeeding. *Discontinuation* is defined as complete cessation of any breast-feeding from 7th to 24th months of age [11]. *Early discontinuation of breast-feeding*, means complete cessation of breast-feeding before 6 months of age.

Data collection

The collection of data on breast-feeding was made by health workers in the IMES during September 15th, 2005 to January 15th, 2006, as previously reported [9]. The Study population included all children less than 2 years of age who lived in urban or rural areas and had been referred to a Health Care Center to receive health care, vaccination or treatment for any disease (in rural areas through home visits by trained interviewers, who were employees of the Ministry of Health (MOH)) [12]. In data about early discontinuation of breast-feeding, only those infants were included, who were between 1 day and 6 months of age at the time of investigation (n=8434). For any breast-feeding, infants between 7-24 month of age (n=52637) were studied.

The data collection was performed by interviewing mothers, using a preprinted questionnaire format. The questionnaire included questions about the name of province, district and Health Care Center; number in the household, mother's age and education, birth date, infant's age, infant feeding pattern during the last 24 hours. Breastfeeding was reported as no breastfeeding or any breastfeeding and in the 0-6 months also as exclusive breastfeeding. Breast milk substitutes or other drinks in bottles (sweetened water, tea, juice, etc) were registered as well as the use of pacifier.

Statistical analysis

IMES data was analyzed using STATA version 8.0 and Survey Analysis commands by using each medical university as a separate stratum and each data collection area as primary sampling unit (PSU) and the proportion of sampled persons to the population of under 2 years of age as weight. The rates of discontinuation in different age groups were calculated with 95% confidence interval (CI). Pearson regression was used to correlate the use of pacifier to breast-feeding duration.

Ethical approval

Approval to conduct this analysis was obtained from the Ethics Committee at the MOH in Iran in February 2007. Informed consent was obtained from mothers before the interview. Ethics approval for the analyses of the results to be conducted in Sweden was obtained in 2009.

Results

Infants 1 day – 6 months of age (n=8434)

The mean age in different regions of the mothers ranged from 24.2 to 29.0 years. The percentage of early discontinuation of breastfeeding before 6 months was 5.3% (Figure 1 A). Early discontinuation of breast-feeding was significantly more common in urban than in rural areas, 6.2% vs. 3.9%, respectively (p=0.001). The mean age of early discontinuation of breast-feeding in the country was 3.2 months ranging 2.4-3.8 months for the different regions (Figure 2 A), without significant differences between rural and urban areas (data not shown). At the age of 6 months, 27.7% of the infants were exclusively breastfed. Reasons for early discontinuation of *exclusive* breast-feeding during the first 6 months of life (72.3%) are given in Table 1 and the main reason given by the mothers was that discontinuation was advised by the physician (54%). Reported causes did not differ between urban and rural areas.

Infants 7-24 months of age (n=52637)

The mean age of the mothers ranged from 25.7 to 29.4 years in the different regions. Discontinuation of any breastfeeding was identified in 11% of the mothers (Figure 1 B).

It was higher in urban areas, 12%, compared to rural areas 9% (p=0.005). The mean age of discontinuation of any breast-feeding was 13.8 months, ranging 12.1- 16.3 months for the different regions (Figure 2 B). It was higher in rural areas, being 13.5 vs. 14.5 months of age (p< 0.001).

The reason given by the mothers for discontinuation of any breast-feeding in infants between 7 to 24 months of age was most often insufficient amount of breast milk (false or true) (Table 2). Return to work outside of home was seldom stated as a reason for discontinuation of breast-feeding.

Formula or other bottle feeding to infants less than one year old was reported in 9% (95% CI: 8-10), and 29% (95% CI: 28-30), respectively. Pacifier was used in 15% (95% CI: 14-16) of the infants. There was a significant negative correlation between use of pacifier and continuation of exclusive or any breast-feeding (r= -0.172, p <0.007 and r= -0.267, p <0.006, respectively). Differences in causes were not significant between urban and rural areas.

Discussion

Our findings show the mean age of early discontinuation of breast-feeding in Iran was 3.2 months of age, while the mean age for discontinuation of breastfeeding above 6 months was 13.8 months. The early discontinuation of breast-feeding was low (5.3%), and also relatively low between 7 to 24 months, being 11%. The prevalence of exclusive breastfeeding at 6 months was 28%. The most common reason for discontinuation of exclusive breast-feeding was according to Table 1, advice from the physician, which did not differ between urban and rural areas. Taveras et al. [13] reported that mothers will not usually discontinue breastfeeding within the first 3 months after delivery if they are encouraged by physicians to continue. The American Academy of Pediatricians recommends physicians to advise mothers on initiation and continuation of breastfeeding and to be knowledgeable about the basics of lactation [14]. It was thus surprising that doctors' recommendations were among the most important reasons to stop exclusive breast-feeding, since the physiological and psychological beneficial effects of breastfeeding should be most acknowledged by them. Similar influences by the doctors

have been reported by others [14, 15]. One study showed that as many as 26% of primary care physicians did not encourage exclusive breast-feeding [16]. Public health physicians are suggested to be more trained to encourage mothers to breast feed [17].

The most common reason for discontinuation of any breast-feeding was insufficient breast milk (Table 2). A previous study from Tehran showed that 74% of mothers started to provide complementary feeding to their infants due to insufficient breast milk [7]. It might be questioned if Iranian mothers really have insufficient breast milk production. True reasons for insufficient breast milk can be divided into primary and secondary, and the causes can be found in mothers as well as infants [18]. The milk production might be enough, but effective sucking or transfer might be absent or absorption may be disturbed in the infant. Breast refusal was reported as a common reason for discontinuation of breast-feeding, and might be related to insufficient milk supply or to psychological factors [18]. One study showed that smoking and medication influenced the duration of both exclusive and any breast-feeding negatively [19], but these reasons could not be confirmed in our study, since data of smoking were missing and medication was only referred to in 11% of the causes of discontinuation.

In our study, family recommendation constituted 20% of causes that influenced mothers to stop exclusive breast-feeding. Also the support of the family has been stressed by others [20], and the lack of support was shown in one study to be one of the reasons for mothers to stop early breast-feeding [15]. In line with the importance of the family, others have reported that mothers perceiving social support found that more important than health service support [21].

Return to work by the mothers and diseases in the infants were rare causes of stopping breast-feeding in contrast to the situation in several Western countries [22]. The introduction of pacifiers has been shown to affect breast-feeding duration negatively [23] and our results support this finding. While maternal age has been shown to have a positive correlation with duration of breast-feeding [23, 24], this correlation was not significant in our study.

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Access to early breast-feeding after delivery influenced the duration of both exclusive and any breast-feeding positively [25], and this is practiced in Iran and would at least be consistent with the long maintenance of any breast-feeding reported in our study.

A limitation of the study is the retrospective approach, using collected data from many different Health Care Centres, although data collection was performed using identical instructions and protocols. Furthermore, 23% of the mothers did not specify the reasons for the cessation of breast-feeding. However, our results are supported by other findings in regards to the importance of support from family and especially from physicians [26].

Conclusion

The cause of early discontinuation of exclusive breast-feeding during infancy was mainly physicians' recommendation and/or insufficient breast milk. Specific reasons, such as mothers' or infants' diseases were rare, indicating that by support and education, the prevalence of exclusive breast-feeding might further increase up to 6 months. The results suggest an important role for the physicians and other health professionals to encourage breast-feeding.
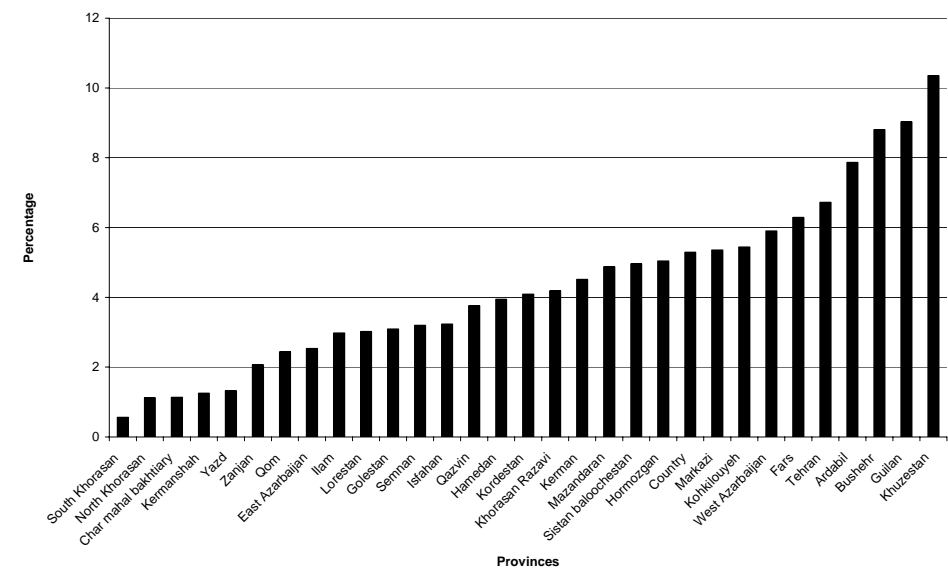
Acknowledgements

References

1. Riordan J: *Breastfeeding and human lactation*. Boston: Jones and Bartlett; 2005.
2. Marshall JL, Godfrey M, Renfrew MJ: **Being a 'good mother': Managing breastfeeding and merging identities**. *Soc Sci Med* 2007, **65**:2147-2159.
3. Kramer MS, Kakuma R: **The optimal duration of exclusive breastfeeding: a systematic review**. *Adv Exp Med Biol* 2004, **554**:63-77.
4. Ip S, Chung M, Raman G, Chew P, Magula N, DeVine D, Trikalinos T, Lau J: **Breastfeeding and maternal and infant health outcomes in developed countries**. *Evid Rep Technol Assess (Full Rep)* 2007:1-186.
5. WHO: **The World Health Organization's Infant Feeding Recommendation. http://www.who.int/nut/. Accessed October 31, 2010.**
6. Yngve A, Sjostrom M: **Breastfeeding determinants and a suggested framework for action in Europe**. *Public Health Nutr* 2001, **4**:729-739.
7. Marandi A, Afzali HM, Hossaini AF: **The reasons for early weaning among mothers in Teheran**. *Bull World Health Organ* 1993, **71**:561-569.
8. Rakhshani F, Mohammadi M: **Continuation of breastfeeding: is this a problem in Southeast Iran?** *Breastfeed Med* 2009, **4**:97-100.
9. Olang B, Farivar K, Heidarzadeh A, Strandvik B, Yngve A: **Breastfeeding in Iran: prevalence, duration and current recommendations**. *Int Breastfeed J* 2009, **4**:8.
10. WOrld Health Organization: **IndIndicators for assessing infant and young child feeding practices, Country profiles. Part III, [cited 2010 DEC]; Availabl from: http://whqlibdoc.who.int/publications/2010/9789241599757_eng.pdf**.
11. World Health Organization: **Nutrition Landscape Information System (NLIS), Country Profile Indicators, Interpretation Guide, [cited 2011 Jan]; Available from:http://whqlibdoc.who.int/publications/2010/9789241599955_eng.pdf**
12. MOH: **Family and Population Health Office, the Ministry of Health IR of Iran, Tehran, Iran**. 2006.
13. Taveras EM, Capra AM, Braveman PA, Jensvold NG, Escobar GJ, Lieu TA: **Clinician support and psychosocial risk factors associated with breastfeeding discontinuation**. *Pediatrics* 2003, **112**:108-115.
14. **Breastfeeding and the use of human milk. American Academy of Pediatrics. Work Group on Breastfeeding**. *Pediatrics* 1997, **100**:1035-1039.
15. Yesildal N, Aytar G, Kocabay K, Mayda AS, Dagli SC, Bahcebasi T: **Breastfeeding practices in Duzce, Turkey**. *J Hum Lact* 2008, **24**:393-400.
16. Leavitt G, Martínez S, Ortiz N, García L: **Knowledge About Breastfeeding Among a Group of Primary Care Physicians and Residents in Puerto Rico**. *J Comm Health* 2009, **34**:1-5.
17. Labarere J, Gelbert-Baudino N, Ayral AS, Duc C, Berchotteau M, Bouchon N, Schelstraete C, Vittoz JP, Francois P, Pons JC: **Efficacy of breastfeeding support provided by trained clinicians during an early, routine, preventive visit: a prospective, randomized, open trial of 226 mother-infant pairs**. *Pediatrics* 2005, **115**:e139-146.
18. Schanler RJ: *Breast feeding 2001, part II: the management of breastfeeding*; 2001.
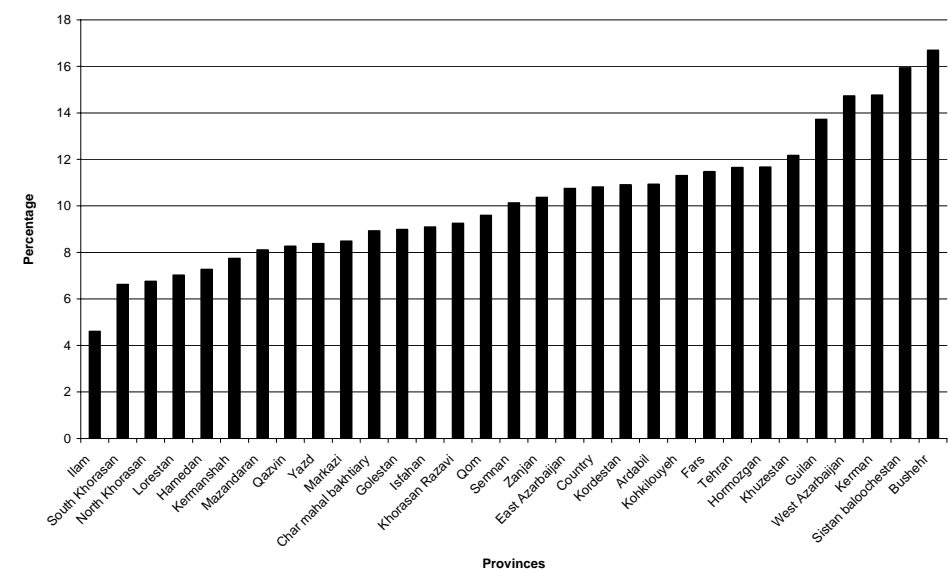
19.   Waldenstrom U, Aarts C, Lane DA, Kauls LS, Ickovics JR, Naftolin F, Feinstein AR: **Duration of breastfeeding and breastfeeding problems in relation to length of postpartum stay: a longitudinal cohort study of a national Swedish sample. Early postpartum discharges. Impact on distress and outpatient problems**. *Acta Paediatr* 2004, **93**:669-676.

20.   Ekstrom A, Widstrom AM, Nissen E: **Breastfeeding support from partners and grandmothers: perceptions of Swedish women**. *Birth (Berkeley, Calif)* 2003, **30**:261-266.

21.   McInnes RJ, Chambers JA: **Supporting breastfeeding mothers: qualitative synthesis**. *J Adv Nurs* 2008, **62**:407-427.

22.   Dennis CL: **Breastfeeding initiation and duration: a 1990-2000 literature review**. *J Obstet Gynecol Neonatal Nurs* 2002, **31**:12-32.

23.   Scott JA, Binns CW, Oddy WH, Graham KI: **Predictors of breastfeeding duration: evidence from a cohort study**. *Pediatrics* 2006, **117**:e646-655.

24.   Roudbari M, Roudbari S, Fazaeli A: **Factors associated with breastfeeding patterns in women who recourse to health centres in Zahedan, Iran**. *Singapore Med J* 2009, **50**:181-184.

25.   Ekstrom A, Widstrom AM, Nissen E: **Duration of breastfeeding in Swedish primiparous and multiparous women**. *J Hum Lact* 2003, **19**:172-178.

26.   Taveras EM, Li R, Grummer-Strawn L, Richardson M, Marshall R, Rego VH, Miroshnik I, Lieu TA: **Mothers' and clinicians' perspectives on breastfeeding counseling during routine preventive visits**. *Pediatrics* 2004, **113**:e405-411.

Figure 1 The percentage of early discontinuation of breastfeeding A (before 6 months), and discontinuation of breast-feeding B (between 7-24 months). Data from IMES.

A

B

Fig. 2 Mean age in months of early discontinuation of breast-feeding A (before 6 months) and discontinuation of breast-feeding B (7 to 24 months). Data from IMES

A

B

Table 1 Percentage distributions of the causes of discontinuation of exclusive breast-feeding before six months of age in 8434 infants in Iran, as given by the mothers. Some mothers have given more than one reason. Mean and 95% Confidence Intervals (CI).

	Physician's recommendation	Insufficient breast milk	Family recommendation	Crying baby	Non-specific reason
Mean (CI)	54 (53-55)	28 (27-29)	20 (19-21)	17 (16-18)	17 (16-18)

Table 2 Percentage distributions of causes for discontinuation of breastfeeding between 7-24 months of age in 52637 infants in Iran. Mean and 95% Confidence Intervals (CI)

| | In mothers | | | | In infants | | Non-specific causes |
	Insufficient breast milk	Disease or medication	Return to work	Time to stop	Breast refusal	Diseases	
Mean (CI)	45 (43-47)	11 (10-12)	3 (2-4)	6 (5-7)	27 (26-29)	6 (5-7)	25 (24-27)

III

Long chain polyunsaturated n-3 fatty acids differ significantly in colostrum in relation to fish intake during pregnancy

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Running title: Fish intake and breast milk

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Abstract

During pregnancy and lactation, the intake of n-3 polyunsaturated fatty acids in fish /seafood may improve maternal and infants' health outcomes. The aim of this study was to estimate fatty acid (FA) concentrations in colostrum in relation to habitual high or low fish intake in two provinces of Iran.

Breast milk was collected early after delivery from 120 consecutively selected mothers, 60 in each province of Guilan and Kermanshah (coastal and inland, respectively). The mothers were interviewed to complete a food frequency questionnaire. The FA composition of colostrum was measured with gas-liquid chromatography.

Mothers in coastal area had significantly higher intake of fish/seafood during pregnancy than those in inland. Consumption of saturated fat was higher in Kermanshah and olives and olive oil intake were significantly higher in Guilan. High fish/seafood intake was associated with significantly higher docosahexaenoic acid (DHA) concentration and lower arachidonic acid (AA)/DHA ratio in colostrum. There were no significant differences in the total polyunsaturated FA status and in linoleic and α-linolenic acid concentrations in colostrums between the provinces. Total n-3 FAs and DHA were significantly higher in Guilan, but total n-6 FAs and AA did not differ. The n-6 FA concentrations were high in both provinces. The ratios of total n-6/n-3 FAs and AA/DHA in breastmilk of mothers from Guilan were significantly lower than those in Kermanshah.

The long chain polyunsaturated FA status in colostrum in two Iranian provinces was generally good and DHA was higher and the ratio AA/DHA were significantly lower in mothers with high fish intake.

Keywords: Essential fatty acids; DHA; food intake; breast milk; Iran.

1. Introduction

In the last 20 years, several studies have examined the maternal intake of fish, seafood and omega (n-) 3 long-chain polyunsaturated fatty acids (LCPUFA) during pregnancy and lactation in relation to the impact on infant's health outcome [1, 2]. Maternal health during pregnancy as well as fetal and child health have the potential to be improved by LCPUFA [3, 4]. The requirement of essential nutrients is especially high during this period of rapid growth for normal development of the fetus and infant and also for long term effects on the child.

The average concentration of arachidonic acid (AA; C20:4n-6) in breast milk is around 0.45% of total fatty acids (FA) [5]. Docosahexaenoic acid (DHA; C22:6n-3) concentration is more modulated by the diet and the content in breast milk has been reported to vary considerably, between 0.1% to 3.8% of total fatty acids [6] and correlates to mother's plasma concentration [7]. Vegetable oils, poultry, meat and fish products (fast food) are today good sources of n-6 PUFA [8, 9]. The amount of n-3 PUFA is high in fish, seafood and fish oils because the marine food chain is based on ocean phytoplankton, which synthesize DHA, and the intake is reflected in the breast milk [10]. Olives and olive oil are rich in monounsaturated fatty acids (MUFA) and it also contains some n-3 PUFA [11].

Fatty acid status in the fetus is dependent on the maternal FA status and dietary intake and a selective transplacental passage of LCPUFA creates a higher LCPUFA concentration in the neonatal circulation [12]. Maternal stores of LCPUFA become available to the fetus in the last trimester in order to meet higher requirements of the fetus during this stage [13, 14]. DHA and AA are accumulated in the fetal retina and brain during pregnancy to manage the need of these LCPUFA for normal neuronal and visual development [15]. Nonesterified fatty acids (NEFA) are considered to be the main source of placental FAs, coming from maternal adipose tissue and hepatic lipoprotein. Even though maternal plasma lipoproteins do not directly cross over the placenta, they play a specific role for the availability of fatty acids to the fetus [14]. The efficient transfer is reflected in lower concentrations of DHA and AA in the mother's plasma as pregnancy progresses [13, 16].

LCPUFA of the n-3 and n-6 series are partly converted from α-linolenic acid (ALA; C18:3n-3) and linoleic acid (LA; C18:2n-6), respectively, but the transformation to LCPUFA of the n-3 series is vey low [17]. The conversion of ALA and LA to LCPUFA is dependent on

competing enzymes (Figure 1). The recommended n-6/n-3 ratio in the diet is 4:1 but is in many countries today much more than 10:1 [18].

AA and DHA are important for the formation of membrane structures in all tissues [19]. The availability of AA has been positively associated with birth weight and growth during the first year of life [20]. Mead acid (ETA; 20:3n-9) and its ratio to AA can be used as a marker for EFA and PUFA status. It has been seen that ETA is negatively related with LA, AA and DHA as well as the sum of n-3 and n-6 PUFA status measured in the phospholipids from cord blood, infant's plasma [7] and cord arterial walls [21]. It usually increases in mother's plasma during the third trimester, which might reflect the surge of PUFA to the fetus.

The amount of DHA in cord blood is correlated to the level of DHA in maternal blood [7, 22]. Maternal supplementation of fish or cod liver oil during pregnancy has been shown to affect the cognitive function of the infants [23, 24]. These studies suggest an association between DHA and neurological functions such as improvement in mental processing at the age of 2 and 4 years [23, 25]. In one study, fish consumption exceeding 340 g/week was necessary to have beneficial effects on child development up to 8 yrs of age [24]. A fast increase of DHA in breast milk was seen during supplementation of fish oil as well as a fast decrease of DHA levels after the supplementation period [10, 26]. It has been suggested that pregnant and lactating women should have an average daily intake of at least 200 mg DHA [19]. The recommended level of DHA can be reached by eating fish and seafood, including fatty fish, approximately twice a week. When advising mothers to consume more fatty fish one should be concerned about the possible contaminants in certain types of fish [25]. Large amount of fish oil, 4 g/day, consumed by pregnant women, resulted in high concentration of n-3 PUFA in blood, but considerably lower concentration of AA in cell membranes of the neonates [25].

Low fish consumption is one of the nutritional problems in Iran [27]. Fish, fish oil and caviar from the Caspian Sea are the most important dietary sources of n-3 PUFA in the north of Iran [28]. The average fish consumption per capita per year is 18 kg in the world; however, it is only 5 to 6.7 kg in Iran [29].

The Caspian Sea is about 422 000 km^2 in area with 6 397 km of coastline, of which more than 900 km are within Iranian borders. The most important fish species in the Caspian Sea are Caspian kutum (R.frisii kutum), mullet (L. aurata and L. saliens) and pikeperch (S.

lucioperca) in this order [30]. Their total PUFA content are 23%, 22% and 20% of total fat, respectively, and the percentage of n-3 fatty acids in pikeperch is 8.5% (g100g^{-1}lipid) [31]. The low fish intake has raised concern about the impact of the low levels of LCPUFA in the maternal diet on the development of the fetus and neonates [32]. The aim of this study was to estimate if EFA and LCPUFA concentrations in breast milk differed in two provinces of Iran, differing in regard to dietary fish intake.

2. Material and methods

2.1 Study design

This study was cross-sectional and performed in collaboration between the National Nutrition and Food Technology Research Institute (NNFTRI) in Iran, the Unit for Public Health Nutrition at the Department of Biosciences and Nutrition at Karolinska Institutet in Sweden and The Faculty of Health, Nutrition and Management at Akershus University College, Norway. The FA composition of breast milk shortly after delivery was determined and the dietary intake of fish and seafood was assessed by structured interview using a food frequency questionnaire (FFQ).

The study was approved by the Medical Ethics Committee of the Kermanshah University of Medical Sciences in 2008. Informed consent was obtained from all mothers. Ethics approval for the statistical analyses of the results to be conducted in Sweden was obtained in 2009.

2.2 Subjects

Two regions of Iran were chosen, the Guilan province close to, and the Kermanshah province far from, the Caspian Sea. One hundred twenty mothers, 60 in each province, were consecutively approached in two public provincial hospitals, Alzahra Hospital in Rasht, the capital of Guilan and Moutazedi Hospital in Kermanshah, the capital of Kermanshah, during July to September 2008. Two mothers in Kermanshah and one mother in Guilan rejected to participate and then the next ones were chosen.

2.3 Breast milk sampling and fatty acid analysis

When the mothers were in the post delivery ward, 8 to 72 hours after delivery, 2 to 5 ml of colostrum was collected by hand pumping into tubes. The tubes were kept in an ice box and sent within half an hour to the provincial health centre for freezing at -20 °C until analysis, which was performed at the laboratory of NNFTRI in Tehran. The milk samples were

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homogenized by Vortex (Heidolph Vortex Shaker REAX 1. 220V. 30W Germany) at 2400 rpm for 30 sec, and 500 μl was mixed with 2 ml KOH in methanol (2N) and 2 ml hexane, and again mixed by use of a Vortex for 2 min. For esterification the sample was maintained for 30 min in ambient temperature and centrifuged (Rotina 35 R, Hettich, Tuttingen, Germany) for 2 min at 5000 rpm at 4°C. Na_2SO_4 (1g) was added to the upper phase and the fatty acid methyl esters (FAME) were removed and 2 μl of the esterified sample was injected into the gas chromatograph (GC-CP3800, Varian, USA) equipped with a fused silica capillary column 50m×0.25mm (I.D. CP-SIL 88, for FAME, Varian Chrompack, USA-Netherlands) supplied with 0.2 μm stationary phase (Varian Chrompack, USA-Netherlands) and a flame ionization detector. Oven temperature was maintained at 45°C for 2 min, then increased by 10 °C/min to 175°C and held there for 15 minutes, followed by increase at 3°C/min to 220°C and kept there for 25 min (total time: 70 min). The injector and the detector temperatures were set at 260°C and 270°C, respectively. Helium was the carrier gas with a split ratio of 30:1 and a column flow of 0.5 ml/min. FA were identified by comparing the retention times of FAME with a standard FAME mixture (SUPELCO 37 Comp. FAME mix, Pennsylvania, USA). All samples were analysed in duplicate. The results are expressed as weight/weight area percent. The values are given as mean ± SEM if not otherwise indicated.

2.4 Dietary measurements

The FFQ used contained 17 items to cover the fish, fish oil, sea food, olive, olive oil and dietary fat consumed during pregnancy. Dietary assessments were completed by first author at an interview with the participants, usually at the same time the breast milk was collected. The response options for the dietary intake were arranged in five categories from "never", "not regularly", "once a week", "twice a week" or "more than twice a week". In the analysis these five categories were reduced to three, named "non/not regularly", "once a week" and "≥ 2 times a week".

2.5 Statistical analysis

Data analysis was performed with Excel and SPSS software 17.0 for Windows. Standard t-test was used to compare the mean differences of total FAs in breast milk and weight gain during pregnancy between the mothers in the two provinces. The fish or seafood intake was categorized. The DHA concentration of total FAs in breast milk and the AA/DHA ratio were compared with the frequency of eating fish or seafood during pregnancy by using Kruskal-Wallis test and Mann Whitney´s U-test. Chi-square test was used to evaluate the difference in

dietary fat consumption including fish or seafood intake between the groups. Differences were considered as statistically significant at p<0.05.

3. Results

3.1 Characteristics of the two study populations

One-hundred-twenty subjects were included in the two provinces. The mean (SD) age was 26.6 (6.7) years in Kermanshah and 26.2 (6.4) in Guilan, ranging 14-40 years and 15-43 years, respectively. The weight before pregnancy was not available in 12 cases (4 in Kermanshah and 8 in Guilan). The mean (SD) did not differ between the groups, being 59.6 (11.6) kg in Kermanshah and 63.7 (13.9) in Guilan, but weight gain during pregnancy was significantly higher in Guilan (p = 0.025) (data not shown).

There were no differences in birth weight, length and head circumference of the babies between 58 infants in Kermanshah and 62 infants in Guilan. Two mothers in Guilan had delivery of twins and anthropometric measurements in two cases were missing in Kermanshah (data not shown). The gestational age was less than 37 weeks in 9 cases (3 in Kermanshah and 6 in Guilan) and in 4 cases data were missing about gestational age.

3.2 Dietary data

Two dropouts occurred in the FFQ because one woman had left the hospital before the interview was completed and one further gave no information about the dietary intake of fish, seafood and fat. The result of the FFQ showed that the subjects in Guilan had a significantly higher intake of fish or seafood during pregnancy compared to the mothers in Kermanshah (p<0.001) (Table 1). Consumption of saturated fat from other animal sources during pregnancy was statistically higher in Kermanshah (p = 0.001) and that of olives and olive oil was significantly higher in Guilan (p<0.001). There were no differences in the intake of other vegetable oils (such as sunflower and corn oil) during pregnancy between the two provinces.

3.3 Fatty acid composition of early breast milk (colostrum)

Table 2 shows the concentrations of the major FAs in breast milk in the two provinces. FAs were similar regarding the EFAs, LA and ALA, but, the total n-3 PUFA and DHA in breast milk were significantly higher in the Guilan compared to the Kermanshah district (p = 0.039 and p<0.001, respectively). Total n-6 PUFA and AA concentrations showed no differences between the two groups. The ratios of total n-6/n-3 fatty acids as well as AA/DHA in breast

milk of the mothers from Guilan were significantly lower (p = 0.013 and p = 0.002, respectively) than those found in mother's milk from Kermanshah. Mead acid (ETA; 20:3n-9) was not identified.

The concentration of DHA was higher in breast milk after premature than after term delivery, being 0.76 (0.26) vs. 0.56 (0.27) (p=0.03), but AA concentration showed no significant differences after term and premature delivery. The AA/DHA ratio was lower after premature delivery 1.91 (0.44) vs. 2.94 (2.10) than after term delivery (p=0.016). There was no difference in the total concentrations of n-6 and n-3 fatty acids. Table 3 only compares early milk after term deliveries in the two provinces in relation to fish and seafood intake. Similar significant differences were obtained when all milk samples were included (data not shown).

According to the average concentration of n-3 and n-6 PUFA of breast milk in relation to maternal age , the youngest tertile (14-23 years) showed the highest concentration of PUFA (p = 0.01), which was mainly related to the n-6 fatty acids (p = 0.03). There was no significant correlation between age groups and fish or seafood intake.

4. Discussion

This study demonstrates that regional differences in Iran regarding fish and seafood intake were reflected in the FA pattern of the early breast milk. The results confirm that dietary fish and seafood intake of the mother influences the DHA content of breast milk. The results also confirm very high concentration of n-6 FA, as earlier reported from Iran [33].

The total n-3 and n-6 PUFA status are dependent on both fish and seafood intake and of the intake of vegetable oils. The higher fish and seafood consumption in Guilan was reflected in significantly higher total n-3 PUFA status (Table 2). The mean values of DHA and AA in colostrum in our study were higher than those reported in a meta-analysis of 16 different studies on colostrum, [34] and much higher than the meta-analysis comparing 106 studies from mature milk, being 0.32±0.22 and 0.47±0.13, respectively [5]. The LCPUFA concentrations of colostrum are usually higher than that in mature milk [5]. The total n-6 PUFA did not show any differences between mother's milk between the two districts and one of the explanations to this similarity would be the high consumption of vegetable oil generally in Iran [35]. These vegetable oils, such as sunflower oil, contain high concentrations of n-6 fatty acids which

would explain why that total n-6 FA did not differ between the groups. High AA concentration in breast milk has previously been reported both from Iran and Iraq [33, 36].

Our study showed high concentrations of total n-3 PUFA in colostrum in both provinces, compared to earlier studies, mainly due to a higher EPA concentration than those previously reported [33, 34, 36]. We did not have a full dietary record, but the high EPA concentration and the relatively small differences between the groups compared to other studies, might be related to a high vegetable intake, as this has shown to highly influence the EPA concentration [37]. On the other hand it is even so high that a co-eluation with another fatty acid can not be excluded, especially as mead acid was not identified in this setting.

The results indicate that mothers with fish or seafood intake at least once a week had the highest DHA concentration and the lowest AA/ DHA ratio. In pregnant women in the republic of Seychelles with high habitual fish consumption, no association was found between breast milk and maternal serum DHA status at 28 weeks of gestation or with the DHA concentrations in breast milk at 1 month post partum [16]. Besides the high habitual fish intake there might also be genetic differences [38], which might explain some of the ethnical differences[39-41] and before full genetic analyses are performed it might be difficult to separate these from regional differences related to diet. A trend to differences in colostrums from mothers living in urban and rural areas has previously been reported from Slovenia [42], but significant differencebb was only found in the concentration of oleic acid. We could not investigate differences between urban and rural individuals since both groups of mothers were collected for big city hospitals with mainly urban populations.

Consistent with other studies, our findings showed a significant positive correlation between DHA intake through fish and seafood and its concentration in the breast milk [22]. Women in Western Australia supplemented with DHA and EPA from week 20 of gestation until delivery showed higher DHA and EPA concentrations in breast milk at day 3 and also 6 weeks after delivery, compared to the controls receiving olive oil, but this difference was no longer detected after 6 months [25] . Other studies have shown that the increased content of LCPUFA in breast milk in early lactation was related to higher infant DHA status at 1 year of age [43]; suggesting that the levels of LCPUFA in breast milk might have a long term effect on the infant's FA status.

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There was no significant difference of mean age between the mothers in the two provinces, but the youngest mothers (14-23 years) had the highest total n-3 and n-6 PUFA concentration in breast milk, not correlated with higher fish or seafood intake. No data were collected about maternal parity but we cannot exclude such influence since it has been shown that DHA decreases with parity [44]. On the other hand, the influence of Western diet, rich in vegetable oils, might be higher in the youngest age group and thereby explain the higher concentrations of n-6 fatty acids in that group. Weaknesses of our study were that we had a small sample size and lack information about several confounding factors regarding maternal habits, which however, would have influenced the results in opposite direction to our finding, i.e. smoking would lower DHA concentrations.

Socioeconomic factors, including family size, income and mothers education, in an urban Iraqian population of mothers have been shown to influence n-3 fatty acid concentrations in the breast milk [45]. However, the relatively high concentration of DHA and n-3 fatty acids in our study, also in the inland, suggests that the socio-economic differences might not have been so important in this study. That does not exclude that such information would be crucial to evaluate if genetic [38] and ethnic [40] differences compared to other countries might affect the relatively good LCPUFA concentrations in colostrum reported in this study.

5. Conclusion

There are accumulating data showing the effect of maternal dietary fatty acid intake on the FA composition of breast milk. This study suggests that Iranian mothers have a good LCPUFA status compared with literature, and that it was further improved by high fish and sea food intake.

6. Acknowledgment

References

1. Cetin I, Koletzko B. Long-chain omega-3 fatty acid supply in pregnancy and lactation. Curr Opin Clin Nutr Metab Care. 2008;11:297-302.
2. Innis SM. Dietary omega 3 fatty acids and the developing brain. Brain Res. 2008;1237:35-43.
3. Fleith M, Clandinin MT. Dietary PUFA for preterm and term infants: review of clinical studies. Crit Rev Food Sci Nutr. 2005;45:205-29.
4. Makrides M. Outcomes for mothers and their babies: do n-3 long-chain polyunsaturated fatty acids and seafoods make a difference? J Am Diet Assoc. 2008;108:1622-26.
5. Brenna JT, Varamini B, Jensen RG, et al. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007;85:1457-64.
6. Innis SM, Kuhnlein HV. Long-chain n-3 fatty acids in breast milk of Inuit women consuming traditional foods. Early Hum Dev. 1988;18:185-9.
7. Sabel KG, Lundqvist-Persson C, Bona E, et al. Fatty acid patterns early after premature birth, simultaneously analysed in mothers' food, breast milk and serum phospholipids of mothers and infants. Lipids Health Dis. 2009;8:20.
8. Whitney EN, Rolfes SR. Understanding nutrition. Belmont, CA: Thomson/Wadsworth; 2008.
9. Sampels S, Strandvik B, Pickova J. Processed animal products with emphasis on polyunsaturated fatty acid content. European Journal of Lipid Science and Technology. 2009;111:481-8.
10. Harris WS, Connor WE, Lindsey S. Will dietary omega-3 fatty acids change the composition of human milk? Am J Clin Nutr. 1984;40:780-5.
11. Crawford M, Galli C, Visioli F, et al. Role of plant-derived omega-3 fatty acids in human nutrition. Ann Nutr Metab. 2000;44:263-5.
12. Coleman RA. The role of the placenta in lipid metabolism and transport. Semin Perinatol. 1989;13:180-91.
13. Al MD, van Houwelingen AC, Hornstra G. Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. Am J Clin Nutr. 2000;71:285S-91S.
14. Herrera E, Amusquivar E, Lopez-Soldado I, et al. Maternal lipid metabolism and placental lipid transfer. Horm Res. 2006;65 Suppl 3:59-64.
15. Ryan AS, Astwood JD, Gautier S, et al. Effects of long-chain polyunsaturated fatty acid supplementation on neurodevelopment in childhood: a review of human studies. Prostaglandins Leukot Essent Fatty Acids. 2010;82:305-14.
16. Bonham MP, Duffy EM, Wallace JM, et al. Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study). Prostaglandins Leukot Essent Fatty Acids. 2008;78:343-50.
17. Larque E, Demmelmair H, Koletzko B. Perinatal supply and metabolism of long-chain polyunsaturated fatty acids: importance for the early development of the nervous system. Ann N Y Acad Sci. 2002;967:299-310.
18. Simopoulos AP. Commentary on the workshop statement. Essentiality of and recommended dietary intakes for Omega-6 and Omega-3 fatty acids. Prostaglandins Leukot Essent Fatty Acids. 2000;63:123-4.
19. Koletzko B, Lien E, Agostoni C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med. 2008;36:5-14.
20. Carlson SE. Arachidonic acid status of human infants: influence of gestational age at birth and diets with very long chain n-3 and n-6 fatty acids. J Nutr. 1996;126:1092S-8S.

21. Dirix CE, Hornstra G, Nijhuis JG. Fetal learning and memory: Weak associations with the early essential polyunsaturated fatty acid status. Prostaglandins Leukot Essent Fatty Acids. 2009.

22. Krauss-Etschmann S, Shadid R, Campoy C, et al. Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. Am J Clin Nutr. 2007;85:1392-400.

23. Helland IB, Smith L, Saarem K, et al. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics. 2003;111:e39-44.

24. Hibbeln JR, Davis JM, Steer C, et al. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet. 2007;369:578-85.

25. Dunstan JA, Simmer K, Dixon G, et al. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. Arch Dis Child Fetal Neonatal Ed. 2008;93:F45-50.

26. Fidler N Fau-Sauerwald T, Sauerwald T Fau-Pohl A, Pohl A Fau - Demmelmair H, et al. Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. J Lipid Res. 2000;41:1376-83.

27. Meester F, Watson RR, Djazayery A, et al. Wild-Type Food in Health Promotion and Disease Prevention [electronic resource] : The Columbus Concept. Totowa, NJ: Humana Press Inc.; 2008.

28. Rajaby M. Experiences of countries with new aquatic industries: the development of aquaculture in Iran. Rev Sci Tech. 2008;27:113-23.

29. Fishery and Aquaculture Country Profiles, access 2010, http://www.fao.org/fishery/countrysector/FI-CP_IR/en [database on the Internet]. Available from: http://www.fao.org/fishery/countrysector/FI-CP_IR/en.

30. Abdolmaleki S, Psuty I. The effects of stock enhancement of pikeperch (*Sander lucioperca*) in Iranian coastal waters of the Caspian Sea Journal of Marine Science. 2007;64:973-80.

31. Hedayatifard M, Jamali Z. Evaluation of Omega-3 Fatty Acids Composition in Caspian Sea Pike Perch (Sander lucioperca). Int J Agri Biol,. 2008;10:235-7.

32. Ministry of Health, Tehran, Iran. Health children Office. 2007.

33. Bahrami G, Rahimi Z. Fatty acid composition of human milk in Western Iran. Eur J Clin Nutr. 2005;59:494-7.

34. Fidler N, Koletzko B. The fatty acid composition of human colostrum. Eur J Nutr. 2000;39:31-7.

35. Nutrition country profiles [database on the Internet] 2010. Available from: http://www.fao.org/ag/AGN/nutrition/IRN_en.stm.

36. Al-Tamer YY, Mahmood AA. Fatty-acid composition of the colostrum and serum of fullterm and preterm delivering Iraqi mothers. Eur J Clin Nutr. 2004;58:1119-24.

37. Welch AA, Shakya-Shrestha S, Lentjes MA, et al. Dietary intake and status of n-3 polyunsaturated fatty acids in a population of fish-eating and non-fish-eating meat-eaters, vegetarians, and vegans and the precursor-product ratio of alpha-linolenic acid to long-chain n-3 polyunsaturated fatty acids: results from the EPIC-Norfolk cohort. Am J Clin Nutr. 2010;92:1040-51.

38. Koletzko B, Lattka E, Zeilinger S, et al. Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. Am J Clin Nutr. 2010.

39.   Fidler N, Salobir K, Stibilj V. Fatty acid composition of human milk in different regions of Slovenia. Ann Nutr Metab. 2000;44:187-93.

40.   van Eijsden M, Hornstra G, van der Wal MF, et al. Ethnic differences in early pregnancy maternal n-3 and n-6 fatty acid concentrations: an explorative analysis. Br J Nutr. 2009;101:1761-8.

41.   Yuhas R, Pramuk K, Lien EL. Human milk fatty acid composition from nine countries varies most in DHA. Lipids. 2006;41:851-8.

42.   Fidler N, Salobir K, Stibilj V. Fatty acid composition of human colostrum in Slovenian women living in urban and rural areas. Biol Neonate. 2001;79:15-20.

43.   Dunstan JA, Mitoulas LR, Dixon G, et al. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: a randomized controlled trial. Pediatr Res. 2007;62:689-94.

44.   Al MD, van Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal docosahexaenoic acid status. Eur J Clin Nutr. 1997;51:548-53.

45.   Al-Tamer YY, Mahmood AA. The influence of Iraqi mothers' socioeconomic status on their milk-lipid content. Eur J Clin Nutr. 2006;60:1400-5.

Mothers' intake	Kermanshah (%)	Guilan (%)	P-value
Fish and sea food	21	60	0.001
Fish oil	10	8	0.5
Olives and olive oil	17	55	0.001
Vegetable oils	76	78	0.4
Animal fat, margarine and hydrogenated oil	84	51	0.001

Table 1. The percentage of mothers eating fish, sea food and other fat products more than once a week during pregnancy in inland (Kermanshah) and coastal (Guilan) provinces of Iran, as reported in the food frequency questionnaire (n=58 and n=60, respectively).

Fatty acids	Kermanshah (n=60)	Guilan (n=60)	p-value
C12:0	1.62±0.10	1.67±0.12	0.73
C14:0	4.12±0.14	4.10±0.13	0.90
C16:0	24.9±0.32	26.1±0.24	0.001
C18:0	6.50±0.14	6.53±0.17	0.87
C20:0	0.25±0.00	0.23±0.01	0.23
C22:0	1.09±0.04	1.10±0.04	0.78
C24:0	0.92±0.05	0.91±0.06	0.92
∑ SFA	39.4±0.8	40.7±0.77	0.16
C14:1ω7	0.24±0.00	0.20±0.00	0.001
C16:1ω7	1.77±0.09	1.81±0.08	0.75
C18:1ω9	30.08±0.30	28.85±0.30	0.001
C20:1ω9	0.83±0.02	0.76±.021	0.04
∑ MUFA	32.9±0.41	31.6±0.4	0.14
C18:2ω6 (LA)	16.30±0.33	16.03±0.38	0.60
C18:3ω3 (ALA)	0.81±0.03	0.81±0.03	0.97
C20:4ω6 (AA)	1.40±0.04	1.32±0.04	0.28
C20:5ω3 (EPA)	1.31±0.06	1.35±0.06	0.60
C22:5ω3 (DPA)	0.35±0.01	0.40±0.02	0.10
C22:6ω3 (DHA)	0.50±0.02	0.66±0.03	0.001
∑ ω3FA	3.04±0.10	3.35±0.11	0.04
∑ ω6FA	17.7±0.36	17.4±0.41	0.53
∑ PUFA	20.8±0.41	20.8±0.42	0.95
LA/ALA	21.2±0.54	20.8	0.60
AA/DHA	3.40±0.31	2.28±0.14	0.001
AA/(EPA+DHA)	0.82±0.03	0.70±0.03	0.01
∑ ω6/∑ω3	6.10±0.20	5.44±0.17	0.01

Table 2. The concentration of major fatty acids in colostrum (weight %) of mothers in inland (Kermanshah) and coastal (Guilan) provinces of Iran. Mean±SEM. SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, LA = linoleic acid, ALA = α-linolenic acid, AA = arachidonic acid, EPA = eicosapentaenoic acid, DPA = docosapentaenoic acid, DHA = docosahexaenoic acid, PUFA = polyunsaturated fatty acids.

Fatty acid	Fish and seafood intake			p-Value
	Non/seldom (n=64)	1/Week (n=25)	≥2/Week (n=17)	
DHA	0.52 (0.23)[#]	0.62 (0.27)	0.69 (0.34)	0.048
AA/DHA	3.2 (2.4) [#, *]	2.4 (1.1)	2.45 (1.6)	0.022

Mann-Whitney´s U-test was used for differences between the groups, # p<0.05 non/seldom compared to >2 times/week, * p<0.05 non/seldom compared to once/week

Table 3. Mean (SD) molar% concentration of docosahexaenoic acid (DHA; C22:6n-3) and the ratio of arachidonic acid (AA; C20:4n-6) to DHA in colostrum after term delivery in relation to the intake of fish and/or seafood during pregnancy, as reported in the food frequency questionnaires. P-values refer to Kruskal-Wallis test.
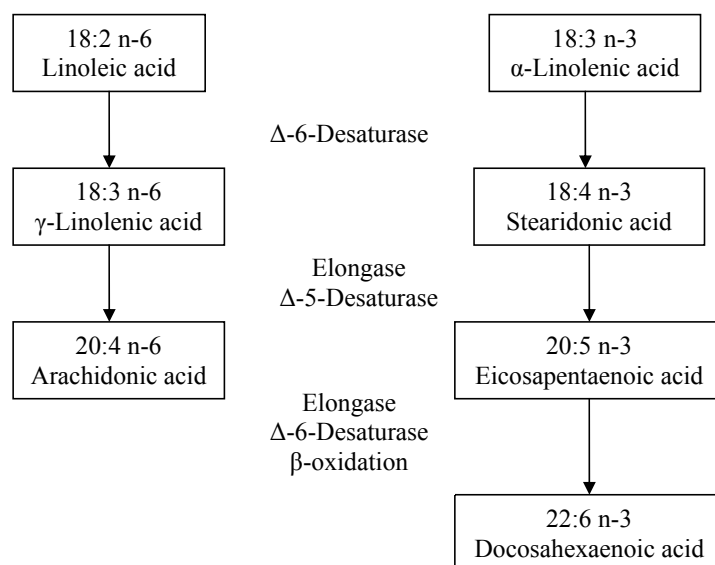
18:2 n-6 Linoleic acid		18:3 n-3 α-Linolenic acid

Δ-6-Desaturase

| 18:3 n-6 γ-Linolenic acid | | 18:4 n-3 Stearidonic acid |

Elongase
Δ-5-Desaturase

| 20:4 n-6 Arachidonic acid | | 20:5 n-3 Eicosapentaenoic acid |

Elongase
Δ-6-Desaturase
β-oxidation

| | | 22:6 n-3 Docosahexaenoic acid |

Figure 1. Simplified scheme of the synthesis of long chain polyunsaturated fatty acids (LCPUFA), arachidonic acid (AA; C20:4n-6), eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3) from their precursor essential fatty acids, linoleic acid (LA; C18:2n-6) and α-linolenic acid (ALA; C18:3n-3), respectively.

IV

REGULAR ARTICLE

Optimal vitamin A and suboptimal vitamin D status are common in Iranian infants

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ABSTRACT

Aim: Investigation of serum concentrations of vitamins A and D in Iranian infants.
Methods: A descriptive cross-sectional study, investigating 7112 infants (15–23 months of age) from all regions of Iran, who attended health care centres from May 25 to June 2, 2001. Unequal clusters with unequal household sizes were sampled. Vitamin A and D levels were analysed with high-performance liquid chromatography.
Results: The mean (SD) concentration of vitamin A was 2.09 (0.83) μmol/L. At a national level, 0.7% of the infants had a level indicating deficiency, and 0.5% of the infants had insufficient concentrations of vitamin A, defined as serum concentrations <0.35 and <0.7 μmol/L retinol, respectively. A total of 88% of infants had optimal concentrations (>1.4 μmol/L). The mean (SD) concentration of vitamin D was 61.3 (31.4) nmol/L. Deficiency was found in 2.8% of infants (<25 nmol/L), and insufficiency in 32.9% (<50 nmol/L). Suboptimal and optimal concentrations were found in 44% and 20%, representing 50–75 and >75 nmol/L, respectively. Girls had lower vitamin D concentrations than boys (p = 0.006).

Conclusion: As in developed countries, vitamin A deficiency was rare in Iranian infants. Vitamin D deficiency was also rare, but 33% of infants had insufficient levels; this was more common in girls than boys.

INTRODUCTION

In several countries, vitamins A and D are provided as supplements for babies during breastfeeding (1,2), as breast milk alone may not meet infants' requirements for these vitamins (3). The vitamin A content of breast milk is dependent on the mother's intake; normally, it is sufficient for the infant soon after birth but decreases during prolonged lactation. Many women experience subclinical vitamin A deficiency during their childbearing years (4), and 10–20% of pregnant women worldwide are vitamin A deficient (5). Young maternal age, multiple pregnancies and minimal antenatal care are associated with low vitamin A concentrations in breast milk (6). Supplementation of lactating women can improve vitamin A concentrations in the breast milk (7). If the vitamin A concentration is initially higher, the infant is less likely to develop a deficiency in subsequent months (7). Vitamin D is usually not significantly secreted in breast milk because of widespread deficiency (8) or very low concentrations (9) in mothers, and deficiency is common in exclusively breastfed infants if they do not receive supplementation (9).

Vitamins A and D are both fat-soluble compounds, and the recommended intake for infants in Iran from 1 month of age, as in most countries, is 450 and 10 μg/day, respectively (10).

Vitamin A is important for vision, protein synthesis and cell differentiation, as well as for growth (11). Plasma retinol concentrations between 0.7 and 1.4 μmol/L are considered suboptimal. More than 1.4 μmol/L is considered optimal. Severe deficiency is defined as <0.35 μmol/L and >0.35 and <0.7 μmol/L is considered insufficient (10). At birth, serum vitamin A concentrations in term infants of well-nourished mothers are generally at least 0.7 μmol/L (10).

The precursor of vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D, and the final product is formed in the kidneys and other tissues by further hydroxylation to 1, 25-dihydroxyvitamin D. Vitamin D can also be formed in the skin in the presence of ultraviolet light and is therefore highly dependent on season and climate. The risk of vitamin D deficiency can be increased by insufficient exposure to sunlight, because of remaining indoors or being fully covered by clothing during daylight hours. In urban centres, insufficient exposure can also result from close tall buildings and pollution.

Recently, the understanding that vitamin D has multiple functions has led to the recommended optimal

concentration of vitamin D being raised to >75 nmol∕L in adults; insufficient concentration is now defined as 25– 50 nmol∕L and deficiency as <25 nmol∕L (12–14).

Limited local and cross-sectional studies provided evidence of micronutrient deficiency in several parts of Iran in 1999 (15). Micronutrient malnutrition was reported to be one of the most significant problems limiting societal and economic progress in Iran in 2000 (15). Health workers, who are employed by the Iranian Ministry of Health, make home visits 3 and 15 days after birth in rural areas and provide parents with vitamin A and D supplements. In urban areas, the health workers and parents meet in primary health care facilities. Supplementary therapy with combined vitamins A and D is started with 15 drops per day at day 15 and increased to 25 drops per day (equivalent to 450 μg vitamin A and 10 μg vitamin D) during the first month and is continued with the same amount during the first year of age. After 6 months of age, iron is added to the supplementation, which continues until 12 months of age. It is recommended that infants are then introduced to family foods, but supplementation is recommended up to 2 years of age (12). The relevance of supplementation with vitamin A is still under debate in several countries (16,17).

The present study is based on a survey in Iran in 2001, in different provinces encompassing both urban and rural areas. The aim of the study was to investigate the status of vitamin A and D among infants aged 15–24 months in Iran. Mothers visiting health care centres within a time frame of 10 days in the spring of 2001 were invited to participate in the study.

SUBJECTS AND METHODS
Iran can be divided into 11 regions with 28 provinces, based on ethnographic, demographic, epidemiological and socioeconomic factors. The study population included infants from 15 months to 2 years of age, from households in all

provinces (Table 1), who came to the health care centres during the period May 25 to June 2, 2001 for the second dose of measles vaccine or for general health care. This sampling method has been considered the best way to select a random sample of this age group. Unequal clusters with unequal household sizes were sampled (18). The clusters were sampled in proportion to the size of the urban and rural population of each district in all regions. The centre of the cluster was selected by systematic random sampling.

There were 880 clusters in 11 regions (Table 1), 504 urban and 376 rural clusters, randomly selected throughout the country. In each cluster, 10 infants were chosen, providing a total of 8800 infants (4400 infants each for measuring vitamin A and vitamin D). The health care staff at the selected health centres informed mothers about the study. Those mothers who agreed to participate also completed a questionnaire about their infant's name, weight, length and head circumference.

Only healthy infants were included. Venous blood was randomly collected for measuring vitamin A or vitamin D. The infants were held on their mothers' lap during the blood sampling. In total, vitamin A and vitamin D samples were collected from 3099 (urban = 1798, rural = 1301) and 4013 (urban = 2035, rural = 1715) infants, respectively. The blood samples were sent to an accredited laboratory within the district for centrifugation, and serum samples were coded and frozen at −20°C until analysis. All samples were transported by a refrigerated vehicle to an accredited laboratory in Tehran and analysed by high-performance liquid chromatography (15). Vitamin A was analysed as retinol, and vitamin D as 25-hydroxyvitamin D. The cut-off for insufficient concentrations of vitamins A and D was set according to published values (see above) (10,13).

Statistical analyses
Data were analysed using STATA version 9 and Social Sciences software version 17.0 (SPSS Inc., Chicago, IL,

Table 1 Mean (SD) of vitamins A and D in different regions of Iran. The number of infants investigated in the different regions was in proportion to the total number of infants in that region (according to the Population and Housing Census conducted in November 1996) (18)

Region	Provinces	Vitamin A n	Retinol Mean (SD) μmol∕L	Vitamin D n	25 (OH) vitamin D Mean, (SD) nmol∕L
1	Gilan, Mazandaran	353	2.01 (0.57)	374	56.2 (19.9)
2	E. Azarbaijan, W. Azarbaijan, Ardebil	334	2.13 (0.99)	347	67.2 (40.2)
3	Golestan, N. Khorasan	275	2.01 (0.65)	332	60.9 (24.2)
4	Semnan, Khorasan Razavi	244	2.10 (0.55)	422	64.2 (31.3)
5	Sistanbalochestan, S. Khorasan	272	1.86 (0.55)	365	55.1 (25.4)
6	Yazd, Isfahan, Chaharmahal and N. Kerman	299	2.09 (0.58)	319	62.9 (36.5)
7	Boshehr, Hormozgan, S. Khuzestan	279	2.02 (0.53)	352	61.4 (19.8)
8	Tehran	299	2.14 (0.59)	393	59.5 (34.5)
9	Zanjan, Ghazvin, Ghom, Markazi	337	2.04 (0.60)	389	55.7 (26.3)
10	Ilam, Kurdistan, Kermanshah, Lorestan, Hamedan, N. Khuzestan	294	2.26 (1.80)	411	64.2 (47.4)
11	Fars, S. Kerman, Kohgiloyeh, Boyer Ahmad	113	2.24 (0.54)	309	67.8, (20.5)
Total	All	3099	2.09 (0.83) Range (0*–25.3)	4013	61.3 (31.4) Range (0*–620.7)

*Content was below the detectable level of analysis for 16 samples for vitamin A and 14 samples for vitamin D.

USA). The results are given as mean and standard deviation (SD). Comparison between groups was made by independent sample test (*t*-test). Significance levels were set at p < 0.05.

Ethical approval
Approval to conduct the study was obtained from the Ethics Committee of the Ministry of Health in Iran. Informed consent was obtained from all mothers. Ethical approval for the analysis to be conducted in Sweden was obtained in 2009.

RESULTS
The mean (SD) concentrations of vitamins A and D were 2.09 (0.83) and 61.3 (31.4) nmol/L, respectively (Table 1). Optimal and suboptimal concentrations of vitamin A were found in 88.4% and 10.4% of the infants (>1.4 and 0.7–1.4 μmol/L), respectively. Only 1.2% of the infants were vitamin A deficient or had insufficient concentrations (<0.35 and 0.35–0.7 μmol/L, respectively). Vitamin A deficiency was identified in nine of the regions, but in only 20 (0.7%) of the 3099 infants (Table 2). There was no difference in vitamin A concentrations by gender or between urban and rural areas.

The distribution of the different concentrations of vitamin A by region is illustrated in Figure 1. The mean vitamin A concentration was highest in region 11, and lowest in region 5 (a region with low socio-economic status) (p = 0.001).

Only 2.8% of infants had vitamin D deficiency (serum concentrations <25 nmol/L) (Table 2), whereas insufficient concentrations (25–50 nmol/L) were found in 32.9%. Suboptimal (50–75 nmol/L) and optimal (>75 nmol/L) concentrations of vitamin D were found in 44.1% and 20.2% of infants, respectively. Deficiency of vitamin D was found in all regions. The distribution of the different concentrations of vitamin D by region is illustrated in Figure 1. There was no difference in vitamin D concentrations by urban and rural areas, but there was a significant difference by gender

(p = 0.006); concentrations were lower in girls than in boys. Low concentrations of vitamin D were most common in regions 1, 5, 8 (representing Tehran) and 9.

DISCUSSION
The important finding of our study was that deficient concentrations of vitamin A and vitamin D were rare during the second year of life (15–23 months of age), the period when supplementation was still recommended. This may indicate that the supplementation recommendations were generally followed, but we do not have any data on compliance. However, a third of the infants had insufficient levels of vitamin D. This could indicate low compliance and/or that the dosage of vitamin D supplementation was too low. It might also be related to insufficient vitamin D status in the mothers; however, this would not explain the gender difference. As the vitamin A and vitamin D data were collected from two different groups of children, we could not use the combined measures to identify A and D supplement users.

The regions are composed of different provinces, but the sample was too small to allow for subanalyses for different provinces. This means that we could not do any analysis of socio-economic variation and its likely impact on vitamin A and D status. There were no significant differences in vitamin deficiency between rural and urban areas; however, this finding may be unreliable as the drop out rate was higher in rural areas. Our study covered only 86% of the rural population, because of the remote location of some villages and difficulties in reaching the nomadic population. The sampling was complete in urban areas.

In April 1995, the World Health Organization categorized countries in terms of whether vitamin A deficiency should be a public health priority. According to this categorization, Iran had a moderate deficiency of vitamin A with subclinical traits, although this could not be verified in our study. A United Nations Children's Fund (UNICEF) report published in 2005 illustrated that 28% of children between

Table 2 The distribution of deficiency, insufficiency and suboptimal and optimal concentrations of vitamins A and D as internationally defined (see text) in the different regions. The number of infants is given for the different regions and percentage distribution for the whole country

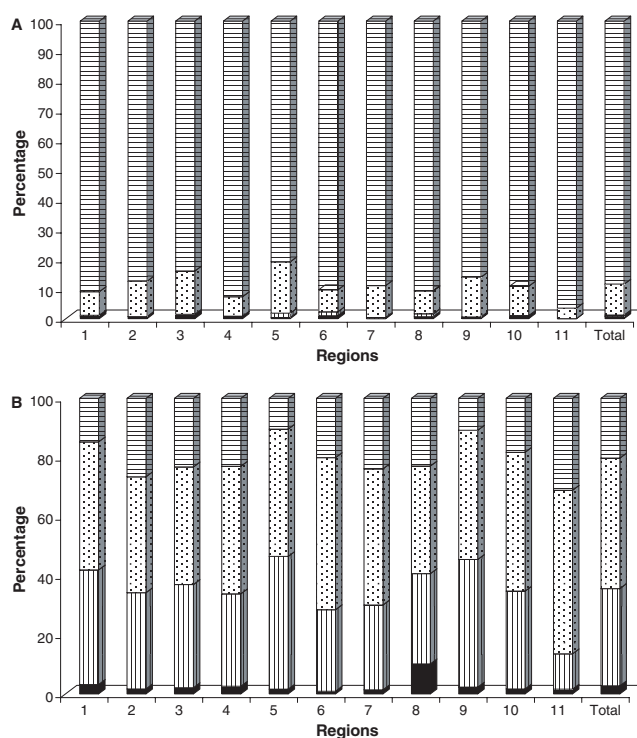| Regions	Vitamin A				Vitamin D			
	Deficiency <0.35 μmol/L	Insufficiency 0.35–0.7 μmol/L	Suboptimal 0.7–1.4 μmol/L	Optimal >1.4 μmol/L	Deficiency <25 μmol/L	Insufficiency 25–50 μmol/L	Suboptimal 50–75 μmol/L	Optimal >75 μmol/L
1	3	1	28	321	12	145	162	55
2	2	0	40	292	6	113	136	92
3	4	0	40	231	7	116	132	77
4	2	0	16	226	11	132	182	97
5	1	4	47	220	6	164	157	38
6	3	4	22	270	3	88	164	64
7	0	1	30	248	5	101	162	84
8	2	3	23	271	40	120	143	90
9	1	1	45	290	9	168	170	42
10	2	1	29	262	7	136	193	75
11	0	0	4	109	5	37	171	96
Total	20	15	324	2740	111	1320	1772	810
%	0.7	0.5	10.5	88.4	2.8	32.9	44.2	20.2

Figure 1 The percentage distribution of deficient (black bars), insufficient (vertical bars), suboptimal (dotted bars) and optimal (horizontal bars) concentrations of vitamins A (A) and D (B) in 15- to 23-month-old infants in different regions of Iran.

6 and 9 months of age were still not receiving vitamin A supplementation (19). These findings do not seem to be relevant to Iran, according to the outcomes of the present study. In less developed countries, like Mozambique, vitamin A deficiency is common and contributes to mortality (20), and in Indonesia, decreasing rates of severe vitamin A deficiency have been seen over the last 25 years, where children, who did not receive vitamin A supplementation, tended to be slightly malnourished altogether (21). In rural areas in India, xerophthalmia is a public health problem and a major cause of blindness in many preschool children (22). It has been shown that periodic vitamin A supplementation is an important intervention to reduce morbidity and blindness among children in developing countries (23). In the group with retinol levels <0.35 μmol/L, we would have liked to be able to assess co-morbidities and follow the levels of acute phase proteins. We are investigating the possibility to do so, in order to evaluate the consequences of vitamin A deficiency in this small group.

The adequacy of providing vitamin A as a supplement is currently under debate. Our data can add to that debate by showing that there does not seem to be a problem with vitamin A deficiency in Iran at the current level and use of supplements. Because our data also point to low levels of vitamin D, compliance with supplementation and possibly also the level of vitamin D in the supplement warrant further investigation before a clear statement can be given on the adequacy of vitamin A and D supplementation in Iran.

We cannot draw any conclusions regarding the vitamin A deficiency situation in the rural parts of Iran, because of low participation rates. In this study, looking at the whole of Iran, 98% had good vitamin A concentrations, and there seems to be very little risk of complications of a low vitamin A status. On the other hand, these infants should not receive a higher dose of vitamin A, as concentrations that are too high can cause toxic symptoms such as increased intracranial pressure (24).

It is sunny in Iran throughout most of the year in many regions. In sunny areas, children can play outside and are exposed to sunlight, which is an important factor for the maintenance of adequate vitamin D status. Region 1, which is located in the north of Iran close to the Caspian Sea, has

more rainy days and high humidity, and our results show that more infants had low vitamin D levels in this region. Several studies indicate lower concentrations of vitamin D by older age, with risk increased by being fully covered by clothing (25) and by spending less time outdoors (25). Dietary habits were not investigated in this study, but oily fish, which is an important source of vitamin D, is not a common food in young infants and children (26).

Living in rural areas has also been implicated as a risk factor for vitamin D deficiency in other countries. The results of a study in China showed severe vitamin D deficiency and rickets among some rural women and their children (27). In a rural region in Bangladesh, an association between low vitamin D status (<29.1 nmol/L) and acute lower respiratory infection (ALRI) was demonstrated among young children (28). ALRI is the most common cause of child mortality globally (29). We had no data about infection because it was beyond the scope of the study.

A study of healthy 8-year-old children from Sweden showed similar vitamin D concentrations to those in this study (30). Sweden is located on a much higher latitude (57°43′N) than Iran, but has vitamin D fortification of low-fat milk and similar clothing is worn by boys and girls. Swedish girls tended to have a better vitamin D status than boys, in contrast to the findings of this study, and a seasonal variation was seen. It is probable that an investigation in Iran after the summer season would give higher concentrations, supporting a better vitamin status in Iranian infants. Vitamin D concentrations in young infants might be lower in winter, and this underlines the importance of close monitoring during the year in Iran.

The high percentage of infants with insufficient concentrations of vitamin D despite recommendation of supplementation implies that more age groups should be investigated and that recommendations of supplementation might be considered above 2 years of age.

CONCLUSION

Iranian infants generally have a very good vitamin A status, but one-third of infants have insufficient concentrations of vitamin D. Based on this study, we cannot make any firm conclusions about the necessity of vitamin A supplementation or the level of vitamin D supplementation. In comparison with other low- and middle-income countries as well as with developed countries, the concentrations of vitamin A were satisfactory, which might be because of infant vitamin supplementation as supported by the Ministry of Health. The concentrations of vitamin D were only satisfactory in 64% of infants, which probably means that vitamin D supplementation dosage is too low or that compliance is poor.

An interventional study is needed to evaluate compliance, the adequacy of dosage and duration of vitamin D supplementation and the effect of sun exposure during the year. The gender difference in vitamin D concentration is of concern and should be monitored.

References

1. Casey CF, Slawson DC, Neal LR. Vitamin D supplementation in infants, children, and adolescents. *Am Fam Physician* 2010; 81: 745–8.
2. Vesel L, Bahl R, Martines J, Penny M, Bhandari N, Kirkwood BR, et al. Use of new World Health Organization child growth standards to assess how infant malnutrition relates to breastfeeding and mortality. *Bull World Health Organ* 2010; 88: 39–48.
3. Schanler RJ. Breast feeding 2001, part II: the management of breastfeeding. Philadelphia: W.B. Saunders, 2001.
4. Omidvar N, Ghazi-Tabatabie M, Harrison GG, Eghtesadi S, Mahboob SA, Pourbakht M. Development and validation of a short food-frequency questionnaire for screening women of childbearing age for vitamin A status in northwestern Iran. *Food Nutr Bull* 2002; 23: 73–82.
5. World Health Organization. Micronutrient deficiencies: combating vitamin A deficiency. Available at: http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf (accessed on July 2010).
6. Katz J, Khatry SK, West KP, Humphrey JH, Leclerq SC, Kimbrough E, et al. Night blindness is prevalent during pregnancy and lactation in rural Nepal. *J Nutr* 1995; 125: 2122–7.
7. Bahl R, Bhandari N, Wahed MA, Kumar GT, Bhan MK, WHI/ICD Immunization-Linked Vitamin A Group. Vitamin A supplementation of women postpartum and of their infants at immunization alters breast milk retinol and infant vitamin A status. *J Nutr* 2002; 132: 3243–8.
8. Lawrence RA, Lawrence RM. Breastfeeding: a guide for the medical profession. State. Louis, MO: Mosby, 2005.
9. Kovacs CS. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. *Am J Clin Nutr* 2008; 88: 520S–8S.
10. Blomhoff R, Vitamin A. In Nordic Nutrition Recommendations 2004: integrating nutrition and physical activity. Copenhagen: Nordic Council of Ministers, 2004.
11. Whitney EN, Rolfes SR. Understanding nutrition. Belmont: Thomson/Wadsworth, 2008.
12. Holvik K, Brunvand L, Brustad M, Meyer H. Vitamin D status in the Norwegian Population. Oslo: General Practice and Community Medicine, 2008.
13. Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25-hydroxyvitamin D deficiency in US children: NHANES 2001–2004. *Pediatrics* 2009; 124: e362–70.
14. Nelson ML, Blum JM, Hollis BW, Rosen C, Sullivan SS. Supplements of 20 microg/d cholecalciferol optimized serum 25-hydroxyvitamin D concentrations in 80% of premenopausal women in winter. *J Nutr* 2009; 139: 540–6.
15. Ministry of Health, Iran. Family and Population Health Office, 2001
16. Gogia S, Sachdev HS. Neonatal vitamin A supplementation for prevention of mortality and morbidity in infancy: systematic review of randomised controlled trials. *BMJ* 2009; 338: b919.
17. Latham M. The great vitamin A fiasco. *World Nutr* 2010; 1: 12–45.

18. Evaluation of population census data through demographic methods [database on the Internet] 2010. Available from: http://www.amar.org.ir/default.aspx?tabid=52.

19. UNICEF. The state of the world's children. [Child survival]. New York: Unicef, 2008.

20. Aguayo VM, Kahn S, Ismael C, Meershoek S. Vitamin A deficiency and child mortality in Mozambique. *Public Health Nutr* 2005; 8: 29–31.

21. Berger SG, de Pee S, Bloem MW, Halati S, Semba RD. Malnutrition and morbidity among children not reached by the national vitamin A capsule programme in urban slum areas of Indonesia. *Public Health* 2008; 122: 371–8.

22. Pal R, Sagar V. Antecedent risk factors of xerophthalmia among Indian rural preschool children. *Eye Contact Lens* 2008; 34: 106–8.

23. Semba RD, de Pee S, Sun K, Bloem MW, Raju VK. Coverage of the national vitamin A supplementation program in Ethiopia. *J Trop Pediatr* 2008; 54: 141–4.

24. Dhiravibulya K, Ouvrier R, Johnston I, Procopis P, Antony J. Benign intracranial hypertension in childhood: a review of 23 patients. *J Paediatr Child Health* 1991; 27: 304–7.

25. Wishart HD, Reeve AM, Grant CC. Vitamin D deficiency in a multinational refugee population. *Intern Med J* 2007; 37: 792–7.

26. Ghassemi H, Harrison G, Mohammad K. An accelerated nutrition transition in Iran. *Public Health Nutr* 2002; 5: 149–55.

27. Strand MA, Perry J, Guo J, Zhao J, Janes C. Doing the month: rickets and post-partum convalescence in rural China. *Midwifery* 2009; 25: 588–96.

28. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. *Acta Paediatr* 2010; 99: 389–93.

29. Bryce J, Boschi-Pinto C, Shibuya K, Black RE. WHO estimates of the causes of death in children. *Lancet* 2005; 365: 1147–52.

30. Eriksson S, Strandvik B. Food choice is reflected in serum markers and anthropometric measures in healthy 8-yr-olds. *e-SPEN, Eur e-J Clin Nutr Metab* 2010; 5: e117–24.