NUTRITIONAL CHARACTERISTICS OF BREAST MILK IN HONG KONG LACTATING WOMEN

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CERTIFICATE OF ORIGINALITY

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<u>Wong Wing-si, Vincy</u>

Abstract

Background

It is well recognized that breast milk provides infants with the best source of nutrients for healthy growth and development. The World Health Organization (WHO) recommends that infants should be exclusively breastfed for the first 6 months of their lives, and from then on be given appropriate complementary foods along with breastfeeding until the age of 2 or beyond. Hong Kong people have a local dietary habit with high seafood consumption. In recent decades, rapid economic development has led to changes in the lifestyle and dietary habits from traditional Chinese towards a more western one. There is a fundamental gap in the research findings on how the dietary patterns of Hong Kong lactating women affect the fatty acid profile and mineral content of breast milk in the past two decades. The aims of this study were (i) to characterize the latest eating patterns of local mothers, (ii) to investigate the nutritional composition of the human milk of local mothers, including trace elements and fatty acid profile and (iii) to examine how the nutritional composition of human milk correlates with local dietary characteristics, particularly the high consumption of seafood.

Method

Breast milk samples from ninety-five healthy lactating women aged 19-40 were collected between May 2014 and August 2015. Among the subjects, 73 of them did not have any supplementation of fatty acids. Maternal dietary intakes of macronutrients and micronutrients were assessed using 3-day diet records. The records were analyzed by the nutritional analysis software The Food Processor Nutrition Analysis and Fitness software to examine the diet quality of lactating mothers. Maternal dietary patterns of the lactating mothers, especially that of fish consumption, were

assessed using Food Frequency Questionnaire (FFQ). In the breast milk samples, the fatty acid profile was analyzed by Gas Chromatography- Flame Ionization Detector (GC-FID) while the mineral content was analyzed by Inductively Coupled Plasma - Mass Spectrometry/ Optical Emission Spectrometry (ICP-MS/OES). All statistical analyses were performed using the SPSS version 23 software. Nonparametric Spearman's rank correlation tests were used to examine the correlations between maternal nutrient intake and the fatty acid profile and mineral content in breast milk. Multiple linear regression was used to determine the correlations after adjusting for different potential confounding factors. $p<0.05$ (two-tailed) was considered statistically significant for all analyses.

Results and Discussion

Our study showed that nearly one-third of the subjects were overweight or obese. Maternal mean protein intake was higher than the Chinese recommended nutrient intake (RNI) for lactating women by 40% while the mean maternal energy intake from fat was also higher than the upper limit of the Chinese AMDR by 23%. As calculated from the 3-day dietary records, the mean intake of vegetables, fruits and dairy products of the lactating mothers were all below the recommendation levels. The findings revealed that the diet quality of the lactating mothers has much room for improvement. The mean maternal energy intake from essential fatty acids, LA and ALA, by lactating mothers were within the AMDR recommended by the Chinese Dietary Reference Intakes (DRIs) 2013.

The mean DHA and total ω-3 fatty acid levels were 0.86% and 3.79% of total fatty acids in the milk samples from mothers who had exclusively breastfed their infants aged within 6 months, respectively. Over 80 percent of the milk samples of lactating mothers who did not take any fatty acid supplements could provide their infants aged from 0-6 months old with adequate level of

DHA. The results of Spearman's rank correlation tests showed that the levels of maternal DHA and ALA intake and their levels in milk were significantly associated. More importantly, the results demonstrated the association between the fatty acid contents in milk of mothers and their specific fish consumptions. They revealed significant associations between DHA content in the milk samples and maternal intakes of both freshwater and saltwater fish, as well as some frequently consumed types of fish in Hong Kong such as Mandarin fish, croaker and salmon. In stepwise linear regression analysis, maternal intake of saltwater fish was the only significant predictor of content of DHA in milk.

The mean dietary intake of selenium of the subjects attained the recommended level suggested by the Chinese DRIs. However, the mean dietary intake of other minerals of the lactating mothers, namely calcium, iodine, iron and zinc, were below the RNI levels. The daily mean dietary intake levels of calcium, iodine, selenium and zinc of exclusively breastfed infants aged 0-6 months old were insufficient to meet the suggested intake levels recommended by Chinese DRIs. Only the mean dietary intake of iron of the 0-6 months old infants could meet the recommendation. The results of Spearman's rank correlation tests showed that the age of infants was negatively correlated with the concentrations of calcium and zinc and positively associated with the concentration of iodine of milk samples. Also, the concentrations of calcium, iron, selenium and zinc in milk were positively correlated with dietary intakes of dairy products of the lactating mothers.

The present study revealed that Hong Kong lactating women and their infants might have inadequate nutrient intakes. Although biological protective mechanisms may prevent lactating mothers and their breast-fed infants from severe health setbacks, persistent micronutrient deficiencies may pose high health risks to the majority of Hong Kong populations. Long term effects on the health of population, especially valuable groups with higher demand on nutrient intake such as infants, women during pregnancy and lactation remain controversial.

Conclusion

The present study has shown the impact of local dietary characteristics, especially high consumption of fish, on breast milk ω-3 polyunsaturated fatty acid concentration in Hong Kong lactating women. The amount of fish consumed is a significant predictor of milk DHA content. Over 80 percent of the breast milk samples from local mothers who had no supplementation of fatty acids would provide adequate DHA to their exclusively breastfed infants aged 6 months or below. The research findings have also revealed that lactating mothers have inadequate intake of essential nutrients including calcium, iron and iodine. In the milk samples, the mean daily dietary intakes of iodine, selenium and zinc could not meet the levels of adequate intakes of 0-6 months old exclusively breastfed infants.

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Abbreviations

BRFS	Behavioural Risk Factors Survey
AA	Arachidonic acid
ALA	Alpha-linolenic acid
AOM	Acute otitis media
BMI	Body Mass Index
Ca	Calcium
CDF	Cation Diffusion Facilitator
CFS	Centre for Food Safety
CNS	Chinese Nutrition Society
CPP	Casein Phosphopeptides
DHA	Docosahexaenoic acid
DMT1	Divalent Metal Transporter-1
EAR	Estimated Average Requirement
ECF	Extracellular Fluid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
Fe	Iron
FPN	Ferroportin
GSHPx	Glutathione peroxidase
HMO	Human milk oligosaccharide
IGF-1	Insulin-like Growth Factor 1
IQ	Intelligence quotient
LA	Linoleic acid
LC-PUFAs	Long chain polyunsaturated fatty acids
MECs	Mammary Epithelial Cells
Mg	Magnesium
NIS	Sodium Iodide Symporter
PMCAs	Plasma Membrane Ca^{2+}-ATPases
RNI	Recommended Nutrient Intake
SCFA	Short-chain fatty acids
SERCAs	Saero(endo)plasmic reticulum Ca^{2+}-ATPases
sIgA	Secretory IgA
sIgG	Secretory IgG
SLPI	Secretory leukocyte protease inhibitors
SPCAs	Secretory Pathway Ca^{2+}-ATPases
T3	Triiodothyronine
T4	Thyroxine
TfR	Transferrin receptor
TRP	Transient Receptor Potential
TSH	Thyroid Stimulating Hormone
UL	Upper Limit level
WHO	World Health Organization

List of publications

Full Paper

Shi-Ying Li, Xiao-Li Dong, Wing-Si Vincy Wong, Yi-Xiang Su, Man-Sau Wong. Long-Chain Polyunsaturated Fatty Acid Concentrations in Breast Milk from Chinese Mothers: Comparison with Other Regions. International Journal of Child Health and Nutrition, 2015, 4, 230-239. (In press)

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Conference Abstracts

Wing-Si Vincy Wong, Yuk-Fan Ng, Suk-Mei Chan, Shi-Ying Li, Chi-Leung Cheung, Hang-Wai Lee, Wing-Yiu Pak, Yi-Xiang Su, Man-Sau Wong. Habitual dietary intake put Hong Kong lactating mothers in a pilot study at risk of calcium deficiency. *10th International Symposium on Nutritional Aspects of Osteoporosis. November 2017, Hong Kong. (Poster)*

Wing-Si Vincy Wong, Yuk-Fan Ng, Suk-Mei Chan, Shi-Ying Li, Chi-Leung Cheung, Hang-Wai Lee, Wing-Yiu Pak, Yi-Xiang Su, Man-Sau Wong. Effect of unique local diet on fatty acid compositions of breast milk in Hong Kong lactating mothers. *13th China Nutrition Science Congress. May 2017, Beijing, China. (Poster)*

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Wing-Si Vincy Wong, Yuk-Fan Ng, Suk-Mei Chan, Shi-Ying Li, Chi-Leung Cheung, Hang-Wai Lee, Wing-Yiu Pak, Man-Sau Wong. Effect of daily diet on the polyunsaturated fatty acids content of breast milk in Hong Kong lactating mothers –pilot findings). *12th nutrition & health symposium. November 2015, Hong Kong. (Poster)*

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Chapter 1 Introduction

In the late 1980s, Prof. David Barker illustrated the concept of developmental programming, a process to investigate whether acting on an environmental stimulus during critical periods of development has permanent effects on its structure and function. In order to survive, retarded growth and altered metabolism are the infants' response to maternal malnutrition (1). A growing body of evidence suggests that different health problems such as cardiovascular disease, type 2 diabetes and hypertension in later life are caused by malnutrition during fetal life and infancy via developmental plasticity (2). Therefore, optimum nutrition is vital for healthy growth and development of infants, resulting in protective effect against a variety of infections and chronic diseases. It is well known that breastfeeding is beneficial for infants and provides proper nutrition for optimal growth and development. Meanwhile, advantages of breastfeeding have also been illustrated on maternal health. Some examples are discussed below:

1.1. Effect of breastfeeding on infant health outcomes

A. Reduced risk of cardiovascular diseases

Breast milk contains higher levels of total cholesterol in comparison to infant formula. Compared with formula-fed infants, Owen et al (3) showed that breast-fed infants had higher blood cholesterol levels than formula-fed infants with a total mean cholesterol difference of 0.64mmol/L. It is suggested that breastfeeding brings long-term beneficial impacts on cholesterol metabolism since breast-fed infants have a lower mean of total cholesterol (with a mean difference of -0.18mmol/L in adulthood compared with formula-fed infants) which in turn reduced the risk of cardiovascular disease.

B. Reduced risk of overweight and obesity

The results of a meta-analysis with 33 studies conducted between 1990 and 2006 showed that subjects who had been breastfed during infancy were less likely to become overweight or obese in childhood and adolescence (4). A possible reason was that infants who were fed with breast milk, which contains lower concentrations of protein and fat than formula, might learn to control the amount of milk consumed and thus had a better self-regulation of energy intake in later life. The lower protein concentration in breast milk may also reduce the release of insulin, and therefore lower fat storage and the risk of obesity (5).

C. Enhanced neurodevelopment

Many studies have demonstrated association between breastfeeding and enhanced neurodevelopment. A meta-analysis by Anderson et al in 1999, which included information from 11 studies conducted in Western countries such as the United Kingdom, Australia and the United States, showed that compared with formula-fed infants, the cognitive function of breast-fed infants was 3.2 points higher after adjustment for maternal intelligence. The better cognitive development was sustained throughout childhood and adolescence (6). In addition, findings of studies from the United Kingdom (7, 8), New Zealand (8) and Brazil (9) showed positive correlation between breastfeeding and attained education level. Studies have revealed that docosahexaenoic acid plays an important role in the development of the brain and retina of infants (10). A more recent study suggested that the beneficial effect of breastfeeding on infants' cognitive development was moderated by a genetic variant in FADS1, an enzyme-encoding gene which is involved in the metabolic pathway of arachidonic and DHA synthesis (11).

1.2. Effect of breastfeeding on maternal health outcomes

A. Reduced risk of breast carcinoma and ovarian carcinoma

Results of a meta-analysis study by Chowdhury et al in 2015 of 98 studies (72 studies in high-income countries and 26 studies in low and middle-income countries) revealed that compared with those who had never breastfed, women who had breastfed had a 22% reduction in breast carcinoma risk. Also, the risk reduction was associated with the duration of breastfeeding. Mothers who had breastfed for less than six months and those for 6-12 months had a risk reduction in breast carcinoma by 7% and 9%, respectively (12). The effect of breastfeeding on risk reduction of ovarian carcinoma is more or less the same as that of breast carcinoma. A meta-analysis which included 29 estimates suggested a 37% risk reduction of ovarian carcinoma among mothers who had breastfed for over 12 months compared with those had not breastfed (12).

B. Reduced risk of type 2 diabetes

In 2013, Aune et al conducted a systematic review of six cohort studies which involved 273,961 participants and which were carried out in Germany, Australia, the United States and China from 1986 to 2011. Results showed a negative association between duration of lifelong breastfeeding and risk of type 2 diabetes, i.e. the longer the breastfeeding duration, the lower the risk. A protection of 9% from type 2 diabetes has been revealed in mothers with more than one -year of total lifetime duration of breastfeeding (12).

C. Lowered postpartum weight retention

Stuebe et al conducted a prospective cohort study involving 570 participants in the United States. The study results showed that women who had exclusively breastfed their infants for over 6 months had the lowest BMI and weight retention at 3 years postpartum compared with who had never exclusively breastfed (13). However, no association between breastfeeding and postpartum weight loss was shown in studies conducted in low and middle income countries (14, 15). Therefore, the effect of breastfeeding on postpartum weight changes is still controversial.

1.3. Breast milk nutrition

The profound beneficial effects of breastfeeding on infants and mothers have been well-studied. The World Health Organization (WHO) recommends that infants should be exclusively breastfed for the first 6 months of their lives, and from then on be given appropriate complementary foods along with breastfeeding until the age of 2 or beyond (16).

Promoting and protecting the nutrition of breast milk is crucial to optimize infant health. Breast milk is a highly variable and very complex biofluid that nourishes infants and confers them immunity to protect them from diseases. The composition of breast milk varies in response to a number of factors and matches the needs of infants according to their age and other characteristics (17, 18). Breast milk nutrition changes along lactation and is commonly classified into 3 categories, colostrum, transitional milk and mature milk (19). It is widely recognized that the nutritional composition of breast milk is specifically tailored by every mother to precisely reveal the personalized requirements of their infants (20).

Breast milk contains a wide variety of complex carbohydrates, with lactose existing predominantly. Human milk oligosaccharides (HMOs) are present in significant amounts in breast milk, on average 20.9g/L at Day 4 post-partum and 12.9g/L in mature milk (21). The function of this specific group of carbohydrates, which cannot be digested by infants, is to nourish the gastrointestinal microbiota (21). As prebiotics to protect infants from colonization by pathogenic bacteria, HMO promotes the growth of certain kinds of beneficial bacteria such as *Bifidobacterium infantis* within their gastrointestinal tracts (22).

There are more than 400 types of breast milk proteins, and they exhibit a variety of functions including provision of nutrition, antimicrobial and immunomodulatory activities, as well as stimulating nutrition absorption (23, 24). These proteins can be divided into 3 main groups, i.e. caseins, whey and mucin proteins. Whey and caseins are differentiated according to their solubility, with the soluble whey proteins found in solution, whilst caseins are suspended in solution (25). The first milk, colostrum, contains high levels of whey protein but undetectable levels of caseins (24). Protein in breast milk gradually decreases from the second month to the seventh month postpartum and becomes stable afterwards. On the other hand, a high concentration of secretory immunoglobulin is present in colostrum compared with mature milk. This reveals that colostrum plays an important role in immunity, rather than in nutrition, and protects infants from environmental pathogens (26).

Lipids are the major source of energy in breast milk, i.e. 40%-55% of total energy (27). Triacylglycerides comprise nearly 98% of the lipid fraction whereas diacylglycerides, monoacylglycerides, free fatty acids, phospholipids and cholesterol predominantly exist in the remaining fraction. Compared with macronutrients of lactose and protein in milk, variation of fat concentration is significantly larger both intra- and inter-individually (28). On average, daily intake of milk lipids of exclusively breastfed infants from birth to 6 months old is about 21.4g (29). Short-chain fatty acids (SCFAs) in milk provide considerable amounts of energy to infants (30) and are necessary for maturation of the gastrointestinal tract (31). Long-chain polyunsaturated fatty acids, which are molecules with a chain length of over 20 carbon atoms and 2 or more double bonds, account for about 2% of the total fatty acids that exist in milk (32). The positions of fatty acids along the glycerol backbone of triglycerides are highly conserved in breast milk, with some fatty acids commonly occur in specific positions (33). Milk fatty acids, oleic, palmitic and linoleic acid, which represent the largest proportions, commonly occur at the positions of sn-1, sn-2 and sn-3, respectively (33). Studies revealed that the positions of fatty acids along glycerol would affect their bioavailability. Importantly, the triglycerides with fatty acids at preferred positions cannot be produced in infant formula (34).

1.4. The fatty acid profile of human milk

a. Saturated fatty acids

Saturated fatty acids account for a large proportion of the cell membrane in phospholipids. The fatty acid composition of cell membrane affects its fluidity, which controls the movement of molecules across the membrane. Some cell signaling molecules, which play important roles in cellular and tissue metabolism and function, contain saturated fatty acids as well. Studies have revealed that dietary saturated fatty acids such as lauric acid, myristic acid and palmitic acid may increase total and LDL cholesterol and induce insulin resistance. High intake of saturated fatty acids is positively associated with risk of cardiovascular disease. A study of 7354 subjects in the UK showed that higher dietary intakes of palmitic acid and stearic acid were positively associated with risk of coronary heart disease (35).

b. Monounsaturated fatty acids

Many cell membrane phospholipids contain large proportion of oleic acid, which is prevalent monounsaturated fatty acid in diet. Studies have revealed the beneficial effects of oleic acid on human health, for example, lower blood pressure (36), improved glucose control and enhanced insulin sensitivity (37). In a 5-year PREDIMED intervention study, subjects who were given Mediterranean diets including extra virgin olive oil had reduced cardiovascular outcomes (38). However, the effects may be attributed to the combination of food or nutrients in the diet, rather than to oleic acid.

c. ω-6 polyunsaturated fatty acids

Linoleic acid and arachidonic acid are the most and second most prevalent ω-6 polyunsaturated fatty acids in diet. Large amounts of these ω-6 PUFAs are present in cell membranes and they play different biological roles. Studies have revealed the lowering effects of blood cholesterol and LDL cholesterol concentrations of linoleic acid, especially when the intakes of saturated fatty acids are replaced by linoleic acid. Also, the insulin sensitivity enhancing effect of linoleic acid has been shown (39). Arachidonic acid not only has a structural role in the brain, but also has roles in cell signaling. It can directly promote inflammation (40).

d. ω-3 polyunsaturated fatty acids

α-linolenic acid, the essential ω-3 polyunsaturated fatty acid, is the precursor of eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Cell membranes contain little α-linolenic acid, but most have large amounts of EPA and DHA. The structure of these ω-3 PUFAs influences the physical properties of cell membrane into which they have incorporated and thus the membrane-generated intracellular signals are affected. Therefore, they play important roles in modulating transcription factor activation and gene expression (41). Also, EPA and DHA are involved in several biological processes such as inflammation, blood clotting and vasoconstriction. The anti-inflammatory action of EPA and DHA is mediated by reduced production of eicosanoids from arachidonic acid (42). After birth, the accumulation of DHA in the brain continues and reaches a total of about 4g DHA in the brain from 2 to 4 years of age (43, 44). Studies showed a positive association between the cognitive development of breastfed infants and supplementation of DHA of lactating mothers (45, 46) Moreover, DHA comprises approximately 50% of total fatty acids of rod and cone outer segments (47). It is the only type of n-3 LC-PUFA found with considerable extent in these body compartments (43). A causal relationship has been shown between intake of DHA at a level of 0.3% of total fatty acids during infancy and visual function achieved at 12 months (48). However, some studies have revealed that excessive consumption of omega-3 fatty acids may lead to reduced body growth and head circumference of infant (49). Therefore, optimum intake of EPA and DHA is vital for infants' growth and development (50).

1.4.1. Concentrations of fatty acids in milk

Breast milk contains more than 200 kinds of fatty acids, of which the majority exists in very low levels, but some are predominantly present in milk such as oleic acid (51). De novo synthesis of fatty acids constitutes about 17% of total fat in breast milk (52). Studies showed that the composition of fatty acids in breast milk changes along lactation, provided that the changes in fatty acids profile are less obvious after the first month of lactation (53). Saturated, monounsaturated and polyunsaturated fatty acids (PUFAs) accounted for approximately 35-40%, 45-50% and 15% of total milk lipids, respectively (54).

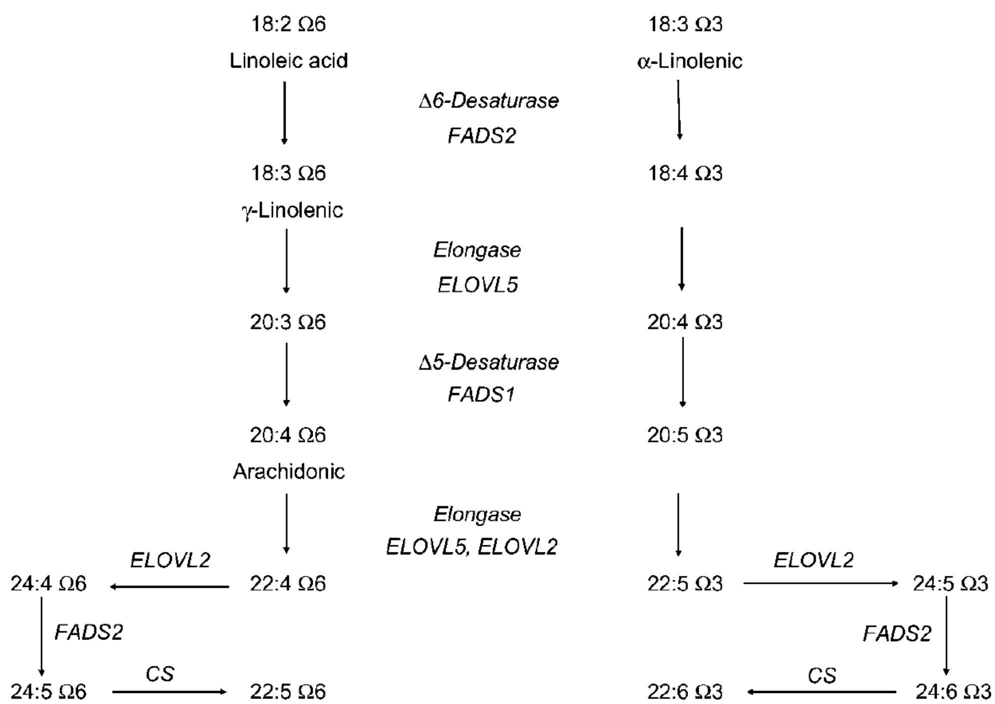
The saturated palmitic acid accounts for the major part of the total saturated fatty acid content. It provides about 25% of total fatty acids in breast milk. There is approximately 70% of milk palmitic acid esterified in the sn-2 position of triacylglycerols which enhance absorption of the fatty acid. The monounsaturated fatty acid oleic acid accounts for 30-40g/100g fat in breast milk and its content is affected by maternal diet (54).

In the group of PUFAs, long-chain polyunsaturated fatty acids (LC-PUFAs) account for about 2% of the total fatty acids that exist in milk (55).

Human milk lipids provide linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3), which are essential PUFAs that cannot be synthesized in human (56). Studies revealed that concentrations of LA and ALA increase with milk maturation (57, 58). About 30% of LA in human milk is derived from maternal dietary intake and the rest comes from the body fat stores of mothers. LA in diet is rapidly transferred into human milk, a process that takes about 12 hours to reach the peak concentration after dietary intake. The level of LA in milk has increased approximately two-fold over the past decades (59) due to changes in habitual diet towards westernized style characterized by high consumption of n-6 fatty acids. Concentrations of LA and ALA in milk vary with dietary intake of lactating mothers in a wide range for 10%-24% and 0.6%-1.9% of fatty acids, respectively. An imbalanced intake of LA and ALA may result in an imbalanced ratio of their metabolites of n-6 and n-3 series since they compete for several active enzymes in the process of fatty acid conversion. Ratio of LA/ALA within 5:1 to 15:1 is suggested by the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) for infant formula feeding. Imbalanced ratio of the essential fatty acids may affect the appropriate function of the immune system of infants (60, 61).

Arachidonic acid (AA; 20:4n-6) and docosahexaenoic acid (DHA; 22:6n-3), members of LC-PUFAs, are converted from LA and ALA, respectively, via a series of chain elongation and desaturation (56). The rate of conversing fatty acids is affected by many factors such as genetics, gender and availability of precursor fatty acids in diet (62). Figure 1 shows the conversion pathways of LA to AA and ALA to DHA.

Figure 1. Metabolic conversion of essential fatty acids Linoleic acid (omega-6 series) and alpha-linolenic acid (omega-3 series) to long chain polyunsaturated fatty acids (LC-PUFAs) (63)



LC-PUFAs in milk originate from various sources including maternal dietary intake, maternal body stores and endogenous synthesis from 18-carbon precursors in the liver, the mammary gland and other tissues. Studies revealed that concentrations of LC-PUFAs of both n-3 and n-6 series reduced significantly by about 38% for AA and about 50% for DHA during first month postpartum (57). The decrement of the fatty acids may be due to decreased maternal stores of LC-PUFAs (64). However, intakes of LC-PUFAs of infants remain more or less the same since the total fat content increases duration lactation (65).

Although the concentrations of LC-PUFAs are affected by maternal diet, increased dietary intake of LA and ALA exerts no significant impact on the contents of their metabolites AA and DHA in mature milk, respectively (66). Less than 1% of dietary ALA would be converted to DHA.

The concentration of AA in milk is relatively stable, ranging from 0.35-0.7 weight percentage of total fatty acids (67). Maternal body fat store accounts for the major portion of AA in milk, and only 1.2% is directly derived from maternal AA intake (68). The turnover of maternal stores is relatively slow. They can buffer short-term changes in dietary intake and to maintain constant supply of essential nutrient to infants.

However, milk DHA concentration is significantly influenced by maternal diets and nutritional status. Thus, it is widely variable among populations (69, 70). Studies revealed that the pattern of milk fatty acids mimics the maternal dietary intake within 2 to 3 days. This implies that dietary fatty acids are rapidly transferred to infants. Positive association has been shown between the intake of marine foods and milk contents of n-3 LC-PUFAs such as EPA and DHA.

The concentration of LC-PUFAs in milk, particularly DHA, has received considerable attention since they are crucial for brain development in early life. Studies have illustrated that DHA begins to accumulate in infant's brain *in utero* and significant deposition occurs in the second half of gestation, when the growth and differentiation of the central nervous system are the most rapid (43, 44, 71). After birth, the accumulation of DHA in brain continues and reaches a total of about 4g DHA in the brain from 2 to 4 years of age (43, 44). DHA in breast milk is readily taken up by the infant brains. Studies showed a positive association between the cognitive development of breastfed infants and supplementation of DHA of lactating mothers (45, 46). Moreover, DHA comprises approximately 50% of total fatty acids of rod and cone outer segments (47). It is the only type of n-3 LC-PUFA found with considerable extent in these body compartments (43). A causal relationship has been shown between intake of DHA at a level of 0.3% of total fatty acids during infancy and visual function achieved at 12 months (48).

1.4.2. Food sources of fatty acids

Animal products such as fatty meats, milk and milk products, including whole-fat milk and cheese, are rich sources of saturated fatty acids. Some plant-based oils such as coconut oil and palm kernel oil contain high proportion of saturated fatty acids as well.

Oleic acid is the most prevalent dietary fatty acid in human diet. Oils are the major food sources of MUFAs. Plant-based oils such as sunflower oil, olive oil and canola oil are rich sources of oleic acid. Nuts and seeds, including almonds, cashers, pumpkin seed and sesame, also provide high contents of MUFAs. Avocados and olives are the only two fruits that contain high MUFAs levels.

The seeds of most plants, except those of coconut, cocoa and palm, are good dietary sources of LA. Soybean and canola oils are the major dietary sources of ALA. Flaxseed oils and some nuts are also good sources of ALA, however, we normally do not consume these foods in large amounts. Phytoplankton and animals are capable of synthesizing DHA and EPA, but not plants. Therefore, all vegetable fats and oils such as those from nuts, grains and seeds contain no DHA or EPA. Ruminant fats such as milk and other dairy products contain only low levels of DHA and EPA. Seafood and fish are the prominent dietary sources of EPA and DHA, small amounts of which could also be provided by poultry and eggs.

1.5. The micronutrients content of human milk

In addition to macronutrients, a number of micronutrients, including trace minerals, are present in breast milk. These elements are crucial for the growth and development of infants. Micronutrient deficiency during early life poses threat to individual and public health (72). It may cause both short-term effects such as increased frequency of infections, and long-term effects such as higher rates of chronic disease. However, excessive intake of micronutrients may also adversely affect infants. For example, a high concentration of iron in infant formula may result in an increased risk of infection in infants due to the increased nutrient bioavailability to pathogenic microorganisms (73). Therefore, it is important to provide sufficient but not excessive micronutrients to infants. Studies showed that to protect newborn infants from both deficient and excessive minerals, the mammary gland is capable of regulating essential elements such as calcium (Ca), iron (Fe) and zinc (Zn) in milk (74), but some essential elements such as iodine (I) and selenium (Se) vary with maternal diet.

Although research effort has been devoted to breast milk concentrations of micronutrients for decades, rapid economic development and changes in dietary patterns demand sustained investigation. To my knowledge, studies on the levels of the essential elements in breast milk of Hong Kong lactating mothers and their dietary intake levels of these nutrients are limited. Determining appropriate intake levels of micronutrients for at-risk groups such as the infants and lactating mothers is of substantial importance for public health recommendation and clinical interventions.

In this study, the maternal intakes of five essential elements (Ca, Fe, I, Se and Zn) and their concentrations in the breast milk of local Hong Kong lactating mothers were examined.

The results of the first Hong Kong Total Diet Study in 2014 showed that a large proportion of interviewees could not meet the recommended daily intake of calcium, iron and iodine. Long term deficiency of minerals may pose high health risk to both lactating mothers and their infants. Seafood is one of the major sources of selenium and zinc, it was expected that the mothers have sufficient intakes of these minerals and be able to provide the recommended amounts of the minerals to their exclusively breastfed infants. We were interested to investigate if the breast milk from local mothers could provide adequate minerals to the infants due to their important health effects. For example, selenium serves as the co-factor for enzymes that are involved in protection against oxidative damage and plays a key role in regulating immune, reproductive, neurological, cardiovascular, and endocrine functions, while zinc is involved in maintaining the body's homeostasis and serves a wide range of bodily functions such as catalyzing metabolic reactions and providing structural support for important proteins.

In the following part, the health impact of those five essential elements and their food sources are discussed. Moreover, concentrations of the elements in breast milk and how maternal diets affect their contents in milk are examined.

1.5.1. The micronutrient in human milk - Calcium

A. Calcium and its role on human health

Calcium is the most abundant mineral in the human body. Almost all (99%) of total body calcium is located in the skeleton. Calcium accretion or bone mineralization is at a high rate during infancy. Positive calcium balance is vital throughout growth, especially during the first 2 years of life. Early bone mass accumulation is crucial for preventing adverse effects on childhood growth and osteoporosis in later life. Rickets is a disease of infants and children characterized by fragile bone and skeletal deformities. It is caused by vitamin D deficiency or inadequate intake of calcium and phosphorus. Bone mass in adulthood is largely dependent on the peak bone mass attained during growth and the rate of subsequent age-related bone loss. Therefore, dietary calcium intake, which is an important determinant of bone mineral density in the growth period, is particularly important. Other than infants, women during puberty adolescents, pregnant women, lactating women and postmenopausal women are the populations at risk for calcium deficiency. Inadequate dietary calcium intake is a severe problem in the world. Low calcium intake negatively influences peak bone mass and increases the risk of osteoporosis. Maintaining adequate dietary intake is important to achieve optimal peak bone mass for populations at high risk of calcium deficiency.

B. Food Sources and calcium intake of Hong Kong populations

Milk and milk products are the best sources of calcium. Soybean and some dark green vegetables such as Chinese flowering cabbage and Chinese spinach are high in calcium as well (Table 1). The WHO suggests that the RNI of calcium for adult female is 1000 mg/day. However, results of the first Hong Kong Total Diet Study in 2014 showed that the highest and mean dietary calcium intakes of female respondents aged between 20 and 59 were 840 and 440 mg/day, respectively. Over 97% of the interviewees in the study could not meet the recommendation. This might be due to the low consumption of dairy products of Hong Kong populations (75).

Table 1 Calcium content of common foods in Hong Kong

Food sources	Calcium content (mg/kg)
Cheese	7525
Yogurt	1950
Chinese spinach	1900
Chinese kale	1875
Beancurd	1763
Crab	1700
Whole milk	1275
Chinese flowering cabbage	1180
Breakfast cereals	1133
Shrimp	695
Mandarin fish	635
Egg	583
Orange	393
Broccoli	365
Kiwi fruit	253
Mutton	61
Beef	47
Rice, white	18

Sources: Centre for Food Safety, HKSAR 2014

Results of a survey on diet and nutrient intake of infants and young children aged 0-48 months in Hong Kong conducted by The Chinese University of Hong Kong and The Department of Health in 2012 revealed that the median dietary intake of calcium of all age groups was higher than the age-specific RNIs, except for the group of young children aged 48 months (76). Milk was the major source of dietary calcium in all groups and contributed to more than 80% of total calcium consumption at or before the age of 12 months, nearly 70% at 18 and 24 months, and about 50% at 48 months. The proportion of respondents with calcium intake below the WHO/FAO average requirement or EAR increased with age. Due to the lower intake of calcium-rich food including milk, the study demonstrated that there were more children in the older age groups (i.e. 18 months or above) who could not meet the level of EAR than in the younger age group (1).

C. Calcium concentration in breast milk

Human milk calcium increases significantly within the first few days postpartum, from less than 6 mM on the first day postpartum to over 8 mM on day five (77, 78). As lactation proceeds, the level of calcium in milk varies less dramatically. Milk calcium concentration declines from 6 to 15 months postpartum (79). The median concentration of calcium in human milk is 252 mg/L, ranging between 84 and 462 mg/L (80). The milk samples from mothers at lactation period within 6 months at extreme calcium concentration, i.e. below 100 mg/L or above 300 mg/L, are rare.

D. Effects of maternal diets on calcium concentration in breast milk

Geographical and cultural diversities among different countries have effects on food composition and consumption. However, studies revealed dietary calcium intake of mothers had no significant effects on milk calcium concentration. Studies have compared the milk of mothers from difference countries such as the US and Nepal (81), rural Mexico (82) and Egypt (83), as well as African countries with Sweden (84, 85). Dietary intake per se was not enough to explain the differences in milk calcium concentration among countries. The dietary intake of calcium of Nepalese mothers was 42% less than that of mothers from the US but no significant differences in milk calcium concentration were shown among the lactating mothers (81).

1.5.2. The micronutrient in human milk - Iron

A. Iron and its roles on human health

Iron is the most abundant trace mineral with approximately 3-4g in the human body. It plays an important role in a wide range of vital functions in the body such as oxygen transport and cellular energy generation. Iron deficiency is defined as an absence of iron stores and insufficient iron being supplied to various tissues in our body. Nutritional iron deficiency implies that the amount of dietary iron intake is inadequate to compensate for the body's physiological needs. It is the most common cause of iron deficiency in the world. Infants, children, adolescents, pregnant and lactating women are populations at high risk for iron deficiency. There are significant high iron requirements in the first 6 to 18 months of postnatal life of infants. Neural development is rapid during this period (86). Iron deficiency alters the morphological and biochemical development of infants. Since iron is vital for optimum neurogenesis and differentiation of a variety of brain cells and brain regions, deficiency in iron either in utero or in early postnatal life may result in brain abnormality (87). Mild deficiency is associated with impaired brain development due to the presence of high content of iron in several compartments in the brain. This results in functional defects such as retarded learning ability and behavioral development. Moreover, the physiological changes cannot be reversed by providing iron at later stages of life and contribute to irreparable damage to brain cells (88).

B. Food Sources and iron intake of Hong Kong populations

Dietary iron can be categorized into two groups: haem iron and non-haem iron (89). Major dietary sources of haem iron are haemoglobin and myoglobin found in meat, poultry and fish. The average absorption of haem iron in a meal with consumption of meat is around 25%. Non-haem is the predominant form of dietary iron and can be obtained from consumption of cereals, pulses, legumes, fruits and vegetables (Table 2) (75).

Chinese Nutrition Society (CNS) suggested that the RNI of iron for adult female is 20 mg/day. However, results of the first Hong Kong Total Diet Study in 2014 illustrated that the dietary intake of iron of female respondents aged between 20 and 49 was 7.9 mg/day. Over 90% of the interviewees in the study could not meet the recommendation (75).

The results of a survey on diet and nutrient intake of infants and young children aged 0-48 months in Hong Kong conducted by The Chinese University of Hong Kong and The Department of Health in 2012 revealed that the median dietary intake of iron of all age groups was higher than the age-specific RNIs, for which milk, grains and vegetables were the major sources of dietary iron (76). A large proportion of infants in the younger age group (i.e. 12 months or below) had dietary intake of iron less than 6mg/day. Although the concentration of iron in human milk is lower than that in infant formula, iron bioavailability in human milk was higher than in infant formula.

Table 2 Iron content of common foods in Hong Kong

Food sources	Iron content (μg/kg)
Pig liver	207500
Breakfast cereals	92750
Oyster	62000
Goose	29250
Beef	25750
Egg	24500
Ear fungus	21250
Chinese Spinach	20250
Crab	17500
Beancurd	13000
Plain bread	12250
Tuna fish	10100
Grey mullet	9350
Pork	9025
Chinese flowering cabbage	8650
Mushrooms	7675
Cheese	3075
Dragon fruit	2725
Rice, white	1253
Whole milk	240

Sources: Centre for Food Safety, HKSAR 2014

C. Iron concentration in breast milk

Iron concentration in breast milk declines throughout the course of lactation. It has been revealed that maternal iron status has insignificant effect on milk iron concentration. Although maintenance of iron concentration in breast milk is a tightly regulated process, a wide range of milk iron concentration from 0.04 to1.92 mg/L has been reported. Since neonate has hepatic reserve of iron, a low level of iron in breast milk can be balanced in breast-fed infants. No proven risk of nutritional iron deficiency has been found in infants during the first 6 months of lactation (90). Although milk iron concentration decreases along the first 4 months of lactation, high efficiency of utilization of iron from hepatic stores and iron bioavailability in milk can adequately support the requirements of breast-fed term infants for optimum growth and development (91).

D. Effects of maternal diets on iron concentration in breast milk

Iron concentration in breast milk has no correlation with maternal dietary iron intake. Some studies suggested that milk iron concentrations are comparable among people from different countries and culture. People with the same culture but different dietary habits have no significant differences in their level of iron in milk (92, 93). Also, there are no significant differences in the iron concentrations in breast milk among mothers with different iron statues. The milk iron concentration from an iron-deficient mother with a serum concentration of 0.34 mg Fe/L was comparable to that from mother of normal iron status with serum concentration of 2.35 mg Fe/L (94). Despite iron supplementation of anemic mothers with enhanced serum iron concentration, no significant differences have been shown in the content of iron in breast milk (95). Neither iron supplementation during pregnancy nor during lactation has any effect on milk iron concentration

(96). There was no increase in the iron concentration in the breast milk from women in Nigeria who had daily 100 mg iron supplementation during the last 6 month of pregnancy (95).

1.5.3. The micronutrient in human milk - Iodine

A. Iodine and its roles on human health

Iodine is necessary for the synthesis of thyroid hormones, major in thyroxine (T_4), by the thyroid gland in human body. Thyroid hormones play a vital role in many physiological actions including growth and development as well as control of metabolic process in the body. From the 15^{th} week of gestation to the first 3 years of life, they are involved in growth and the development of the brain and central nervous system. Furthermore, thyroid hormones play a critical role in other important metabolic processes in the body such as metabolism of carbohydrates, protein, fat, vitamins and minerals. (97). However, people at all stages of life, from the intrauterine stage to old age, are affected by iodine deficiency. Several populations are at high risk of deficiency including pregnant women, lactating women, women at child-bearing age and young children under 3 years old. In many developing countries, especially in areas where there is a lack of widespread use of iodized salt, infants are at significant high risk of iodine deficiency. Iodine deficiency in infants may result in thyroid hormone deficiency and thus contributes to irreversible derangement in the development of the brain and the central nervous system. Iodine deficiency leads to mental retardation and even cretinism in severe condition. On the other hand, both the iodine status of mothers during and after pregnancy and uptake of iodine by the mammary gland during lactation affect iodine nutrition of lactating mothers. Maternal iodine pool may reduce during pregnancy and lactation, resulting in adverse effects on mothers (98).

B. Food Sources and Iodine intake of Hong Kong populations

The content of iodine in food is influenced by the iodine content of the soil where plants grow. Therefore, there are wide variations in the iodine content in food among different geographic locations. Seaweed and reef fish are major sources of the nutrient (Table 3). In Hong Kong, seaweed is the main source of dietary iodine and contributes to 46.2% of the total iodine intake. Fish soup is another important source of iodine from diet, contributing to 7.5% of the total intake due to the high amounts of iodine in seawater fish.

The Chinese Nutrition Society suggested that the estimated average requirement, the recommended nutrient intake (RNI), and the UL of iodine to be 120, 150 and 1,000 μg/day, respectively for Chinese adults aged 18 years or above. Results of the first Hong Kong Total Diet Study in 2011 illustrated that the median of iodine intake in female population in Hong Kong was only 43 μg/day. Over 50% of the population had daily iodine intake lower than 50 μg, which is the threshold for normal thyroid functioning. Furthermore, approximately 91% of respondents of the study had iodine intake below EAR (75). However, in the survey, iodized salt was not included to estimate the iodine intake of Hong Kong populations. The pattern of iodized salt consumption of local population was unknown. To accurately determine the dietary intake of iodine, both salt intake and type are necessary for iodine intake assessment.

Table 3 Iodine content of common foods in Hong Kong

Food sources	Iodine content (μg)
Mussels 100g	140
Prawn 100g	44
Golden thread (fish) 100g	36
Horsehead (fish) 100g	35
Seaweed snack 1g	34
Yoghurt 100g	29
Skimmed milk 250ml	20
Canned sardines 100g	19
1 Chicken egg (63g)	18
Big Eyes (fish) 100g	18

Sources: Centre for Food Safety, HKSAR 2011

C. Iodine concentration in breast milk

Colostrum has the highest iodine concentration ranging from 200-400 μg/L (99). The concentration declines during the next several weeks and remains steady in mature milk (100-102). The iodine concentration in human milk varies markedly among populations in the world as dietary iodine intake varies. Studies have revealed that milk iodine concentration ranges from 20 to 330 μg/L in Europe whereas it ranges from 30 to 490 μg/L in the US (103, 104). The concentration is as low as 12 μg/L in some countries where iodine deficiency is prevalent.

D. Effects of maternal diets on iodine concentration in breast milk

Studies have revealed that iodine concentration in human milk is correlated with maternal dietary intake of iodine. Milk iodine concentrations are higher in countries with the consumption of iodized salt such as the US, Sweden and China, in which the median iodine concentration in milk are 146, 92 and 146 μg/L, respectively (105-107). Moreover, lactating mothers with iodine supplementation has increased milk iodine concentration (108), but the effect is not significant in well-nourished mothers (109). Addition of iodine into foodstuffs such as bread and dairy products also increases maternal iodine intake and thus the milk iodine concentration (110). However, mothers with iodine deficiency have not been revealed as having a significant lower milk iodine concentration (106).

1.5.4. The micronutrient in human milk - Selenium

A. Selenium and its roles on human health

Selenium plays an important role in modulation of growth and development, maintenance of defense against infection and protection of body tissues from oxidative stress. Glutathione peroxidase (GSHPx), selenium-containing enzymes, is involved in the protection of cells from the damaging impacts of hydrogen peroxide or oxygen-rich free radicals during stress, infection or tissue injury. Insufficient selenium would cause different diseases such as Keshan disease and Kaschin-Beck disease. Keshan disease is common in children aged 2-10 years and women in childbearing age. It would cause pathological changes such as a multifocal myocardial necrosis and fibrosis. Studies have revealed that distribution of Keshan disease is highly affected by geochemical variables. Prevalence of the disease is high in areas where extremely low selenium contents are found in staple crops. (111). Kaschin-Beck disease is another disease attributed to insufficient selenium intake. It commonly occurs in areas where the content of selenium in soil available for crop growth is low. The disease is prevalent in children and is characterized by joint necrosis. It results in epiphyseal degeneration of arm and leg joints and thus structural shortening of fingers and long bones. It causes retarded and stunted growth in children (112).

B. Food Sources and selenium intake of Hong Kong populations

The contents of selenium in foods are influenced by the selenium levels of soils and crops in the natural environment. Results of the first Hong Kong Total Diet Study in 2014 illustrated that the food group of “fish and seafood and their products” contributed to 33% of the total intake of the population. It is followed by the food groups of “meat, poultry and game and their products” and “cereals and their products”, which contributed 28% and 19% of dietary sources of selenium to the population, respectively (Table 4) (75). The WHO suggested that the RNI of selenium for adult female is 26 μg/day. The study has shown that the upper and mean levels of dietary selenium intake of female respondents aged between 20 and 49 were 240 and 130 μg/day, respectively. Less than 2% of female interviewees in this study failed to meet the recommendation. (75).

Table 4 Selenium content of common foods in Hong Kong

Food sources	Selenium content (μg/kg)
Pig liver	1300
Mandarin fish	1255
Oyster	933
Pomfret	683
Egg	535
Scallop	483
Pork	388
Chicken	325
Cheese	290
Salmon	265
Peanut	238
Beef	123
Pasta	99
Mushrooms	49
Whole milk	30
Chinese Spinach	24
Banana	20
Rice, white	14

Sources: Centre for Food Safety, HKSAR 2014

C. Selenium concentration in breast milk

Infants are born with selenium reserve but they also depend on selenium provided in breast milk. Milk selenium exists in the forms of seleno-proteins and seleno-amino acids in milk proteins, which are well-tolerated by breast-fed infants even at high concentrations. A wide variety of milk selenium content among people in different countries is associated with maternal intake of selenium in natural foods. As lactation progresses, total selenium content in colostrum is the highest which then decreases throughout lactation. Milk median concentration of selenium of lactating mothers with intake of selenium from natural food is 26, 18, 15 and 17 μg/l for colostrum (0-5d), transitional milk (6-21d), mature milk (1-3 months) and late lactation (over 5 months), respectively. The overall median concentration of selenium in breast milk is 10 μg/l.

D. Effects of maternal diets on selenium concentration in breast milk

Since milk proteins account for the total amount of selenium in breast milk, milk selenium level declines with protein content as lactation progresses. Maternal dietary intake of selenium affects the concentration of selenium in breast milk. The selenium content in soils where cereal crops grow affects the selenium contents of foods (113, 114). In some areas with selenium fertilization of soils, levels of selenium in foods and breast milk were higher. In Finland, selenium fertilization has improved the selenium status of the populations and contributed to higher concentration of selenium in breast milk (115). Maternal dietary habits would be affected by seasonal changes and so would the milk selenium concentration. Studies have shown a lower milk selenium during the rainy season in Gambia (116).

1.5.5. The micronutrient in human milk - Zinc

A. Zinc and its roles on human health

Zinc ranks the second most abundant trace element in the body and the most abundant in cells with an amount of 1.5-2.5g present in average adult. It is one of the crucial micronutrients for normal growth and development of human (117). It acts as a cofactor to over 300 enzymes that are involved in a significant array of catalytic, structural and regulatory biological processes (118). Prolonged dietary inadequacy of zinc cannot be compensated and may lead to accommodation and alterations in biological processes. Prevalence of moderate zinc deficiency is high in pregnant women, infants and children worldwide. It is mainly due to low dietary intake of zinc or reduced zinc bioavailability (119). Food and Agricultural Organization reported that in low-income regions, deficiency of zinc is a common phenomenon (120). Zinc deficiency is now known as a significant risk factor for morbidity and mortality and it leads to about 4% of child mortality (121). Zinc deficiency may be attributed to inadequate zinc intake or absorption, increased requirement of significant loss of zinc from body (122).

B. Food Sources and zinc intake of Hong Kong populations

Lean red meat, whole-grain cereals, pulses and legumes are rich sources of zinc (Table 5). The WHO suggested that the normative requirement of zinc for an adult female with moderate zinc availability (i.e. mixed diet with animal or fish protein) is around 3.3 mg/day for a 55.7 kg adult female. Results of the first Total Diet Study in 2014 revealed that the highest and mean dietary zinc intake of female respondents were 14 and 7.9 mg/day, respectively. Zinc intake of less than 3% of the interviewees was lower than the requirement (75).

Results of a survey among 1272 infants and young children aged 0-48 months in Hong Kong conducted by The Chinese University of Hong Kong and The Department of Health in 2012 revealed that their dietary intakes of zinc increased with age (76). The median intake of dietary zinc was higher than the age-specific RNIs while 6.4% of them had zinc intake lower EAR. Compared with the Population Zinc Inadequacy Indicator of > 25% defined by the International Zinc Nutrition Consultative Group, the ratio of zinc inadequacy was low in Hong Kong. Inadequate dietary zinc intake was more common in the group of infants aged 9 months, in which about 21% had dietary zinc intake lower than EAR. This might be due to their low consumption of meat or fish (123).

Table 5 Zinc content of common food in Hong Kong

Food sources	Zinc content (μg/kg)
Oyster	315000
Beef	64250
Crab	60500
Cheese	37000
Peanut	33250
Pork	28250
Breakfast cereals	28000
Egg	21250
Fermented bean products	18250
Chicken	14000
Grouper	10175
Grey mullet	8625
Chinese Spinach	7500
Mushrooms	6725
Rice, white	5575
Chinese flowering cabbage	5500
Whole milk	4450
Banana	2200
Kiwi fruit	1050

Sources: Centre for Food Safety, HKSAR 2014

C. Zinc concentration in breast milk

Many studies have suggested that the content of zinc is the highest in colostrum, with a concentration of 2-3mg/L (31-46 μmol/L), but declines rapidly to 0.9mg/L (14μmol/L) after 3 months and becomes steady beyond 12 months postpartum (124-126). Maternal zinc store is also depleted during lactation. Large amounts of zinc are transferred to mammary gland after delivery. Compared with the longitudinal changes in concentrations of other nutrients in breast milk, zinc level is one of the nutrients that exhibits the most significant change during the first 4 months of lactation. The mean amount of milk zinc transferred to exclusively breastfed infants decreases rapidly from about 4mg/day during the first few days of life to about 1.75mg/day by 1 month, and deceases gradually to about 0.7mg/day by 6 months (127). The WHO suggested that the requirement for absorbed zinc is 1.3mg/day and 0.7mg/day during the first 3 months and 3 to 5 months of life, respectively (128). Based on the assumption of the presence of zinc reserve at birth that could meet the demand of normal biological processes for term infants and no consistent benefits have been revealed in exclusive breastfed term infants with the consumption of zinc supplement, the Steering Committee of the International Zinc Nutrition Consultative Group (IZiNCG) suggested that breast milk could provide sufficient amount of zinc to exclusively breastfed infants for approximately 6 months (129).

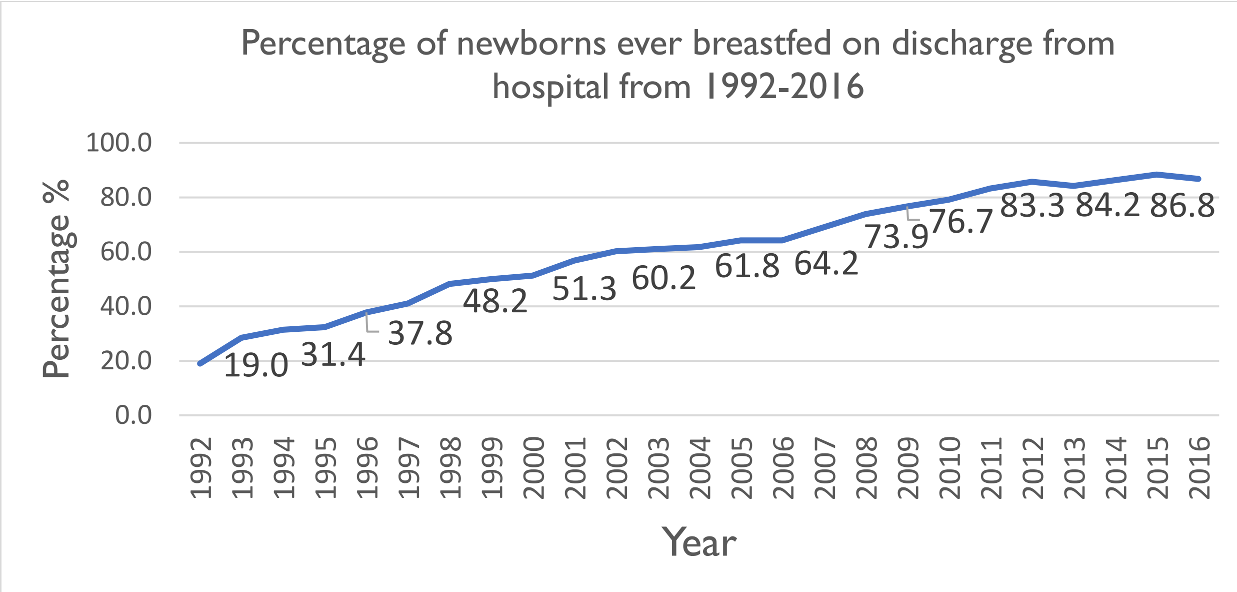
D. Effects of maternal diets on zinc concentration in breast milk

Maternal zinc status and supplementation were also shown to have no significant effects on milk zinc concentration. Zinc concentration in human milk is maintained over a wide range in dietary intake. Supplementation of zinc increases plasma zinc level but not milk zinc level. In a study, supplementation of pharmacologic doses of zinc (50-150mg/day) was given to lactation mothers of term infants at early weeks postpartum. The zinc concentration in milk declined continuously(130). Some trials were conducted to examine the effects of long-term supplementation of zinc during lactation on the level of zinc in breast milk. In a study, lactating mothers who had daily zinc intake of about 1 mg were supplemented with 15mg/d of zinc for a period of nine months. Compared with both the control and non-supplemented groups, results showed that zinc concentration in milk of lactating mothers in zinc-supplementation group declined slowly after a few months postpartum (124).

1.6. Breastfeeding rate in HK

Although the advantages of breastfeeding have been largely revealed and mentioned in chapter 1.1 and 1.2, the global average duration of exclusive breastfeeding is less than 6 months and the percentage of mothers who exclusively breastfed their infants for 6 months is as low as 36% (131). The proportion of women in Hong Kong who choose to breastfeed their infants has increased substantially over the past two decades, from 19% in 1981, 37% in 1997 (132) and 64% in 2007, to over 85% of mothers now who initiate breastfeeding upon discharge from hospital (Figure 2) (133).

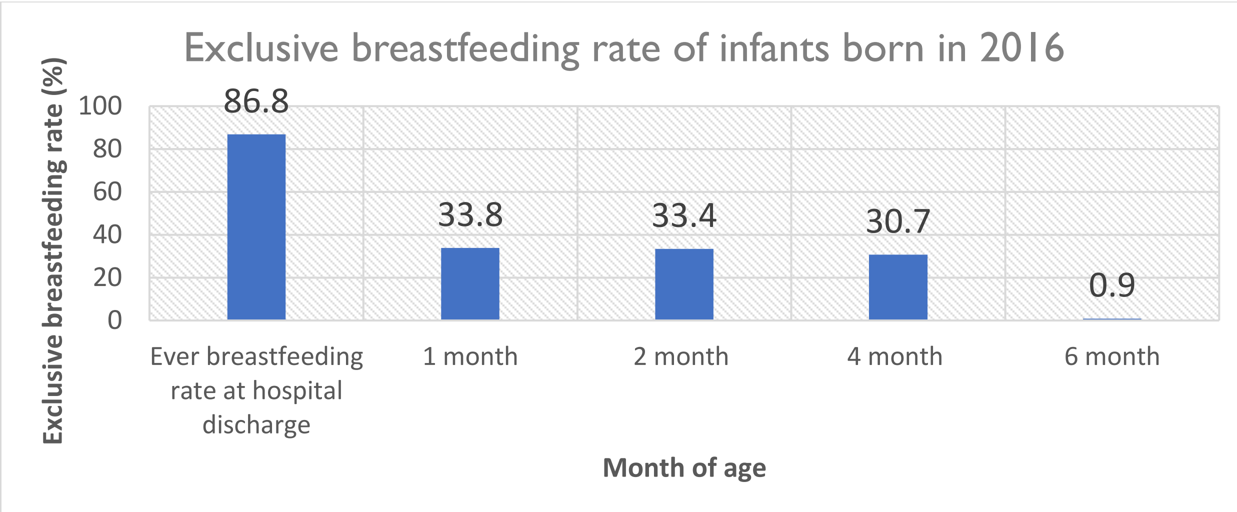
Figure 2. Breastfeeding rate of infants upon discharge from hospital in Hong Kong, 1992-2016 (133)



The increment of breastfeeding rate in the city might be attributed to different policies adopted by the government to support breastfeeding mothers in the community. For example, Advisory Guidelines on Babycare Facilities and Practice Note on the Provision of Babycare Rooms in Commercial Buildings were issued in 2008 and 2009, respectively. Increasing number of Babycare facilities are provided in community premises such as libraries and shopping malls for the convenience of lactating mothers. Public events such as the World Breastfeeding Week are organized regularly to enhance public awareness on the benefits of breastfeeding. The Department of Health has also collaborated with community partners and other stakeholders to promote the advantages of breastfeeding via various media channels including television advertisements and newspaper feature articles (134). On the other hand, in 2013, the Secretary for Food and Health suggested the senior management of Government Bureaux/ Department to adopt a Breastfeeding Friendly Workplace Policy for employees. The Policy recommended several means to support lactating staff to continue lactation. For example, refrigerating facilities should be provided to keep expressed breast milk, a private area with comfortable chairs and electric sockets to operate breast pumps should be provided too. In the meantime, private enterprises are encouraged to adopt the Policy to support lactating mothers (134). Support is also provided to women during pregnancy and in the postpartum period. The Department of Health encourages mothers to breastfeed their infants in its Breastfeeding Policy by launching workshops and developing educational materials for parents; providing guidelines to breastfeeding women in the Maternal and Child Health Centres (MCHCs); providing trainings to maternal and child healthcare professionals and launching public activities to enhance the public awareness of the benefits of breastfeeding (134).

Despite the high breastfeeding rate among local mothers, the rates of exclusive breastfeeding at 4 and 6 month of age dropped to about 30% and 1% respectively in 2016 (Figure 3) (135). The rate of exclusive breastfeeding at 6 month of age is comparable to that in the UK (1%) and Singapore (1%) but much lower than that in the US (16.4%) and Australia (15%). To the majority of infants in Hong Kong and other countries, both the duration of exclusive breastfeeding and the total duration of breastfeeding do not meet the WHO recommendation (136).

Figure 3. Exclusive breastfeeding rate of infants born in Hong Kong, 2016 (135)



1.7. Factors affect breastfeeding frequency in Hong Kong

There are demographic, socio-economic and obstetric factors that contribute to the suboptimal duration of exclusive breastfeeding in Hong Kong (137, 138). The factors are discussed below:

1.7.1. Employment status

High workforce participation rates among childbearing women and unfriendly workplace environment are reasons attributed to cessation of breastfeeding. Returning to work may hinder a mother's intention to continue breastfeeding, especially in the first six weeks after giving birth. Studies showed that the duration of breastfeeding of mothers who are housewives or part-time employees is longer than those who work full-time (139). Hong Kong mothers are entitled to have a maternity leave for a maximum of 10 weeks. Early return to work is a crucial reason to wean breastfeeding as long working hours result in maternal tiredness and inadequacy of support in working environment (139).

1.7.2. Family income

Women who have lower monthly family income (i.e. less than HK$ 10,000) tend to have longer duration of breastfeeding. Compared with formula feeding, breastfeeding is more cost-effective. In contrast, due to their economic advantage, mothers who have higher monthly family income could afford the expense of infant formula and shorter duration of breastfeeding results.

1.7.3. Tiredness and fatigue

After giving birth, mothers commonly feel tired and fatigue and adverse parenting behaviors (140) and postnatal depression symptoms may result (141). This might lower the willingness of breastfeeding of mothers. Providing information to pregnant women about problems they might face in the postpartum period may help them to reduce adverse emotional changes.

1.8. Dietary patterns in Hong Kong Populations

Due to urbanization and rapid economic growth, unsatisfactory diet and unhealthy lifestyle have become more common in Hong Kong population. People have a sedentary lifestyle and westernized dietary habits characterized by high consumption of refined carbohydrates, meat and sugary foods. Many working people usually eat out for lunch or even dinner due to their busy working schedule and irregular working hours. People normally spend more than one-third of the daily life at work. The Behavioural Risk Factors Survey (BRFS) conducted by the Department of Health in 2015 revealed that among the 2000 interviewees, nearly half of them ate out for lunch five or more times a week (142). Foods from fast food outlets contain very few vegetables, too much oil and sodium (143). Traditional Chinese eating pattern also means that low consumption rates of dairy products are common (144). Increasing trend of overweight and obesity has greatly been attributed to undesired eating habits and low consumption of fruit and vegetables. To prevent chronic disease, the WHO recommends that adult should intake 400g of fruit and vegetables daily. The BRFS revealed that among about 3300 interviewees, over four-fifths (81.0%) aged 18-64 failed to meet the WHO's recommendation of daily consumption of five servings of fruit and vegetables, and the inadequacy was more prevalent among males (145).

On the other hand, Hong Kong is a coastal city and people have a high consumption of seafood and fish. Reports revealed that in 2011, over 5 million tonnes of seafood were consumed in Hong Kong. In average, 71.2kg of seafood was consumed per capita, which was around 3.8-fold more than the global average (i.e. 18.9 kg per capita) and double the per capita intake in mainland China. Hong Kong ranked the second largest per capita seafood consumption in Asia and the seventh in the world (146). The seafood consumption is relatively higher than the recommendation of weekly intake of 330g in the United States (US Food and Drug Administration of United States/ US

Environment Protection Agency 2004) and of 300-450g in Australia and New Zealand (Food Standards Australia and New Zealand 2011) (147). More specifically, surveys showed that the average daily fish consumption was 57.48 g in Hong Kong (148). People consume many different kinds of both sea water and fresh water fish such as grass carp, mud carp, mandarin fish, grey mullet and yellow croaker.

1.9. Objectives and Hypothesis

Changing eating patterns to western diets may pose detrimental impact on the long-term health in the city's populations. However, there is a fundamental gap in the research findings on how the dietary patterns of Hong Kong lactating women affect the fatty acid profile and mineral content of their breast milk in the past two decades. There is an urgent need for further research on the nutritional characteristics of human milk from Hong Kong lactating women. Such information will provide scientific evidence for devising appropriate public health measures for improving the well-being of both exclusively breast-fed infants and lactating mothers in Hong Kong.

In the present study, we aim:

1. to characterize the latest eating patterns of local mothers
2. to investigate the nutritional composition of the human milk of local mothers, including trace elements and fatty acid profile
3. to examine how the nutritional composition of human milk correlates with local dietary characteristics, particularly the high consumption of seafood

Information on the relationship between the dietary patterns of Hong Kong lactating mothers, especially high consumption of seafood and fish, and the milk fatty acid proportions and mineral content is limited. In the present study, we hypothesized that the nutritional composition of breast milk, including the fatty acid profile and mineral content, is affected by dietary habits of Hong Kong lactating women.

Chapter 2 Methods

2.1. Study design

2.1.1. Sample size calculation

The optimal sample size was estimated based on the variability of the level of polyunsaturated fatty acids in the breast milk of Chinese women (mean±SD: 25.5±5.27 mol%, n=82) (149). These values were adopted as the subjects of the study (149) are ethnically, geographically and culturally most similar to subjects of this research. Cochran's sample size formula was applied to determine the optimal sample size: $n \geq (ZS/E)^2$

where n = sample size, S = standard deviation, Z = confidence level (95% confidence = 1.96) and E = range of possible random error

The possible random error of 4~5% for the mean value and power of 80% were assumed in the present study, so E was (25.5 x 4/5%) = 1.02/1.275. Using these values, the optimal sample size $N \geq (1.96\times5.27/1.02\sim1.275)^2 = 65\sim102$.

Therefore, a sample size of 102 was deemed to be sufficient to limit random error of the mean value to 4-5%. To allow for a margin of 20% refusal rate, we planned to recruit 120 subjects in this study.

2.1.2. Subject recruitment

Subjects were recruited via convenience sampling through posters (Appendix I) and e-mail announcements. The promotion materials were mailed to all PolyU colleagues and breastfeeding-supporting groups including Leche League and Hong Kong Breastfeeding Mothers' Association and uploaded to online social networking platforms such as Facebook. Subjects' eligibility was checked by research staff with a screening questionnaire (Appendix II) via phone call. Both inclusion and exclusion criteria for subjects to participate in this study are listed below. To those who are eligible for participation, study details were explained and appointments for 1.5-hour interview in PolyU were made on the basis of first-come-first-serve accordingly. A maximum of 4 appointments were scheduled a day. In order to remind subjects to sign an informed written consent form and fill in a 3-day dietary record, research staff contacted them 4 days ahead of the date of appointment.

Application for ethics approval was submitted to The Hong Kong Polytechnic University Human Subjects Ethics Sub-committee prior to the commencement of the study (ethics approval reference number: HSEARS20150306002).

A. Inclusion Criteria for subjects

- Healthy Chinese lactating mothers (18-40 years old)
- 60 days postpartum who breastfeed exclusively or partially
- Hong Kong residents having resided in Hong Kong for a continuous period of not less than 18 months
- Having delivered at full term (>37 gestation weeks)
- Having given birth to singleton infant within normal birth weight of > 2500g
- Baby has no known abnormality.

B. Exclusion criteria for subjects

- Concurrent participation in any clinical trial or study
- Use contraceptive medication after giving birth

- Suffer from renal, liver or thyroid dysfunction, cognitive impairment, or any other indication of a major medical or psychological illness

2.2. Data collection and analysis

2.2.1. Anthropometric data collection

Subjects' weight was measured with the lightest clothing and the use of a digital electronic balance (REX TE856, REX PRODUCTS LTD), ranging from 0.1-150 kg, to the nearest 0.1 kg. Their height was measured with a measuring tape, ranging from 0-200 cm, to the nearest 0.1 cm. Subjects were asked to take off shoes, stand straight with their back and heel touching the tape, and look at the horizontal level. Their Body Mass Index (BMI) (kg/m^2) was then calculated as body weight (kg) divided by body height (m) squared.

2.2.2. Demographic data collection

Demographic and other lifestyle habits, such as subject's age, occupation, family income and smoking status and alcohol use were collected using a standardized questionnaire (Appendix III). Information on other maternal characteristics, such as the number of pregnancies, number of births, and previous history of lactation, breastfeeding practice and pattern of current lactation was also collected.

2.2.3. Dietary record collection

Since the present cross-sectional study aims to examine the effect of maternal dietary intake on breast milk nutrition, assessments of diet habits and nutrient intakes of lactating women are necessary. Quantitively accurate dietary assessments are necessary to determine whether respondent's intakes of nutrients and food groups meet recommendations. The targeted population was young lactating mothers who had attained certain education levels. Assuming they had the ability of record keeping, 3-day dietary record was used to collect their detailed dietary information and also captured day to day variation of diet. On the other hand, information of dietary intake of each respondent was needed to determine the correlation between maternal dietary intake and contents of some nutrients especially DHA in breast milk. Dietary record reflects few days of respondent's intake and could not reflect the dietary habits. Therefore, food frequency questionnaire was used to assess both frequency and amount of intakes of specific groups of food such as DHA-rich sources in past 3 months.

A. 3-day dietary record

Scheduled face-to-face interviews with subjects were conducted at the Department of Applied Biology and Chemical Technology, PolyU. The subjects' dietary records of three consecutive days prior to the interview were collected using 3-day dietary record sheets. Record sheets were mailed to subjects upon appointment of interview had been made. Subjects were asked to keep their usual diets. Detailed guidelines were provided to subjects to assist them to complete the record (Appendix IV). The time and place of consumption, types and quantities of both food and beverage, condiments and oils used as well as cooking method were recorded. Also, their dietary practices such as removal of fish and chicken skin and consumption of any other ingredients in soup were asked to maximize the accuracy of consumption estimation. To facilitate the diet interview, food photo booklets consisting of local commonly consumed foods, guidelines of food portion size and containers' volume as well as feeding utensil models including bowls, plates and spoons of different standardized portion size were shown to subjects for clarification of any unclear or missing food items. Any consumption of snacks and drink between main meals would be further confirmed to ensure no information was missing. After the diet interviews, dietary records were checked again if there were any queries about all food items such as unrealistic intake quantities and abnormal intake pattern. Subjects were interviewed again over the phone to clarify any incomplete items if necessary.

B. Food Frequency Questionnaire

A validated Food Frequency Questionnaire (FFQ), which has been modified by addition of PUFA-rich food items in accordance with the study purpose, was used to assess subjects' dietary patterns and determine their average dietary intakes of specific nutrients in the past 3 months (Appendix V). The questionnaire comprising of 11 food categories with a total of 153 food items that included vegetables and beans, mushrooms and algae, fishes and seafood, fish soups and soup remains, eggs, dairy products and beverages, snacks, soups as well as condiments and oils. The category of fish and seafood includes items on the list of "Advice for pregnant women, women planning pregnancy and young children on fish consumption", issued by the Center for Food Safety (CFS), HKSAR in 2013 (150). As it is not possible to include all food items in the questionnaire, rows of 'others' were provided to the respondents to record other consumed foods in each part. The part of consumption frequency consists of 9 options ranging from never to everyday in the past three months. To minimize assessment error, our subjects were reminded to average the consumption amounts of seasonal foods in past 3 months. Furthermore, to avoid double counting of dietary intakes, subjects were reminded the way of recoding of some food items such as seaweeds and sushi. Of all items in the questionnaire, frequency, portion size and the method of preparation (e.g. roasting, grilling, frying) were recorded. During interview, food photo booklets, guidelines of food portion size and containers' volume as well as feeding utensil models were provided to subjects to facilitate the record process. Consumption details of supplements and traditional Chinese medicine, including brand, frequency and dosage of use were collected. The mean daily intake of different kinds of foods were calculated by dividing the total amount by 90 days whereas that of different groups of food were calculated by sum of mean daily intake of foods in that group.

C. Food item coding of dietary records

To assess the dietary intakes of subjects by nutritional analysis software, all items of the records were coded by our collaborators at The Chinese University of Hong Kong (CUHK). They were well-trained coders so that error due to unfamiliarity with the task could be minimized. Food codes were assigned to all food and beverage items of the collected dietary records. Also, the quantity of salt and cooking oil intake was estimated by standardized estimation. Records were then further checked by an experienced coder to ensure there were no missing and incorrect items. The coded dietary records were input to the nutritional analysis software The Food Processor Nutrition Analysis and Fitness software version 10.13.1 (ESHA Research, Salem, USA), with details shown in Appendix VI by our well-trained research staff. Their input was further checked by some senior research staff members to ensure there were no missing and incorrect items. As the database of the software was US-based, many traditional Chinese and local foods could not be found in the system. Our collaborators at CUHK provided us with a self-developed list of local food with detailed nutrition contents, which was developed based on information from China and the Centre for Food Safety, Hong Kong. The list of food items has been input into our database. Thus, nutritional content of maternal dietary intakes could be assessed with greater accuracy. Results of nutrients intake were generated from the nutritional analysis software and cross-checked by our collaborators.

2.2.4. Breast milk collection

On the day of interview, after checking of dietary record, subjects were asked to supply a specimen of milk from one breast of fully expressed in a private interview room. They were allowed to use a provided electric breast pump (mini electric breast pump, MEDELA Inc, USA) or hands to express milk into sterile polystyrene containers without preservatives. The samples were stored in ice buckets and then transported to the laboratory within 1 hour of collection. All milk samples were divided into aliquots on receipt. One aliquot was stored at -20°C and batched for fatty acid analysis. The remaining aliquots were stored at -80°C until further analysis.

2.3. Laboratory methods

2.3.1. Milk fatty acid profile

The methods used in this study to analyze fatty acids profile of collected milk samples were modified from AOAC 966.06. Details are as follows:

A. Digestion and extraction

1g of milk sample was weighed into a labeled Mojonnier flask. 100mg pyrogallic acid, 2mL (5mg/mL C11 triglyceride) internal standard solution and a few boiling granules were then added to the flask. 2mL ethanol was added and the solution was mixed well. 4mL H_2O and 2mL NH_4OH were added to the mixture. The flask was placed into a basket in a shaking water bath at 70°–80°C at a moderate agitation speed for 10 minutes. The solution was mixed every 5 minutes. The flask was then cooled down to room temperature. Ethanol was added to fill the bottom reservoir of the flask and mixed gently. Then, 20mL diethyl ether and 20mL petroleum ether were added to the flask and then the mixture was shaken gently. The ether (top) layer was decanted into a 150mL boiling tube. Addition of diethyl ether and petroleum ether was repeated twice. Ether was evaporated with the aid of a nitrogen blow concentrator (Eyela MGS2200).

B. Methylation

Extracted fat residue was dissolved in 2–3mL chloroform and 2–3mL diethyl ether. The mixture was transferred to a test tube and then evaporated to dryness using a nitrogen blow concentrator. 2.0mL 7% BF_3 reagent, and 1.0mL toluene were added to the test tube. The screw-capped vial was sealed and then heated in an oven for 45 min at 100°C. The vial was gently shaken every 10 minutes. The vial was then cooled down to room temperature. 5.0mL H_2O, 1.0mL hexane, and 1.0g Na_2SO_4 were added and then shaken for 1 min. After layers separation, the top layer was transferred to another vial containing 1.0 g Na2SO4.

C. Gas Chromatographic Determination

Fatty acids in breast milk would be converted into their corresponding fatty acid methyl ester (FAMEs). With added internal standard (FAME C11:0), samples were injected into a gas chromatography (GC system, 7890A, Agilent Technologies), separated by capillary column (Supleco, SP 2560) and analyzed by a flame ionized detector (FID). Commercially available FAME standard (Nu-Check- Prep, Cat. No.GLC- Nestle-36) was injected to establish a calibration curve for peak identification by comparing the retention time of sample peaks. Specific fatty acid was then quantified by calculating from the calibration curve with reference to an internal standard. Relative retention times (vs FAME of triglyceride internal standard solution) and response factors of individual FAMEs would be obtained by GC analysis of individual FAME standard solutions and mixed FAME standard solution. FAME in sample was determined via against the standards to identify each fatty acid.

D. Calculations

Response factor (Ri) for each fatty acid was calculated as follows:

$$R_i = \frac{Ps_i}{Ps_{C11:0}} \times \frac{W_{C11:0}}{W_i}$$

where Psi = peak area of individual fatty acid in mixed FAMEs standard solution; PsC 11:0 = peak area of C11 : 0 fatty acid in mixed FAMEs standard solution; W C11:0 = weight of internal standard in mixed FAMEs standard solution; and Wi = weight of individual FAME in mixed FAMEs standard solution

The amount of individual (triglycerides) (W TG) was calculated in samples

$$W_{FAMEi} = \frac{Pt_i \times Wt_{C11:0} \times 1.0067}{Pt_{C11:0} \times R_i}$$

$$W_{TGi} = W_{FAMEi} \times f_{TGi}$$

where Pti = peak area of fatty acids in test portion; WtC11:0 = weight of C11:0 internal standard added to test portion, g; 1.0067 = conversion of internal standard from triglyceride to FAME; PtC11:0 = peak area of C11:0 internal standard in test portion; and fTGi = conversion factor for FAMEs to triglycerides for individual fatty acids

wt% in sample was calculated as follows

$$= \frac{WTGi}{\sum WTGi} \text{ x } 100\%$$

2.3.2. Milk micronutrients

Methods used to analyze different micronutrients of collected milk samples are shown as follows:

A. Milk Calcium, Iron, Selenium and Zinc

1g of breast milk samples was weighed into microwave digestion vessels. 4 ml of nitric acid and 1 ml of hydrogen peroxide were added into each vessel. The vessels were closed tightly and digested by microwave digestion system (Milestone ETHOS One) with the parameters stated as below. After digestion, the vessels were cooled down to room temperature and the solution was transferred to 25 ml volumetric flasks and marked up with Millipore Milli-Q water. Samples were then measured against Iron and Zinc standards by inductively coupled plasma - mass spectrometry (ICP-MS) (Agilent 7500ce); measured against Calcium standards by inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent).

Parameters of microwave oven digestion:

Parameters	Phase 1	Phase 2
Power (W)	1000	1000
Ramp time (min)	5	10
Final temperature (℃)	120	200
Hold time (min)	20	30

B. Milk Iodine

1 g of breast milk samples was weighed into 50ml Polypropylene tubes. 5ml of 8% tetramethylammonium hydroxide (TMAH) and 0.75ml of Millipore Milli-Q water were added into each tube. The solution was mixed well in tightly capped tubes. The tubes were then placed in fume hood and left overnight. On the following day, the samples were heated and shaken at 90℃ for 1 hour in a water bath. They were then cooled down to room temperature. The samples were marked up to 40ml with Millipore Milli-Q water and filtered by cellulose syringe filters. Samples were measured against Iodine standards by inductively coupled plasma - mass spectrometry (ICP-MS) (Agilent 7500ce).

2.4. Statistical methods

All analyses were performed using the SPSS version 23 software. Normality of data was tested by Shapiro-Wilk test. Normal distributed data were shown as mean (standard deviation) whereas non-normally distributed data were shown as median (interquartile range).

According to the lactation stages, the subjects were divided into three groups. Mothers with infants aged 0-6 months who were exclusively breastfed were assigned to group 1. Mothers with infants aged between 7-12 months who were partially breastfed were assigned to group 2, and mothers with infants aged over 12 months who were partially breastfed were assigned to group 3.

One-way analysis of variance (ANOVA) for normally distributed data or Kruskal-Wallis H test for non-normally distributed data were used to compare the means between three groups of subjects.

To examine the effects of maternal diets on milk fatty acid profile and mineral content, Spearman's rank correlation tests were performed to analyze the effects of maternal diets on milk fatty acid profile amongst subjects without fatty acid supplementation as well as on milk micronutrients level amongst subjects without mineral supplementation.

After examining the correlations, multiple linear regression was used to determine the relationship after adjusting for different potential confounding factors such as subject age, household income and education level. $p<0.05$ (two-tailed) was considered statistically significant for all analyses.

Chapter 3 Results

3.1. Subjects' characteristics

The anthropometric and socioeconomic characteristics of the subjects, namely their age, body weight and height at lactating period, BMI at lactating period, education level, household income and occupation, are shown in Table 6. The subjects were young women with a mean age of 32, and a mean BMI of 22kg/m^2. Nearly one-third of the subjects were overweight (BMI ≥ 23kg/m^2) or obese (BMI ≥ 25kg/m^2). The majority were working full-time. Two-thirds of them had tertiary or above education level and high monthly household income (> HK$ 30,000). Positive correlation was shown between the maternal education level and household income. Among the 73 subjects, the infants of 29 of them were aged between 0 and 6 months and were exclusively breastfed.

Table 6 Anthropometric and socioeconomic characteristics of 73 lactating women

Variable	Mean ± SD / n (%)
Age, years	32.1 ± 3.7
Body weight, kg	56.9 ± 8.5
Body height, m	1.60 ± 0.05
BMI[a], kg m^{-2}	22.2 ± 3.1
Underweight, n (%)	3 (4.1)
Normal, n (%)	46 (63.0)
Overweight, n (%)	13 (17.8)
Obese, n (%)	11 (15.1)
Maternal education level[b]	
Low, n (%)	17 (23.3)
High, n (%)	56 (76.7)
Monthly household income[c]	
Low, n (%)	23 (31.5)
High, n (%)	50 (68.5)
Occupation	
Housewife, n (%)	16 (21.9)
Working full-time, n (%)	57 (78.1)
Duration of lactation, month(s)	
≤0 - 6, n (%)	29 (39.7)
>6 - 12, n (%)	20 (27.4)
≥12 - 24, n (%)	24 (32.9)

Data are given as mean ± SD
[a] Underweight: BMI< 18.5; Normal: BMI 18.5-<23.0; Overweight: BMI 23-<25.0; Obese: BMI ≥25
[b] High: Tertiary education or above
[c] High: Monthly household income> HK$ 30,000 (151)

3.2. Dietary intakes of lactating mothers

3.2.1. Energy and macronutrients

The mean dietary intakes of macronutrients of the 73 lactating women, as calculated from the 3-day dietary records, are shown in Table 7. The energy intake of the lactating mothers was 2400 kcal/day while the percentage energy intake of mothers from carbohydrates, protein and fat was 44, 18% and 37% respectively. The mean dietary intakes of the macronutrients of the subjects were out of the acceptable macronutrient distribution range (AMDR) according to the recommended value of the Chinese Dietary Reference Intakes (DRIs) 2013 (152). The diets of these lactating women were characterized by high consumption of protein and fat, and low consumption of carbohydrates. Their maternal mean protein intake was higher than the Chinese recommended nutrient intake (RNI) for lactating women by 40%. The mean maternal energy intake from fat was also higher than the upper limit of the Chinese AMDR by 23%. Eighty percent of their energy intake from carbohydrates could not meet the Chinese AMDR of 50-65% of total energy intake. Their mean dietary intake of carbohydrate was 44% of total energy intake.

Table 7 Dietary intakes of macronutrients of 73 lactating women

Energy and nutrients	Dietary intakes (g/d)	Recommendation from Chinese DRIs 2013
Energy (kcal)	2370.2 ± 407.1	2300
Carbohydrates (g)	258.6 ± 49.5	
%kcal	44.0 ± 6.6	50-65 (AMDR)
% subjects <AMDR	80.0	
% subjects within range	20.0	
% subjects >AMDR	0.0	
Protein (g)	112.1 ± 27.2	80g (RNI)
%kcal	18.9 ± 3.1	
% subjects <AMDR	0.0	
% subjects within range	10.0	
% subjects >AMDR	90.0	
Fat (g)	98.1 ± 24.4	
%kcal	36.9 ± 5.0	20-30 (AMDR)
% subjects <AMDR	0.0	
% subjects within range	10.0	
% subjects >AMDR	90.0	

Data are given as mean ± SD

3.2.2. Food group consumption

The intakes of different food groups, namely grains, vegetables, nuts, fruits, meats, red meats, poultry, seafood, eggs and dairy products, of the lactating mothers are shown in Table 8. The consumption of the five major food groups by the subjects, i.e. grains, vegetable, fruits, meats and dairy products, were compared with the suggested daily intake recommended by the Department of Health, Hong Kong SAR. The median of daily intake of grains (6.8 servings) and the mean of daily intake of meats (9.0 servings) exceeded the recommended ranges. However, the intake levels of vegetables, fruits and dairy products were all below the suggested intake levels. The daily intakes of vegetables and fruits by the subjects were about 1.5 and 0.9 servings, respectively, which only attained about one-third of the recommended values. The intake of dairy products like milk and cheeses by lactating mothers reached only one-tenth of the suggested consumption per day i.e. 2 servings. Among all the food groups, nuts and seafood are rich sources of fatty acids.

Table 8 Food group consumption of 73 lactating women

Food groups	Intake (g/d)	Servings/day [a]	Suggested servings per day from Hong Kong (153)
Grains	510.3 ± 238.8	6.8 ± 3.2	4-5
Vegetables	119.3 ± 93.0	1.5 ± 1.2	4-5
Nuts	0.0 ± 6.7		
Fruits	68.3 ± 120.6	0.9 ± 1.5	3
Meats, mean ± SD	268.9 ± 109.7	9.0 ± 3.7	6-7
Red meats	91.2 ± 99.6		
Poultry	51.7 ± 74.8		
Seafood	87.5 ± 92.0		
Eggs	25.8 ± 34.4		
Dairy products	41.7 ± 161.6	0.2 ± 0.7	2

Data are given as median ± IQR unless otherwise stated

[a] One serving: Grains: 75g; Vegetables: 80g; Fruits: 80g; Dairy products: 240ml; Meats: 30g (cooked)

3.3. Fatty acids

3.3.1. Maternal intakes of fatty acids

The maternal dietary intakes of fatty acids are shown in Table 9. The average intakes of saturated fatty acids (SFA), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) accounted for 9.1%, 30.9% and 17.7% of the total energy intake, respectively. The Chinese DRIs recommended that the AMDR of SFA intake of lactating mothers should be within 10% of their total energy intake. Therefore, the consumption of SFA of our subjects was within the suggested range.

The consumption of essential fatty acids, LA and ALA, by lactating mothers were within the AMDR recommended by the Chinese DRIs. Among the various ω-3 fatty acids, the lactating mothers' median intake of the summation of EPA and DHA was 0.28g/day, which fulfilled the recommended value of 0.25g/day. Moreover, their median daily intake of DHA only attained the suggested amount of 0.2g.

Table 9 Dietary intakes of fatty acids of 73 lactating women

Fatty acids	Dietary intakes (g/d)	Recommendation from Chinese DRIs 2013
SFAs (g)	24.3 ± 7.5	
%kcal	9.1 ± 2.0	<10 (AMDR)
MUFAs (g)	30.9 ± 10.5	
%kcal	11.6 ± 2.9	
PUFAs (g), median ± IQR	17.7 ± 9.0	
%kcal	7.1 ± 1.6	
LA (g)	10.8 ± 4.1	
%kcal	4.1 ± 1.3	4.0 (AI), 2.5-9 (AMDR)
ALA (g)	1.4 ± 1.4	
%kcal	0.54 ± 0.59	0.6 (AI), 0.5-2 (AMDR)
AA (g), median ± IQR	0.26 ± 0.19	
EPA (g), median ± IQR	0.08 ± 0.12	
DHA (g), median ± IQR	0.20 ± 0.29	0.2 (AI)
EPA+DHA (g), median ± IQR	0.28 ± 0.42	0.25 (AI)

Data are given as mean ± SD unless otherwise stated
AMDR Acceptable Macronutrient Distribution Range RNI Recommended Nutrient Intake AI Adequate Intake
SFAs Saturated fatty acids MUFAs Monounsaturated fatty acids PUFAs Polyunsaturated fatty acids LA Linoleic acid AA Arachidonic acid ALA Alpha-linolenic acid EPA Eicosapentaenoic acid DHA Docosahexaenoic acid

3.3.2. Breast milk fatty acid composition of lactating mothers

The fatty acid composition in the breast milk of the group of 73 lactating women is shown in Table 10. The mean of total fat content of the 73 milk samples was 41.3g/l. Three categories of fatty acids, SFAs, MUFAs and PUFAs, represented approximately 42%, 36% and 22% of total fatty acids, respectively. Palmitic acid (C16:0) accounted for the largest proportion of SFAs (19%) while oleic acid (18:1 ω-9) and linoleic acid (18:2 ω-6) accounted for the largest proportion of MUFAs (33%) and PUFAs (17%), respectively. The mean DHA level reached approximately 0.9% of the total fatty acids in the milk from these mothers. On the other hand, the mean ω-6/ω-3 PUFAs and LA/ALA ratios in the milk samples were 5.8 and 9.1, respectively.

As the sole source of nutrition for infants aged 6 months or below, the fatty acid composition of breast milk of the group of 29 lactating women who were exclusively breastfeeding is also shown in Table 5. Assuming that the average volume of milk consumed by infants aged within 0-6 months is 750ml/day, the daily intakes of fatty acids were calculated and compared with the Chinese DRIs recommendation.

Table 10 The milk fatty acid composition of the 73 lactating women at different stages of lactation

Fatty acids (%wt/wt of all FAs)	All subjects (n=73)	Subjects who had exclusively breastfed their infants aged within 6 months or below (n=29)
SFAs		
10:0	1.18 ± 0.40	1.29 ± 0.41
12:0	6.02 ± 1.78	5.08 ± 1.80
14:0	8.03 ± 6.14	8.44 ± 9.48
15:0	0.19 ± 0.11	0.18 ± 0.08
16:0	19.0 ± 2.43	18.32 ± 3.04
17:0	0.25 ± 0.06	0.26 ± 0.07
18:0	5.89 ± 1.13	5.61 ± 1.12
20:0	0.05 ± 0.09	0.12 ± 0.09
21:0	0.40 ± 0.33	0.20 ± 0.22
22:0	0.29 ± 0.24	0.19 ± 0.19
24:0	0.03 ± 0.06	0.07 ± 0.09
Total SFAs	41.43 ± 6.07	39.86 ± 7.77
MUFAs		
14:1 ω-5	0.05 ± 0.08	0.09 ± 0.08
15:1	ND	ND
16:1 ω-7	2.48 ± 0.74	2.67 ± 0.75
17:1 ω-7	0.13 ± 0.10	0.05 ± 0.09
18:1 ω-9	33.52 ± 3.64	33.60 ± 4.09
20:1 ω-9	0.15 ± 0.27	0.38 ± 0.32
22:1 ω-9	0.02 ± 0.05	0.06 ± 0.06
24:1 ω-9	0.07 ± 0.11	0.05 ± 0.08
Total MUFA	36.42 ± 3.66	36.91 ± 4.35

ω-6 PUFAs		
18:2 ω-6 (LA)	17.16 ± 3.50	17.89 ± 4.09
18:3 ω-6	1.07 ± 0.77	1.66 ± 0.87
20:2 ω-6	0.08 ± 0.15	0.21 ± 0.17
20:3 ω-6	0.09 ± 0.16	0.23 ± 0.19
20:4 ω-6 (AA)	0.71 ± 0.22	0.74 ± 0.24 (AI: 0.15g/d) (mean daily intake: 0.21 g/d)
22:2 ω-6	0.02 ± 0.07	0.06 ± 0.11
Total ω-6 PUFAs	18.65 ±3.60	19.44 ± 4.29
ω-3 PUFAs		
18:3 ω-3 (ALA)	1.49 ± 1.12	2.20 ± 1.02
20:3 ω-3	0.01 ± 0.03	0.03 ± 0.04
20:5 ω-3 (EPA)	0.29 ± 0.29	0.36 ± 0.31
22:5 ω-3	0.41 ± 0.56	0.34 ± 0.18
22:6 ω-3 (DHA)	0.80 ± 0.65	0.86 ± 0.66 (AI:0.1g/d) (mean daily intake: 0.25 g/d)
Total ω-3 PUFAs	3.54 ± 1.41	3.79 ± 1.54
Total PUFAs	22.14 ± 4.27	23.23 ± 4.99
ω-6/ω-3 PUFAs	5.84 ± 1.87	5.68 ± 1.90
18:2 ω-6 /18:3 ω-3	9.12 ± 2.35	8.95±2.73
20:5 ω-3+22:6 ω-3	1.09 ± 0.83	1.22 ± 0.86
Total fat (g/l)	41.34 ± 13.67	39.13 ± 14.69

Data are shown as mean ± SD
ND Not detectable
AI Adequate Intake
SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, LA: Linoleic acid, AA: Arachidonic acid, ALA: Alpha-linolenic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid

3.3.3. Correlation between maternal dietary intake and fatty acid composition of breast milk

Spearman's rank correlation tests were conducted between the maternal characteristics and the levels of different fatty acids in the milk samples. However, no correlations were found among the characteristics of lactating mothers, namely maternal BMI, age, monthly household income andeducation level. Correlation tests were conducted between maternal dietary intakes of fatty acids and their levels in breast milk. Only the maternal dietary intakes of some ω-3 fatty acids were correlated with their levels in breast milk (Fig. 1). Results showed that the levels of DHA, EPA and ALA in milk samples and maternal intakes of the corresponding fatty acids were positively correlated, both maternal ALA and DHA intake and their levels in milk were significantly associated.

Correlation tests were also conducted between maternal intakes of different food groups and the content of fatty acids in milk samples. Maternal consumption of nuts was significantly correlated with total PUFAs content in breast milk (R=0.281, p=0.018). The results of the correlation tests revealed significant associations between DHA content in the milk samples and maternal intakes of both freshwater and saltwater fish, as well as some frequently consumed types of fish in Hong Kong, including mandarin fish, salmon and croaker (Table 11). In stepwise linear regression analysis, we studied the association of milk DHA concentration with a number of variables, namely maternal intake of freshwater fish, maternal intake of saltwater fish, age, BMI, education level and monthly household income. Maternal intake of saltwater fish was the only significant predictor of the content of DHA in milk ($R^2 = 0.242$, $p < 0.001$) but the other variables were not significant predictors. With the effect size of 0.49, the ad power of the present study is 99.7%.

Table 11 Spearman's correlation coefficient between dietary intake of freshwater and saltwater fish species consumed in Hong Kong and contents of DHA and EPA in breast milk. Significant correlations are highlighted in bold.

Dietary intake (g/d)	Breast milk fatty acids (%wt/wt of all FAs)	
	EPA	DHA
Freshwater fish	0.158	**0.285****
Grass fish	0.093	0.194
Mandarin fish	**0.250***	**0.268***
Largemouth bass	-0.123	-0.007
Mud Carp	0.0.39	0.105
Bid Head fish	0.011	0.023
Saltwater fish	**0.284****	**0.404****
Salmon	0.165	**0.214***
Pomfret	0.004	0.167
Croaker	0.177	**0.241****
Golden thread	0.098	0.161
Grouper	-0.029	0.093

*: Correlation is significant at the level of $p < 0.05$ (two tailed)
**: Correlation is significant at the level of $p < 0.01$ (two tailed)

3.3.4. Comparison of the fatty acid profiles of breast milk for infants aged 6 months or below from Hong Kong lactating women with those from other countries

The fatty acid concentrations of milk from local mothers who had exclusively breastfed their infants aged from 6 months or below (29 samples) were compared with those from mother in other countries including Asian countries (China and Japan) and European countries (Greece, Finland, Sweden and Germany) (Table 12). Concentrations of fatty acids that were compared include SFAs, MUFAs, PUFAs, total ω-3 fatty acids, total ω-6 fatty acids, LA, AA, ALA, EPA and DHA.

Compared with the breast milk of mothers in other countries, contents of PUFAs in the breast milk of Hong Kong mothers were relatively high (23% of weight percentage of total fatty acids). More importantly, DHA content in the milk of local mothers, which accounted for 0.86% of weight% of total fatty acids, was significantly higher than those in other countries, except Japan.

Compared with the study conducted in Hong Kong two decades ago, concentrations of all fatty acids, except MUFAs, in milk samples of the present study were higher. The concentration of total ω-3 fatty acids was significantly increased from 2.17% to 3.79%, which could be explained by the significant increments of milk EPA concentration (0.08% to 0.36%) and milk DHA concentration (0.56% to 0.86%).

Breast milk of mothers in the Mainland had higher total PUFAs concentration than that from Hong Kong mothers (28% compared with 23% of total weight% of fatty acids). Significant differences were shown in milk total ω-6 fatty acids concentration between China populations and Hong Kong populations (24% compared with 19% of total weight% of fatty acids), which was partially due to the higher milk LA concentration of mothers in China (22% of total weight% of fatty acids). Although the total ω-3 fatty acids concentration of milk from mothers in the two populations did not show significant difference, milk ALA concentration of mothers in the Mainland was higher than those in Hong Kong (2.8% compared with 2.2% of total weight% of fatty acids).

The total ω-3 fatty acids of milk from local mothers was higher than those from Japan mothers, and they were 3.79% and 3.11% respectively. The significant difference between them was attributed to the milk ALA content. The milk samples of Hong Kong mothers had a mean ALA concentration of 2.20%, which was higher than that of Japan mothers (1.33%). Milk DHA content of Hong Kong mothers (0.86%) was comparable to that of Japan mothers who had habitually high consumption of fish (0.99%). The total ω-6 fatty acids concentrations of milk from local mothers (19%) were also significantly higher than those from Japanese populations (14%). More specifically, the milk samples of local mothers had higher LA and AA concentrations than those of Japan mothers (18% compared with 13% and 0.74% compared with 0.40%, respectively).

The levels of milk EPA and DHA of subjects in the 1997 study were significantly lower than those in our present study. The increased consumption of seafood may explain the significant differences in the level of LC-PUFAs concentration in their milk. In the present study, the median daily dietary intake of seafood, including fish consumption, was 87.5g, while the mean daily fish intake of subjects in the 1997 study was only 26g.

The levels of most fatty acids in the breast milk of mothers in European countries were significantly different from those in the present study. Generally, milk samples from European lactating mothers contained higher concentrations of SFAs and MUFAs and lower concentrations of PUFAs. The content of LA, which is an ω-6 fatty acid, and the content of total ω-3 fatty acids of milk from local mothers were significantly higher than those from mothers in European countries. The levels of EPA and DHA in milk samples from Greece (0.15%, 0.45%) and Finland (0.18%, 0.46%) populations attained only one half of the levels in milk samples of the present study (0.36%, 0.86%).

Table 12 Comparison of the fatty acid profiles of breast milk for infants aged 6 months or below in Hong Kong lactating women with those in other countries

			wt% total fatty acids									
Regions	Infant age	Subject	SFA	MUFA	PUFA	total n-3	total n-6	LA	AA	ALA	EPA	DHA
Hong Kong (Present study)	0-6 months	29	39.86 ± 7.77	36.91 ± 4.35	23.23 ± 4.99	3.79 ± 1.54	19.44 ± 4.29	17.89 ± 4.09	0.74 ± 0.24	2.20 ± 1.02	0.36 ± 0.31	0.86 ± 0.66
Hong Kong (25)	6 weeks	51	38.07 ± 4.56	40.42 ± 16.29	20.14 ± 3.03*	2.17 ± 0.74*	17.97 ± 2.94	15.80 ± 3.01*	0.41 ± 0.16*	1.24 ± 0.54*	0.08 ± 0.09*	0.56 ± 0.23*
China (27)	42 days	477	35.13 ± 6.50*	36.88 ± 5.25	27.55 ± 6.21*	3.86 ± 2.93	23.91 ± 5.97*	22.00 ± 5.89*	0.62 ± 0.19*	2.81 ± 2.83*	0.41 ± 0.96	0.38 ± 0.31*
Japan (15)	3 months	51	41.51 ± 0.46	38.43 ± 0.41	17.37 ± 0.29*	3.11 ± 0.14*	14.26 ± 0.25*	12.66 ± 0.25*	0.40 ± 0.01*	1.33 ± 0.05*	0.26 ± 0.02	0.99 ± 0.08
Greece (14)	3 months	39	45.29 ± 7.97*	35.77 ± 10.18	18.19 ± 5.72*	0.76 ± 0.72*	17.42 ± 5.66	15.15 ± 5.01*	0.89 ± 0.35*	0.16 ± 0.21*	0.15 ± 0.25*	0.45 ± 0.15*
Finland (40)	3 months	49	43.60 ± 6.00*	40.10 ± 4.00*	14.80 ± 4.00*	2.70 ± 1.10*	12.10 ± 3.40*	11.10 ± 3.20*	0.39 ± 0.09*	2.00 ± 0.70	0.18 ± 0.22*	0.46 ± 0.54*
Sweden (38)	3 months	19	40.72 ± 1.04	45.15 ± 0.74*	14.14 ± 0.57*	1.95 ± 0.14*	12.19 ± 0.47*	10.93 ± 0.45*	0.38 ± 0.02*	1.60 ± 0.13*	0.06 ± 0.01*	0.25 ± 0.01*
Germany (41)	6th weeks	462	47.12 ± 9.12*	39.33 ± 6.91*	13.57 ± 5.97*	1.15 ± 0.92*	11.48 ± 4.76*	10.09 ± 4.41*	0.46 ± 0.32*	0.62 ± 0.42*	0.04 ± 0.07*	0.17 ± 0.23*

Data are given as mean ± SD

*: Significant at the level of $p < 0.05$

SFAs Saturated fatty acids MUFAs Monounsaturated fatty acids PUFAs Polyunsaturated fatty acids LA Linoleic acid AA Arachidonic acid ALA Alpha-linolenic acid EPA Eicosapentaenoic acid DHA Docosahexaenoic acid

3.4. Minerals

3.4.1. Maternal intakes of minerals

According to the 3-day dietary records, the daily dietary intakes of minerals, namely calcium, iodine, iron, selenium and zinc, of 62 lactating mothers were summarized in Table 13. The recommended dietary intakes of the minerals as suggested by the Chinese DRIs were shown as well. Among all the minerals, only the mean dietary intake of selenium of the subjects (145.6μg/day) attained the recommended level of 78μg/day. Dietary intakes of other minerals of the subjects were well below the RNI levels. The median dietary intake of calcium was 691.2mg/day only. Nearly 90% of the subjects could not meet the suggested intake level of 1000mg/day. The median dietary intake of iodine was 48.8 μg/day, which fulfilled only 20% of the suggested intake level. The median dietary intake of iron was 13.3 mg/day, which satisfied about one half of the suggested intake level. The dietary intakes of iodine and iron of over 90% of the subjects were below the recommendations of 240 μg/day and 24mg/day, respectively. Although the mean dietary intake of zinc of the subjects met the suggested level of 12mg/day, over 50% of them had inadequate intake of zinc.

Table 13 Dietary intakes of micronutrients of 62 lactating women

Micronutrients	Daily dietary intakes	Recommendation from Chinese DRIs 2013
Calcium (mg), median ± IQR	691.2 ± 295.3	1000 (RNI)
% subjects <RNI	87.1	
% subjects >RNI	12.9	
Iodine (μg), median ± IQR	48.8 ± 54.8	240 (RNI)
% subjects <RNI	96.8	
% subjects >RNI	3.2	
Iron (mg), median ± IQR	13.3 ± 6.6	24 (RNI)
% subjects <RNI	91.9	
% subjects >RNI	8.1	
Selenium (μg)	145.6 ± 40.1	78 (RNI)
% subjects <RNI	3.2	
% subjects >RNI	96.8	
Zinc (mg), median ± IQR	11.1 ± 4.9	12 (RNI)
% subjects <RNI	56.5	
% subjects >RNI	43.5	

Data are given as mean ± SD unless otherwise stated

EAR estimated average requirement *RNI* Recommended nutrient intake *AI* Adequate intake

AMDR Acceptable macronutrient distribution ranges *EI* Energy intake

3.4.2. Breast milk content of minerals of lactating mothers

The results in Table 14 showed the median concentrations of different minerals in milk samples, namely calcium, iodine, iron, selenium and iodine, which were grouped on the basis of the age of the infants (i.e. mothers of infants aged 0-6 months, 7-12 months 13-24 months were allocated to groups 1, 2 and 3, respectively). Assuming that the daily milk consumption of exclusively breast-fed infants from 0-6 months old is 750ml, the dietary intakes of minerals of infants from breast milk of mothers in group 1 were compared with the levels of adequate intakes recommended by the Chinese DRIs.

The mean concentration of calcium in milk samples was 242.7mg/L. The results revealed a decreasing trend in milk calcium content along lactation. The mean daily dietary calcium intake of 0-6 –month-old infants who were exclusively breastfed was 199.9mg, which is close to the amount of adequate intake, i.e. 200mg, as suggested by the Chinese DRIs. One half of the milk samples provided adequate calcium to the infants.

The concentration of iodine in milk increased along lactation, and there were significant differences in the median iodine content between milk samples from mothers in group 1 and those in group 3. The mean dietary iodine intake (80.7μg/day) of the infants of subjects in group 1 was slightly below the recommended level of 85μg/day by Chinese DRIs, resulting in about 54% of the infants who had inadequate dietary intake of iodine.

The median milk iron concentration of subjects in group 1 (0.56mg/L) was higher than that in groups 2 and 3 (0.38mg/L), but the differences were not significant. The median iron concentration of all milk samples was 0.49mg/L. Over 80% of the exclusively breastfed infants aged 6 months or below had adequate intake of iron of 0.3 mg/day as recommended by the Chinese DRIs.

The content of selenium in milk decreased along lactation, with the median concentration of all samples being 8.1μg/L. The Chinese DRIs suggested the daily dietary selenium intake of 0-6 –month-old infants at 15μg. In our study, however, nearly 80% of the infants had not attained the level of adequate intake, with the mean dietary selenium intake being 9.4μg/day only.

The median zinc concentration of all milk samples was 1002.1μg/L. Significant differences in the milk zinc concentration were shown among the 3 groups of subjects, with the highest mean concentration of 1154μg/L in samples from mothers in group 1. The mean dietary zinc intake of infants of subjects in group 1 was 866 μg/day only. Although the zinc content of milk samples for infants aged 0-6 months was relatively higher than that for infants aged over 6 months, more than 85% of infants could not reach the suggested dietary zinc intake of 2000 μg/day for 0-6 months old and exclusively breastfed infants.

Table 14 Concentrations of minerals in breast milk of lactating women and the daily dietary intakes of minerals of exclusively breastfed infants aged within 6 months

Concentration of minerals in milk	All subjects n=62	Group 1[A] n=28	Group 2[B] n=18	Group 3[C] n=16
Calcium (mg/L)	242.7 ± 42.9	263.4 ± 34.8[a]	232.7 ± 48.5	213.8 ± 30.9[a]
Iodine (μg/L) , median ± IQR	128.8 ± 82.9	111.7 ± 58.4[a]	132.4 ± 86.0	171.8 ± 97.4[a]
Iron (mg/L) , median ± IQR	0.49 ± 0.22	0.56 ± 0.16	0.38 ± 0.23	0.38 ± 0.30
Selenium (μg/L) , median ± IQR	8.1 ± 11.1	10.9 ± 9.2	7.3 ± 17.6	5.7 ± 11.0
Zinc (μg/L) , median ± IQR	1002.1 ± 482.0	1154.7 ± 877.9[ab]	965.9 ± 527.5 [a]	794.8 ± 353.7[b]
Daily dietary intake of minerals[#]			AI for 0-6 months old infants[1]	
Calcium (mg)	199.9 ± 38.8		200	
% subjects <AI	50.0			
% subjects >AI	50.0			
Iodine (μg)	80.7 ± 41.6		85	
% subjects <AI	53.6			
% subjects >AI	46.4			
Iron (mg)	0.41 ± 0.12		0.3	
% subjects <AI	17.9			
% subjects >AI	82.1			
Selenium (μg)	9.4 ± 7.5		15	
% subjects <AI	78.6			
% subjects >AI	21.4			
Zinc (μg)	866.1 ± 671.5		2000	
% subjects <AI	85.7			
% subjects >AI	14.3			

Data are given as mean ± SD unless otherwise stated

[ab]: Means or median in the same row with different superscripts letters are significantly different at the level of $p<0.01$

[A] Group 1: Subjects who had exclusively breastfed their infants aged within 6 months

[B] Group 2: Subjects who had breastfed their infants aged between 7 and 12 months

[C] Group 3: Subjects who had breastfed their infants aged between 13 and 24 months

[#] Assumed volume of daily breast milk consumption of infants from 0-6 months old is 750ml

[1] Adequate Intake according to Chinese Dietary Reference Intakes 2013

3.4.3. Correlation between maternal dietary intake and content of minerals of breast milk

Spearman’s rank correlation tests were conducted between mineral content in breast milk (calcium, iodine, iron, selenium and zinc) and infant and maternal characteristics (Table 15).

Some studies have shown that infant age is associated with the levels of some kinds of minerals in breast milk (127). In the current study, the results revealed that the age of infants was negatively correlated with the concentrations of calcium (r_s= -0.595) and zinc (r_s= -0.434) and positively associated with the concentration of iodine (r_s= 0.446) of milk samples.

Some studies have illustrated that no significant correlation was found between milk mineral content and the age and BMI of lactating mothers (154). The results of Spearman’s rank correlation tests of the current study revealed that, in line with those previous studies, no statistically significant correlations were found between the concentration of minerals in milk and maternal age and BMI. However, maternal education level was positively associated (r_s= 0.277) with milk selenium content.

Spearman’s rank correlation tests were conducted between milk mineral content and maternal dietary intakes of different food groups as well (Table 15).

The concentrations of calcium (r_s= 0.276), iron (r_s= 0.383), selenium (r_s= 0.275) and zinc (r_s= 0.371) in milk were positively correlated with the dietary intakes of dairy products of lactating mothers. However, maternal intakes of grains, vegetables, fruits and meats were not associated with the levels of minerals in milk.

Table 15 Spearman's correlation coefficient between maternal characteristics, infant characteristics and maternal dietary intakes of different food groups and content of minerals in breast milk. Significant correlations are highlighted in bold text.

	Breast milk minerals				
	Calcium	Iodine	Iron	Selenium	Zinc
Maternal characteristics					
Age	0.006	-0.121	-0.093	0.109	-0.049
BMI	-0.123	-0.249	0.078	0.148	0.116
Education level	0.150	-0.220	0.067	**0.277***	0.170
Infant characteristic					
Infant age	**-0.595****	**0.446****	-0.220	-0.157	**-0.434****
Maternal dietary intakes					
Grains	0.065	0.024	-0.016	0.108	-0.107
Vegetables	0.082	-0.084	0.033	0.018	0.050
Fruits	0.192	-0.196	0.103	0.069	-0.013
Meats	0.012	-0.064	-0.065	0.026	0.120
Dairy products	**0.276***	0.048	**0.383****	**0.275***	**0.371****

*: Correlation is significant at the level of $p < 0.05$ (two tailed)

**: Correlation is significant at the level of $p < 0.01$ (two tailed)

Chapter 4 Discussion

Hong Kong populations are changing towards a more modern westernized diet, which is characterized by high proportion of energy-dense food but insufficient amounts of essential nutrients. The results of the present study provided some updates on the nutrition of the breast milk of Hong Kong lactating mothers and their habitual dietary intakes. Also, correlations between local dietary characteristics and the nutritional composition of human milk were found.

The present study revealed that the high level of polyunsaturated fatty acids in milk samples could meet the suggested daily intake of exclusively breastfed infants aged 6 months or below as recommended by the Chinese DRIs. Fish is a well-known source of ω-3 PUFAs, especially DHA, which are important for infant brain development. High fish consumption with a large variety was shown in the study group. The average fish intake of the lactating mothers in our study was 10 servings per week, which is higher than the suggested weekly consumption recommended by USDA (155). Correlation has been shown between maternal fish intake and milk DHA concentration. Also, maternal intake of saltwater fish is a significant predictor of the content of DHA in milk.

The study results showed that the content of most of the examined essential micronutrients in breast milk, namely calcium, iodine, selenium and zinc, was too low to meet the suggested levels of adequate intake of 0-6-month-old infants. Infants are born with limited mineral store, therefore persistent deficiency in dietary intake would pose severe and irreversible adverse health impact on them. Not only the dietary nutrient intakes of infants, but also those of the lactating women are worthy of our attention.

The present study revealed that the mean dietary fat intake (98 g/d) of the lactating mothers in this study is 69% more than that in a study conducted in Hong Kong a decade ago (58 g/d) (156). Subjects had overconsumption of meat products with a mean intake of 269g/day. Ninety percent of subjects had excess intakes of fat and protein which accounted for 18% and 37% of total energy intake, respectively. Hong Kong people have long working hours and thus no time to prepare meals. They often go to fast food outlets for lunch and even dinner. Therefore, it is not surprising that the populations had inadequate consumptions of nutrient-dense foods such as vegetables and fruit. The median consumptions of vegetables (119g/d) and fruit (68g/d) of the subjects fulfilled only a third of the suggested intake amounts. Chinese populations have habitually low consumption of dairy products. Parents tend to stop feeding milk to their children after the age of one. The lactase production of the children decreases significantly and thus making it difficult for them to digest dairy products by the time they reach adulthood. As a result, lactose intolerance is a severe problem in Hong Kong. As expected, the median consumption of dairy products of subjects was very low (42g/d), which fulfilled only one-tenth of the recommended level. Due to the imbalanced diet with limited food variety, the lactating mothers had insufficient intakes of several key nutrients.

Optimum consumptions of various food groups by lactating mothers are necessary to maintain good health for them and their infants. However, there is a range of structural barriers that may hinder the intention to achieve nutritious diets for people in Hong Kong, such as busy lifestyles, culturally low intakes of dairy products and gaps in knowledge on healthy choices. On the other hand, we may think that subjects who have high education level and higher monthly household income should have acquired adequate knowledge about healthy eating and be able to afford and choose nutritious foods. However, such phenomenon was not revealed in the present study. There was a high proportion of subjects who were overweight or obesity. We did not examine the correlations between the quality and adequacy of the diet of lactating mothers and their education level and household income. To effectively develop public education programme on adequate and balanced diet in the city, the correlation information may help. In our future study, we may consider studying subjects' diet quality via using different diet quality indices e.g. DQI (Diet Quality Index), HDI (Healthy Diet Indicator) to figure out the correlations.

4.1. Fatty acid profile of breast milk

The last study on the breast milk fatty acid components in Hong Kong dates back to 1997 (156). Previous studies have reported different breast milk fatty acids profiles in mainland Chinese women living in urban, rural, coastal and inland regions and maternal diet impacts on the fatty acids in milk have been shown (149, 157-159). Hong Kong people have a unique dietary habit with high seafood consumption. The present study confirms the association between fatty acid contents in breast milk of Hong Kong lactating mothers and their consumption of aquatic foods. More importantly, for the first time, the study demonstrates the associations between the fatty acid contents in the milk of mothers and their specific fish consumptions.

The present study showed that the concentrations of DHA (0.86 %) and ω-3 fatty acids (3.79%) in the breast milk of subjects who had exclusively breastfed their infants aged 6 months or below were significantly higher than those observed in Hong Kong in 1997 (0.56 %, 2.17%) (156), and those from coastal areas of China (0.41-0.61%, 1.68-3.34%) (149, 157, 158). Moreover, the content of DHA (0.86 %) in the breast milk of Hong Kong lactating mothers was not only higher than the world mean (0.32%) (160), but also comparable to the milk of mothers from some island countries and regions such as Japan (0.99%), Korea (0.96%) and Taiwan (0.98) (161). DHA is synthesized limitedly in infants from the age of 0-6 months, and it is regarded as a conditional essential fatty acid for infants (162). Therefore, it is crucial to provide sufficient DHA to infants for normal brain development. Assuming that daily milk consumption is 750ml, among the 29 milk samples of lactating mothers with infants aged from 0-6 months old, 24 could provide their infants (i.e. more than 80 percent) with adequate level of DHA (100 mg) as recommended by the Chinese DRIs 2013 (152).

Although studies have suggested that the effects of maternal diets on the lipid profile of mature milk are insignificant (159), there is increasing evidence that demonstrates the effect of maternal diets on milk ω-3 PUFAs to a certain extent (59). It is well-known that both seafood and fish are prominent dietary sources of ω-3 fatty acids, especially DHA and EPA (29). In this study, as expected, total PUFAs content in breast milk correlated with both the amount and types of fish consumed. The average fish intake of lactating mothers in our study was 10 servings per week, which is higher than the weekly consumption suggested by USDA (155). The findings are in line with other studies, suggesting a positive association between DHA concentration in milk of lactating mothers and the amount of their fish consumption (163). Also, populations in Hong Kong consume a wide range of fish. The most frequently consumed type of fish of the subjects in our study was salmon, which is regarded as a fatty fish that contains high levels of EPA and DHA (164). Among the different types of local fish, maternal intakes of salmon, croaker and mandarin fish were significantly correlated with their milk DHA content. A significant positive correlation between the levels of EPA and DHA has been shown in the milk samples. However, the results only showed significant association between intake of saltwater fish, but not freshwater fish, and milk EPA. The impact of maternal consumption of saltwater fish on DHA content (r=0.404) was more significant than that on EPA content (r=0.284) in milk. Studies have revealed that milk EPA content is less sensitive to maternal dietary intakes compared with milk DHA content (159). The DHA content in the breast milk from Hong Kong mothers is higher than that from mainland Chinese mothers, while the EPA content in the breast milk from Hong Kong mothers is comparable to that from mainland Chinese mothers (Table 7). The significant difference in milk DHA content might be attributed to the frequent consumption of saltwater fish of Hong Kong mothers. Moreover, consumption of fish soup is a special dietary practice in Chinese postpartum women as it is believed that fish soup could promote the production of breast milk. In our study, the average daily fish soup intake of mothers who breastfed for over 3 months was 100 ml (165). We believe that this kind of diet would affect the milk DHA concentration, but the extent of its contribution remains to be determined.

On the other hand, significant differences in the EPA concentration of milk samples were found between our study and the study in 1997 (156). However, the disparity might not be due to the choice of analytical methods as both studies employ similar GC-FID method in analyzing fatty acid composition. Further investigation is required to figure out the possible factors that contributed to the difference.

High breast milk SFA concentration as a result of high-fat diets of mothers in this study is in accordance with observations in other studies conducted in European populations such as in Spain (166) and Sweden (167). Studies have illustrated that milk AA is relatively stable (168). Although significant correlation between maternal intake of AA and milk AA has not been shown in the present study, the impact of long-term changes in eating habits of the local population on milk AA concentration could not be neglected. Since both DHA and AA play an important role in maintaining body health, studies have illustrated that imbalanced dietary intake of ω-6/ ω-3 might bring about adverse health effects to both mothers and infants, such as impaired immune system function and cognitive development (169). The results of the present study revealed that, in line with results of some previous studies, the content of AA in breast milk in Chinese population was higher than that in European populations (159). An AA concentration of 0.71% in milk samples is within the recommended range of 0.35-1.00% (170), but higher than that of the world mean (0.47%) (160).

The use of different cooking oils could help explain the wide variations of MUFAs and PUFAs content in the milk of mothers from different countries. The demand for olive oil and canola oil, prominent dietary sources of oleic acid, is growing rapidly due to the increasing trend of health consciousness in Hong Kong (171). Studies have shown that dietary intake of oleic acid, which comprises a large proportion of milk MUFA, is positively associated with its level in breast milk (166). However, such association was not shown in our study though the majority of our subjects (over 60%) used olive oil or canola oil as their household cooking oils. Compared with countries like Finland (172) and Germany (173), where olive oil is the common cooking oil (174) and MUFA constitutes at least one third of the total fatty acid consumption (175), the overall olive oil consumption in Hong Kong is relatively low. Also, nearly 80% of the subjects were working mothers and ate out for lunch and even dinner. Foods from fast food outlets are commonly cooked with vegetable oils such as peanuts oil and corn oil. This resulted in lower milk MUFA concentration in this study as compared with the milk samples from other European countries.

This study shows that LA and ALA contents in the milk from Hong Kong mothers are significantly higher than those from some European and Japanese mothers, but significantly lower than those from mainland Chinese mothers (Table 7). There is a common Chinese dietary practice of consuming the traditional Chinese dish "Pig Knuckles and Ginger Stew" after giving birth. The main ingredients of this dish include eggs, gingers and pig trotters, which are believed to facilitate the recovery process of mothers after delivery. Therefore, Chinese women during the postpartum period often consume many eggs, of which the egg yolk is a well-known source of LA (165) and the special dietary practice results in enhanced milk LA concentration compared with women in other countries. On the other hand, the fact that the usage of LA- and ALA-rich vegetable oils such as soybean oil and rapeseed oil is prevalent on the mainland (176) could explain the significantly higher proportions of essential acids in the breast milk of mainland Chinese mothers than Hong Kong mothers. The differences in cooking oil usage may also explain the higher total ω-3 LC-PUFAs concentration in milk from Hong Kong mothers (3.79%) than that in milk from Japan mothers (3.11%). The milk DHA content of Hong Kong mothers (0.86%) was comparable to that of Japan mothers who had habitually high consumption of fish (0.99%). The significant difference was attributed by the milk ALA content. The milk samples of Hong Kong mothers had a mean ALA concentration of 2.20%, which was higher than that of Japan mothers (1.33%). The prevalence of ALA-rich vegetable oils usage such as soybean oil and canola oil in Hong Kong populations may explain the high concentration of ALA in milk, but further research is needed to figure out other possible factors.

Since precursors of ω-3 and ω-6 LC-PUFAs compete for enzymes during the elongation-desaturation process, an imbalanced dietary LA to ALA proportion may exhibit adverse effects on the neural and retinal functions of infants. Therefore, the ratio of dietary intakes of LA and ALA is more important than the absolute amounts of consumption (166). The LA/ALA ratio in this study was 9.12, which is in agreement with the range of 5-15 recommended by The European Society for Pediatric Gastroenterology and Nutrition (177).

The present cross-sectional study examines the dietary patterns and fatty acid profile of breast milk in Hong Kong mothers. It is likely that there are factors other than diet that affect the breast milk composition. Many women in Hong Kong follow traditional Chinese cultural practices during the postpartum period, with restrictions on their diet, hygiene and physical activities (178), but the impact of these practices on breast milk composition is not well studied.

4.2. Concentrations of minerals of breast milk

Studies have revealed that the concentrations of most minerals in breast milk remain fairly constant throughout the course of lactation and are not affected by maternal diets (179). Among several food groups, namely grains, vegetables, fruits, meats and dairy products, only the maternal intake of dairy products was correlated with the concentrations of some minerals of milk (i.e. calcium, iron, selenium and zinc). Compared with people from Western countries, Chinese populations have habitually low intake of dairy food such as milk and yogurt. Mothers who have high consumption of dairy products might be more health-conscious. Therefore, the nutritional value of breast milk from mothers who have higher consumption of dairy products might be higher than those who have lower constipation of dairy products. A long-term balanced diet would help optimizing breast milk nutrition.

The results of the present study revealed that in line with results of previous studies, infant age was negatively correlated with milk calcium (r=-0.595). Milk calcium decreases along the lactation period due to the reduced concentrations of citrate and casein in the Golgi apparatus of the mammary epithelial cells. Formation of calcium citrate and calcium-casein complex is reduced and thus the efficiency of transportation of calcium into milk is lowered (77). Previous publications have reported a range of mean calcium concentration in human milk between 84 and 462mg/L, with a median concentration of 252mg/ L (80). The mean concentration of calcium of milk samples collected in the present study was 243mg/L, which is comparable to the reported mean level.

A survey conducted in 1995 on the dietary intake of calcium of local population revealed that female adults had a mean calcium consumption level of 560mg per day (180). Although the dietary intake of calcium of subjects in our study increased more than 30 per cent in recent decades (737.4mg/day), it still failed to meet the suggested intake level of women during lactation (1000mg/day). Calcium is transferred from the maternal store to breast milk to ensure infants have sufficient dietary calcium intake for normal growth. Women would experience a notable drop in bone density during pregnancy and lactation, though the significant loss of calcium in maternal store could be regained. The long-term effects of calcium deficiency on maternal bone health and infant development remain controversial.

Infant age was also negatively correlated with the milk zinc content (r=-0.434). The decreasing trend in milk zinc concentration along lactation was consistent with observations in some previous reports. Compared with the longitudinal changes in the concentrations of other nutrients in breast milk, zinc level is one of the nutrients that exhibits the most significant changes during the first 4 months of lactation. Studies have suggested that the content of zinc is the highest in colostrum, with a concentration of 2-3mg/L (31-46μmol/L), then declines rapidly to 0.9mg/L (14μmol/L) after 3 months and becomes steady beyond 12 months postpartum (124-126). The present study revealed that the median zinc concentrations in the collected milk samples of the first 6 months of lactation was higher than that of the second 6 months, which was in turn higher than that of the following 12 months. The median zinc concentration of milk provided by mothers of infants aged 0-6 months old was 1.15mg/L. Although nearly 80% of the milk samples in group 1 could not provide the recommended amount of daily dietary zinc intake to infants who were exclusively breastfed, the presence of zinc reserve at birth could meet the demand for normal biological processes for term infants and thus no symptoms of zinc deficiency were found in infants (129). The discrepancy between maternal intake of zinc and its level in milk shown in our study may be attributed to the nature of the maternal diet. Zinc absorption would be lowered by increased phytate consumption. Data on phytate concentrations in food items is always lacking, so it is difficult to accurately estimate the bioavailability of these minerals from dietary assessments. Studies have revealed the association between maternal plasma Zn content and milk Zn concentration but no consistency between maternal dietary Zn intake and plasma zinc concentration. To accurately determine maternal Zn status, collection of blood samples from lactating mothers may be considered in future study.

Among all the trace elements examined in the study, only the median iron level of the collected milk samples could meet the suggested daily amount of intake of exclusively breastfed infants aged 6 months or below with the assumption of daily milk intake of 750ml. The iron content of milk samples (0.49mg/L) was within the reported wide range of iron concentration of 0.04-1.92 mg/L in breast milk. Low level of iron in breast milk can be balanced by hepatic reserve of iron in breast-fed neonate. High efficiency of utilization of iron from hepatic stores and iron bioavailability in human milk can adequately support the requirements of breast-fed term infants for their optimum growth and development (91). Therefore, the content of iron in itself is not of importance since no proven risk of nutritional iron deficiency has been found in infants during the first 6 months of lactation (90). However, more than 90% of lactating mothers in our study had insufficient dietary intake of iron. People with low iron consumption had been revealed in the first Hong Kong Total Diet Study conducted in 2014 (75). Inadequate iron consumption is a public health concern in the city for decades. The continuum of optimum maternal status from the periconceptional period through lactation is crucial. Take iron as an example. Many studies have revealed that iron stores at conception are an important predictor of maternal iron status and risk of anemia in later pregnancy and postpartum (90, 181). It is difficult to replenish depleted iron stores once pregnancy is in progress. Optimum iron status in populations, especially in women at childbearing age, shall be promoted to ensure adequate store throughout pregnancy and during the postpartum period.

Infants are born with selenium stores, but they also depend on the nutrient provided in human milk (182). There were no statistically significant differences revealed in milk selenium levels among the 3 groups of collected milk samples. The result was in line with previous studies showing that milk selenium level varies less from 4 months onwards (183). Although the mean daily selenium intake of subjects met the recommended level, nearly 40% exclusively breastfed infants aged within 6 months old could not obtain adequate selenium from human milk. The scarcity of associations between maternal dietary intakes and Se concentration may be attributed to the bioavailability of selenium. Selenium bioavailability changes with the selenium content of foods and the chemical forms of the element that are absorbed and metabolized. Overall, absorption of all forms of Se is relatively high (70%–95%) but varies according to the source and the Se status of the subject. Studies showed that maternal serum selenium level during postpartum correlated with milk selenium and infant selenium level at the age of 6 months. Since blood samples had not been collected from lactating women for examining their serum selenium levels, the prevalence of selenium deficiency could not be determined. A growing number of studies have revealed the adverse effect of selenium deficiency on health such as increased risk of infection in infancy, detrimental neurocognitive outcomes in childhood (183) and cardiovascular diseases in later adulthood (184). Maternal selenium supplementation during pregnancy or postpartum effectively increased maternal selenium levels (183), therefore it might be a possible public health measure to reduce its prevalence in populations.

Studies have suggested that maternal eating habit exerts considerable effect on milk iodine content. However, no significant correlation was found between milk iodine level and the food groups consumed, which may be attributed to two possible sources of error. Firstly, iodine level in human milk varies throughout the day. In the present study, single milk sample was collected from each subject. As a result, it might provide an imprecise measurement of daily iodine output or maternal iodine sufficiency. Secondly, the sample size of the study is small. Generalizability of results to the wider breastfeeding population should be made with further validation of the results.

In our study, only 3 per cent of the lactating mothers, with the mean daily iodine intake of 48.8μg, had a dietary iodine intake that met the level of recommended nutrient intake. However, about 50% exclusively breastfed infants aged within 6 months old could obtain adequate iodine from human milk. The discrepancy between maternal iodine intake and the mineral level in milk could be explained by the regulatory protective mechanisms that control the amount of iodine excreted into the breast milk of iodine-deficient lactating women (185). Studies have revealed that 40%–45% of the iodine ingested by the mother appears in breast milk. During lactation, the expression of the sodium iodide symporter in mammary gland increases, thus iodine is strongly concentrated in human milk (186). Therefore, even the lactating mothers have inadequate iodine intake or iodine deficiency, the mineral would be concentrated and transferred from maternal store to human milk.

Although the biological systems of mothers may protect infants against iodine deficiency, insufficient maternal iodine intake may exert harmful effects to the lactating women (185). In areas of high prevalence of iodine deficiency such as Hong Kong, salt iodization programme should be launched and strong efforts should be made to ensure that iodized salt is distributed widely. It has been proven as a cost effective way to relieve the public health problem in many countries (187). Women during pregnancy and postpartum have higher demand on iodine to prevent the foetus and infants from the adverse impact of iodine deficiency, especially on brain development. Iodine supplementation should be given to this at-risk group to ensure sufficient iodine would be provided to meet the iodine requirement of infants.

4.3. Recommendations for nutrition education

To make effective healthcare policy for local population, it is necessary to understand local adaptations. The present study revealed that Hong Kong lactating women and their infants might have inadequate nutrient intakes. There is a need for widespread and culturally appropriate nutrition interventions. The findings of this study would help the government to formulate public health care policies. Through community education, the importance of maintaining a healthy and balanced diet shall be promoted extensively by different sectors including the government, health care professionals and the academia.

Based on the findings of the present study, several main strategies to optimize maternal dietary intakes of nutrients are recommended. Firstly, increased consumption of nutrient-dense foods such as fat-free dairy products, fruits and vegetables and reduced consumption of energy-dense foods such as meat should be encouraged. Dietary guidelines have been made according to local dietary habits to optimize diet quality and improve health outcomes of lactating women.

The study results have shown that subjects had inadequate dietary intake of various micronutrients. Dietary advice on balanced diet and food variety should be provided to the lactating women. The dietary sources of minerals recommended for lactating mothers are suggested below:

Calcium: Milk, cheese and yogurt are main food sources of calcium, and the low fat varieties among these foods are recommended. Calcium is also found in tofu, dark green vegetables such as choy sum and kale, canned fish and nuts.

Iron: Dietary iron can be categorized into two groups: haem iron and non-haem iron. Major dietary sources of haem iron are found in meat, poultry and fish while non-haem is the predominant form of dietary iron and can be obtained from consumption of cereals, pulses, legumes, fruits and vegetables. Ascorbic acid found in both food sources such as fruits and vegetables or in fortification form has significant enhancing effect on the absorption of non-haem iron.

Iodine: Food is a major source of iodine , especially seaweeds, seawater fish, and shellfish. To lower the risk of iodine deficiency in the population, dietary iodine intake with the recommended range of RNI and upper limit via usage of iodized salt should be promoted.

Selenium: The contents of selenium in foods are influenced by the selenium levels of soils and crops in the natural environment. Grains, meats and seafood are good sources of selenium.

Zinc: Lean red meat, whole-grain cereals, pulses and legumes are rich sources of zinc.

Supplementation of minerals would be a second option as long as people cannot acquire sufficient nutrients from diets. Generally, the public who do not use iodized salt may have insufficient intake of iodine from their normal diets. Therefore, the vulnerable populations such as women who are at pregnancy or lactation may consider taking iodine-containing supplements.

As WHO suggests, appropriate complementary foods should be given to infants along with breastfeeding from 6 months until the age of 2 or beyond. Although the levels of all minerals, except iodine, in milk showed decreasing trend along lactation in the present study, mothers shall be advised to keeping breastfeeding beyond 6 months post-delivery. Breast milk provides infants not only with nutrients, but also bioactive compounds such as antibody which are beneficial to their health.

Studies suggested that human milk contains low level of vitamin D. Sunlight exposure increases the production of vitamin D in infants. However, Hong Kong people have insufficient sunlight exposure due to their lifestyle. Therefore, vitamin D supplementation to infants may be considered.

There are some limitations of the present study. The recruitment of subjects, including promotion conducted on campus via poster and email, or via online social platforms and the voluntary participation of the women may have resulted in selection bias, as women using online platform are expected to have higher educational level and economic status. Another potential limitation was that the presence of two research staff might have caused an inter-observer variation; however, effort had been made to minimize this. They were trained on how to conduct the dietary record interview. An instruction guide and examples to the questionnaire with detailed information about each item had been provided to them as well. Besides, the actual intakes of trace elements of the lactating mother in our study might have been higher than reported here, since some local food items were unavailable in the food database. They were replaced by other food items with more or less the same nutritional value. Also, the cross-sectional design of our study did not allow us to measure changes in milk nutritional composition over the period of lactation. We recruited subjects in a wide range of postpartum period to examine the content of nutrient at different stages of lactation. However, the generalizability of the current results is limited by the small sample size. The preliminary results of the present study are able to add information about fatty acid profile and the level of essential micronutrients in milk and thus support us to design a further study with a larger sample size.

Chapter 5 Conclusions

The present study has shown the impact of local dietary characteristics, especially high consumption of fish, on breast milk ω-3 polyunsaturated fatty acid concentration in Hong Kong lactating women. The amount of fish consumed is a significant predictor of milk DHA content. Over 80 percent of the breast milk samples from local mothers who had no supplementation of fatty acids would provide adequate DHA to their exclusively breastfed infants aged 6 months or below. The research findings have also revealed that lactating mothers have inadequate intake of essential nutrients including calcium, iron and iodine. The concentrations of iodine, selenium and zinc are low in breast milk of Hong Kong mothers. Thus, the mean daily dietary intakes of the minerals could not meet the levels of adequate intakes of infants aged 0-6 months old who are exclusively breastfed. Poor diet quality of women during lactation would not only bring about adverse health impact on themselves in the long run, but also detrimental effect on the infants' development. Long-term follow-up study with a larger sample size will help evaluate and optimize the effect of local dietary patterns on the nutrition of breast milk of mothers and health status of the Hong Kong population. The research findings could help the government to formulate and execute health policy and thus reduce the financial burden of health care in Hong Kong.

Appendices

Appendix I: Subject recruitment poster

Appendix II: Questionnaire for screening of subject

參加者者編號: ______________

基本資料記錄（篩選研究對象）

您好！我們現正研究香港哺乳期婦女的膳食習慣對母乳內鈣、硒、鐵、鋅 DHA 及 EPA 的影響。現正招募 18-40 歲、生產後 60 天的中國健康婦女，如有閣下有興趣參與研究，請用數花鐘回答以下問題，一切資料只會用作篩選對象之用，絕對保密。謝謝!

1) 您的年齡是否介乎 18-40 歲?

a) □ 是 b) □ 否

2) 請問您是否在香港連續居住不少於 18 個月的香港居民?

a) □ 是 b) □ 否

3) 請問您有沒有同時參與任何臨床試驗或研究?

a) □ 有 b) □ 沒有

4) 您是否現正全時間或部間時間餵哺母乳並生產達 60 天?

a) □ 是 c) 否，將於___月後達到生產 60 天
b) □ 否

5) 請問您是否在足月(37 孕週以上)時分娩?

a) □ 是 b) □ 否

6) 您這次生產是否雙胞或多胞胎？

a) □ 是 b) □ 否

7) 您的孩子出生時的體重是否正常(2500 克以上)?

a) □ 是 b) □ 否

8) 在懷孕和哺乳期間，請問您有沒有服食任何鈣、硒、鐵、鋅、DHA 和 EPA 的補充劑如魚油?

a) □ 有 b) □ 沒有

9) 分娩後，請問您有沒有服食避孕藥物?

a) □ 有 b) □ 沒有

10) 請問您有否患有腎，肝或甲狀腺功能減退，認知障礙或任何其他藥物或心理疾病?

a) □ 有 b) □ 沒有

11) 請問您是否願意向我們提供您的聯絡方法，以供日後聯絡之用？

a) □ 願意
b) □ 不願意

姓名：__________________________

簽名：__________________________

聯絡電話：__________________________

日期：__________________________

問卷完

Appendix III: Questionnaire for demographic data collection

Personal Information of the Volunteers (for Recruitment)

Questionnaire No. 問卷編號: _______________

參加者之個人資料 – 徵集志願者時適用

Date of questionnaire received 問卷收集日期: _____/______/ ______

Part A:甲部:

1) Gender of the child 孩子的性別: ☐ Boy 男 ☐ Girl 女

2) Was the baby delivered at full term (≥ 37 gestation weeks)? ☐ Yes 是 ☐ No 否

   您的孩子是否足月出生 (≥ 37 周)?

3) Was the baby a singleton infant? ☐ Yes 是 ☐ No 否

   這次是否只有一胎?

4) What was the birth weight of the baby? _______ kg / _______ pounds

   孩子出生時的體重是多少?

5) How often does the baby consult doctor? ☐ Never 從未 ☐ <1 Time per Month 一月少於一次

   過去孩子看醫生的次數? ☐ Once per Month 一月 一次

   ☐ 2 Times or more per Month 一月二次或以上

6) Does the baby have any known abnormality? 孩子是否有任何異常? ☐ Yes 是 ☐ No 否

7) DOB of the child 孩子出生年及月: _____ / _____ /_____ (dd /mm/ yyyy)

Part B: 乙部:

1) Name 姓名: ___________________________________ (in English) (英文)

____ (in Chinese) (中文)

2) Age 年齡: __________

3) Height 身高: 1st: __________ cm 2nd: __________ cm

4) Weight 體重: 1st: __________ kg 2nd: __________ kg

5) Blood Pressure 血壓: 1st: ______(systolic 上壓) 2nd: ______(diastolic 下壓) 脈率 Pulse: ______

6) 1st: ________(systolic 上壓) 2nd: __________(diastolic 下壓) 脈率 Pulse: ______

7) Marital status: 婚姻狀況 ☐ Single 單身 ☐ Married 已婚 ☐ Widowed 寡居 ☐ Divorced 離婚 ☐ Separated 分居

8) Start breastfeeding immediately after postpartum 生產後立刻開始餵哺母乳給孩子 ☐ Yes 是 ☐ No 否

9) Frequency of Breast-feeding (Please choose one) : 餵哺母乳的習慣 (請只選擇一個答案) ☐ Exclusively 完全 ☐ Two-third 三分二 ☐ Half-half 一半 ☐ One-third 三分一

10) Education Level 教育程度: ☐ No schooling completed 沒有接受教育 ☐ Primary-school or less 小學或以下 ☐ High-school 中學 ☐ Matriculate 預科 ☐ Tertiary 大專 ☐ Bachelor's degree or higher 大學或以上

11) Occupation 職業: ____

12) Family Income 家庭收入: (Please choose one (請只選擇一個答案) ☐<$5k ☐$5k-$10k ☐$10k-$20k ☐$20k-$30k ☐$30k-$40k ☐$40k-$50k ☐>$50k

13) Years of residence in Hong Kong 居港年期: __________ Yrs

14) Lifestyle (Could take all)生活模式 (可全部揀選): ☐Smoking 吸煙 ☐Drinking 飲酒 ☐Regular Exercise 定時運動

15) Volume of breast milk provided 所提供人奶的容量: ________ml

Appendix IV: Template of 3-day dietary record

Food Diary and Activity Log Sheet

飲食及活動記錄表

參加者編號: ______________

填寫飲食紀錄指引

Guidelines for Completion of Food Diary and Activity Log Sheet

1. 請依照例子填寫_____年____月____日 至 ____月____日的飲食及活動紀錄。
2. 請不要因為填寫此飲食及活動紀錄而改變你日常的飲食/活動習慣。
3. 請緊記寫下你進食或飲用過的每一樣食物, 包括水! 亦請記下你在家以外進食過的零食及任何食物。
4. 可以的話請記下食物的重量/份量。如果那是一件預先包裝好的食物你可以記下標籤上的重量。如果難以做到, 你可以日常的簡單方式記低份量, 如 2 茶匙糖, 2 湯匙蔬菜, 一碗飯等。
5. 請在「食物或飲品種類」一欄填寫食物的生產商名稱。
6. 請填寫煮食方法, 例如煎, 蒸, 炸或煮等。
7. 請將任何自製食物的食譜簡單地記在後面一頁。
8. 你簡單記下日常活動及運動情況, 並睡眠時間. 如散步 30 分鐘, 晚上 11 時睡覺.

進食及活動記錄表 (例子)

Diet Record & Activity Log Sheet (Example)

記錄日期： 2013 年 1 月 3 日 (星期四)

時間 Time	地點 Place	食物/飲品名稱 Food/Drink Consumed	份量 Amount
上午 7:10	屋企	白方飽 (連皮)	1 片
		煎雞蛋	1 隻
		低脂奶	1 盒
10:00	小食店	芝士餅	1 包
		棉花糖	2 粒
12:30	茶餐廳	鱆魚雞粒鳳爪飯	1 盒
		鳳爪	2 隻
		鱆魚雞粒	4 湯匙
		水	1 杯
4:30	屋企	可樂	1 支
		朱古力糖	3 粒
晚上 7:30	屋企	飯	1 碗
		牛肉	1 兩
		菜心	3 條
		節瓜 , 豆腐卜	2 件
		燒肉	3 件
		冬瓜瘦肉湯	1 碗

		瘦肉 (湯渣) (如有吃)	2 片
8:30		橙	半個
9:00		雪糕	1 杯

整日喝了幾多清水：約___6_____杯 / 毫升 營養補充劑 (如維他命丸/鈣片) (如有)：___無______

屋企用什麼油煮食：____芥花籽油______

煮食鹽的用量 (鹽, 豉油, 蠔油): ☐淡味 ☑ 普通 ☐ 咸

日常活動及運動: __散步半 小時

睡眠時間: ___晚上 11:00

進食及活動記錄表 (Diet Record & Activity Log Sheet)

請記錄進食之所有食物，包括早餐、午餐、晚餐、湯水、飲品、零食及消夜。請以碗、杯、湯匙、茶匙、大/小塊、大/小件或尺吋大小等，以作記錄之單位。

記錄日期：________年________月________日 (星期________)

				(不用填寫)	
時間	**地點**	**食物/飲品名稱**	**份量**	**編號**	**三天份量**

整日喝了幾多清水：約________ 杯 / 毫升

營養補充劑 (如維他命丸/鈣片) (如有)：________________

屋企用什麼油煮食：______________

煮食鹽的用量 (鹽, 豉油, 蠔油): ☐淡味 ☐ 普通 ☐ 咸

日常活動及運動: ________________________________

睡眠時間: ______________________________

Appendix V: Template of food frequency questionnaire

香港理工大學

The Hong Kong Polytechnic University

飲食問卷調查

Food Frequency Questionnaire

(Subject Number)參加者編號: ____________________

(Date)日期: ____________________

(Interviewer's Number)訪問員編號: ____________________

身高: __________ 體重: __________

蔬菜類/豆類 Vegetables & Beans (1)

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
菜心 Choy Sum	1202										碗 bowls	1 bowl = 150g
芥蘭 Chinese Kale	1245										碗 bowls	1 bowl = 150g
西蘭花 Broccoli	1207										碗 bowls	1 bowl = 150g
莧菜 Chinese Spinach	1211										碗 bowls	1 bowl = 150g
西洋菜 Watercress	1237										碗 bowls	1 bowl = 150g
菠菜 Spinach	1270										碗 bowls	1 bowl = 150g
通菜 Water Spinach	1209										碗 bowls	1 bowl = 150g
韭菜 Chinese Chives	1247										碗 bowls	1 bowl = 150g
豆苗 Pea Shoots	1242										碗 bowls	1 bowl = 150g
蘆筍 Asparagus	1238										碗 bowls	1 bowl = 150g
西芹 Celery	1203										碗 bowls	1 bowl = 150g
蕃薯苗 Sweet potato-green leaves	1250										碗 bowls	1 bowl = 150g
白菜 Chinese white cabbage	1253										碗 bowls	1 bowl = 150g
豆角 Asparagus Bean	1274										碗 bowls	1 bowl = 150g

鮮黃豆 Fresh Soybeans	1244										份 servings	1 serving = 50g
硬豆腐 Tofu, Hard	1552										份 servings	1 serving = 50g
布包豆腐 Tofu, Soft	1552										磚 cubes	1 cube = 300g
腐竹 Dried Tofu Sheets	1553										份 servings	1 serving = 50g
油炸豆腐 Deep Fried Tofu	1576										份 servings	2 pieces = 120g
腐皮 Tofu Skin	1556										份 servings	1 serving = 50g
芥花子 Canola	1501										份 servings	1 serving = 35g
合桃 Walnut	1503										份 servings	1 serving = 35g
亞麻籽 Flaxseed	1502										湯匙 spoons	1 spoon = 35g

蔬菜類/豆類 Vegetables & Beans (2)

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
栗子 Chestnut	1566										粒 pieces	1 piece = 10g
腰果 Cashew Nuts	1564										份 servings	1 serving = 35g
花生 Peanut	1568										份 servings	1 serving = 25g
其他 Others												

菇菌類/藻類 Mushrooms & Algae

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
木耳 Jew's Ear	1147										碟 plates	1 plate = 100g (Wet Weight)
銀耳/雪耳 White fungus	1140										碟 plates	1 plate = 100g (Wet Weight)
黃耳 Golden tremell	1149										碟 plates	1 plate = 100g (Wet Weight)
香菇 Dried mushroom	1144										碟 plates	1 plate = 100g (Wet Weight)
杏鮑菇 King oyster Mushroom	1142										碟 plates	1 plate = 100g (Wet Weight)
金針菇 Winter mushroom	1141										碟 plates	1 plate = 100g (Wet Weight)
紅菇 Vinous Russula	1143										碟 plates	1 plate = 100g (Wet Weight)
海藻/昆布 Seaweed	1211										碟 plates	1 plate = 100g
海帶 Sea-tangle	1182										碟 plates	1 plate = 100g
海苔 Sedge	1185										碟 plates	1 plate = 100g
其他 Others												

魚類及海產類 Fishes & Seafood

1. 請問你在過去三個月有否進食淡水魚? 如有, 請到第三頁。 有 沒有

Did you eat any freshwater fish within the past three months? If yes, please go to page 3

2. 請問你在過去三個月有否進食咸水魚? 如有, 請到第四頁。 有 沒有

Did you eat any saltwater fish within the past three months? If yes, please go to page 4.

3. 請問你在過去三個月有否進食魚生? 如有, 請到第六頁。 有 沒有

Did you eat any sashimi/sushi within the past three months? If yes, please go to page 6.

4. 請問你在過去三個月有否飲魚湯? 如有, 請到第七頁。 有 沒有

Did you have any fish soup within the past three months? If yes, please go to page 7.

淡水魚 Fresh Water Fishes

食物種類 Type of Food	編碼 Code	煮法 Cooking Method	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
			從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
鯇魚 Grass Fish	952											份 servings	1 serving = 200g
鯪魚 Mud Carp	985											份 servings	1 serving = 100g
鰱魚 (大魚) Silver carp (Big Head Fish)	959											份 servings	1 serving = 100g
生魚 Snake Head	984											份 servings	10 slices = 50g
鯽魚 Crucian carp	278											份 servings	1 serving = 100g
桂花魚 Mandarin fish	621											份 servings	1 serving = 100g
金山鯛、非洲鯛 Tilapia, Nile tilapia	561											份 servings	1 serving = 100g
加州鱸、大口鱸 Large mouth bass, Largemouth black bass	1714											份 servings	1 serving = 100g
寶石魚 Jade Perch	937											份 servings	1 serving = 100g
其他 Others													
其他 Others													

咸水魚 Sea Water Fishes (1)

食物種類 Type of Food	編碼 Code	煮法 Cooking Method	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
			從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
烏頭 Grey mullet	981											份 servings	1 serving = 200g
鱔魚 Eel	989											份 servings	10 slices = 50g 1 thick slice =30g
鱸魚 Bass/Perch	982											份 servings	1 serving = 100g
紅衫魚 Golden Thread	953											份 servings	1 serving = 100g
牙帶魚 (斬件) Ribbon Fish	987											份 servings	1 serving = 100g 1 thick slice =30g
三文魚 Salmon	990											件 pieces	1 serving = 100g
九肚魚 Bombay duck	254											份 servings	1 serving = 100g
吞拿魚 Tuna Fish	962	罐頭(大罐)										罐 cans	1/3 can = 50g

吞拿魚 Tuna Fish	963	罐頭(小罐)										罐 cans	1 can = 90g
盲鰽 Barramundi	581											份 servings	1 serving = 100g
石斑 Grouper	914											份 servings	1 serving = 100g
魚立 魚 Seabream	2023											份 servings	1 serving = 100g
沙丁魚、沙甸魚 Sardine and pilchard	1740	非罐頭										份 servings	1 serving = 100g
大眼雞 (Big-eye perch, Red bigeye, Bulls-eye perch	601											份 servings	1 serving = 100g
紅鮪 Snappers	620											份 servings	1 serving = 100g
粗鱗撻沙、撻沙 Largescale tonguesole	630											份 servings	1 serving = 100g
懵仔 (斑腩) Black bonito, cobia	571											份 servings	1 serving = 100g
黃花魚, 白魚或 Croaker	586, 1731											份 servings	1 serving = 100g
鯧魚、鱠魚 Pomfret	2306											份 servings	1 serving = 100g

咸水魚 Sea Water Fishes (2)

食物種類 Type of Food	編碼 Code	煮法 Cooking Method	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
			從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
蘇眉 Humphead wrasse	973											份 servings	1 serving = 100g
馬友 Fourfinger threadfin, Blind tasselfish	1734											份 servings	1 serving = 100g
泥艋 Rabbitfish	1732											份 servings	1 serving = 100g
鯖魚 Mackerel	991											份 servings	1 serving = 100g
其他 Others													
其他 Others													
其他 Others													

魚生 Sashimi

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
日本池魚 Japanese jack mackerel, Atlantic horse Mackerel	1706										片 pieces	1 pieces = 15g
吞拿魚 Tuna	311										片 pieces	1 pieces = 15g
三文魚 Salmons	990										片 pieces	1 pieces = 15g
油甘魚 Yellowtail kingfish, Yellowtail amberjack	587										片 pieces	1 pieces = 15g
章雄 Purple amberjack, Greater amberjack	617										片 pieces	1 pieces = 15g
白鱔 Japanese eel	989										片 pieces	1 pieces = 15g
其他 Others												
其他 Others												
其他 Others												

魚湯/湯渣 Fish Soups & Soup Remains

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
生魚 Snake Head	984										份 servings	1 serving = 100g
鯽魚 Crucian carp	278										份 servings	1 serving = 100g
狗棍 Lizardfish	580										份 servings	1 serving = 100g
牛鰍，沙鰍 Flathead, Bartail flathead	992										份 servings	1 serving = 100g
沙鑽 Japanese sillago	993										份 servings	1 serving = 100g
石狗公 Rockfish	1702										份 servings	1 serving = 100g
紅衫魚 Golden Thread	953										份 servings	1 serving = 100g
大眼雞 Big-eye perch	601										份 servings	1 serving = 100g
其他 Others												
其他 Others												
其他 Others												

海產類 Seafood

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
魷魚 Squid	955										份 servings	7 slices = 50g
生蠔 Oysters	967										份 servings	6 pieces = 50g
蠔豉 Dried Oysters	968										份 servings	10 pieces = 50g
蝦 Prawns	964										份 servings	2 pieces = 25g
蟹 Crabs	969										隻 pieces	1 piece = 100g
帶子/瑤柱 Scallops/Dried Scallops	970										份 servings	3 pieces = 20g
海參 (乾) Sea Cucumbers	976										份 servings	1 serving = 5g
魚丸/魚腐 Fish Balls	958										份 servings	5 pieces = 100g
魚片 Fish Cakes	957										份 servings	4 slices = 50g
墨魚 Cuttlefish	986										份 servings	7 slices = 50g
鯪魚球 Mud Carp Fish Balls	985										份 servings	2 pieces = 50g
罐頭沙丁魚 Canned Sardines	961										件 pieces	1 piece = 50g
罐頭豆豉鯪魚 Fried Dace with Black Bean Sauce	974										件 pieces	1piece = 50g
咸魚 Salted Preserved Fish	977										片 slices	1 slice = 5g
海蜇 Jelly Fish	978										份 servings	1 serving = 50g
其他 Others												
其他 Others												

蛋類 Eggs

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
烚熟雞蛋 Hard Boiled Eggs	1154										隻 pieces	1 piece = 50g
煎雞蛋 Pan Fried Eggs	1155										隻 pieces	1 piece = 50g
炒蛋 Stir Fried Eggs	1153										隻 pieces	1 piece = 50g
奄列 Omelette	1159										隻 pieces	1 piece = 50g
蛋白 Egg White	1152										隻 pieces	1 piece = 35g
蛋黃 Egg Yolk	1151										隻 pieces	1 piece = 15g
皮蛋 Century Eggs	1157										隻 pieces	1 piece = 50g
咸蛋 Salted Duck Eggs	1156										隻 pieces	1 piece = 50g
鵪鶉蛋 Quail Eggs	1158										隻 pieces	1 piece = 10
其他 Others												
其他 Others												
其他 Others												

奶類及飲料 Dairy Products & Beverages

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
全脂牛奶 Whole Milk	76										杯/盒 Cups/packs	1 cup = 250ml 1 pack = 236ml
低脂牛奶 Low-fat Milk	77										杯/盒 Cups/packs	1 cup = 250ml 1 pack = 236ml
脫脂奶 Skimmed Milk	79										杯/盒 Cups/packs	1 cup = 250ml 1 pack = 236ml
朱古力奶 Chocolate Milk	78										杯/盒 Cups/packs	1 cup = 250ml 1 pack = 236ml
全脂奶粉 Whole Milk Powder	82										湯匙 tablespoons	1 Tablespoon = 7g
低脂奶粉 Low-fat Milk Powder	84										湯匙 tablespoons	1 Tablespoon = 7g
脫脂奶粉 Skimmed Milk Powder	83										湯匙 tablespoons	1 Tablespoon = 7g
煉奶 Condensed Milk	80										湯匙 tablespoons	1 Tablespoon = 20g
花奶 Evaporated Milk	81										湯匙 tablespoons	1 Tablespoon = 15g
椰子汁 Coconut Juice	2293										杯 cups	1 cup = 250ml
維他奶 Vitasoy	2261 (s)										盒 packs	1 pack = 250ml
豆漿 Soy Milk	2292										杯/盒 Cups/packs	1 cup = 250ml 1 pack = 236ml
可口可樂 Coca Cola	2281										杯/罐 Cups/cans	1 cup = 250ml 1 can = 330ml
百事可樂 Pepsi	2282										杯/罐 Cups/cans	1 cup = 250ml 1 can = 330ml
七喜 Seven Up	2283										杯/罐 Cups/cans	1 cup = 250ml 1 can = 330ml
忌廉 Cream Soda	2284										杯/罐 Cups/cans	1 cup = 250ml 1 can = 330ml
咖啡 Coffee	2285										杯 cups	1 cup = 250ml
奶茶 Milk Tea	2286										杯 cups	1 cup = 250ml
其他 Others												

小食類 Snacks

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
魷魚絲 Dried Squid Strings	956										包 packs	1 pack = 20g
豆腐花 Tofu Fa	1555										碗 bowls	1 bowl = 200g
合桃酥 Walnut Short Cakes	2040										件 piece	1 piece = 150g
曲奇 Cookies	201										件 piece	1 piece = 20g
蛋卷 Egg rolls	207										條 piece	1 piece = 10g
西餅 Assorted cakes	202										件 piece	1 piece = 80g
薯片 Potato chips	205										包 packs	1 pack = 20g
雪糕 Ice-cream	203										杯 cups	1 cup = 120g
乳酪 Yogurt	204										杯 cups	1 cup = 120g
牛奶布甸 Milk Pudding	120										杯 cups	1 cup = 120g
蛋撻 Egg Tart	302 (l)										件 piece	1 piece = 60g
其他 Others												
其他 Others												
其他 Others												

湯水類 Soups (1)

請問你在過去三個月有否飲用幫助製造乳汁的湯水?　　有　沒有

Did you drink any soup which helps producing breast milk within the past three months?

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
眉豆花生雞腳湯 Split Peas, Peanut & Chicken Paws Soup	J1										碗 bowls	1 bowl = 200ml
節瓜章魚湯 Hairy Melons & Octopus Soup	J1										碗 bowls	1 bowl = 200ml
青菜滾豆腐湯 Vegetable & Tofu Soup	J1										碗 bowls	1 bowl = 200ml
章魚木瓜湯 Octopus & Papaya Soup	J1										碗 bowls	1 bowl = 200ml
章魚蓮藕魚湯 Octopus , Lotus Root & Fish Soup	J1										碗 bowls	1 bowl = 200ml
木瓜花生大棗湯 Papaya, Peanut & Date Soup	J1										碗 bowls	1 bowl = 200ml
章魚花生雞湯 Octopus, Peanut & Chicken Soup	J1										碗 bowls	1 bowl = 200ml
疏籮魚仔木瓜湯 Apogonidae & Papaya Soup	J1										碗 bowls	1 bowl = 200ml
王不留行豬手湯 Semen Vaccariae & Pig Hock Soup	J1										碗 bowls	1 bowl = 200ml
鯽魚木瓜湯 Tilapia & Papaya Soup	J1										碗 bowls	1 bowl = 200ml

黃耆豬腳湯 Astragalus & Pettitoes Soup	J1										碗 bowls	1 bowl = 200ml
通草章魚木瓜湯 Ricepaperplant Pith, Octopus & Papaya Soup	J1										碗 bowls	1 bowl = 200ml
通草豬展湯 Ricepaperplant Pith & Pig Hock Soup	J1										碗 bowls	1 bowl = 200ml
蘋果雪耳山班魚湯 Apple, Snow Fungus & Snakehead Soup	J1										碗 bowls	1 bowl = 200ml

湯水類 Soups (2)

食物種類 Type of Food	編碼 Code	過去三個月的次數 How Often Within the Past Three Months?									每次有多少 How much each time?	參考份量 Reference Portion
		從未 Never	一月少於一次 <1 Time per Month	一月一次 Once per Month	一月二至三次 2 – 3 Times per Month	一星期一次 Once per Week	一星期二次 Twice per Week	一星期三至四次 3 – 4 Times per Week	一星期五至六次 5 – 6 Times per Week	每日 Every day		
豆腐鯽魚湯 Tofu & Tilapia Soup	J1										碗 bowls	1 bowl = 200ml
蕃茄薯仔魚湯 Tomatoes, Potatoes & Fish Soup	J1										碗 bowls	1 bowl = 200ml
黑豆湯 Black Soy Beans Soup	J1										碗 bowls	1 bowl = 200ml
其他 Others												
其他 Others												
其他 Others												

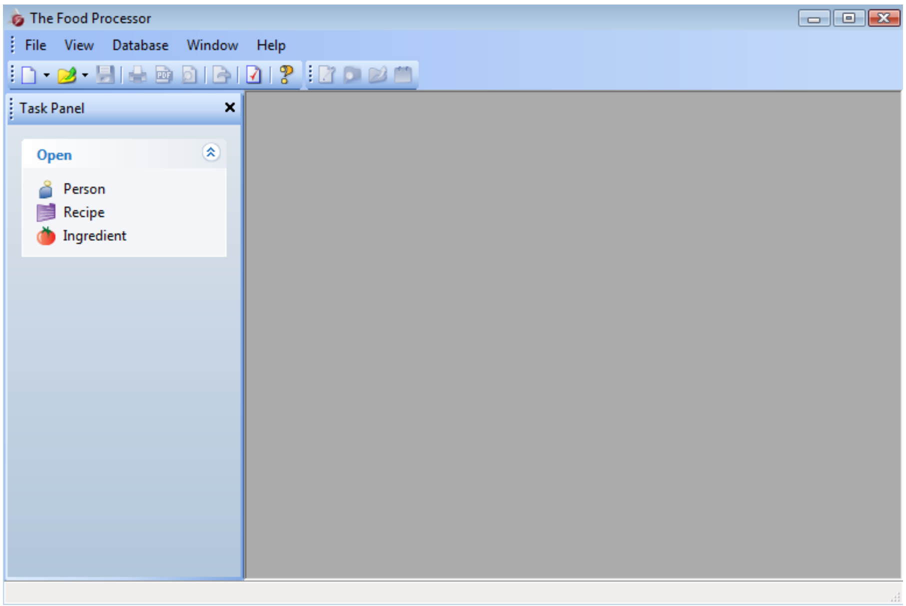
調味料和油 Condiments and Oils

煮食油的用量: 早餐: 如搽麵包或炒飯麵( ) 茶匙 午餐: 如炒□或蒸魚 ( ) 茶匙 晚餐: 如炒□或蒸蛋 ( ) 茶匙 總共: ( ) 茶匙 用那一種油? (請選擇) 粟米 2205 植物油 2203 花生油 2206 芥花子油 2213 橄欖油 2208 豬油 2210 牛油 2201 植物牛油 2202 其他:__________	Cooking Oil Usage: Bkf: eg: Butter on Toast or Fried rice/noodles ( ) tsp Lunch: eg: Stir-Fried or Steam fish ( ) tsp Dinner: eg: Stir-Fried or Steam fish ( ) tsp Total: ( ) tsp Which type of oil? (Please Circle) Corn oil 2205 Vegetable oil 2203 Peanut oil 2206 Canola oil 2213 Olive oil 2208 Lard 2210 Butter 2201 Margarine 2202 Others:__________
煮食鹽的用量 (鹽, 豉油, 蠔油): □ 喜歡食物少許味道 □ 喜歡食物比較咸 □ 喜歡撈汁食飯或外出用膳 □ 喜歡加鹽, 豉油, 蠔油	Cooking Salt Usage: (Salt, Soy sauce, Oyster sauce) Like foods with very light taste Like foods that are salty Like to have sauces over rice or like to eat out Like to add salt, soy sauce and oyster salt

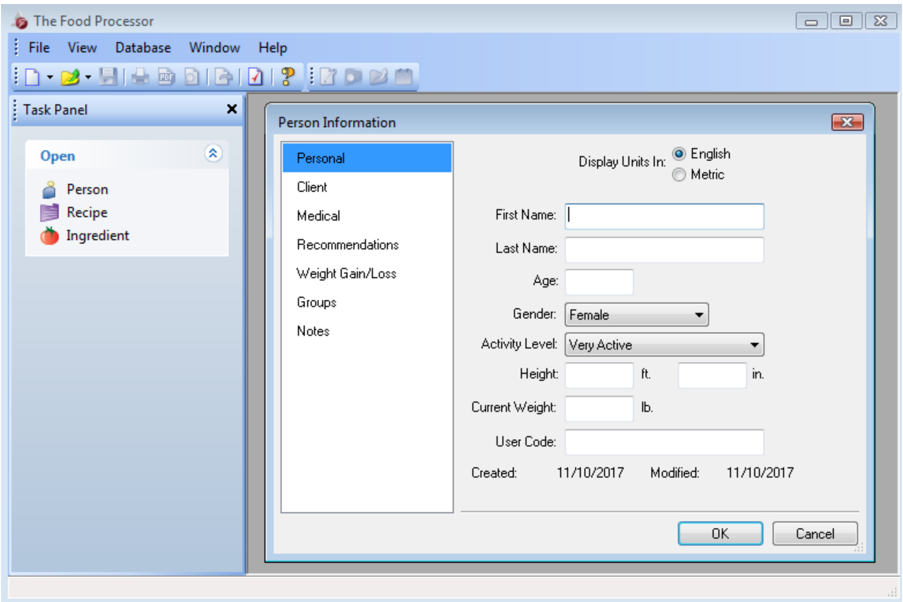
補充劑 品牌 _________ 一星期次數 _________ 每次數量 _________ 種類 _________	Supplements Brand _________ How many times per week? _________ How much each time _________ Which Type _________
中藥 一星期次數 ________ 每次數量 ________ 種類 ________	Traditional Chinese Medicine How many times per week? _________ How much each time _________ Which Type _________

Appendix VI: Procedures of data entry of dietary record to the nutritional analysis software The Food Processor Nutrition Analysis and Fitness software

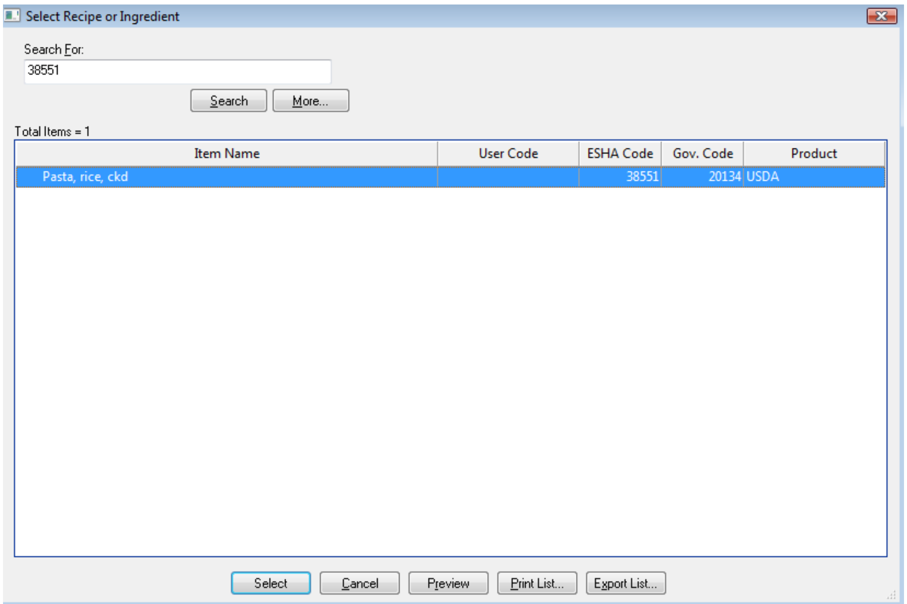
Step 1 Create files for every subjects in the software

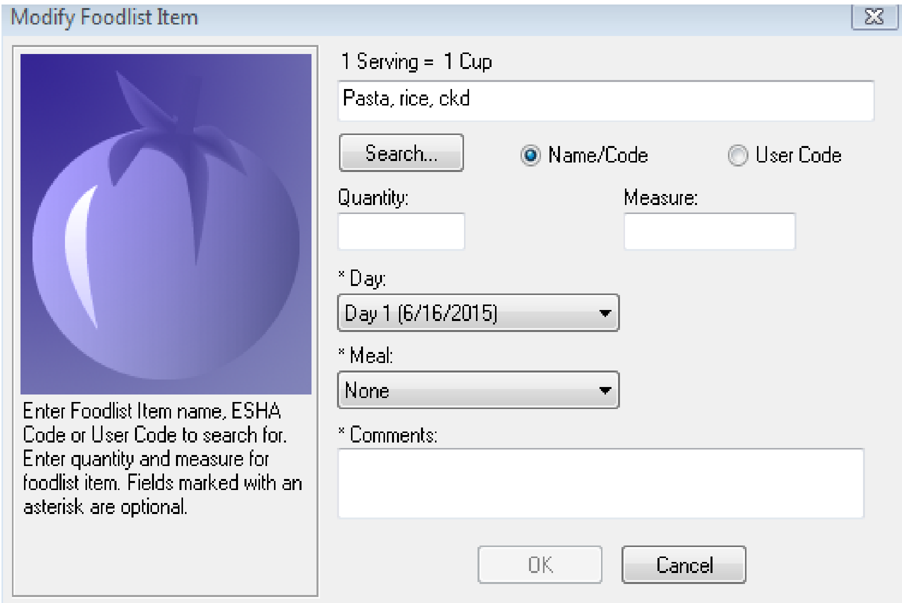


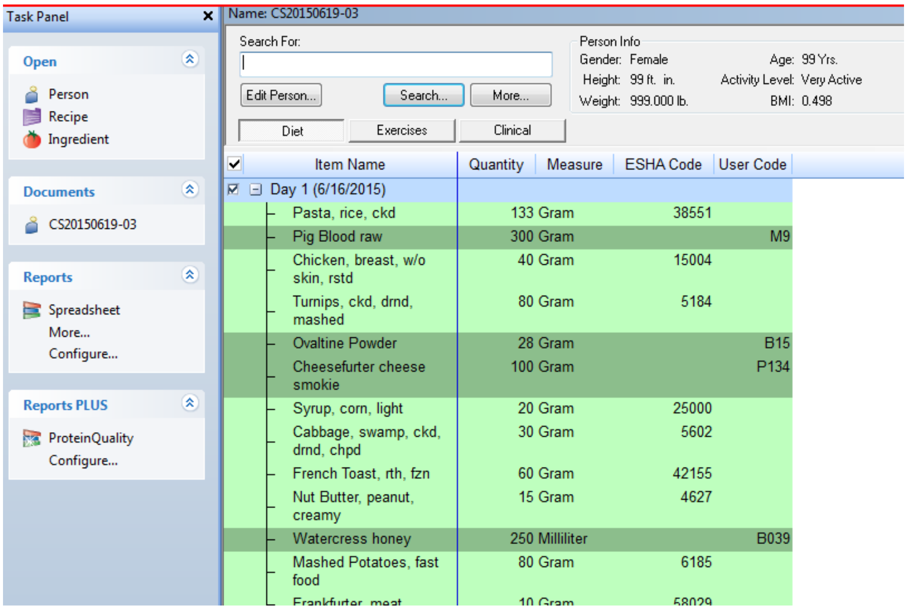
Step 2 Enter subject's personal information



Step 3 Enter subject's 3-day dietary record

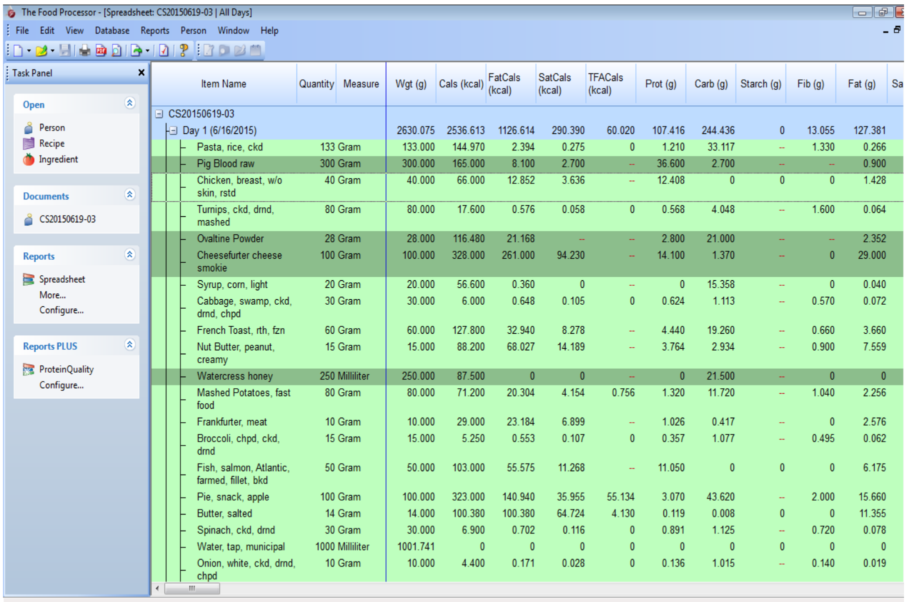






Item Name	Quantity	Measure	ESHA Code	User Code
Day 1 (6/16/2015)				
Pasta, rice, ckd	133 Gram		38551	
Pig Blood raw	300 Gram			M9
Chicken, breast, w/o skin, rstd	40 Gram		15004	
Turnips, ckd, drnd, mashed	80 Gram		5184	
Ovaltine Powder	28 Gram			B15
Cheesefurter cheese smokie	100 Gram			P134
Syrup, corn, light	20 Gram		25000	
Cabbage, swamp, ckd, drnd, chpd	30 Gram		5602	
French Toast, rth, fzn	60 Gram		42155	
Nut Butter, peanut, creamy	15 Gram		4627	
Watercress honey	250 Milliliter			B039
Mashed Potatoes, fast food	80 Gram		6185	

Step 4 Report of 3-day dietary record



Item Name	Quantity	Measure	Wgt (g)	Cals (kcal)	FatCals (kcal)	SatCals (kcal)	TFACals (kcal)	Prot (g)	Carb (g)	Starch (g)	Fib (g)	Fat (g)
CS20150619-03												
Day 1 (6/16/2015)			2630.075	2536.613	1126.614	290.390	60.020	107.416	244.436	0	13.055	127.381
Pasta, rice, ckd	133 Gram		133.000	144.970	2.394	0.275	0	1.210	33.117	--	1.330	0.266
Pig Blood raw	300 Gram		300.000	165.000	8.100	2.700	--	36.600	2.700	--	--	0.900
Chicken, breast, w/o skin, rstd	40 Gram		40.000	66.000	12.852	3.636	--	12.408	0	0	0	1.428
Turnips, ckd, drnd, mashed	80 Gram		80.000	17.600	0.576	0.058	0	0.568	4.048	--	1.600	0.064
Ovaltine Powder	28 Gram		28.000	116.480	21.168	--	--	2.800	21.000	--	--	2.352
Cheesefurter cheese smokie	100 Gram		100.000	328.000	261.000	94.230	--	14.100	1.370	--	0	29.000
Syrup, corn, light	20 Gram		20.000	56.600	0.360	0	--	0	15.358	--	0	0.040
Cabbage, swamp, ckd, drnd, chpd	30 Gram		30.000	6.000	0.648	0.105	0	0.624	1.113	--	0.570	0.072
French Toast, rth, fzn	60 Gram		60.000	127.800	32.940	8.278	--	4.440	19.260	--	0.660	3.660
Nut Butter, peanut, creamy	15 Gram		15.000	88.200	68.027	14.189	--	3.764	2.934	--	0.900	7.559
Watercress honey	250 Milliliter		250.000	87.500	0	0	--	0	21.500	--	0	0
Mashed Potatoes, fast food	80 Gram		80.000	71.200	20.304	4.154	0.756	1.320	11.720	--	1.040	2.256
Frankfurter, meat	10 Gram		10.000	29.000	23.184	6.899	--	1.026	0.417	--	0	2.576
Broccoli, chpd, ckd, drnd	15 Gram		15.000	5.250	0.553	0.107	0	0.357	1.077	--	0.495	0.062
Fish, salmon, Atlantic, farmed, fillet, bkd	50 Gram		50.000	103.000	55.575	11.268	--	11.050	0	0	0	6.175
Pie, snack, apple	100 Gram		100.000	323.000	140.940	35.955	55.134	3.070	43.620	--	2.000	15.660
Butter, salted	14 Gram		14.000	100.380	100.380	64.724	4.130	0.119	0.008	0	0	11.355
Spinach, ckd, drnd	30 Gram		30.000	6.900	0.702	0.116	0	0.891	1.125	--	0.720	0.078
Water, tap, municipal	1000 Milliliter		1001.741	0	0	0	0	0	0	0	0	0
Onion, white, ckd, drnd, chpd	10 Gram		10.000	4.400	0.171	0.028	0	0.136	1.015	--	0.140	0.019

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