University of Southampton

DIETARY IODINE INTAKE DURING PREGNANCY AND BIRTH OUTCOME

by

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ABSTRACT

FACULTY OF MEDICINE
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Humans need iodine to make thyroid hormones which control the body's metabolism and are essential for normal growth and development. The relationship between iodine intake during pregnancy and birth outcome has not been determined. To assess this relationship, a prospective cohort study of 361 women was undertaken at Chiang Mai Province, Thailand. Casual urine samples collected several times throughout pregnancy, and urinary iodine excretion (μg/g creatinine) was used to assess the dietary iodine intake during pregnancy. Casual urine samples were collected from these pregnant subjects at some time from 9 to 12 A.M. and analyzed for creatinine and iodine. Methods for assessing urinary iodine and creatinine excretions were developed. The methods were reproducible and accurate. Birth outcome [birthweight, length, head circumference (HC), chest circumference (CC) and the percentage of head circumference to length (RHL %)] and other factors which may affect birth outcome were recorded. These included social economic status, nutrient intakes and food frequency, maternal age, maternal anthropometry and blood pressure, and gestational age. The results from this study showed that dietary iodine intake (during 11-15 weeks of gestational age) was related to the infant's RHL %. There is a clear mechanistic basis to explain these epidemiological findings.

List of Publications

Abstracts

1. Pruenglampoo S, Leelapat P, Nimsakul S, Tansuhaj A, Sethawanit S, Rugpao S, Chiowanich P, Margetts BM and Jackson AA. (1994). The relationship between dietary iodine intake during pregnancy and birth outcome. *The Proceedings of the Nutrition Society* **53**, 256 A.

2. Pruenglampoo S, Leelapat P, Vongchak T, Likit-ekarat V, Woranuj S and Kumrin T. (1994). The relationship between urinary iodine excretion in 24 h urine collection and casual urine samples in Thai pregnant women. *The Proceedings of the Nutrition Society* **53**, 245 A.

3. Pruenglampoo S, Silprasert A, Leelapat P, Kumrin T, Suwannarach C, Nimsakul S, Likit-Ekaraj V. (1995). Iodine content in foods available in districts of Chiang Mai province, Thailand. *Presented at the Third APFAN Conference on Food Analysis, 22-25 May, Manila, The Philippines*

4. Pruenglampoo S, Leelapat P, Kumrin T, Woranuj S, Nimsakul S, Likit-Ekaraj V, and Silprasert A (1995). Iodine content in rice. *Presented at the 13rd Annual Health Sciences Meeting, 25-26 July, Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand.*

5. Pruenglampoo S, Leelapat P, Kumrin T, Suwannakitti S, and Silprasert A (1995). Iodine content in breast milk at different stages of lactation. *Presented at "Mahidol Day", Annual Scientific Meeting, 22 September, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

In Thailand, infant mortality rate **(IMR)** and the prevalence of goitre are high by international standards. IMR provides an indicator of health risks during the first year of life when an infant is very susceptible to adverse health conditions such as malnutrition and poor sanitation. IMR during 1975-1979, 1980-1984 and 1985-1989 are estimated to have been about 56, 37 and 28 per 1,000 live births respectively in Thailand, compared to rates of 14, 11 and 9 in The UK (*World Population Prospect*, 1990). The term goitre means a bigger thyroid than normal as a result of iodine deficiency and the total goitre rate is the parameter used to assess the iodine deficiency **(IDD)** situation in an area. The IDD survey (1989-1991), covered 252 districts of 53 provinces, conducted by health officials, school health teachers, nutrition officers from Regional Health Promotion centres and the Nutrition Division, indicated that nearly 15 million people in all districts with over 10 % IDD were at risk, while 4.2 million were within the highest risk category (Wanaratna, 1992). Areas where the prevalence of goitre is high tend to be areas where the IMR is also high. Whether there is an etiological link between the two is not clear.

Areas which have high IMR also have a high prevalence of birthweights below 2500 g (**LBW**) (Oruamabo *et al*, 1988; Ales *et al*, 1988 & *World Health Statistics Annual*, 1986). Thus, a decrease in the incidence of **LBW** may also lead to a decrease in IMR. There are also a number of risk factors which are associated with low birthweight in infants. Nutrition is one such factor. Dietary intake during pregnancy influences birth outcome (for example, birthweight), although not in a simple linear relationship (Smith, 1947; Stein & Susser, 1975; Lechtig *et al*, 1975).

This thesis explores the relationship between iodine intake during pregnancy and birth outcome. Before the relationship can be properly explored there is a need to consider the methodological issues involved in assessing iodine intake.

This thesis will be presented in six chapters. **Chapter 1** outlines the hypothesis, background of Thailand, rationale of the study and objective of the study. **Chapter 2** reviews the relevant literature: particularly that concerned with iodine, iodine metabolism, creatinine metabolism, iodine intake and urinary iodine excretion, the correlation between dietary iodine intake and 24-hour urinary iodine excretion, the correlation between 24-hour urinary iodine excretion and casual urinary iodine excretion, low birthweight infant and causation and the evidence for a relationship between iodine and birth outcome. **Chapter 3** describes the methods used in this thesis. It explains the development, validity and reliability of the methods used to determine creatinine and iodine contents in urine samples and iodine content in foods. It describes five experiments which have been done to show whether urinary iodine excretion from a casual urine sample can be used as an indicator of dietary iodine intake and whether there are any factors which may affect the concentration of the ratio of urinary iodine and urinary creatinine (for example, dietary iodine intake, urine volume, dietary creatinine, and the level of protein intake). Finally, **Chapter 3** considers the assessment of birth outcome, the assessment of other factors which may effect birth outcome, the study design and data analysis. **Chapter 4** presents the main findings of the study. **Chapter 5** discusses the results obtained in chapter 4. **Chapter 6** presents the conclusions of this thesis and future work.

CHAPTER 1
INTRODUCTION

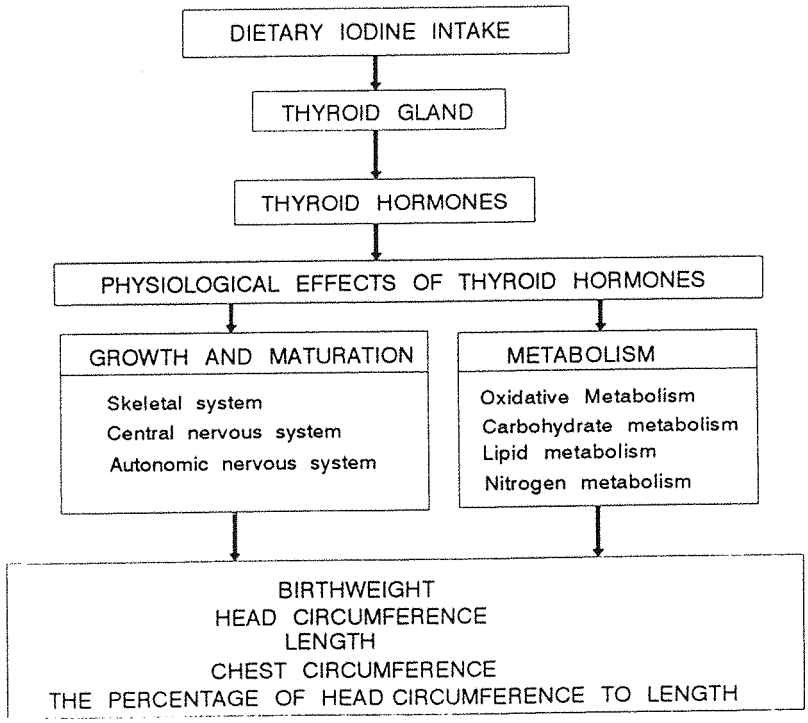
1.1 HYPOTHESIS

GENERAL HYPOTHESIS

Low maternal iodine intake during pregnancy adversely affects the size and shape (birth outcome) of the offspring.

SPECIFIC HYPOTHESES

(1) The lower the maternal dietary iodine intake during pregnancy the lower the birthweight (size) of the offspring.
(2) Lower maternal dietary iodine intake during pregnancy leads to specific effects on the growth of long bones, leading to shorter babies at birth.
(3) Low maternal iodine intake during pregnancy leads to differential effects on differential aspects of fetal growth; head size relative to length.



1.2 BACKGROUND

Thailand is a country in the heart of South-Eastern Asia. It is bordered by Burma to the west, Cambodia to the east, Laos to the north and Malaysia to the south. Thailand is divided into four regions: the mountainous north, the north-east plateau, the central plain and peninsular south. It is a tropical country with three seasons; summer, the rainy season and the cool season. To investigate the hypothesis, a research project was conducted in Chiang Mai, a province in the northern region.

1.3 RATIONALE OF THE STUDY

Good health may be defined as the state of being well, without disease. The development of each country is helped by having a healthy population. The government of Thailand has recognised this relationship. Thus national socio-economic plans have been established to improve the quality of life of the population under the WHO announcement "Health for all by the year 2000". One of the objective indicators of improving health is a decrease in the infant mortality rate (IMR). There is a close relationship between the IMR and birth outcome (for example, the proportion of low birthweight in the population). In addition, it has been found that the lower the birthweight the higher the infant mortality rate (Kadar H, 1983; Raju, 1986). Developed countries have lower IMRs and lower rates of LBW than developing countries (see **Table 1.1**). Shapiro *et al* (1980) reported that infants with LBW have neonatal mortality rates that are 37.5 per 1000 live births, some 1.3 times greater than those with normal birthweight **(NBW)**. Since a LBW infant reflects a small size baby, a LBW infant may have a small head circumference, be shorter in length and have a smaller chest circumference. The LBW rate in

northern Thailand, in 1982, was 12.79 per cent live births (Chaturachinda *et al.*, 1987): the highest rate when compared with the other regions in Thailand (see **Table 1.2**). Annual Provincial Health Report (1989) showed that the incidence of LBW infants in Chiang Mai was 13.63 per cent.

Table 1.1: IMR (per 1000 live births) and the proportion of LBW (per 100 live births) in developed and developing countries

COUNTRY	IMR	LBW
SWEDEN 1980	4.9	4.1
JAPAN 1980	4.9	5.3
U.S.A 1981	7.1	6.8
SINGAPORE 1981	8.9	11.4
MALAYSIA 1980	15.6	11.9
INDONESIA 1981	26.2	18.4
THAILAND 1983*	45.3	12.3
UNITED KINGDOM 1983*	10.0	6.7

Source: Kadar H, 1983.
* = World Health Statistics Annual, 1986

Table 1.2: LBW (per 100 live births) by region (in 1982)

REGION IN THAILAND	LBW
NORTHERN	12.8
NORTHEAST	10.4
CENTRAL	9.3
SOUTHERN	8.2

Source: Chaturachinda *et al.*, 1987.

However, the figures in **Table 1.2** might underestimate the real pattern because some mothers deliver their infants at home or some place other than hospitals or health centres and some parents report neither births nor deaths to the

authorities. These rates of LBW infant are high compared with developed countries and suggest the need for further studies, especially in the northern part of Thailand.
The north of Thailand not only has a high incidence of LBW but it also has a high rate of iodine deficiency. In 1988 a survey of the nutritional status in the north (Thainour, 1989) showed 12.07 per cent of school children had goitre and the prevalence in the endemic area was 43.11 per cent. Since humans need iodine to make thyroid hormones, iodine deficiency may lead to thyroid hormone deficiency. Lack of thyroid hormone produces varying effects, goitre, hypothyroidism and cretinism, depending on the age at which the iodine deficiency occurs. The very severe consequences of hypothyroidism occurring during fetal and neonatal life are called cretinism. Cretins have irreversible mental retardation and many have several characteristics, for example, deaf, mutism, short stature, and retarded development of the musculoskeletal system. Moreover, there is evidence to show that women in severely iodine deficient areas have more miscarriages, stillbirths, and other problems of pregnancy and reproduction than do women in iodine sufficient areas. The above data suggest that there may be a relationship between iodine intake during pregnancy and birth outcome (including birthweight, head circumference, chest circumference, length and the percentage of head circumference to length). To date the possibility of this relationship has not been investigated in detail.

1.4 AIM OF THE STUDY:

To investigate whether there is a positive relationship between dietary iodine intake during pregnancy and birth outcome.

CHAPTER 2

REVIEW OF THE LITERATURE

2.1 INTRODUCTION

The first step of this study was to review the literature concerned with dietary iodine intake and birth outcome. The literature is reviewed in the following sections: iodine metabolism, the relationship between dietary iodine intake and birth outcome, creatine-creatinine metabolism, the relationship between dietary iodine intake and 24-hour urinary iodine excretion, the relationship between 24-hour urinary iodine excretion and urinary iodine excretion from a casual urine sample. Finally, risk factors for low birthweight have also been reviewed.

2.2 IODINE

Iodine is an essential element for normal growth and development in animals and man. Iodine is referred to as a trace element because it occurs in the human body in only small amounts (0.00005 % of the body, dry weight basis). Humans need iodine to make thyroid hormones. These hormones are produced by the thyroid gland. Thyroid hormones control many chemical processes in different parts of the body. They are essential not only for maintenance of body heat and energy but also for normal development and function of the brain and nervous system.

There are two abnormalities of thyroid function. First, **hypothyroid states**, a deficiency of thyroid hormone produces a number of clinical states depending upon the degree of the deficiency and the age at which it occurs, for example, cretinism, juvenile myxoedema, Hashimoto's disease and simple goitre (the enlargement of thyroid gland plus the low level of thyroid hormone). Second, **hyperthyroid states**, toxic goitre (Graves'disease) is enlargement of the thyroid gland which is accompanied by

the secretion of excessive amounts of thyroid hormone. The term 'toxic' refers to the toxic symptoms which include nervousness, fatigue ability, loss of weight, increased body temperature with excessive sweating, and increase in the heart rate.

2.2.1 Source of iodine

2.2.1.i Iodine in water

The level of iodine in water reflects the iodine content of the rocks and soils of a region and also reflects the iodine content of locally grown foods or feeds. Iodine also has an association with goitrous and nongoitrous areas. This relationship is illustrated by the fact that the iodine content of water from goitrous areas is lower than that from nongoitrous areas. For example, Karmarkar *et al.* (1974) showed that the water from goitrous areas in India, Nepal, and Ceylon to range from 0.1 to 1.2 μgI/litre, compared with a nongoitrous area in Delhi of 9.0 μgI/litre. These were the same as in Thailand (Wanaratana, 1992), see **Table 2.1**. It was found that the iodine concentration in water of goitrous areas (northern and northeast) was smaller than that found in nongoitrous areas (central and south). Therefore, iodine concentration in water may be a useful index to the location of goitrous areas.

Table 2.1: The iodine concentration in water and soils, by region in Thailand.

Region/province	Water (μg/l)	Soils (μg/kg)
Northern		
Chiangmai (Ping river)	2.0	295.0
Nan (Nan river)	2.4	240.0
Lampang (Wang river)	4.7	86.5
Prae (Yom river)	3.4	162.0
Northeast		
Nongkai (Mae Kong River)	4.0	128.0
Kon-gan	3.7	80.5
Central		
Bangkok (Chaopraya river)	12.0	1,780.0
Thonburi (Chaopraya river)	11.5	1,890.0
Nakorn-Pratom	8.6	1,060.0
Ayudthaya	11.0	1,232.0
Southern		
Tarad	31.0	3,920.0
Rayong	36.5	5,965.0
Pang-nga	19.5	1,865.5
Prajuab	21.5	1,468.0

Source: Wanaratana, 1992.

2.2.1.ii Iodine in foods

Generally, Foods of marine origin are rich in iodine whereas for the other foods, the amount of iodine in food stuffs depends on the concentration of iodine in the soil of the region in which they are produced (see **Tables 2.2, 2.3**). It also depends on the amount and nature of the fertilizers applied, for example, Chilean nitrate of soda, the only mineral fertilizers naturally rich in iodine, can double or triple the iodine content of food crops when applied in the amounts required to meet their nitrogen needs (Underwood, 1977).

Table 2.2: Iodine content (μg/kg wet weight) of raw vegetables grown in different areas of Britain.

VEGETABLES	BOSTON	CAMBRIDGE	TRURO	PRESTON
Potato	20	<10	<10	80
Cabbage	<10	<10	<10	40
Broccoli	<10	20	-	50
Carrots	10	<10	70	10

Source: Wenlock *et al*, 1982.

Table 2.3: The comparison of iodine content (μg/kg dry weight) in some vegetables between Prae province (Northern region) and Bangkok (Central region).

SPECIMENS	PRAE	BANGKOK	RATIO
Squash (tops and young leaves)	99.2	925	1:13.4
Chilli pepper	58.5	915	1:15.6
Ivygourd (leaves)	70.7	632	1:8.9
Yard-long beans (green)	77.3	461	1:6.0

Source: Wanaratana, 1992.

Iodine content in some vegetables in **Table 2.3** are higher than those in **Table 2.2**. Because first, iodine content of vegeatables in **Table 2.3** are expressed as μg per kg dry weight (water content in vegetable samples were evaporated by a lyophilyzer before they were analyzed for iodine content), and second the vegetables in **Table 2.3** were not the same as the vegetables in **Table 2.2**.

In 1981, the basis of a food composition table of iodine in British food was developed by Wenlock *et al*.(1982), see **Table 2.4**. They reported that milk was the most variable, as well as the most important, individual source of iodine, as shown in **Table 2.5**. Milk products, including butter and cheese, and eggs were also rich in iodine.

Table 2.4: Iodine content of British Foods

FOOD GROUP	IODINE CONTENT (μg/kg) Mean	Range
Cereal	140	50-280
Meat	240	70-580
Fish	750	320-1440
Milk	230	50-550
Fats	220	80-420
Root vegetables	80	<20-280
Other vegetables	80	<20-260
Fruit and sugars	550	<30-3800
Beverages	20	<10-120

Source: Wenlock et al, 1982.

Table 2.5: Estimated average daily iodine intake in Britain, 1977-1979

FOOD GROUP	ESTIMATED WEIGHT OF FOOD EATEN (kg/d)[1]	ESTIMATED IODINE INTAKE (μg/person/d)[2]
Cereal	0.23	31
Meat	0.15	36
Fish	0.02	15
Milk	0.40	92
Fats	0.08	17
Root vegetables	0.18	15
Other vegetables	0.11	8
Fruit and sugars	0.17	93
Beverages	0.12	16
TOTAL	**1.46**	**323**

Sources:
1. Buss et al, 1978.
2. Wenlock et al, 1982.

Thailand still lacks data on the iodine content of foods; however, iodine content in some crops, fruits and vegetables in Bangkok have been analyzed, as shown in **Appendix 1**.

2.2.2 Dietary iodine intake

It is difficult to assign normal limits to the daily dietary intake of iodine, because this varies widely throughout the world, depending on the iodine of soil, land and water and upon culturally established dietary preferences. For example (Labhart, 1974), in most areas of the United States, dietary iodine intake now approximates 500 μg daily whereas in Japan, intakes were as high as a few milligrams per day. The average daily intake of iodine in Britain was estimated by Wenlock *et al.*(1982), using their food analysis table and some data of Buss and Lindsay (1978), to be around 323 μg/person per day (**Table 2.5**). A recent analysis of British diets based on seven day weighed inventories (Gregory *et al.*, 1990) showed mean levels of iodine intake for men of 243 μg/d and for women 176 μg/d. Iodine intakes in other areas of the world were substantially less than those of the United States and United Kingdom. In regions of iodine deficiency, it might be as low as 10 μg per day.

2.2.3 General distribution

The healthy human adult body contains a total of 15-20 mg, of which 70-80 per cent is present in the thyroid gland. The remainder is distributed in the skeletal muscles and other tissues, for example, brain, testes, ovary, lymph nodes, kidney, lung and liver.

Both inorganic and organic forms of iodine were found in

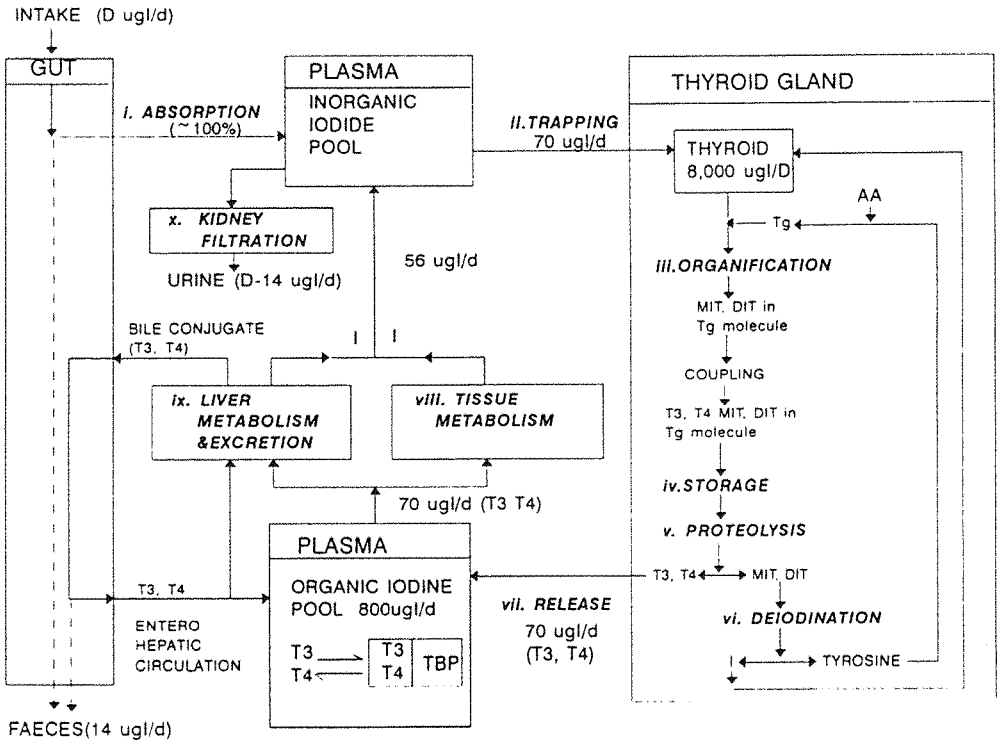
blood. Wayne *et al.*(1964), stated that the normal range of plasma inorganic iodide (PII) to be 0.08-0.60 μg/100 ml. The organic iodine of the blood is present mainly as thyroxine bound to the plasma proteins. Only 0.05 per cent is free in human serum (Sterling & Bremner, 1966).

The iodine concentration of milk was greatly influenced by dietary iodine intake. It was shown that, in an animal experiment, at an intake of 1.6 mg I/cow/day the milk contained 28 ± 6 μg I/litre, at 12.7 mg/day it was 78 ± 18, and at an intake of 20 mg/day it was 267 ± 55 μg/litre (Underwood, 1977).

2.2.4 Iodine metabolism in the normal adult

Iodine balance (supply and demand) is maintained from dietary sources, i.e food and water. Iodine may enter the body via medications, diagnostic agents and food supplements. Iodine metabolism is shown in **Figure 2.1**. Ten key steps are described in more detail: these key steps are highlighted in italics in the figure.

Figure 2.1: Iodine metabolism and thyroid hormone synthesis



2.2.4.i Iodine absorption

Iodide from food and drinking water is absorbed by the small intestine as inorganic iodide (Small *et al.*, 1961).

But the other forms of iodine are reduced to iodide prior to absorption (Cohn, 1932). Iodide administered orally is rapidly and almost completely absorbed from the tract, with little appearing in the faeces (Vough *et al.*, 1963). From the blood stream the iodide rapidly diffuses into the extracelluar fluid (ECF) of the tissues. The concentration of iodide in the ECF is approximately 1.0-1.5 μg/100 ml, indicating a peripheral pool of approximately 250 μg. This is due to the volume of the ECF of about 20 litres.

2.2.4.ii Iodide trapping

Inorganic iodide of the extracelluar fluid (ECF) is actively transported across the basal thyroid cell membrane against an electrochemical gradient. This active transport is known as the iodide pump and is a carrier-mediated, energy requiring mechanism linked to the Na-K-dependent ATPase system (Slaunwhite, 1988). Hence, anything that interferes with ATP production, such as hypoxia, hypothermia or ingestion of cyanide will inhibit uptake. The iodide pump can be competitively inhibited by similar-sized monovalent anions such as thiocyanate, nitrate and perchlorate whereas TSH enhances active iodide transport after a lag of several hours (Tong, 1974). Iodide removed from the plasma by the thyroid is reversible because it can be free to diffuse out of the cell into the circulation. It does so at the rate of approximately 40-50 μg daily in normal subjects.

Under normal circumstances, the thyroid contains the largest pool of body iodine approximately 8,000 μg with daily turnover of about 70 μg/d (Wayne *et al.*, 1964; Colard *et al.*, 1965; Oddie *et al.*, 1970; Ingbar and Woeber,1981).

2.2.4.iii Organification

Organification refers to the conversion of inorganic iodide to an organic form. There are five steps as follows:

(a) Iodide oxidation

First, iodide must be oxidized to some more electrophilic intermediate substance before it can be incorporated into thyroglobulin. Molecular iodine [I] (Mayberry *et al.*, 1969), a free iodine radical [I*] (Nunez & Pommier, 1969) and iodinium ion [I+] (Harington, 1944) are all possible intermediates. Then it is released at the cell/colloid interface, which is presumably where the subsequent iodinations occur. This oxidation occurs at microvilli under the action of a membrane-bound peroxidase enzyme with hydrogen peroxide acting as the electron acceptor. This stage may be blocked by carbimazole, methimazloe, thiourea and related compounds (Whitby, 1988).

(b) Thyroglobulin synthesis

Thyroglobulin is synthesized within the follicular cells. It is a glycoprotein (mol.wt.660,000) and sometimes it is called 'prethyroglobulin' as it is not yet iodinated.

(c) Exocytosis

This is the process where membrane-bound vesicles containing prethyroglobulin pass from the follicular cells through the apical cell membrane into the colloid.

(d) Iodination

The iodinating intermediate from (a) is rapidly converted

into thyroglobulin. The iodination takes place on the 3 and 5 positions of tyrosyl residues of the protein and not on the free amino acid tyrosine (Taurog, 1974). This process forms monoiodotyrosine (MIT, at the 3 position)). A further iodination of MIT at the 5 position yields di-iodotyrosine (DIT). The cell membrane contains numerous microvilli, which might be organelles involved in iodination. This stage can be blocked by thiouracil and by sulphonamide (Whitby, 1988). Taurog (1970) and Pommier *et al.*(1973) reported that excess iodide can inhibit iodination.

(e) Coupling

Thyroid peroxidase catalyses intramolecular coupling of MIT and DIT to produce thyronines. The coupling of two DITs produces T_4 (thyroxine) whereas MIT and DIT residues interact to form T_3 (3,5,3'-triiodothyronine) with smaller amounts of rT_3 (3,3',5' triiodothyronine) and 3, 3'- T_2 (3,3'diiodothyronine). The T_3 appears to be a minor pathway of triiodothyronine biosynthesis. A large part of T_3 is produced peripherally by the monodeiodination of thyroxine.

2.2.4.iv Storage

The thyroglobulin molecule containing the iodothyronines, T_4 and T_3, is sequestered within the follicular lumen. This colloid serves as a storage depot for thyroid hormone and provides prolonged protection against depletion of circulating hormone even in the absence of thyroxine biosynthesis.

2.2.4.v Proteolysis

Under the stimulation of TSH, formation of pseudopodia is evident at the apical surface of the follicular cell,

followed by endocytosis of colloid to yield colloid droplets. These droplets are called phagosomes. Fusion of phagosomes with lysosomes yields phagolysosomes. Hydrolysis of thyroglobulin is thought to occur in the phagolysosomes. It appears that hydrolysis is facilitated by reduction of disulfide bonds in thyroglobulin. This is effected by a transhydrogenase that uses reduced glutathione (GSH). The availability of GSH, in turn, depends on the activity of a second enzyme, glutathione reductase, that uses NADPH to reduce oxidized glutathione. Finally, this process releases amino acids (which may be reincorporated into new thyroglobulin), MIT, DIT, T_4 and T_3.

2.2.4.vi Deiodination of iodotyrosines

After proteolysis, the free iodotyrosines (MIT, DIT) are deionized by microsomal iodotyrosine dehalogenase. The iodine liberated from the iodotyrosines may contribute to a second iodide pool within the thyroid.

2.2.4.vii Secretion

Secretion of T_3 and T_4 occurs by diffusion down the concentration gradient from cell to plasma whereas MIT, DIT and thyroglobulin do not. The thyroid gland secretes a total of about 70 μg of T_4 and T_3 per day. In blood, there are 2 important points about the transport of T_4 and T_3. Firstly, normally > 99.5 per cent of hormone is protein bound. Secondly, only the free (unbound) hormone can cross cell membranes and affect intracellular metabolism.

Thyroid hormones are transported in plasma (T_4, 4-12 μg/dl; T_3, 0.06-0.18 μg/dl), almost entirely, reversibly, to plasma proteins. They are thyroxine binding globulin (TBG, with very high binding affinity, binds about 75 per cent T_4 and

75 per cent T_3), thyroxine-binding prealbumin (TBPA, low binding affinity, binds 15 per cent T_4) and albumin (very low binding affinity, binds 10 per cent T_4 and 25 per cent T_3). Absolute concentrations of free T_4 and T_3 are 1.2-3.6 ng/dl and 0.2-0.5 ng/dl respectively.

2.2.4.viii Peripheral conversion of T_4 into T_3 and deiodination of thyroid hormones by peripheral cells

In plasma, protein-bound hormone is in equilibrium with free hormones which enter cells of the liver and other tissues. In tissues, 80 per cent of the T_4 is deiodinated to yield T_3. About half the deiodinated T_4 joins the tissue pool of T_3.

The liver is the most important organ for the deiodination of T_4. It is thought that, in the liver, a single microsomal enzyme is responsible for the production of T_3 or rT_3 (the hormonally inactive 3,3,'5'triiodothyronine). There is strong evidence that T_3 is the biologically active thyroid hormone (Whitby *et al.*, 1988). The iodide from deiodination is excreted in the urine or recirculated to the thyroid.

2.2.4.ix Conjugation of thyroid hormones with glucuronide in the liver

About 20 per cent of the organic iodine leaving the plasma is excreted in the bile , either free or after conjugation in the liver with glucuronate or sulphate. A proportion of the free or conjugated T_4 and T_3 entering the gut in the bile are reabsorbed (the enterohepatic circulation of T_4 and T_3), the rest is lost in the faeces. Therefore most of the iodine in faeces is in organic form. Some may come from food stuffs which have not been liberated in absorbable form during digestion. Wayne *et al.*(1964) reported that six subjects, with no evidence of thyroid disease, had a

mean value of faecal excretion of iodine of 17.5 ± 4.6 μg/d and a range of 4-33 μg.

However, faecal loss of organic iodine may be excessive when GI absorption is impaired (Hiss & Dowling, 1962), for example, chronic diarrhoeal states or under the influence of certain dietary constituents, such as soya bean products (Pinchera *et al.*, 1965).

2.2.4.x Excretion by kidneys

Iodine is excreted mainly in the urine. Wayne *et al.* (1964) demonstrated that in normal adults the greater part of iodine entering the body is excreted in the urine and it is almost entirely in the inorganic form.

Regarding the clearance of iodine from the blood by kidneys, renal iodine clearance is the rate at which the plasma is cleared of iodine by the kidneys. Generally, the renal clearance is 5 to 55 ml/min, with an average of 35 ml/min (Labhart, 1974).

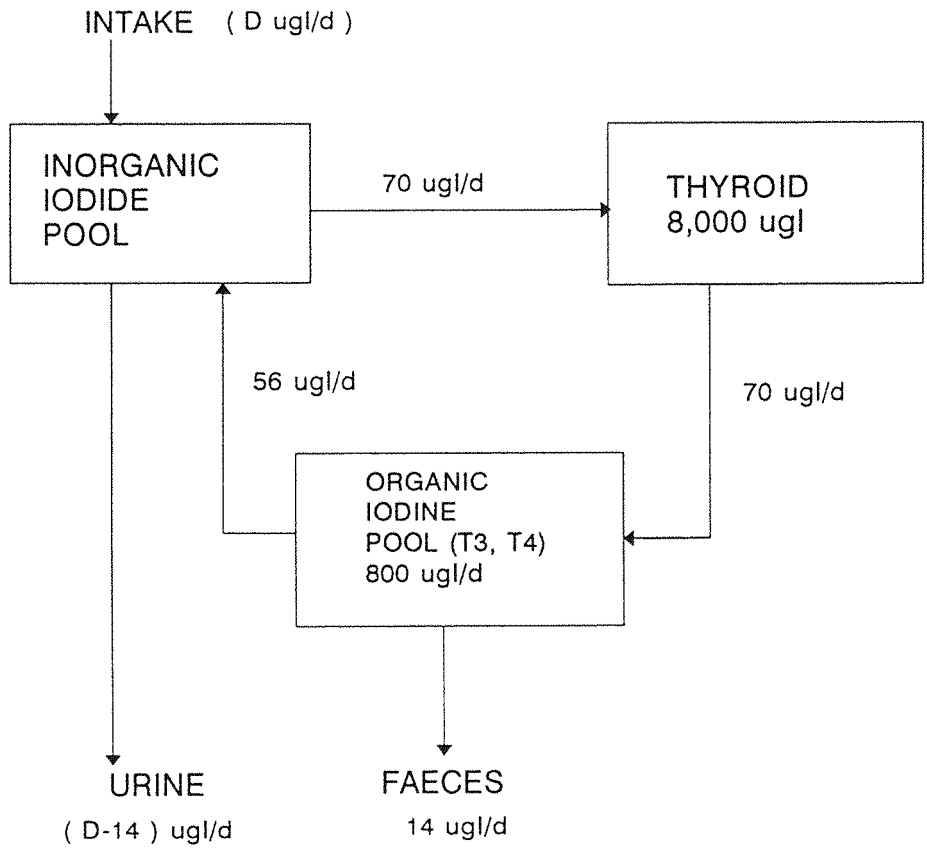
The plasma inorganic iodine bears an inverse relation to the renal clearance of iodine. Plasma iodide is filtered in the renal glomerular and partially reabsorbed in both proximal and distal portions of the renal tubule (Williamson *et al.*, 1962). Thus the iodine in the urine of normal persons is mostly in **inorganic form**.

Under abnormal circumstances, substantial losses of iodine may occur. For example, in nephrosis syndrome or other protein uric states, T_4 and T_3 are excreted in the urine associated with their transport proteins. Iodinated tyrosine is lost in the urine in the disorder in which the enzyme iodothyrosine dehalogenase is lacking from both the

thyroid and peripheral tissues.

A simplified schematic diagram of iodine metabolism is shown in **Figure 2.2**.

Figure 2.2: Iodine metabolism



Source: Stather and Greenhalgh, 1983

In brief, the average normal adult gland contains about 8,000 μg I with a daily turn over of about 70 μg/d. Most of the organic iodine (mainly as thyroxine) which enters the blood from the thyroid is metabolised in tissues and is returned to the plasma pool as inorganic iodide. Thus, some of the iodine will be recycled back to the gland.

The average value of the organic iodine level in the body is 800 μg (Wayne *et al.*, 1964; Colard *et al.*, 1965; Ingbar and Woeber, 1981). About 20 per cent of iodine in the organic form is excreted in the faeces; it is derived from thyroxine which has been conjugated with glucuronic acid and sulphuric acid in the liver and secreted into the gastrointestinal tract in the bile (Stather and Greenhalgh, 1983). Finally, the major part of the iodine entering the body is excreted in the urine and it is mostly in the inorganic form.

2.2.5 Regulation of thyroid function

2.2.5.i Thyroid stimulating hormone (TSH)

The output of TSH from the anterior pituitary is controlled by thryrotrophin-releasing hormone (TRH), a tripeptide secreted by the hypothalamus. Hormone synthesis in the thyroid is controlled predominantly by plasma TSH. In turn, TSH output is regulated by the negative feed back effects of free T_3 and free T_4 acting on both the hypothalamus and the pituitary (see **Figure 2.3**).

2.2.5.ii Temperature

Exposure to cold causes a release of thyroid hormone, but this is probably mediated through TSH release by the pituitary.

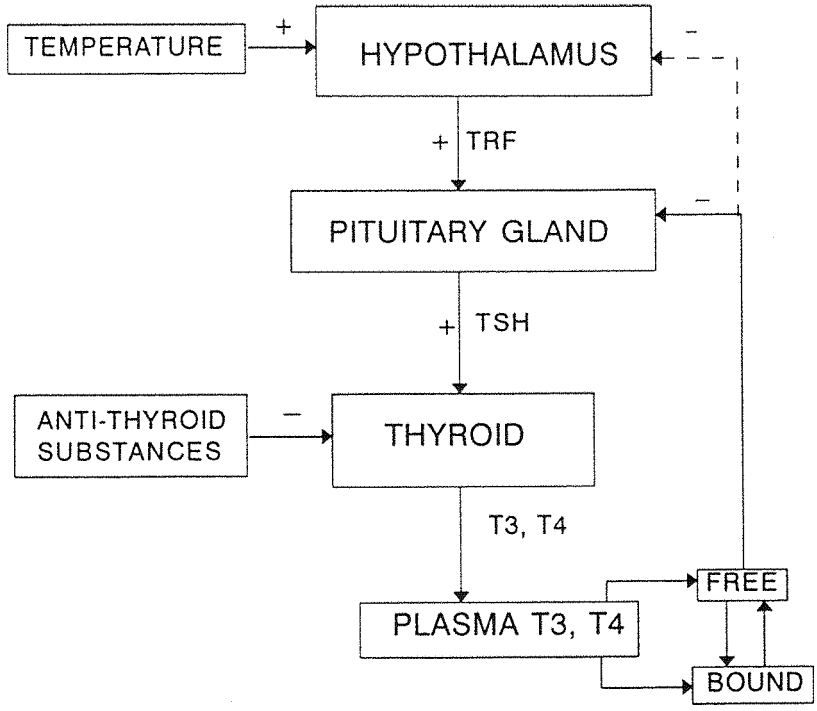
2.2.5.iii Iodine

Iodine itself is an important autoregulator in thyroid gland function. Decreased iodide rapidly increases subsequent iodine uptake and enhances breakdown of thyroglobulin to T_4.

2.2.5.iv Antithyroid substances

Antithyroid drugs can inhibit the production of thyroxine both at the organification and at the coupling steps. Examples of these antithyroid drugs are the goitrogens; propylthiouracil, methylthiouracil, thiouracil, carbimazole, thiourea and methimazole.

Figure 2.3: The hypothalamic-pituitary-thyroid axis.



Source: Adapted from Hawker, 1978.

2.2.6 Iodine requirement

2.2.6.i Iodine requirement in normal adult

There are various methods of determining the requirements of iodine, for example, determinations of the average daily urinary losses of iodine, balance studies and calculation of iodine requirements based on quantitative studies of iodine metabolism.

Calculations based on average daily losses of iodine in the urine give an adult human requirement of 100-200 μg/day (Curtis *et al.*, 1937).

For balance studies, if the intake is known and the excretion in the urine, faeces, sweat and breath can be measured, a balance can be calculated. The results of balance studies (Cole and Curtis, 1935; Flickinger, 1951; Harrison *et al.*, 1965) indicate that equilibrium or positive balance could be achieved between 44 to 162 μg/day.

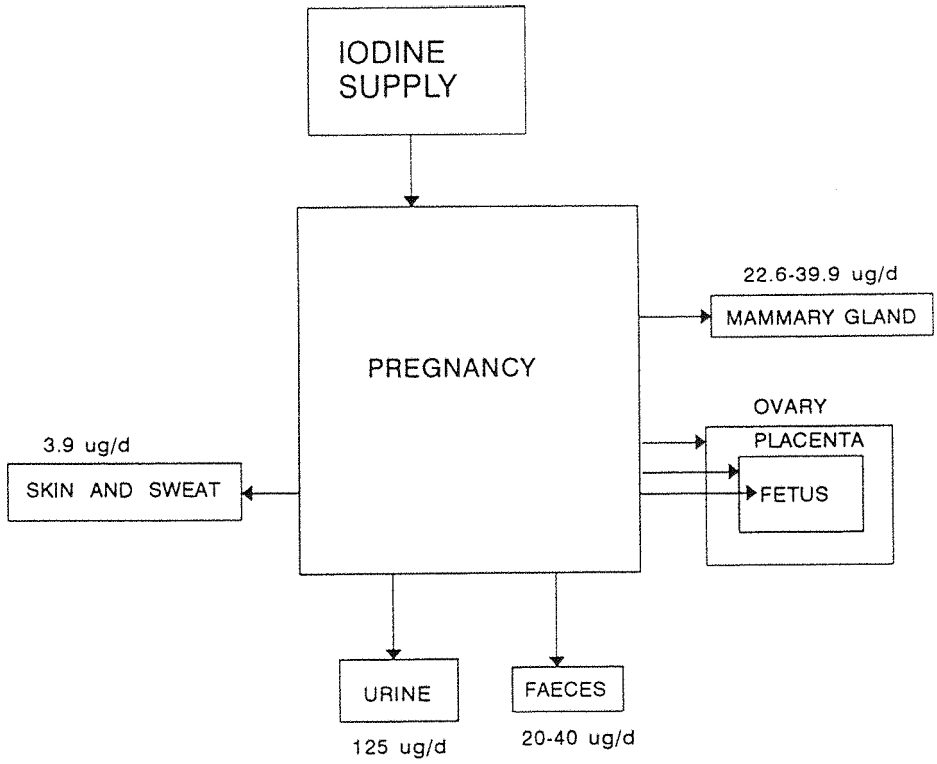
By calculating the iodine requirements based on quantitative studies of iodine metabolism, Wayne *et al.* (1964), concluded that the minimum safe amount of iodine which had to be in the individual's diet was 160 μg/day. The definition of the minimum iodine requirement in this calculation was the amount of iodine intake needed to maintain the plasma inorganic iodide (PII) within the range of 0.08-0.60 μg per cent, and so avoid the formation of an iodine deficiency-goitre. Although, the lower limit of the normal PII was 0.08 μg per cent, there was overlap between the normal range and that observed in iodine deficiency-goitre. Thus the minimum PII value which protected a person from iodine deficiency-goitre was increased to be 0.10 μg per cent. With a renal clearance of iodide of 34 ml per minute (Wayne *et al.*, 1964), the 24-hour urinary excretion of iodine at PII of 0.10 was 50 μg (0.10x34x24x60/100). If faecal iodine excretion was 20 μg/day, the minimum iodine requirement should be 70 μg/day. Since both the renal clearance of iodide and the faecal iodine excretion had a wide normal range. The renal clearance of iodide and faecal iodide excretion might reach 55 ml per minute and 40 μg/day respectively. Therefore, a minimum iodine intake of 120 μg/day is required to maintain

the PII at the lower levels of normal range (0.10 μg per cent). However, considering the absolute rather than the safe minimum, Wayne *et al.* (1964) suggested that, it should be better to aim at PII level of 0.15 μg or more.
Therefore, the safe level of iodine intake was 160 μg per day.

2.2.6.ii Iodine requirement in normal pregnancy

The iodine requirements of the normal, healthy, resting adult might be greatly increased in various functional activities and disturbances (Underwood, 1977). For example, strenuous physical exercise, fever, and infection could increase the demand. There is evidence that the requirement is greater during pregnancy and lactation. In pregnancy, the renal clearance may reach 58 ml per minute (Aboul-khair *et al.*, 1964). A significant rise in the protein-bound iodine of the serum of pregnant women has been reported (Dowling, Freinkel and Ingbar, 1959; 1960). Moreover, there are the additional demands of the foetal thyroid gland and other tissues, and of the mammary glands. Thus, the iodine requirement during pregnancy may rise to 200 μg per day although this has not been objectively determined in balance studies. The fate of iodine during pregnancy is shown in **Figure 2.4**.

Figure 2.4: The fate of iodine in normal pregnancy



Iodine requirements are also influenced by the presence of goitrogens and other elements in diet (Underwood, 1977). The goitrogenic substances, for example, thiocyanates, perchlorates are known to interfere with iodine uptake by the thyroid gland. The elements, for example, rubidium arsenic, fluoride, calcium and selenium may be associated with iodine metabolism.

The United States National Research Council (1980) and Dietary Reference Values for Food Energy and Nutrients for the United Kingdom (1991) recommend iodine intake for healthy people, which depends on age, gender and physiological status as shown in **Table 2.6**.

Table 2.6: Recommend iodine intake for healthy people
$\mu g/d$ ($\mu mol/d$)

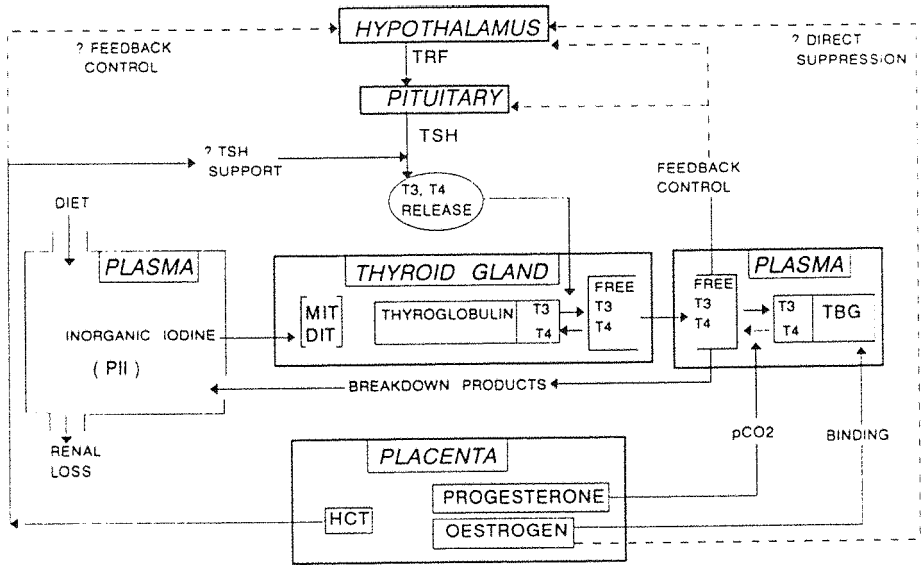
SUBJECTS	US RDA (1)		UK RNI (2)	
	age	iodine	age	iodine
Infants (months)	<3	40	0-3	50(0.4)
	3-5	40	4-6	60(0.5)
	6-8	50	7-9	60(0.5)
	9-11	50	10-12	60(0.5)
Children (years)	1-3	70	1-3	70(0.6)
	4-6	90	4-6	100(0.8)
	7-9	120	7-10	110(0.9)
Boys (years)	10-19	150	11-14	130(1.0)
			15-18	140(1.1)
Girls (years)	10-19	150		
Men (years)	20-60+	150	19-50	140(1.1)
			50+	140(1.1)
Women (years)	20-60+	150		
Pregnant		+25	-No increment	
Lactating		+50	-No increment	

Source: (1) Food and Nutrition Board, National Research Council, 1980.
(2) Dietary Reference Values for Food Energy and Nutrients for the United Kingdom, 1991.

2.2.7 Iodine metabolism in normal pregnancy

In **section 2.2.3**, the details of the metabolism of iodine and of the chemistry and behaviour of the thyroid hormone were explained. This section focuses on the changes in thyroid physiology during pregnancy. A scheme with particular attention to the special relations of pregnancy is shown in **Figure 2.5**

Figure 2.5: Iodine metabolism and thyroid metabolism in normal pregnancy



Source: Adapted from Hytten and Leitch, 1971.

Considering **Figure 2.5**, iodine is the essential inorganic for thyroid hormone manufacture. Over the day, the plasma inorganic iodine (PII) fluctuates widely with food intake. How the fasting level is controlled is not known (Hytten and Leitch, 1971). Losses of iodine in urine vary with dietary intake, and uptake by the gland varies inversely with customary intake, as intake rises above deficiency level (Oddie *et al.*, 1968; Fisher and Oddie, 1969; Oddie *et al.*, 1970). In pregnancy, renal clearance is greatly increased, but the increase is balanced by a low fasting level of PII; so that the absolute loss of iodine is not changed (Aboul-Khair, 1964; Hyntten and Leitch, 1971).

There are three placental hormones which play an important part in thyroid metabolism during pregnancy; oestrogen, progesterone and chorionic thyrotrophin. **Oestrogen** raises the globulin content of the plasma. Because of this, the total thyroxine binding capacity of TBG may be doubled, from a mean 23 to 50 μg per 100 ml (Keane, Pegg and Johnson, 1969), increasing the carrying capacity for the extra hormone released. Thus, both the total circulating hormone and the concentration of bound hormone (PBI) are raised by something between 50 to 100 per cent (Dowling, Freinkel and Ingbar, 1960). Since the total circulating hormone is raised in pregnancy it might be expected that the rate of uptake of iodine by the gland would be raised also (Aboul-Khair *et al.*, 1964). **Progesterone** induces overbreathing in pregnancy which correlates to a state of low alveolar and arterial partial pressure of carbon dioxide (pCO_2). The low pCO_2 is associated with low levels of free hormone (Malkasian and Tauxe, 1965; Tulloch, 1966; Standeven, 1969). Low levels of pCO_2 and free hormone are found as early as the second month of pregnancy (Hytten and Leitch, 1971). A low level of free thyroxine may permit a rise in the secretion of TSH, but a rise is not always apparent (Raud and Odell, 1969; Lemarchand-Be'raud and Vannotti, 1969). In addition, Hershman *et al.*(1970) demonstrated that oestrogen injected in non-pregnant subjects could reduce the circulating level of TSH, and the high oestrogen supply in pregnancy might act against the effect of progesterone. The role of **chorionic thyrotrophin** (HCT) is obscure. Since it resembles TSH in immunoassay, it may support the action of TSH (Hennen, Pierce and Freychet,1969).

It is widely known that the thyroid enlarges during pregnancy, but the extent of the enlargement and the variation in enlargement between subjects are not known.

Some investigators show that in areas where goitre was not endemic, the proportion of enlargement varied from 9 to 30 per cent of pregnant women (Crooks *et al.*, 1964; 1967).

2.2.8. Thyroid function during growth and development

The interaction between dietary iodine intake and the thyroid function during a pregnancy in the mother and the fetus needs to be considered.

Human fetal thyroid development begins about the third to the fourth week of gestation and the gland has reached its site in the neck by week seven (England, 1990).

Iodine uptake by human fetal thyroid during various stages of development has not been quantitated but fragmentary radioactive iodine uptake data suggests that iodine uptake gradually increases toward term and is then transiently accelerated in the neonatal period (Fisher *et al.*, 1962; Hodges *et al.*, 1955). In addition, Hall and Besser (1989) have published that by the 11th week of intrauterine life, the fetal thyroid can accumulate iodine and synthesize colloid; iodotyrosines and iodothyronines are produced; both T_4 and TBG have been demonstrated in the fetal circulations; the levels of TBG rise rapidly and serum T_4 levels parallel the rise in TBG. The concentration of free T_4 also rise, to plateau levels found at term by the end of the second trimester. Fetal serum T_3 levels are low before 24 weeks but both total and free T_3 levels rise after 24 weeks and remain lower than maternal levels even at term. Reverse T_3 (rT_3) levels are elevated in the fetal circulation but after birth normal adult rT_3 are found by about five days. TSH has been identified in the fetal pituitary as early as 14 weeks and at 11 weeks in the circulation, correlating well with the time of onset of

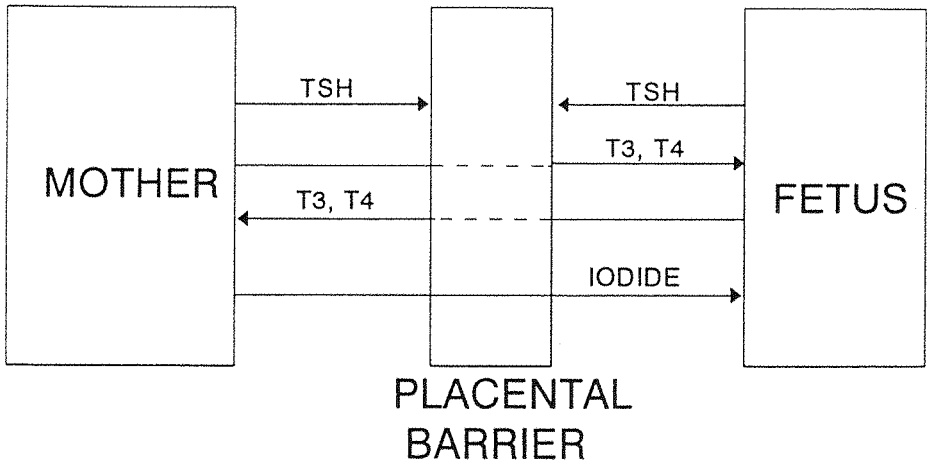
thyroid hormone synthesis in the fetal thyroid. The TSH levels increase rapidly to approach terms values by 16 weeks, showing a relationship with free thyroxine. TSH values in the fetus are higher than those in maternal serum or in normal children, possibly due to the lower T_3 level.

Regarding placental transfer, the bulk of the evidence suggests that there is a partial placental barrier to the passage of T_4 and T_3 in both animals and in man (D'Angelo *et al.*, 1967; Fisher *et al.*, 1964 and 1977; Hall and Besser, 1989). The quantitative aspects of placental T_4 and T_3 transport remain to be precisely delineated.

Thyroid stimulating hormone (TSH) does not cross the placenta during pregnancy and the fetal and maternal autoregulatory mechanisms for thyroid function operate independently (Fuchs and Klopper, 1971). Data derived from experiments in animals (D'Angelo *et al.*, 1967; Fisher *et al.*, 1977) indicate that TSH does not cross the placenta. In contrast, iodide and even some organic iodine-containing compounds appear to cross the placenta readily (Burrow *et al.*, 1965; Hawe, 1965).

Summary of transplacental transport is illustrated in **Figure 2.6**.

Figure 2.6: The transplacental transport of TSH, T_3, T_4 and iodide in the human



Source: Fuchs and Klopper, 1971

Summary of the changes of maternal and fetal thyroid physiology are demonstrated below:

MOTHER	FETUS
1. TSH normal 2. Thyroid enlarged, function normal 3. TBG capacity increase 4. PBI increase 5. Free T4 and T3 normal 6. BMR increase (Maternal+fetal)	1. TSH normal at term 2. Thyroid begins to function 2-6 weeks, normal at term 3. TBG capacity increases at term 4. PBI increases at term 5. Free T4 and T3 normal at term

Source: Adapted from Fuchs and Klopper, 1971.

It is not clear whether the fetus requires its own thyroid

hormones during pregnancy or whether the hormone can be supplied by the mother. However, producing thyroid hormones both in a pregnancy and in a fetus requires iodine as a raw material. Therefore, iodine should be critically important in fetal development, particularly of the neural and skeletal systems.

2.3 ASSESSMENT OF DIETARY IODINE INTAKE

The dietary iodine intake can be evaluated by direct or indirect methods.

2.3.1 Direct measurement of iodine intake

There are 2 steps for direct measurement of 24-hour iodine intake. First, dietary sampling, the best technique consists in collecting a duplicate of foods ingested by the patient during the period of study (Koutras *et al.*, 1970). Second, iodine analyses, the iodine content in food samples are analyzed by using available standard methods (Vough and London, 1963; Wayne *et al.*, 1964; Moxon *et al.*, 1980). Then 24-hour dietary iodine intake is calculated.

2.3.2 Indirect evaluation of iodine intake

From section 2.2.4, the iodine metabolism shows that the inorganic iodine is either accumulated in the thyroid gland or excreted in urine. Since the faecal excretion of iodine is relatively constant and small, the 24-hour urinary iodine excretion may satisfactorily reflect the dietary iodine intake. The problem of this method is to be sure of the accuracy of the 24-hour collection. The excretion of iodine per gram creatinine in a single specimen may be an alternative method to evaluate the level of 24-hour dietary iodine intake. Therefore, we need to consider the

relationship between total dietary iodine intake (μg/day) and 24-hour urinary iodine excretion, the relationship between 24-hour urinary iodine excretion and urinary iodine excretion as μg/g creatinine from a casual urine sample, and the creatine-creatinine metabolism.

2.3.2.i Iodine intake and 24-hour urinary iodine intake

A number of studies have demonstrated a positive relationship between iodine intake and 24-h urinary iodine excretion.

Broadhead *et al* (1965) investigated the effect of change in iodine intake on 24-h urinary iodine excretion. In their experiment, six subjects consumed a meal of 180 g, of haddock (iodine content; the mean was 130 μg/100 g). No other fish was taken for two days before and during experiment. The result was that in three of the six subjects the excretion of iodine in the urine was greatest on the day when the fish was eaten. In addition they also investigated the effect of the addition of a known iodine supplement on the 24-hour urinary iodine excretion. In five subjects 500 μg of iodine in the form of iodine solution of potassium iodide was taken at breakfast at the beginning of 24-hour collection. The mean increase in urinary iodine excretion on the day of the supplement was 304 μg; representing 61 per cent of the dose. The values had returned to the normal range on the second day after the supplements had been taken.

Vought *et al*. (1964) demonstrated a correlation between dietary iodine intake and 24-hour urinary iodine excretion. Iodine intake and excretion were studied in 19 subjects (9 females and 10 males), living in five households. Subjects were observed for five days. Total collections of

food and 24-hour urine were made. Then total iodine in diet and in urine were analyzed. They found that the correlation coefficient between mean (five days) dietary iodine intake and mean urinary iodine excretion was high ($r = 0.88$).

Nelson *et al.* (1987) investigated the association between dietary iodine intake and urinary iodine excretion of 56 women in two British towns (Preston and Southampton). They found a moderate positive association between individual estimates (by questionnaire) and 24-h urinary iodine excretion (determined by the dry-ashing method). Those correlations were as follows, Preston: $r = 0.50$, $P< 0.01$, $n = 31$; and Southampton: $r = 0.24$, $P> 0.05$, $n = 25$.

Brug *et al.* (1992) found a significant association between the iodine status of Dutch adults (men = 376, $r=0.21$, $P<0.01$; women = 440, $r =0.11$, $P<0.01$) by using urinary 24-hour iodide excretion and total dietary iodine intake by using food frequency questionnaire with food compositions table.

Given this, it may be concluded that although 24-h urinary iodine excretion can not be used for calculating iodine intake directly, it may be used for discriminating between levels of iodine intake (for example, low, medium and high). The more dietary iodine intake, the more urinary iodine excretion.

2.3.2.ii The relationship between urinary iodine from a casual urine sample and 24-hour urinary iodine excretion

So far, the most precise estimation of the iodine supply of the populations has been obtained through measurements of the urinary iodine in 24-hour collections (Maisterrena *et al.*, 1968; Vought *et al.*, 1964). Since the feasibility and

the completeness of 24-hour collections are often in doubt, it has been proposed that the ratio between the concentrations of iodine and creatinine in a casual urine sample may be used.

Follis *et al.* (1964) employed the I/Cr ratio in single urine specimens as an indicator of iodine intake of school age girls in Thailand and the USA. They showed differences in the iodine excretion between groups within Thailand, and also differences between Thai and USA school girls. Vought *et al.* (1963) investigated the reliability of estimates of daily urinary iodine excretion from the I/Cr ratio of a single casual specimen which depends on the assumption that the average 24-hour urinary creatinine excretion is relatively constant. The 24 hour urinary iodine excretion (UIE, μg/24hr) was calculated as followed:

$$\text{UIE } (\mu g/24hr) = \frac{\text{Iodine } (\mu g/l)}{\text{Creatinine } (g/l)} \times 1.0 \text{ (females) or } 1.5 \text{ (males)}$$

They concluded that the I/Cr of a single specimen was a reasonably good approximation of the 24-hour iodine excretion, (n=6 normal euthyroid volunteers) since the means of daily urinary iodine excretion estimated from all casual specimens are not statistically different from the means of the observed values.

They also suggested that estimates of urinary iodine excretion derived in this manner were satisfactory for field study purpose, particularly if sex difference in the excretion was taken into account and non fasting specimens were used. Jolin & Escobar del Rey (1965) have evaluated the I/Cr ratios of casual urine samples as indices of daily urinary iodine output during field studies. The following simple calculation was used:

$$\text{Urinary iodine } (\mu g/d) = \frac{\text{I (casual sample) } (\mu g/l) \text{ x K}}{\text{Creatinine (casual sample) (g/l)}}$$

where K = Creatinine (g/d)
= 1.0 for women
= 1.7 for men
= 0.018 x body wt (kg) girls
= 0.020 x body wt (kg) boys

They found that there was no statistically significant difference between means and standard deviations of data of the daily iodine output and those derived by calculation from I/Cr in a casual urine sample (n = 97 healthy volunteers); there was a positive correlation between the determined and calculated values ($r = 0.74$, $P < 0.001$).

However, the estimation of 24-h urinary iodine excretion by the methods of Vought *et al.* (1963) and Jolin & Escobar del Rey (1965) seemed to be valid only if the daily urinary creatinine excretion by a given subject was fairly constant. Therefore, some of the investigators established regression equations for estimating the 24-h urinary iodine excretion from the I/Cr in a single urine specimen.
Frey *et al.* (1973) examined the relationship between urinary iodine in 24 hour urine collection and urinary iodine in single specimen (collected between noon and 6 p.m. on the same day). In their study, 33 males and 29 females were examined. They found that the correlation between 24-hour iodine excretion (Y) and the I/Cr in a single afternoon specimen (X) was positive in both sexes. The straight line as calculated by the method as least squares was described by the following equations:

Males $Y = 0.50X + 7.34$, $r = 0.69$ $(p = 0.001)$
Females $Y = 0.76X + 7.40$, $r = 0.68$ $(p = 0.005)$

They concluded that it was possible to use regression equations on the basis of the urinary iodine per gram of creatinine in single afternoon specimens for estimation of the average 24-h iodine excretion in population studies when dealing with sample sizes of 30 or more.

Hass *et al.* (1988) also estimated 24-hour iodine excretion on the basis of iodine and creatinine determinations using regression equations determined in a pilot study of 50 men and women. Contrary to Frey *et al.* (1973), they did not use a single urine specimen but collected urine samples over a period of 5 hours, from 17.00 to 22.00 h. The calculated linear regression equations were

Males $Y = 26.0 + 1.16X$; $r = 0.70$
Females $Y = 2.54 + 1.16X$; $r = 0.89$
where X = urinary iodine excretion (μg/g creatinine) in 5-hour specimens
Y = 24-hour urinary iodine excretion (μg/day)

The studies (Frey *et al.*, 1973; Hass *et al.*, 1988) showed that, in order to estimate the 24-hour urinary iodine excretion, using regression equations on the basis of the urinary iodine per gram of creatinine (including its standard deviation) seemed to be more correct and accurate than that calculated by multiplying the individual level of the I/Cr ratio by a factor representing a theoretical average creatinine excretion (Vought *et al.*, 1963; Jolin *et al.*, 1965). Thus casual urinary iodine excretion (I/Cr) may be used to estimate the levels of dietary iodine intake. Since not only is there positive relationship between dietary iodine intake and 24-hour urinary iodine excretion but there is also a positive relationship between 24-hour urinary iodine excretion and casual urinary iodine excretion (I/Cr). However, because the coefficient

correlation of these regression equations were not perfectly correlated (r=1). This might lead to misclassification bias because either a casual urine sample with high iodine content might be a 24 hour urine sample with low iodine content and vice versa. Thus it is still possible to have a distorsion between the relationship between 24-hr urinary iodine excretion and casual urinary iodine excretion (I/Cr). For example, if the average of these coefficient correlations (r=0.74) was converted to percent correctly classified, it was 70.1 % indicating that 29.9 % of 24-hr urine samples were misclassified (Clayton and Gill, 1991).

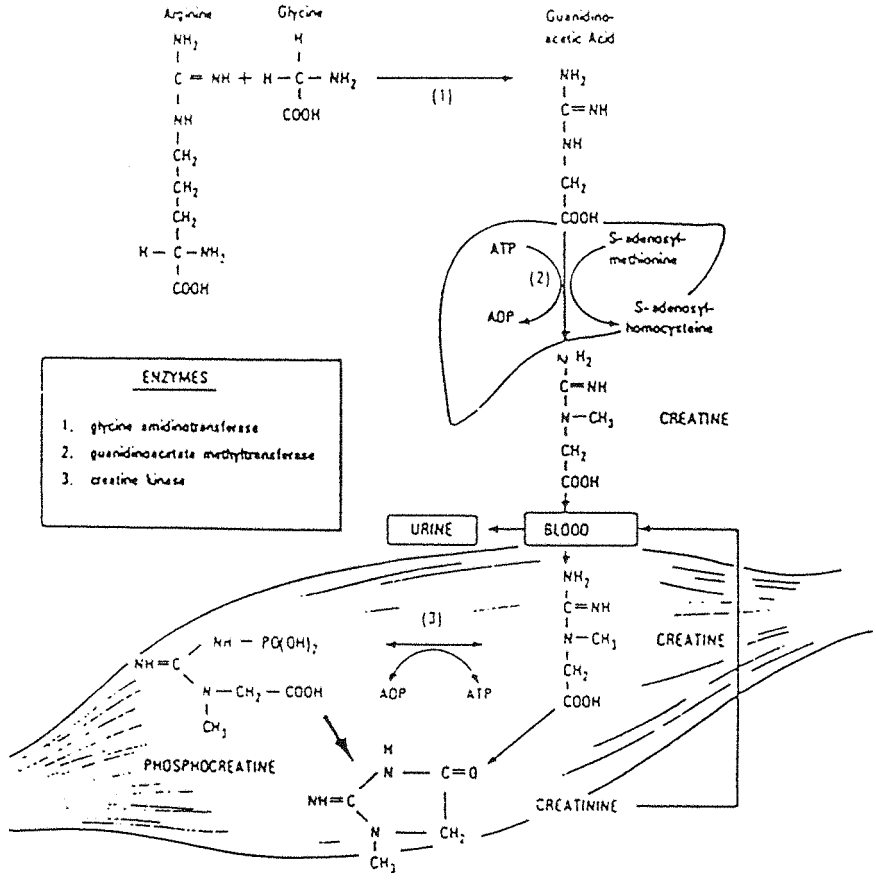
2.3.2.iii Creatine-creatinine metabolism

Creatine and creatinine metabolism needs to be reviewed because creatinine is widely used as a ratio of another interested substances in urine, for example, I/Cr when the 24-hour urine sample is difficult to collect; under the assumption that creatinine is excreted via kidneys constantly per day. A number of factors may influence creatine-creatinine metabolism.

2.3.2 iii.a Biosynthesis of creatine

Creatine is a nitrogenous organic compound, which participates in cellular energy metabolism. Bloch and Schoenheimer (1941) first proposed that creatine was derived from three amino acids, namely, arginine, glycine and S-adenosyl methionine as shown in **Figure 2.7**.

Figure 2.7: Principle pathway in creatine metabolism



Source: Heymsfield et *al*, 1983

Borsook and Dubnoff (1947) showed the first of two biosynthetic steps: synthesis of glycocyamine (guanidoacetate) from glycine and arginine. This reaction is reversible and controlled by enzyme transamidinase (glycine amidinotransferase) and is found in human kidneys, liver, pancreases, brain, spleen and mammary gland (Walker, 1979). The kidney is the predominant biosynthetic site in human and the rate of creatine synthesis is regulated by

feedback inhibition of transamidinase. In the second step, creatine is formed by transfer of methyl group from S-adenosyl-methionine to guanidoacetate. This step is irreversible and occurs primarily in liver tissues.

The synthesized creatine is released into the circulation, where the next step is active uptake by muscle and other tissues (Walker, 1979; Haughland, 1975). About 98 per cent of the total body creatine pool is within muscle (120 g in a 70 kg adult man) and < 2 per cent is within other tissues, for example, brain, liver and kidney and other fluids, e.g. blood and urine (Heymsfield *et al.*, 1983). Urinary losses of creatine are usually negligible, unless renal tabular reabsorption is impaired or blood levels become excessively high (Heymsfield *et al.*, 1983).

Within muscle, creatine exists in 2 forms, namely, creatine and phosphocreatine (Saks *et al.*, 1978). Both of them are converted to creatinine. The spontaneous loss of phosphoric acid from phosphocreatine in muscle under physiologic conditions is the major reaction that produces creatinine. The nonenzymatic slower reaction consisting of loss of water from creatine also forms creatinine in muscle. Phosphocreatine converts to creatinine at a rate 2.6 per cent while creatine dehydrates converts to creatinine at a rate of 1.1 per cent (Heymsfields *et al.*, 1983). Since urinary creatinine is derived from these two sources, the in vivo measurement of creatine turn over rate provides a value between 1.1 and 2.6 per cent.

2.3.2.iii.b Urinary creatinine excretion

Creatinine diffuses from the cell and ultimately appears in the urine after glomerular filtration (Bjornsson, 1975). Urinary output of creatinine correlates better with muscle

mass than with body weight (Clark *et al.*, 1951). A lean individual would be expected to have higher creatinine excretion (per kilogram of body weight) compared with an obese individual (Garns *et al.*, 1955).

Folin (1905) found little day-to-day variation in the amount of creatinine excreted in the urine of a healthy individual (CV 6.8 per cent). Careful studies in reliable subjects showed a daily variation from four to eight per cent in creatinine excretion (Cryer, 1970; Greenblatt, 1976). However, Edwards *et al.* (1969) reported the CV for creatinine excretion among individuals to vary from 5.6 to 22.3 per cent and Bailey *et al.* (1970) demonstrated a variation from 4.1-28.2 per cent. The variations between studies may be explored , at least in part, by differences in dietary habits.

2.3.2.iii.c Factors which influence daily creatinine excretion

A number of investigators have shown that there are other factors which influence daily creatinine excretion. They are as follows:

(a) Exercise

It was found that extremely strenuous exercise can increase urinary output of creatinine by 5 to 10 per cent (Hobson, 1939; Srivastava, 1957).

(b) Emotional stress

Scrimshaw *et al.* (1966) concluded that the variability in creatinine output increases during stress, however the causes of this phenomenon are poorly understood.

(c) Menstrual cycle

A rise and fall in creatine and creatinine excretion during the menstrual cycle has been demonstrated by Smith (1942).

(d) Aging

With increasing age, fat free body mass declines in the adult and most of this loss in lean tissue can be attributed to the atrophy of skeletal muscle. Creatinine excretion also declines with age, and this presumably reflects the diminution in muscle mass (Thomlinson *et al.*, 1969).

(e) Infection, fever and trauma

Creatinine turnover in rats suffering from lung abscesses was increased (Waterlow *et al.*, 1972). Schiller *et al.* (1979) demonstrated a rise in creatinine excretion with both fever and severe trauma. An increase of 20 to 100 per cent in urinary creatinine excretion in some patients occurred in the immediate post traumatic period (Schiller, 1979; Geiger, 1981; Threlfall, 1981).

(f) Renal disease

There is a decrease in the excretion of urinary creatinine if glomerular rate falls in chronic renal failure or renal insufficiency (Goldman, 1954; Jones & Burnett,1974; Mitch & Walser, 1978; Mitch & Collier, 1980). They suggested that it was due either to reduced creatinine production or an alternate excretory pathway.

(g) Diet

There are three dietary constituents namely protein, creatine and creatinine, which affect urinary creatinine excretion.

Firstly, dietary protein is the main source of the amino acid precursors (glycine, arginine, methionine) of creatine. Therefore a decrease in dietary protein intake may lead to a decrease in creatine production. In addition the activity of transamidinase is influenced by dietary protein intake. An 85 per cent reduction in transamidinase activity was found when rats were fed with a protein-free diet for 12 days (Van pilsum, 1957). However, some investigators (Bleiler & Schedl, 1972; Lykken et al, 1980) showed that there was a small effect of protein intake *per se* on urinary creatinine excretion .

Secondly, the amount of dietary creatine directly influences the size of the creatine pool, which in turn is proportional to the output of creatinine in urine. Crim *et al.* (1975) fed normal volunteers with diets which shifted from a 9-day low creatine diet (0.23 g/d), to a 10-day high creatine diet (10 g/d). They found that urinary creatinine excretion increased about 10 to 30 per cent.

Thirdly, the dietary creatinine influences creatinine excretion since it undergoes intestinal absorption and prompt renal excretion (Hunter, 1922; Shaffer, 1908).

2.4 IODINE AND BIRTH OUTCOME

There is some evidence to suggest a relationship between iodine intake during pregnancy and birth outcome.

2.4.1 Iodine and birthweight

There are only a few studies which have assessed the relationship between iodine intake and birthweight.

Thilly (1981), in a controlled trial, studied the effect of an injection of iodized oil, compared to no treatment, on birthweight,perinatal and infant mortality and development quotient in Zaire and Papua New Guinea. The findings indicate substantial improvement in birthweight of infants with reductions in perinatal and infant mortality and also improvement in the development quotient in those given iodized oil (see **Table 2.7**).

Table 2.7: Effect of injection of iodized oil given during pregnancy in Zaire.

OUTCOME	NOT TREATED	TREATED
Birthweigt (g)	2634 ± 552 (98)	2837 ± 542 (112)
Perinatal mortality (:1000 live births)	188 (123)	98 (129)
Infant mortality (:1000 live births)	250 (263)	167 (252)
Developmental quotient	104 ± 24 (66)	157 ± 16 (72)

All differences were significant ($p<0.05$). Sample size in brackets

Source: Thilly, 1981.

Filteau *et al.* (1994) conducted a study in Bangladesh with high goitre prevalence and without an iodization programme in order to determine the effect of a single oral dose (400 mg I as iodized poppyseed oil (IPSO)) during pregnancy on maternal and infant thyroid hormones and iodine status. All women recruited in the first trimester (n=38) were

given IPSO and, of women recruited later in pregnancy, 113 were given IPSO while 114 served as controls.

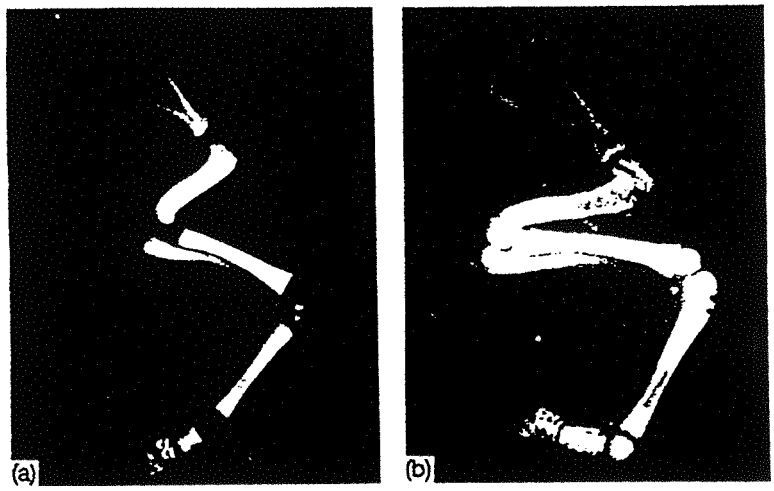
Filteau *et al.* (1994) found that birthweight was increased slightly but significantly ($p<0.05$) by IPSO treatment from 2.4 kg (SD 0.42) to 2.53 kg (SD 0.41) and gestational age at supplementation was not a significant covariate.

2.4.2 Iodine and the length of an infant

Since length or height is determined by the length of the skeleton, we might anticipate an effect of thyroid hormone on bone growth.

At birth or within the first few months of life, children with severe hypothyroidism show retarded bone development, as first reported by Dorff (1934). The distal ossification centre of the femur is absent or appears very small and porous. If the hypothyroidism is not corrected, the development of the skeleton is severely retarded, growth is impaired and immature body proportions and features, such as short legs and the flat broad base of the nose are retained (Falkner, 1966). From animal studies, the iodine deficient lambs show misshapen skull, and partial dislocation of the leg joints in comparison to the controls. X-rays of the bones confirms this and provides evidence of delayed ossification of the growing bone centres or epiphyses as shown in **Figure 2.8** (Potter *et al.*, 1981).

Figure 2.8: X-rays of the left forelimbs of an iodine deficient (A) and a control (B) fetus of 140 days gestation showing epiphyseal dysgenesis as a result of iodine deficiency



Source: Hetzel, 1989.

2.4.3 Iodine, head circumference and chest circumference

No studies have looked at the relationship between dietary iodine intake during pregnancy and head or chest circumferences in infants.

2.4.4 Iodine and the other birth outcome

McMichael *et al.* (1980) demonstrated that the hypothyroid guinea pig (produced by surgical removal of the thyroid) had a three to four fold increase in abortions and stillbirths which were eliminated by replacement therapy with thyroxine during pregnancy.

Morreale de Escobar *et al.* (1986) compared pregnant, iodine deficient rats with rats which had maternal thyroidectomies before pregnancy. Both groups had a reduction in the number of embryos and in their individual body weight following maternal iodine deficiency, more severe effects were seen following maternal thyroidectomy.

Endemic cretinism is another major effect of foetal iodine deficiency. This term, cretinism refers to the very severe consequences of hypothyroidism.

Pharoah *et al.* (1971) had a study in pregnancy in the Western Highlands of Papua New Guinea to see whether endemic cretinism could be prevented by using iodized oil. They concluded that an injection of iodized oil given prior to pregnancy could prevent the neurological syndrome of endemic cretinism.

Man *et al.* (1969) compared previous reproductive histories of 97 hypothyroxinemic women with 97 control women who had normal BEI (butanol extractable iodine, represents concentration of plasma thyroxine hormone) before and after 24-gestational week. The hypothyroxinemic women had a significantly higher incidence of abortions, prematurity, stillbirths, major anomalies, and progeny with subsequent retardation.

Goitres occur more frequently during pregnancy. For example, Crooks *et al.* (1964) established the frequency of goitre during pregnancy in the Aberdeen area of Scotland. Goitre was considered present if the thyroid gland was both visible and palpable. With these criteria, 70 per cent of pregnant women (n = 184) and only 38 per cent (n=116) of nonpregnant women had goitre.

From these findings it may be concluded that the effects of iodine deficiency on pregnancy outcome are abortion, stillbirth, varying manifestations of cretinism, congenital anomalies and there may be a decrease in birthweight and length of an infant, but little data exist on the effects on other aspects of fetal growth. Hetzel, B.S. (1989) have summarized iodine deficiency (see **Table 2.8**).

Table 2.8: The spectrum of iodine deficiency disorders.

FOETUS	Abortions Stillbirths Congenital anomalies Increased perinatal mortality Increased infant mortality Neurological cretinism Myxoedematous cretinism Psycomoter defects Foetal hypothyroidism
NEONATE	Neonatal hypothyroidism Neonatal goitre
CHILD and ADOLESCENT	Goitre Juvenile hypothyroidism Impaired mental function Retarded Physical development
ADULT	Goitre with its complications Hypothyroidism Impaired mental function

Source: Hetzel, 1989

2.5 RISK FACTORS FOR LOW BIRTHWEIGHT

Low birthweight has been defined as a birthweight of less than 2500 g. It can be caused either by premature delivery (short gestation) or by/ fetal growth retardation and a combination of both (Villar and Belizan, 1982). Many risk factors for low birthweight have been reported (**Table 2.9**). The investigation of the relationship between the dietary iodine intake during pregnancy and birthweight outcome needs to take into account these other risk factors.

Table 2.9: Risk factors associated with LBW

RISK FACTORS	EXAMPLES
1. Demographic risks	1.1 Age (<18, >35 years) 1.2 Race (black) 1.3 Low socioeconomic status 1.4 Unmarried 1.5 Low level of education
2. Medical risks predating pregnancy	2.1 Parity 2.2 Low weight for height 2.3 Genitourinary anomalies/Surgery 2.4 Selected diseases -diabetes -chronic hypertension 2.5 Nonimmune status for select infections -rubella 2.6 Poor obstetric history, including previous LBW infant, multiple spontaneous abortions 2.7 Maternal genetic factors
3. Behavioral and environmental risks	3.1 Smoking 3.2 Under nutritional status 3.3 Alcohol and other substance abuse 3.4 Dietylstilbestrol and other toxic exposures, including occupation hazards 3.5 High altitude

RISK FACTORS	EXAMPLES
4. Evolving concepts of risks	4.1 Stress -physical -psychosocial 4.2 Uterine irritability 4.3 Events triggering uterine contractions 4.4 Cervical changes detected before onset of labour 4.5 Selected infections -mycoplasma -chlamydia trachomatis 4.6 Inadequate plasma volume expansion 4.7 Progesterone deficiency
5. Health care risks	5.1 Absent or inadequate prenatal care 5.2 Iatrogenic prematurity
6. Medical risks in current pregnancy	6.1 Multiple pregnancy 6.2 Poor weight gain 6.3 Short interpregnancy interval 6.4 Hypotension 6.5 Hypertension 6.6 Selected infections -symtomatic bacteria -rubella -cytomegalo virus 6.7 First, second, or trimester bleeding 6.8 Placenta problem -placenta previa -abruption placenta 6.9 Hyperemesis 6.10 Oligohydraminos/polyhydraminos 6.11 Anemia/abnormal haemoglobin 6.12 Isoimmunization 6.13 Fetal abnormalies 6.14 Incompetent cervic 6.15 Spontaneous premature rupture of membranes

Source: Berhman, 1985

2.6 SUMMARY

From this chapter, it might be inferred that:

1. Iodine is an essential element which is used by the thyroid gland to produce thyroid hormones.

2. There are two abnormalities of thyroid function, namely hypothyroid states and hyperthyroid states.

3. Dietary iodine intake varies widely throughout the world depending on the iodine in soil, land and water and upon the culturally dietary preferences. We can receive iodine from drinking water and foods. Foods of marine origin are rich in iodine.

4. From a review of iodine metabolism, it shows that the kidney is the main pathway of excretion of iodine and thyroid hormones control many chemical processes in different parts of the body.

5. There are some changes of thyroid function during pregnancy. The thyroid gland in pregnancy may be hyperaemic and the clearance high. The renal clearance is increased. Oestrogen raises both the globulin content of the blood and the carrying capacity of TBG. The rise in binding capacity of the protein results in an increase circulating T_4. Progesterone induces overbreathing and the pCO_2 of plasma falls. The concentration of free hormone is also low because pCO_2 controls the binding of T_3 and T_4 proteins. Because of the low concentration of free thyroid hormone, secretion of TSH would be expected to rise. However, oestrogen acts as a suppressor, the level of TSH in plasma may be within normal range.

6. There is evidence to show that TSH does not cross the placenta whereas iodide can cross the placenta. A number of investigators have demonstrated that there is a partial placental barrier to the passage of T_3 and T_4 in both animals and man.

7. Adequate fetal thyroxine levels are necessary for normal fetal growth and development. Regarding Wayne 's study (1964), iodine intake in pregnancy might be raised to 200 μg per day.

8. To measure dietary iodine intake, the indirect method using excretion of iodine from a casual urine sample as μg/g creatinine can be used as an index to estimate the levels of dietary iodine intake, depending on:

(a) The 24-hour urinary iodine excretion in each subject may be estimated by multiplication I/Cr of a casual urine sample with the mean of the urinary creatinine excretion (K, g/d) of the target population. Then the levels of dietary iodine intake will be evaluated from those data of 24-hour urinary iodine excretions.

(b) Extrapolate the correlation equation between 24-hour urinary iodine and casual urinary iodine excretions (I/Cr) in subsamples of the target population. By that equation, the individual 24-hour urinary iodine excretion is calculated from excretion of iodine as μg/g creatinine. Then the levels of dietary iodine intake will be evaluated from those data of 24-h urinary iodine excretions.
However, physiological change during pregnancy may effect urinary iodine excretion, and this has not been studied.

There are variations of urinary creatinine excretion from day-to-day (CV 4-8 per cent), within subjects (CV 10 per

cent) and between subjects (4.1-29.0 per cent). There are a number of factors which have an effect on urinary creatinine excretion, for example, exercise, emotional stress, menstrual cycle, aging, infection, fever and trauma, renal disease and diet (protein, creatine and creatinine intake).

9. Finally there are some risk factors which may be associated with birth outcome. These factors should be considered when the relationship between urinary iodine excretion from casual urine samples and birth outcome is investigated.

CHAPTER 3

METHODOLOGY

3.1 INTRODUCTION

To provide a background of this investigation into whether there is a relationship between dietary iodine intake during pregnancy and birth outcome, this chapter describes the methodologies used to measure dietary iodine and birth outcome.

In order to develop the methods, and test their reliability, and the technical competence of the researcher in using the methods, a series of pilot studies were conducted. These small scale studies were not intended to provide new data on the methodology, but to set as a quality control for the main study. Seven studies have been conducted to address the following issues:

First, to evaluate the reliability and the accuracy of the methodology of measuring urinary creatinine (**3.2.1**).

Second, to evaluate the reliability and the accuracy of the methodology of measuring urinary iodine excretions (**3.2.2**).

Third, in this study, only one male was asked to collect 24 hour urine samples for three days at specific times. The objectives of the study were to investigate: the diurnal variation of urinary iodine and urinary creatine excretions; whether a casual urinary iodine give the same result as a 24-hour urinary iodine measurement; and the percentage recovery of a known amount of iodine intake from a 24-hour urine sample (**3.2.3**). Although, this study had only one subject, the results of the study, at least, were helpful for the main study to confirm whether urinary iodine excretion from casual urine samples were related to 24-hr urinary iodine excretion and total dietary iodine intake. The size of the within person variation of the

urinary iodine excretion from casual urine samples also affected the way to calculate the sample size in the main study.

Fourth, 24-hr urine samples were collected as part of another study (Fernando 1991) which had the objective to investigate the effect of dietary creatine supplementation on 24-hr urinary creatinine excretion of vegetarians. The objective of determining iodine content in these 24-hr urine samples was to investigate the effect of dietary creatine supplement on 24-hr urinary iodine excretion (μg/g creatinine) (**3.2.4**). The results of this study, at least, may give an idea about the effect of dietary creatine on the ratio of urinary iodine and creatinine excretions (expressed at μg/g cratinine) which was used to investigate dietary iodine intake in the main study. Dietary iodine intake was not controlled duing the study (before and after supplementation) and there were only six female vegetarian subjects recruited.

Fifth, we got 24-hr urine samples were collected from a study (Daneilson, 1991) investigating the limits of adaptation to a diet low in protein in normal men (n=6). The objective of determining iodine content in these 24-hr urine samples was to investigate the effect of the levels of protein intake (low: N-intake 68 mgN/kg/d; high: N-intake =165 mgN/kg/d) on the 24-hr urinary iodine excretion (**3.2.5**). Again, during the periods of adequate protein and low protein intakes, dieatry iodine intake of these subjects was not controlled. The results of this study, at least, showed whether there was any effect of the level of protein intake; it also gave an idea as o whether we should take the level of protein intake into account for the analysis of the relationship between exposure and outcome in the main study.

Sixth, in this pilot study, the objective was to investigate the percent recovery of iodine supplement together with how long it took for the iodine to be recovered in urine (**3.2.6**). Although, we had only one subject, the results in this study could confirm whether the 24-hr urinary iodine excretion was a good index of dieatry iodine intake on the day which it was eaten.

Seventh, to evaluate the reliability and the accuracy of the methodology of measuring iodine content in foods (**3.2.7**). This study was conducted becuase we needed an accurate and precise method of determining the iodine content in food items. This method was used in a pilot study in Thailand about the relationship between urinary iodine excretion in 24-hour urine collection and casual urine samples in Thai pregnant women (**3.3**)

Regarding 24-hr urine sample collections, all subjects in the studies metioned above were educated to realize the importance of collecting urine sample completely.

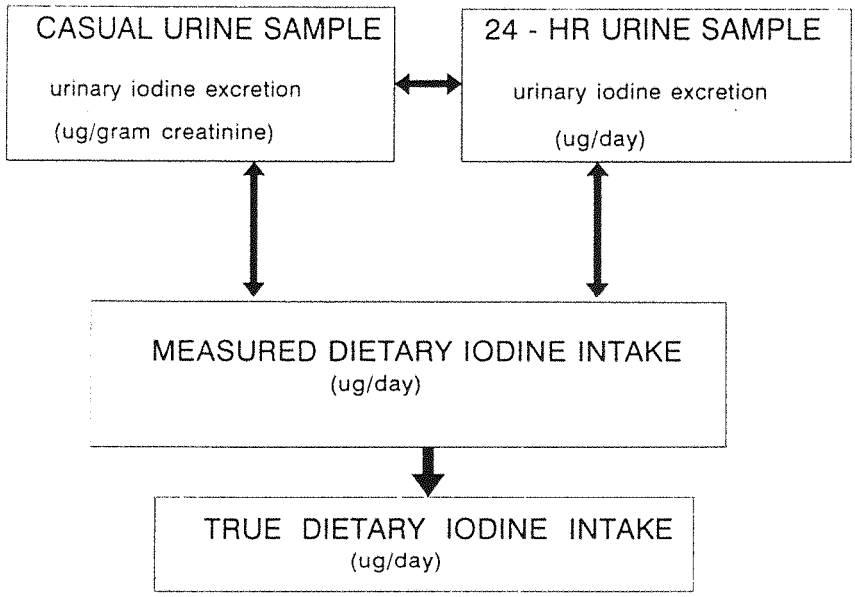
Finally, there is a description of the methods used in the main study: the study design, dietary assessment, urinary analysis, birth outcome measure, and the assessment of other factors which may affect birth outcome and data analysis.

3.2 DEVELOPMENT OF METHODS

According to the basic principles of iodine metabolism, inorganic iodine is either accumulated in the thyroid gland or excreted in urine. Since the renal iodine clearance rate is relatively constant, urinary iodine excretion fluctuates according to the plasma concentration of iodide which, in turn, depends on the amount of iodide absorbed

from the gut. It is assumed that the faecal excretion of iodine is relatively constant and that, therefore, the 24-hour urinary iodine excretion reflects the dietary iodine intake. The 24-hour urinary iodine excretion is an index commonly used for assessing the levels of iodine intake (Vought *et al.*, 1964; Broadhead *et al.*, 1965; Nelson *et al.*, 1987; Brug *et al.*, 1992). However, this index may not always be practical for large field studies. Most field studies have used urinary iodine excretion in casual urine samples (μg iodine/g creatinine) to estimate the 24-hour urinary iodine excretion and then evaluated the level of iodine intake, as shown in **Figure 3.1**.

Figure 3.1: The model shows the relationship between urinary iodine excretion in casual urine samples (μg/g creatinine), urinary iodine excretion in 24-hour urine samples and dietary iodine intake (The optimal numbers of days of recording for each method needs to be calculated to determine the ratio of witin and between person variation).



3.2.1 The methodology of measuring urinary creatinine excretion

The objective of this section is to check the validity and reliability of a method of determining urinary creatinine and to establish a reliable method of determining iodine content in urine samples.

3.2.1.i Principle of the method

Creatinine reacts with alkaline picrate to produce a red colour (Jaffe's reaction). The reaction is not specific, and various methods have been used to increase the specificity. One of them is the additional purification step of absorption on Lloyd's reagent (Fuller's earth). Lloyd's reagent is frequently used to absorb creatinine from a specimen, to separate it from non specific chromogenic substances. Terms such as "total chromogen" , "alkaline picrate reactive material", "apparent creatinine" have been employed to distinguish creatinine and true creatinine. By using Lloyd's reagent in conjunction with the Jaffe's reaction it is possible to estimate true creatinine. In this thesis the urinary creatine excretion (UCE) of each urine sample was determined by using Jaffe's reaction which was modified from Taussky's method (Bonsnes & Taussky, 1945; Henry, 1964).

As regards the materials and method for measuring urinary creatinine excretion, it was shown in **appendix 2**.

3.2.1.ii Reproducibility and percentage recovery

One urine sample was analyzed for creatinine 20 times. It was found that the coefficient of variation (CV) was 3.57 per cent (Mean±SD = 78.14±2.79 mg/dl). Added creatinine may be recovered quantitatively from the urine. It was

found that the percentage of creatinine recovered varied from 95.5-107.6 per cent.

3.2.2 The methodology of measuring urinary iodine excretion with stopping the reaction between Ce^{4+}/As^{3+} by brucine sulphate.

3.2.2.i Principle of the method

This method was modified from Wayne's method (1964) by which iodine in urine samples can be determined by using the catalytic action of iodine on the ceric-arsenite system. The method relies on the fact that the reduction of Ce^{4+} by As^{3+} in acid medium is catalyzed by iodide. Since the reduction of Ce^{4+} to Ce^{3+} changes the optical absorbance at 420 nm, the progress of the reaction is best monitored by following the decolorization of ceric ammonium sulphate. The colorimetric estimation of iodine by catalytic action on the ceric-arsenic system can be measured by stopping the reaction with brucine sulphate. As regards the materials and method for measuring urinary iodine excretion, it was shown in **appendix 3**.

3.2.2.ii Reproducibility and percentage recovery

One urine sample was analyzed for iodine 20 times. The coefficient of variation (CV) was 4.49 per cent (Mean±SD = 14.04±0.63 μg per cent). Added iodine may be recovered quantitatively from the urine. Some typical results are shown in **appendix 3**. The percentage recovery varied from 94.4 per cent to 104.4 per cent.

It can be seen from **sections 3.2.1 and 3.2.2** that the methodology of measuring both urinary creatinine and urinary iodine excretions are accurate and precise. Therefore we used these measurements in this study.

3.2.3 An experimental study of urinary iodine and urinary creatinine excretions

Experimental studies to investigate:

(1) the diurnal variation of urinary creatinine excretion (UCE, g per cent) and urinary iodine excretion (UIE, μg per cent and μg/g creatinine) to determine whether time of collection influences the estimated daily excretion;

(2) whether a casual urinary iodine gives the same result as a 24-hour urinary iodine measure;

(3) the percentage recovery of a known amount of iodine intake from a 24-hour urine sample.

The 24-hour urine samples were collected from one adult male on day 1 (d1), day 8 (d8) and day 9 (d9). On d1, urine samples were collected every hour from 8 a.m. to 12 a.m., every two hours from 0 p.m. to 12 p.m. and then from the period of 0 a.m. to 8 a.m.. For d8 and d9 urine samples were collected every two hours from 8 a.m. to 8 a.m.. These urine samples represented the casual urine sample. A plastic bottle (250 ml) was used as a container with 2 or 3 drops of toluene as a preservative and kept in the refrigerator. The volume of each collection was measured and recorded. Urinary creatinine excretion (UCE) and urinary iodine excretion (UIE) were determined by the methods mentioned in **3.2.1 and 3.2.2**. The 24-hour urinary iodine (TUIE) and creatinine (TUCE) excretions were calculated by summating those values from all urine samples together. Dietary intake in each day was recorded and the iodine intake was approximately estimated by using the food table of Wenlock *et al.* (1982).

It was found that:

(1) In casual urine samples of the same subject, there was a diurnal variations in UCE (g per cent) and UIE (μg per cent and μg/g creatinine). The per cent CV of UIE (μg per cent) was higher than that of UIE (μg/g creatinine), see **Table 3.1**.

(2) From this study it can be concluded that if UIE (μg/g creatinine) from a casual urine sample is used for estimating TUIE (μg/d), it will give a better estimate than that obtained using UIE (μg per cent) from a casual urine sample, although the minimum and maximum values cover a wide range for both measures (see **Table 3.2**).

(3) The 24-hour urinary iodine excretion (TUIE, μg/d) provides a good index of dietary iodine intake. For example, the higher the dietary iodine intake, the higher the urinary iodine excretion. From this study the mean of TUIE was 79.69 per cent of the total dietary iodine intake (TDII, μg/d) and appeared reasonable constant, at least across the range of intakes measured here (see **Table 3.3**).

Table 3.1: The per cent CV of rate of urine excretion (RUE), urinary creatinine excretion (UCE) and urinary iodine excretion (UIE)

Casual urine sample	**Day 1**	**Day 8**	**Day 9**
RUE (ml/hr)	57.50	48.10	57.40
UCE (g/hr)	12.28	8.77	14.29
UCE (g %)	55.88	54.74	47.14
UIE (μg/hr)	25.04	27.06	26.70
UIE (μg%)	52.11	54.64	34.93
UIE (μg/g creatinine)	26.82	22.89	27.87

Table 3.2:* The per cent error of using UIE (μg per cent and μg/g creatinine) of casual urine samples to estimate TUIE (μg/d)

Day	μg%		μg/g creatinine	
	max	min	max	min
1	+151.1	-35.0	+49.9	-34.3
8	+186.0	-49.8	+44.4	-36.0
9	+86.3	-59.9	+53.6	-43.6

* see the detail in **appendix 4**
max = per cent error of over estimate
min = per cent error of under estimate

Table 3.3: The value of 24-hour liquid intake (TLI), 24-hour urine volume (TUV), 24-hour urinary creatinine excretion (TUCE), total dietary iodine intake (TDII) and 24-hour urinary iodine excretion (TUIE) on days 1, 8, 9

24-hour data	Day 1	Day 8	Day 9
TLI (ml/d)	~1575	~1700	~ 1700
TUV (ml/d)	924	1843	1248
TUCE (g/d)	1.33	1.36	1.35
TDII (μg/d)	~125	~210	~210
TUIE (μg/d)	95.91	172.76	168.14
TUIE(%of TDII)	76.73	82.27	80.07

3.2.4 The effect of dietary creatine supplementation on the urinary iodine excretion (μg/g creatinine) of 24-hour urine collection in vegetarians

Non vegetarians receive their creatine supply mostly from the diet (because all meats contain creatine 120 - 130 mmoles/kg dry weight of muscle) whereas vegetarians must rely on glycine and arginine to satisfy their need for creatine. Fernando (1991) reported that after six female

vegetarians were supplemented with dietary creatine, their 24-hour urinary creatinine excretions (TUCE, g/d) tended to increase but the increase was not statistically significant when compared with the TUCE of those vegetarians before dietary creatine supplementation. In field studies, when urinary iodine excretion (UIE) was investigated, most of the results were shown as μg/g creatinine. Therefore, the objective of this study was to investigate the effect of creatine supplementation on the urinary iodine excretion, especially when expressed as μg/g creatinine.

Six female vegetarian volunteers (subjects: A, B, C, D, E and F) were recruited. Mean and S.D of their age, weight and height were 21±2.19 years, 53.04±4.18 kg, and 164.67± 5.24 cm respectively. They were all in normal health and there was no restriction on their food intake. 24-hour urine samples were collected from the volunteers. The collection was divided into two parts. The first part consisted of the subjects acting as their own controls for the first three days (d1-d3) of the study. The second part, each morning before starting the urine collection of the day, the subjects were given a supplement of one gram of creatine a day dissolved in water for the next three days (d4-d6). All of the urine samples were preserved by using 25 ml of 6 M HCl. These samples were measured daily and aliquots stored separately in the refrigerator until they were analyzed. 24-hour urinary creatinine excretion (TUCE) and 24-hour urinary iodine excretion (TUIE) of these samples were analyzed by the methods mentioned in **sections 3.2.1 and 3.2.2**.

When the study was finished, there were only three subjects (subjects: A, B and C) whose 24-hour urine samples were completely collected. Over all, from six subjects, there were 16 of 24-hour urine samples both during the period

before creatine supplementation and during the period after creatine supplementation.

From this study it may be inferred that:

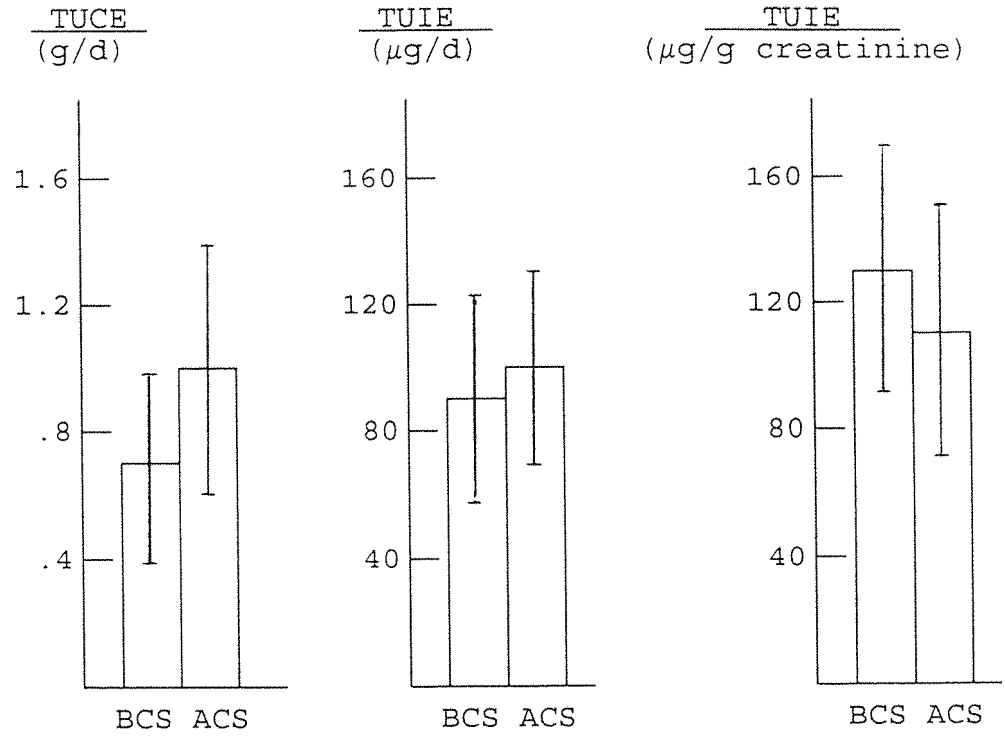
(1) When the TUCE (g/d), the TUIE (μg/d) and the TUIE (μg/g creatinine) on d6 of subjects A, B and C were compared with those on d1, d2 and d3, the results did not show any statistically significant differences (paired T-test).

(2) Overall, there was a slight increase in the TUCE (Mean±SD = 0.985±0.431 g/d, n=16) during the period after creatine supplementation when compared with the TUCE (Mean±SD = 0.703±0.284 g/d, n=16) measured before creatine supplementation (see **Figure 3.2**).

(3) Considering the mean of 24-hour urinary iodine excretion (TUIE, μg/d), the TUIE (Mean±SD = 97.65±30.44 μg/d, n=16) during the period after creatine supplementation tended to be higher than that (Mean±SD = 86.74±28.26, n=16), measured before creatine supplementation (see **Figure 3.2**). This may be due to variation in subjects food intake during the study period.

(4) With regard to the mean of 24-hour urinary iodine excretion (TUIE, μg/g creatinine), overall, the mean of TUIE (Mean±SD = 131.99±40.97, n=16) during the period before creatine supplementation tended to be higher than that (Mean±SD = 112.28±43.11, n=16) during the period of after creatine supplementation. This was due to the increase of urinary creatinine excretion after creatine supplementation. As shown in **Figure 3.2**, comparison of TUIE (μg/d) and TUIE (μg/g creatinine) revealed a discrepancy. This discrepancy showed clearly the effect of creatine supplementation, which resulted in increasing the urinary creatinine excretion which then gave a misleading assessment of iodine status.

Figure 3.2: The Mean ± SD of pooled data of TUCE (g/d), TUIE (μg/d) and TUIE (μg/g creatinine) before creatine supplementation (BCS) and after creatine supplementation (ACS)



3.2.5 The effect of low protein intake on the 24-hour urinary iodine excretion in normal adults males

Generally high protein diets have higher iodine contents, because sources of foods rich in protein (for example, milk, eggs and fish) also tend to be rich in iodine. Therefore low protein intakes may result in low iodine intakes and may change the levels of urinary creatinine excretion. This study investigated how the urinary iodine excretion change during a short period of low protein intake (4 days, 0.425 g/kg/d).

Six normal adult males were recruited (Danielsen, 1991). Mean and S.D of their age, weight and height were 20.5±1.4 years, 72.0±2.8 kg and 179.2±4.2 cm respectively. They were all in good health, had been consuming their normal habitual diet and had not been taking any medication prior to participation in the study. The experiment was divided into two periods and each period was for four days. During the first period, subjects ate a diet which had an adequate protein and energy content (N-intake = 165 mgN/kg/d and energy intake = 148 kj/kg/d), whereas in the second period, subjects ate a diet which had low protein, but adequate energy content (N-intake = 68 mgN/kg/d and energy intake = 148 kj/kg/d). The 24-hour urine samples were collected from these subjects throughout each period (8 days). The total volume of urine in each container was measured by weighing the full container and subtracting the initial weight of both the container and acid (assuming the density of urine is approximately 1 g/ml). Following this, a 60 ml aliquot of urine was taken and stored frozen ready for analysis. The 24-hour urinary creatinine excretion (TUCE) and 24-hour urinary iodine excretion (TUIE) of each sample was analyzed by the methods mentioned in **3.2.1 and 3.2.2.**

The results of the study showed that:

(1) Overall, the mean of 24-hour urinary creatinine excretion (TUCE, Mean±SD = 1.70±0.20 g/d, n=24) during the period of low protein intake (LPI) was slightly lower than that (Mean±SD = 1.73±0.17 g/d, n=24) during the period of adequate protein intake (API), but the difference was not statistical significant.

(2) There were significant differences ($p<0.05$) when the mean of 24-hour urinary iodine excretion (TUIE, μg/d) on d4 (LPI) was compared with the means on d1, d2, d3 and

d4 (API). The lower the protein intake, the lower the TUIE (μg/d).

(3) The average 24-hour urinary iodine excretion (TUIE, μg/g creatinine) of all subjects on d4 of LPI period was significant difference ($p<0.05$) when compared with those on d1, d2, d3 and d4 of the adequate protein period. Again the lower the protein intake, the lower the TUIE (μg/g creatinine).

3.2.6 Urinary iodine excretion: Addition of known amount of iodine supplement

In this study, a single supplement of iodine was taken by mouth to investigate the percent recovery of iodine supplement together with how long it took for the iodine to be recovered in urine.

One normal adult male (body weight 55 kg) ate a controlled diet for five days, receiving protein 57.72 g/d (1.05 g/kg/d) energy 2,606.50 kcal/d (47.39 kcal/kg.BW/d) and iodine 152.50 μg/d. The total liquid intake was also controlled. It was about 1,560 ml/d. A single supplement of iodine (472.88 μg/d in the form of aqueous iodine oral solution, B.P) was taken by mouth at the beginning of a 24-hour urine collection on day 3. Thus, the total iodine intake on day 3 was 625.38 μg/d. The 24-hour urine samples were collected each day. For each day, the time of collecting the specimens started at 8 a.m. and was divided into 6 periods. Each period was four hours, for example, >8-12, >12-16,....., >4-8. Thus, during day 1 to day 5, there were 30 periods. Urinary creatinine excretion (UCE) and urinary iodine excretion (UIE) of each period was determined by the methods mentioned in 3.2.1.i and 3.2.1.ii. The 24-hour urinary iodine and creatinine excretions were calculated by summating those values from

all urine samples together. Total dietary iodine intake (TDII) was calculated by using food tables and the amount of foods that had been eaten.

From Table 3.4, and Figures 3.3 and 3.4, it was found that:

(1) The majority of dietary iodine intake was excreted in urine on the day in which it was eaten;

(2) As a whole the mean of the per cent of recovered iodine in urine (per cent RIU) of d1-d5 was 62.26 per cent of total dietary iodine intake;

(3) With regard to the iodine supplement (472.88) of d3, the per cent of recovered iodine in urine (per cent RIU) was {(365.47-95.46)x100}/472.88 = 57.10 per cent of the dose.

Table 3.4: Total liquid intake (TLI), total urine volume (TUV), 24-hour urinary creatinine excretion (TUCE), 24-hour urinary iodine excretion (TUIE), total dietary iodine intake (TDII) and percent of recovered iodine in urine (per cent RIU) of days 1, 2, 3, 4 and 5.

Data	Day 1	Day 2	Day 3	Day 4	Day 5
TLI (ml/d)	1560	1560	1560	1560	1560
TUV (ml/d)	1232.9	1064.4	1112.2	1334.3	1535.0
TUCE (g/d)	1.529	1.566	1.508	1.484	1.453
TUIE(μg/g Cr)	62.92	60.96	242.35	74.66	57.21
TDII (μg/d)	152.50	152.50	625.38	152.50	152.50
TUIE (μg/d)	96.20	95.46	365.47	110.79	83.13
% RIU	63.08	62.60	58.44	72.65	54.51

Figure 3.3: UIE (μg/g creatinine) in each period during d1-d5

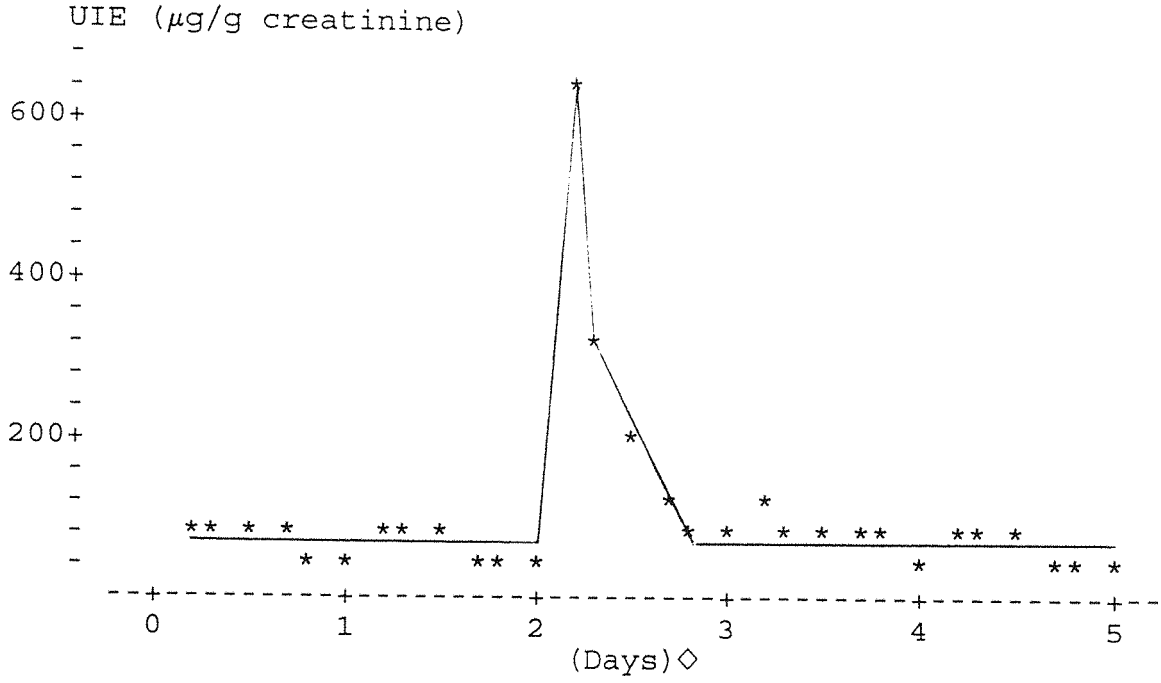
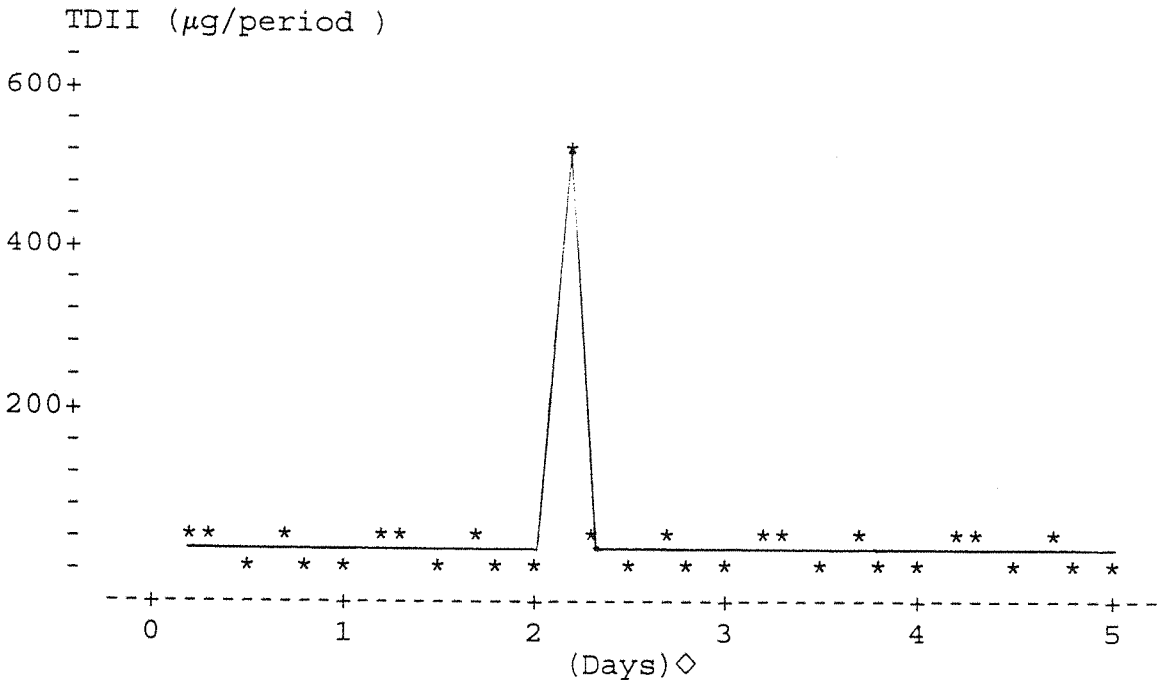


Figure 3.4: TDII (μg/period) in each period during d1-d5



◇ = One day had six periods and one period had four hours
* = One period

3.2.7 The methodology of measuring iodine content in foods

To estimate dietary iodine intake from estimated weighed food intakes requires the availability of data on the iodine content of the food being eaten.

To date in Northern Thailand there are no data on the iodine content of foods. The objective of this section was to check the validity and reliability of a method of determining the iodine content (modified from the Moxon's method, 1980) in food samples.

3.2.7.i Principle of the method

In brief, there are three steps for the determination of total iodine in food.

(a) Destruction of organic matter in food

Dry alkaline ashing is used to prepare samples of food for determination of iodine. Samples are ashed in a muffle furnace at 550°C for one hour in the presence of potassium carbonate and zinc sulphate.

(b) Extraction of iodide from the ash residue

The organic residue is dissolved in distilled water and carbonaceous matter is removed by centrifugation.

(c) Colorimetric reaction

This method is suitable for measuring the iodide content of solutions prepared. The colorimetric reaction is based on the destruction of the intensity of the orange ferric thiocyanate complex by nitrite in the presence of iodide.

The reduction in the intensity of the orange colour is proportional to the iodide concentration in the solution and can be read off against a standard curve to assess the iodine concentration.

Regarding the materials and method for measuring iodine content in foods, it was as shown in **appendix 5**.

3.2.7.ii Reproducibility and percentage recovery

One milk powder sample was analyzed 17 times for iodine content. The Coefficient of variation (CV) was 8.30 per cent, which is within the acceptable range, although rather high, suggesting some variability in the measurement. A known amount of iodide was added into a milk powder sample and the recovery of iodide measured. It was found that the percentage of iodide recovered varied from 94.7-104.4 per cent.

3.2.8 To summarize the results of development of methods and five experiments:

(1) The methods of determining urinary creatinine, urinary iodine and iodine content in foods described above appear to be satisfactory, in terms of both the reproducibility and the percentage of recovery;

(2) The results of the study comparing the total dietary iodine intake (TDII) and 24-hour urinary iodine excretion (TUIE) suggested that the higher the dietary iodine intake, the higher the 24-hour urinary iodine excretion. However, the 24-hour urinary iodine excretion (TUIE) did not represent 100 per cent of the total dietary iodine intake. From **chapter 2**, there were a number of studies which confirmed this conclusion, for example, Broadhead *et al.* (1965), Vough *et al.* (1964), Nelson *et al.*

(1987) and Brug *et al.* (1992). **On this basis, it may inferred that although 24-hour urinary iodine excretion can not be used for calculating iodine intake directly, it may be used for discriminating between the levels of iodine intake;**

(3) Creatine supplementation (1 g creatine a day for three days) tends to increase the 24-hour urinary creatinine excretion (g/d). This suggests that urinary creatinine excretion should be considered when urinary iodine excretion (μg/g creatinine) from a casual urine sample is used to estimate 24-hour urinary iodine excretion;

(4) The levels of protein intake may affect indirectly or directly the 24-hour urinary iodine excretion (μg/d). From **section 3.2.5**, an adequate protein intake (nitrogen = 165 mgN/kg/d,) could be related to higher urinary iodine excretion than a low protein intake (nitrogen = 68 mgN/kg/d, 4 days). This may have been related to the high protein content of foods such as, whole milk, eggs and fish fingers, which also contain more iodine than foods of low protein origin. Levels of protein intake may affect urinary iodine excretion (μg/d) directly since animal experiments have shown that a diminished renal iodine clearance (the rate at which the plasma is cleared of iodine by the kidneys) is found in rats after protein starvation (Wayne *et al*, 1960). Therefore, the lower the renal iodine clearance values, the lower the 24-hour urinary iodine excretion. Moreover, some amino acids, for example, tyrosine are needed for synthesis of thyroglobulin which is essential for producing thyroid hormones. Therefore, a low protein intake may lead to a restriction of some amino acids (for example, tyrosine deficiency) and finally may have an effect on 24-hour urinary iodine excretion (TUIE, μg/d);

(5) From **section 3.2.3**, in the same subject, the per

cent CV of urinary iodine excretion expressed per gram creatinine was smaller than that of urinary iodine excretion expressed μg per cent. As a result, urinary iodine excretion (μg/g creatinine) from a casual urine sample gave a better estimate of 24-hour urinary iodine excretion (μg/day) than that obtained using urinary iodine excretion (μg per cent).

Although 24-hour urine specimens are preferred to estimate the level of total dietary iodine intake (TDII), they are not practical for large-scale surveys. Moreover, the completeness of 24-hour collections is in doubt, especially without the use of markers such as para amino benzoic acid (PABA). It has been proposed that the ratio between the concentrations of iodine and creatinine (μg/g creatinine) may be a reasonable estimate of intake.

3.3 PILOT STUDY IN THAILAND: THE RELATIONSHIP BETWEEN URINARY IODINE EXCRETION IN 24-HOUR URINE COLLECTION AND CASUAL URINE SAMPLES IN THAI PREGNANT WOMEN

From the results in **section 3.2.3**, the urinary iodine excretion (UIE, μg/g creatinine) of a casual urine sample was used for estimating the 24-hour urinary iodine excretion (TUIE, μg/d). It was found that the urinary iodine excretion (UIE, μg/g creatinine) from a casual urine sample was a reasonable approximation of the 24-hour urinary iodine excretion (TUIE). However, the estimation of TUIE seemed to be valid only if the daily urinary creatinine excretion by a given subject was fairly constant. Some investigators (Frey *et al.*, 1973; Hass *et al.*, 1988) have previously established a positive correlation equation for estimating the 24-hour urinary iodine excretion (TUIE) from the urinary iodine excretion expressed per gram creatinine (UIE, μg/g creatinine) in a single urine specimen (see **detail in chapter 2**). They concluded that to estimate the 24-hour urinary iodine excretion, using the correlation equation on the basis of the I/Cr ratio (including its standard deviation), seemed to be better than the calculation by multiplication of the individual I/Cr with by factor representing a theoretical average creatinine excretion (g/d).

So far, it may be inferred that there was a positive relationship not only between the total dietary iodine intake and the 24-hour urinary iodine excretion, but also between the 24-hour urinary iodine excretion and urinary iodine excretion (μg/g creatinine) from a casual urine sample. Obviously, the levels of iodine intake are not the 'truth' but will give the same pattern of intake.

To investigate whether urinary iodine excretion (μg/g creatinine) from a casual urine sample can be used to estimate the pattern of iodine intake in Thai pregnant women, a pilot study was done in Thailand. As far as I am aware no such study has been done before. In this study, the correlation between iodine excretions in 24-hour urine collection (TUIE, μg/d) and casual urine samples (UIE, μg/g creatinine) and the correlation between iodine levels in 24-hour urine collection (TUIE, μg/d) and total dietary iodine intake (TDII, μg/d) were investigated in 10 well-educated Thai pregnant women at various gestational age (8-33 weeks).

Casual urine samples were collected from subjects separately for each voiding from 6.00 a.m. of the study day until 6.00 a.m. on the following day. These urine samples were analyzed for iodine and creatinine by using the method mentioned in **sections 3.2.1 and 3.2.2**. The 24-hour urinary iodine (TUIE) and creatinine (TUCE) excretions were calculated by summating those values from all urine samples together.

The quantity of food intake of each subject was also recorded on the day of urine collection. These foods were collected and analyzed for iodine content using the modification method of Moxon (1980) which was explained in detail in **section 3.2.7 and appendix 5**. The total dietary iodine intake (TDII) of each subject was then calculated.

It may be inferred that:

(1) There was a positive and statistically significant relationship between total dietary iodine intake (TDII, Mean±SD = 129.59±71.17 μg/d, n=10) and 24-hour urinary iodine excretion (TUIE, Mean±SD = 104.93±48.66 μg/d, n=10).

The correlation was r=0.81 (P<0.05, n=10). The average per cent of recovered iodine in urine was 93.65 per cent (with per cent CV = 42.99), as shown in **Table 3.5.**

Table 3.5: The mean of total dietary iodine intake (TDII, μg/d), 24-hour urinary iodine excretion (TUIE, μg/d) and percent recovered iodine in urine (per cent RIU) of 10 Thai pregnant women

	TDII (μg/d)	**TUIE (μg/d)**	**(%) RIU**
Mean	129.59	104.93	93.65
SD	71.17	48.66	40.26
%CV	54.92	46.37	42.99
N	10	10	10

The results for the percent recovery of iodine in urine (per cent RIU) among Thai pregnant women were similar to those reported in the study of Dworkin *et al.* (1966). Dworkin *et al.* (1966) studied the relationship of iodine ingestion to iodine excretion in 4 pregnant women. Their results show a positive correlation between total dietary iodine intake (μg/d) and 24-hour urinary iodine excretion (μg/d) (r = 0.69, P<0.05). However, the percent recovery of iodine in urine (per cent RIU) of their study (mean = 121.9 per cent, S.D = 62.1 per cent and per cent CV = 50.9) is higher than that in this study. This may be due to the number of subjects for the study and the difference in gestational age of pregnant subjects in our study and Dworkin's study.

(2) It was found that there was a positive significant correlation between the urinary iodine excretion in casual urine sample (UIE, μg/g creatinine) of the first voiding (>6-12 a.m.) and the 24-hour urinary iodine excretion (TUIE, μg/d). It was r = 0.68 (P<0.05, n=10). There were also positive significant correlations between the 24-hour

urinary iodine excretion (TUIE, μg/d) and the means of urinary iodine excretion in casual urine samples (UIE, μg/g creatinine) during the periods >6 to 12 a.m., >12 to 18 p.m., >18 to 24 p.m., and >0 to 6 a.m. at r= 0.80, 0.86, 0.97 and 0.76 ($p<0.05$, n=10) respectively (see **Table 3.6**).

Table 3.6: The correlation between urinary iodine excretion (UIE, μg/g creatinine) from casual urine samples and 24-hour urinary iodine excretion (TUIE, μg/d)

SUBJ	TUIE (μg/d)	UIE (μg/g creatinine) in casual urine samples				
		1st void. >6-12 a.m.	Mean >6-12 a.m.	Mean >0-6 p.m.	Mean >6-12 p.m.	Mean >0-6 a.m.
1	129.18	102.5	102.5	182.0	119.8	63.7
2	85.84	73.5	73.5	109.8	94.6	74.2
3	86.19	65.2	91.7	129.9	90.6	66.2
4	136.33	60.9	60.9	156.2	172.6	138.9
5	86.02	74.6	83.6	93.9	84.2	81.8
6	93.25	63.6	63.6	104.0	113.2	109.4
7	223.85	104.6	144.4	190.7	263.3	142.2
8	65.55	50.2	50.2	51.5	66.1	62.4
9	54.19	65.6	65.6	70.8	81.4	63.1
10	88.93	87.3	87.3	91.2	99.6	91.3
Mean	104.93	74.8	82.3	118.0	118.5	89.3
SD	48.66	18.0	27.0	46.2	58.6	30.8
%CV	46.37	24.1	32.8	39.2	49.4	34.4
r	-	**0.68**	**0.80**	**0.86**	**0.97**	**0.76**
P	-	**<0.05**	**<0.05**	**<0.05**	**<0.05**	**<0.05**

We also investigated the correlation between total dietary iodine intake (TDII, μg/day) and urinary iodine excretion

from casual urine samples. The similar results were achieved. The correlation between the UIE (μg/g creatinine) in casual urine samples of the first voiding (>6-12 a.m.) and the TDII (μg/day) was 0.69 ($P<0.05$, n=10).

(3) In this study, the result also showed that the average of the coefficient variation (per cent CV) in urinary iodine excretion from casual urine samples expressed μg per cent (Mean$\pm$SD = 55.22 $\pm$ 17.07, N=10) was higher than that expressed per g creatinine (Mean$\pm$SD = 27.27$\pm$12.11, N=10).

In conclusion, it may be suggested that urinary iodine excretion (UIE, μg/g creatinine) from casual urine samples can be used as a biomarker for discriminating between the levels of iodine intake. However, there are some factors which can affect urinary creatinine output (for example, dietary protein, creatine, and creatinine intakes; section **2.3.2.iii.c**) and also urinary iodine excretion (μg/g creatinine) (for example, goitrogens and gestational age at urine samples collected) which need to be considered in the analysis of the results. In addition, the CV of urinary iodine excretion (μg/g creatinine) from casual urine samples is also quite large and suggests within subject variation which may reduce the statistical power of the study. For example, from equations; $N = 2\sigma^2 (Z_{\alpha/2} + Z_{\beta})^2/d^2$ and CV = σ/MEAN, it can be seen that if CV is high, the standard deviation (σ) will be high and it will reduce the statistical power of the study (β). In this case, the number of subjects in a study should be considered with the most conservative estimate of the accuracy of the measures used.

3.4 METHODS FOR THE MAIN STUDY

3.4.1 Study design

This **prospective cohort study** derived some basic data from **the Low Birthweight (LBW) project** which was in cooperation between the Research Institute for Health Sciences and other faculties of Chiang Mai University (for example, Medicine, Nursing and Social sciences), Chiang Mai, Thailand. The research was supported by the Ford Foundation, New York. The objective of the LBW project was to verify the probable risk factors contributing to LBW in Northern Thailand. Risk factors (i.e. suspected effects on the incidence of LBW) were recorded. They were biomedical factors (maternal factors and fetal factors), socio-economic factors and nutritional factors. Since the LBW was a prospective cohort study, the sample size required for each factor was calculated using the following formula:

$$N = [Z_{\alpha} (2pq)^{\frac{1}{2}} + Z_{\beta} \{p1 \{1+R-p1(1+R^2)\}\}^{\frac{1}{2}}]^2 / \{p1 (1-R)\}^2$$

Where:

N = the sample size required for each factor

p1 = the incidence of LBW among the non-exposed population

p2 = the incidence of LBW among the exposed population

R = the relative risk of LBW which is equal to p2/p1

p = 1/2 p1(1+R)

α = type I error rate

β = type II error rate

Z_{α} and Z_{β} = unit normal deviate corresponding to types I and II error rates.

In calculating the sample size, the LBW project used data from a study of factors affecting rural LBW in Thailand, conducted in Yala Province from November 1979 to August

1981 by the Family Health Division, Department of Health, Ministry of Public Health. Seven factors were studied, namely: mother's education; family income; birth intervals; first prenatal visit; amount of food intake; history of previous obstrical complications and the age of the mother. **Table 3.7** showed the sample size required for each factor using the formula mentioned above with 95 per cent power of the test and 0.05 per cent type 1 error rate (two side test).

Table 3.7: The sample size (N) required for each factor

Factors	p1	p2	R	N
Mother's education	0.318	0.347	1.090	7,196
Family income	0.412	0.642	1.559	120
Birth interval	0.338	0.284	0.840	1,912
First prenatal visit	0.320	0.468	1.463	280
Amount of food intake	0.257	0.458	1.780	145
History of previous obstetrical complication	0.332	0.347	1.046	29,072
Age of mother	0.314	0.475	1.513	237

If family income, first prenatal visit, amount of food intake and the age of the mother were studied in the LBW project, at least 196 LBW index cases {(120+280+145+237)/4} needed to be recruited. Since the incidence of LBW in Northern Thailand was approximately 13 per cent, the number of index cases (196) could be obtained from the number of infant cases, or 1508 (100x196/13). Assuming that the drop out rate is 30 per cent, then the number of infant cases should be 1,960. If the percentage of abortions is 10 per cent, then the number of recruited cases should be 2,156. The LBW project started in 1990 at Chiang Mai province, Thailand and the criteria for inclusion and exclusion were as follows:

3.4.1.i Criteria for inclusion

Pregnant women attending antenatal clinics at the two study centres (Maharaj Nakorn Chiang Mai Hospital, centre 1; and Health Promotion Centre, Region 5, Ministry of Public Health; centre 2) and having gestational age less than or equal to 24 weeks.

3.4.1.ii Criteria for exclusion

Those pregnant women who have been recruited in the study but do not give birth at the two selected study centres.

In the LBW project, all those pregnant women who attended the antenatal clinic and fulfilled the inclusion criteria were recruited. These subjects were followed up periodically until delivery. At the end of the follow up period (August, 1991) there were 2,623 pregnant subjects who delivered their infants at the centres. In centre 1, there were 1,415 pregnant subjects; 88.55 per cent of them lived in Chiang Mai province and 11.45 per cent of them lived in the other provinces next to Chiang Mai. In centre 2, there were 1,208 pregnant subjects; 86.67 per cent and 13.33 per cent of them lived in Chiang Mai province and in the other provinces next to Chiang Mai respectively.

The present study was initiated when approximately 85 per cent of the total Thai pregnant subjects had already been recruited in the LBW project. Thus, the last 15 per cent of Thai pregnant women of the LBW project (centre 1, n=192 and centre 2, n=169) were recruited in this study. Of these pregnant women (n=361), casual urine samples, information about dietary intake, and other factors which may affect birth outcome of their infants were collected, along with their addresses. This is shown in **Table 3.8**;

Table 3.8: Address of pregnant subjects in centre 1 and centre 2

Address	Centre 1		Centre 2		Total	
	N	%	N	%	N	%
Chiang mai	172	89.6	144	85.2	316	87.5
Others*	20	10.4	25	14.8	45	12.5
Total	192	100.0	169	100.0	361	100.0

(* means Lampoon and Chiang Rai provinces)

From **Table 3.8**, it can be seen that the distribution of pregnant subjects in each centre of this study was similar to that of the LBW project.

With regard to the objective of this thesis, we need to investigate the relationship between dietary iodine intake (assessed by the level of urinary iodine excretion from casual urine samples) and birth outcome, rather than to investigate the risk factors of low birthweight infants.

Therefore, the sample size of casual urine samples required to compare the means of three groups of urinary iodine excretion (low, medium and high) were calculated from the following formula:

$$N = 2\sigma^2 (Z_{\alpha/2} + Z_{\beta})^2/d^2$$ (Cole *et al.*, 1991)

Where: N = sample size required for each group

σ = the standard deviation of the variable

α = type I error

β = type II error

d = the difference between the groups to be detected

Z_α and Z_β = unit normal deviate corresponding to types I and II error.

From **section 3.3** (see **Table 3.10**), the Mean±SD of urinary iodine excretion from casual urine samples of Thai pregnant women was 74.8±18.0 μg/g creatinine (n=10). Assuming it needed to be able to detect a 10 per cent difference of the mean with 80 per cent power at five per cent significance requires;

$$N = 2X18^2X(1.96+0.84)^2/(7.5)^2$$

$$= 91 \text{ subjects in each group.}$$

Therefore if there were three groups of urinary iodine excretion (low, medium and high), the number of total subjects should be 273.

By using the same formula, the sample size for each group (low, medium and high) of birth outcome variables were calculated, as shown in **Table 3.9**

Table 3.9: The sample size (N) required for each group of birth outcome variables (at α = 0.05)

Birth outcome	Mean ± SD	Power (80 %)		Power (90%)	
		d=10%	d=5%	d=10%	d=5%
BWT(g)	3006 ± 403	29	116	38	152
HC (cm)	33.2 ± 1.3	3	12	4	16
Length (cm)	49.4 ± 2.1	3	12	4	16
RHL (%)	67.2 ± 3.0	4	16	5	20
CC (cm)	31.5 ± 1.8	6	24	7	28

BWT = Birthweight, HC = Head circumference, CC = Chest circumference and
RHL (%) = (head circumference/length)*100
d = The difference between the groups to be detected

From **Table 3.9**, for example, if we want to detect a 10 per cent difference of mean of birth outcome variables with 80 per cent power at five per cent significance, the number of total subjects for birthweight, head circumference, length, RHL and chest circumference should be 87 (29x3), 9 (3x3), 9 (3x3), 12 (4x3) and 18 (6x3) respectively.

With 361 subjects there should be sufficient statistical power to detect biologically meaningful differences, if they exist.

3.4.2 Urinary analysis

Casual urine samples: Since it was not possible to collect 24-hour urine samples in these subjects and we have shown that there was a positive relationship between 24-hour urinary iodine excretion (TUIE, ug/d) and urinary iodine excretion (ug/g creatinine) from a casual urine sample, their casual urine samples were collected. Pregnant

subjects attending ANC were asked to collect their casual urine samples (usually during 9 a.m.-12 a.m.) in a clean plastic bottle which had a few drops of toluene as a preservative. These urine samples were collected at approximately 12, 24, 32 and 36 weeks of gestational age. However, the exact time was dependent upon when they were recruited in the study and how often they followed the attended ANC. Therefore, some subjects gave five samples but some subjects gave only one casual urine sample.

Given this, there were 1126 casual urine samples among 361 pregnant women. These urine samples were kept in a deep freeze refrigerator (-30°C) until they were analyzed for creatinine and iodine contents by the methods mentioned in **sections 3.2.1 and 3.2.1**.

3.4.3 Dietary survey

In this study, there were 2 methods of dietary survey; a 24-hour recall method; and food frequency method. In each method, there was a questionnaire form for interviewing pregnant subjects. Each pregnant subject was interviewed three times; during the first trimester (10-12 weeks), second trimester (22-24 weeks) and third trimester (32-34 weeks). However, by the criteria for inclusion (pregnant subjects having gestational age ≤ 24 weeks), the first interview (or the first visit) may have been in the second trimester. The number of interviews was dependent upon both the follow up and when a pregnant subject was recruited into this study. The process for interview of both questionnaire forms was about 15 minutes per subject.

3.4.3.i The 24-hour recall method

The 24-hour recall was used as an attempt to quantify food intake during a specific day just before the interview. Each pregnant subject was asked to recall all food consumed during the previous day and to estimate quantities in ordinary measures or servings. In order to increase the accuracy of quantities of food consumed, the pregnant subject was provided with measuring cups or other devices to aid the estimation.

The 24-hour recall is not valid as an accurate measure of food intake of one individual. But when large numbers of subjects are involved, the method is considered to be indicative of the dietary pattern characteristic of the group.

3.4.3.ii Food frequency method

The aim of the food frequency approach is to assess average long term diet, for example, intake over weeks, months, or years, and is the conceptually important exposure rather than the intake on a few specific days.

In this study, food frequency was used to determine the overall pattern of food consumed over a one month period. Thus the multiple-choice response format, with the number of options provided. There were 6 categories: every day, 4 times per week, 1-2 times per week, 1-2 times per month, rarely and never. For the food list, foods were divided into 5 categories depending on the nutrients contained in each food item e.g. carbohydrate, protein, fat, minerals and vitamins. They were as follows:

1) Starch and sugar e.g. glutinous rice, rice, noodle, sugar, bread, and potatoes

2) Meats and pulses e.g. pork, chicken, duck, beef, buffalo, fresh fish, fermented fish, kapi, sea fish, squid, prawn, egg, beans, fresh milk, and soya milk
3) Foods cooked with vegetable oil, animal fat and coconut milk.
4) Vegetables
5) Fruits.

The reasons for dividing foods into these five categories were as follows: first, it was easy to interview; second, it was important to select carefully the most informative items for the food list to avoid fatigue and boredom that could impair concentration and accuracy of the interviewee; and third, to make sure that the food had a substantial content of the nutrient(s) of interest. Thus food frequency will give information about frequency of some food groups consumed by pregnant subjects which may be relevant to dietary iodine intake during pregnancy. Finally, energy intake and the other nutrient intake such as protein, fat and carbohydrate intake were calculated from the 24-hour recall data using the Thai food composition tables. There were no iodine values in the Thai food tables. In order to provide an estimate of iodine intake, the iodine content of the most commonly consumed foods was assessed.

3.4.4 Assessment of birth outcome

The size at birth of the infants who were delivered by those pregnant subjects was recorded by specially trained nurses. These nurses were trained to measure birth outcome by an expert in anthropometric measurement and then their ability to assess birth outcome was standardized by measuring the birth outcome of a number of infants. The results of the repeatability study showed no statistically significant differences between repeated measures in the

same child by the interviewers. The methods used for each measure are described below.

3.4.4.i Birthweight (BWT)

Special scales (Seca) that allow infants to lie or sit were used to measure the infant's weight. The capacity of the weighing machine was 10 kg and the minimum scale was 20 g. Scales were sandardized using a known weight.

3.4.4.ii Length

It was measured by using a measuring board (HEALTH-O-METER, made by CONTINENTAL) that had a fixed headboard and movable footboard contacts. It took two people to obtain an accurate measurement; one to hold the infant's head against the headboard and keep the leg straight, and the other to do the measuring. The infant's length was measured as in centimetres, the minimum scale was 0.1 cm.

3.4.4.iii Head circumference (HC)

It was measured by using a nonstretchable tape (made of fibre glass, the minimum scale was 0.1 cm) which was placed so as to encircle the largest part of infant's head, just above the eyebrow ridges, just above the point where ears attach, and around the occipital prominence at the back of the head.

3.4.4.iv Chest circumference (CC)

To measure chest circumference, a nonstretchable tape (made of fibre glass, the minimum scale is 0.1 cm) was used. It was measured at the level of the nipple line and after breathing out.

3.4.4.v The percentage of head circumference to length [RHL (%)]

This percentage was calculated by:

RHL % = (Head circumference/Length)X100

3.4.5 Other factors

The other factors which may affect birth outcome were recorded and divided into three groups. They were as follows;

3.4.5.i Social economic status

Education, occupation of pregnant subjects and their total income were recorded by questionnaire.

3.4.5.ii Maternal status

Age and height of pregnant subjects were recorded at the first admission whereas weight, mid arm circumference, triceps skinfolds, systolic blood pressure and diastolic blood pressure during the pregnancy were measured by standard instruments and methods at the first admission and every visit.

3.4.5.iii Gestational age

The gestational age at the time of each casual urine sample was collected (GAI). Further, the gestational age at birth (GABI), gestational age at food frequency and 24-hour recall interviewed (GAFOOD) and also gestational age (GA) at each follow up were recorded.

3.4.6 Data analysis

The following methods were used to analyze the data in the study. Prior to this, the information was organized into an SPSS computer file.

3.4.6.i Descriptive statistics

It is concerned with techniques that are used to describe or characterize the data. It may be divided into 2 types, namely, the measurement of central tendency (e.g. average, geometric mean, harmonic mean, mode, median, quartiles, deciles and percentiles) and the measurement of dispersion (e.g. range, average deviation, quartile deviation, standard deviation, coefficient of dispersion and shape of distribution).

3.4.6.ii Cross-tab tables and Chi-square

Cross-tab tables are contingency tables, which list cell frequencies for data classified by at least two variables. The table also show row and column frequencies and percentages. Chi-square is a statistic used for evaluating whether there are associations between variables in cross-tab tables. Chi-square (X^2_{obt}) is calculated from the equation: $X^2_{obt} = \Sigma(f_o-f_e)^2/fe$,

where fo = observed frequency in cell,
fe = expected frequency in the cell, and Σ is over all the cells.

After evaluating X^2_{obt}, then X^2_{crit} (df = k-1, α = 0.05) must be determined from a statistical table, where df is degree of freedom and k equals the number of groups or categories. If $X^2_{obt} \geq X^2_{crit}$, then the null hypothesis (there is no

association between the variables) will be rejected using the 5 per cent nominal level of statistical significance.

3.4.6.iii Analysis of Variance (ANOVA)

The analysis of variance is a statistical technique employed to analyze multigroup comparisons. By using the F test (F_{obt}), it is possible to assess whether there are significant differences between the means of the groups. F_{obt} is calculated from the equation:

$$F_{obt} = \frac{\text{between-groups variance estimate } (S_B^2)}{\text{within-groups variance estimate } (S_W^2)}$$

F_{obt} is evaluated by comparing it with F_{crit} ($df_b = k-1$, $df_w = N-k$, $\alpha = 0.05$) from a statistical table,

where df_b = between-groups degrees of freedom,
df_w = within-groups degree of freedom,
k = the number of groups,
$N = n_1+n_2+n_3+\ldots n_k$.

If $F_{obt} \geq F_{crit}$, then the null hypothesis (H_0)will be rejected. Here, H_0 means that there is no a significant difference between the means of the groups. The analysis of variance (ANOVA) is used in both independent groups and repeated measures designs. It also used when one or more factors (variables) are investigated in the same experiment.

3.4.6.iv Correlation

Correlation techniques are used to study relationships. A number representing the strength of a relation can be calculated and it is called the correlation coefficient. The Pearson Product Moment Correlation Coefficient (**r**) is the most usual method by which the relation between two variables is quantified. The correlation coefficient may range for +1.00 through 0.00 to -1.00. A +1.00 indicates a perfect positive relationship and -1.00 indicates a perfect negative relationship. However, a correlation, which shows that two variables are related, does not mean that one variable caused the other. The formula for the correlation coefficient is

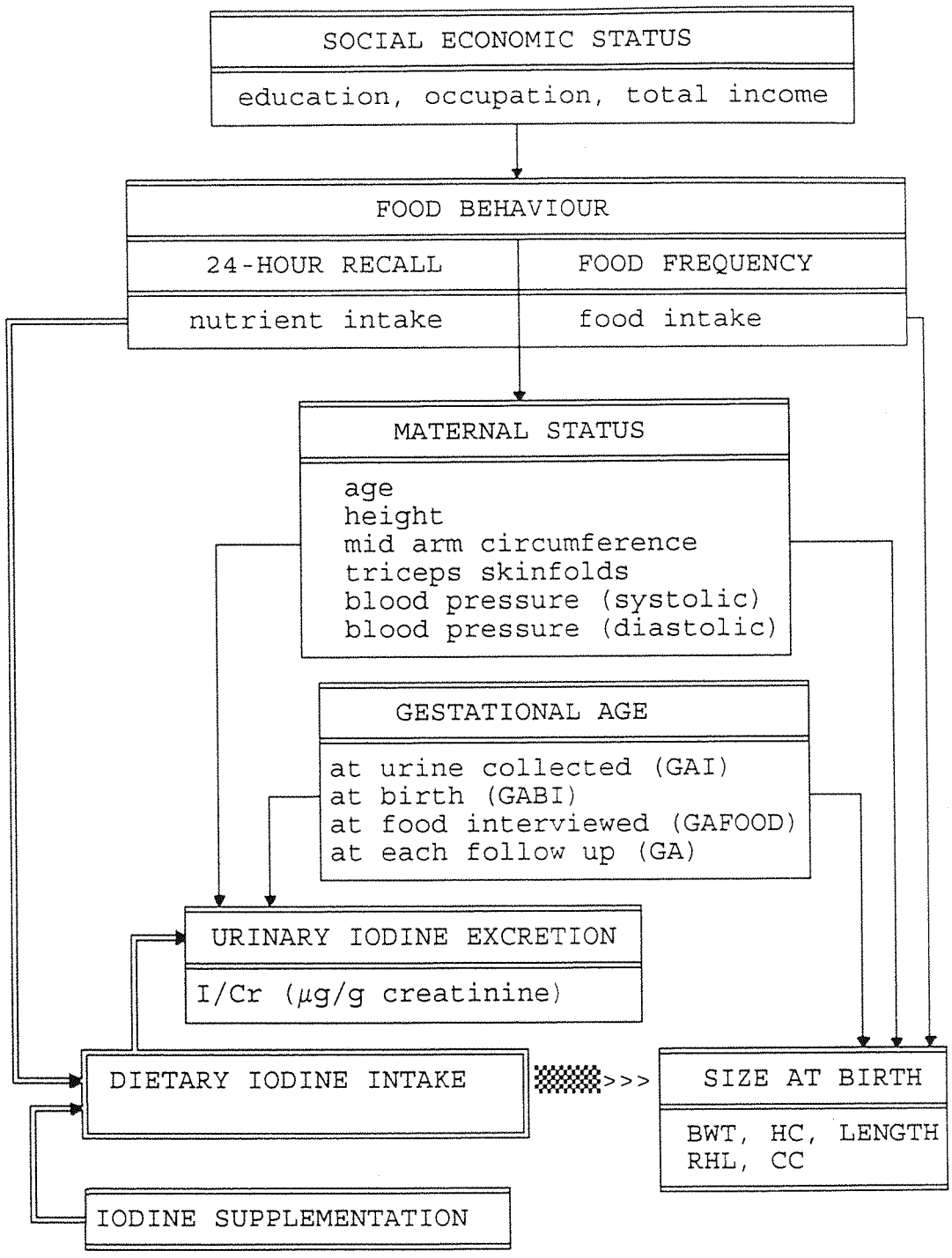
$$r = \frac{\Sigma XY - (\Sigma X)(\Sigma Y)/n}{[\{\Sigma X^2 - (\Sigma X)^2/n\}\{\Sigma Y^2 - (\Sigma Y)^2/n\}]^{1/2}}$$

where; X = data of variable x
Y = data of variable y
n = sample size of variables x and y.

Generally, the statistical significance of the correlation can be determined by using the table in a statistical text book.

In brief, the possible relationship between dietary iodine intake, iodine supplementation, social economic status, food behaviour, marital status, gestational age and birth outcome are shown in **Figure 3.5**.

Figure 3.5: Factors which may effect birth outcome.



CHAPTER 4
RESULTS OF MAIN STUDY

4. RESULTS

This chapter is divided into six sections. Firstly, it covers general information, including the location and the characteristics of Thai pregnant subjects, maternal anthropometry and blood pressure, food intakes, food behaviour, urinary iodine excretion of Thai pregnant subjects during this study and the results of birth outcome. Secondly, it describes the variables which may affect either urinary iodine excretion or birth outcome/ and or both of them. It is also concerned with how to stratify urinary iodine excretion and birth outcome by these variables. Thirdly, the correlation between urinary iodine excretion and birth outcome is investigated. Fourthly, it presents the results of the interaction between urinary iodine excretion and birth outcome. Fifthly, it shows the results of the effect of iodine supplement on birth outcome. Finally, it describes the effect of changing urinary iodine excretion during pregnancy on birth outcome.

4.1 GENERAL INFORMATION

4.1.1 Location

There were 361 Thai pregnant women recruited in this study of which 192 and 169 pregnant subjects delivered their babies at Maharaj Nakorn Chiang Mai hospital (centre 1) and Health Promotion, region 5 (centre 2) respectively. Both centres are in amphur Muang (amphur=district). Most subjects lived in Chiang Mai province (87.5 per cent). The distribution of location of pregnant subjects in each centre was similar (see **Table 4.1**).

Table 4.1: Location of pregnant subjects at Maharaj Nakorn Chiang Mai hospital (C1) and Health Promotion, Region five (C2)

Address	C1	%	C2	%	Total	%
Chiang Mai	172	89.6	144	85.2	316	87.5
Others*	20	10.4	25	14.8	45	12.5
Total	192	100.0	169	100.0	361	100.0

(* = Lampoon and Chiang Rai provinces)

4.1.2 The characteristics of Thai pregnant subjects

Table 4.2 shows the characteristics of Thai pregnant subjects at first admission (the first day when a pregnant woman is recruited to be a subject). It can be seen that the total average age of these pregnant subjects was 25.3 years (SD = 4.5). Pregnant subjects in centre 1 had higher average age than those in centre 2 (P=0.0001). About 20.0 per cent of these pregnant subjects were agricultural workers. In centre 1, the percentage of pregnant subjects, who were agricultural workers, was higher than that of pregnant subjects in centre 2 (LR =0.0003). The mean total income was 4110 Baht per month (about £110). Pregnant women's average total income in centre 1 seemed to be higher than that in centre 2. However it was not normally distributed. After the data were transformed into the log form, it was found that pregnant women's average total income in centre 2 (Mean = 3.54, SD = 0.27) was significantly higher ($P < 0.05$) than that (Mean =3.47, SD = 0.30) in centre 1. There was no difference in the length of education received by the subjects in centres 1 and centre 2, and 73.6 per cent of them had been educated according to the Thai government's 6 years of compulsory education.

Table 4.2: Characteristics of Thai pregnant subjects in the study at first admission

Characteristics	Centres		
	1	2	Total
Total subjects:			
Numbers	192	169	361
per cent	53	47	100
Age (years):			
Mean	26.1[a]	24.2[b]	25.3
SD	4.5	4.2	4.5
Occupation (%)			
Agricultural working	27.7	10.8	19.7
Non-agricultural working	72.3	89.2	80.3
Total income (Bht/month)*:			
Mean	4303	4196	4110
SD	5405	2972	4418
Length of education (%):			
≤ 6 years	74.1	73.0	73.6
> 6-12 years	15.7	18.9	17.2
>12 years	10.2	8.1	9.2

(* 38 Bht = £1)
Variables with different superscript are significantly different between centres (P=0.0001)

4.1.3 Maternal anthropometry and blood pressure

Table 4.3 illustrates maternal anthropometry at first admission and before labour. At the first admission, the average gestational age, height, weight, mid arm circumference, triceps skinfold, systolic blood pressure, and diastolic blood pressure were 13.1 wks, 151.9 cm, 49.5 kg, 24.3 cm, 19.1 mm, 113.2 mmHg and 72.4 mmHg respectively. Before labour, only gestational age at birth, weight and blood pressure were able to be evaluated, because the time and circumstances of the delivery meant that the research team staff could not be present to make all the desired measurements. The mean weight of pregnant subjects before labour was 59.6 kg. The mean gestational age at birth of their infants was 39.0 wks. The mean

systolic and diastolic blood pressure of the Thai pregnant subjects were 117.8 mmHg and 77.8 mmHg respectively. The results showed that the average values of weight and blood pressure (both systolic and diastolic) of Thai pregnant subjects before labour were increased significantly (*P*=0.0001) when they were compared with those at first admission.

Table 4.3: Gestational age, maternal anthropometry and blood pressure at the first admission and before labour

Maternal anthropometry & blood pressure	**1 st Admission**	**Before labour**
	Mean (SD) *n*	**Mean (SD)** *n*
Gestational age (wks)	13.1 (4.5) *361*	39.0 (1.7) *361*
Height (cm)	151.9 (5.1) *361*	-
Weight (kg)	49.5[a] (7.4) *361*	59.6[b] (7.4) *216*
Mid arm circumference (cm)	24.3 (2.6) *360*	-
Triceps skinfold (mm)	19.1 (5.7) *360*	-
Blood pressure: -Systolic -Diastolic (mmHg)	113.2[a] (9.4) 72.4[a] (7.0) *357*	117.8[b] (10.9) 77.8[b] (9.1) *353*

For each variable separately, groups with different superscript are significantly different (*P* = 0.0000)

In addition, maternal anthropometry was divided into three groups, namely first (≤12 weeks), second (>12 to 24 weeks) and third trimesters (>24 weeks), see **Table 4.4**.

The mean weight of Thai pregnant subjects increased significantly from the first to the third trimesters. The change in weight between the third and the first trimesters was about ten kilograms.

The mean mid arm circumference (24.9 cm) and triceps skinfold (21.1 mm) of Thai pregnant subjects in the third trimester were significantly higher than those in the first and the second trimesters (24.4 cm for mid arm circumference, 19.1 and 19.5 mm for triceps skinfold).

The mean blood pressure (diastolic) of Thai pregnant subjects in the second trimester (71.2 mmHg) was found to be significantly lower than those in the first (72.4 mmHg) and the third (72.9 mmHg) trimesters.

Table 4.4: Maternal anthropometry and blood pressure at the first, second and third trimesters

Maternal anthropometry & blood pressure	Trimesters		
	1st	2nd	3rd
	Mean (SD) *n*	Mean (SD) *n*	Mean (SD) *n*
Weight (kg)	48.7[a] (7.4) *178*	51.5[b] (7.0) *355*	58.0[c] (7.5) *356*
Mid arm circumference (cm)	24.4[a] (2.5) *176*	24.4[a] (2.4) *293*	24.9[b] (2.3) *256*
Triceps skinfold (mm)	19.1[a] (5.9) *176*	19.5[a] (5.3) *293*	21.1[b] (5.3) *256*
Blood pres. -systolic -diastolic (mmHg)	113.0 (8.4) 72.4[a] (6.6) *176*	111.9 (9.4) 71.2[b] (7.1) *355*	112.4 (9.7) 72.9[a] (7.8) *359*
Gestational age (wks)	9.8 (2.0) *178*	19.7 (3.5) *355*	34.4 (4.1) *359*

For each variable separately, groups with different superscript are significantly different (Least significance difference test; $P < 0.05$)

4.1.4 Food intake and food behaviour

Subjects were interviewed three times throughout their pregnancy about their food behaviour. From 361 pregnant subjects recruited in this study, only 344 subjects (gestational age varied from 4-37 weeks) could be interviewed at the first time. Then 340 subjects (gestational age varied from 17-37 weeks) and 130 subjects (gestational age varied from 27-37 weeks) were interviewed at the second and the third times respectively. There was a decrease in the number of subjects because either they missed the follow-up or some of subjects had delivered their babies before they could be interviewed at either the

second or the third time. From these three interviews, pregnant subjects were divided into three groups which were dependent upon trimesters when they were interviewed as show in **Table 4.5**.

Table 4.5: The number of pregnant subjects (N) in each interview and the number of interviews (n) after they were grouped into trimesters

Interview	Trimesters			N
	1st (≤ 12 wk)	2nd (>12-24 wk)	3rd (> 24 wk)	
1st	112	206	26	344
2nd	-	122	218	340
3rd	-	-	130	130
n	112	328	374	

With regard to the Thai food tables, nutrient intakes at the first, second and third trimesters were calculated, namely, energy intake, protein intake, fat intake and carbohydrate intake. **Table 4.6** showed the results of nutrient intakes at each trimester. It was found that the mean of energy, protein, fat and carbohydrate intakes at the second trimester were significantly higher than those at the first trimester. The mean of energy and carbohydrate intakes at the third trimester were significantly higher than those at the first trimester but they did not show any significant difference when they were compared with those at the second trimester. The mean of protein intake at the third trimester was significantly higher than that at the first trimester but it was significantly lower than that at the second trimester. For fat intake, the mean at the third trimester was not significantly different from the mean at either the first or the second trimester.

Table 4.6: Nutrient intakes at the first, second and third trimesters

Nutrient intakes	Trimesters		
	1st	2nd	3rd
	Mean (SD) *n*	**Mean (SD)** *n*	**Mean (SD)** *n*
Energy (kcal/d)	2496[a] (805) *112*	2829[b] (799) *328*	2728[b] (714) *374*
Protein (g/d)	72.6[a](26.0) *112*	83.2[b] (27.6) *328*	78.7[c] (23.2) *374*
Fat (g/d)	51.9[a](29.4) *112*	59.7[b] (30.1) *328*	56.6 (27.4) *374*
Carbohydrate (g/d)	438[a] (157) *112*	493[b] (150) *328*	481[b] (144) *374*
Gestational age (wks)	10.9 (1.6) *112*	22.6 (2.7) *328*	31.8 (2.1) *374*

For each variable separately, groups with different superscript are significantly different (Least significance difference test; $P < 0.05$) and *n* was the number of interviews.

Similar results were observed if only the nutrient intakes of the pregnant subjects who were interviewed at all three trimesters (N=105) were analyzed, as showed in **Table 4.7**.

Table 4.7: Nutrient intakes at the first, second and third trimesters of pregnant subjects who were interviewed during all three trimesters

Nutrient intakes	Trimesters		
	1st	2nd	3rd
	Mean (SD) *n*	Mean (SD) *n*	Mean (SD) *n*
Energy (kcal/d)	2518[a] (819) *105*	2803[b] (761) *105*	2722 (679) *105*
Protein (g/d)	73.1[a] (25.7) *105*	83.7[b] (26.5) *105*	78.6 (21.7) *105*
Fat (g/d)	52.9[a] (30.2) *105*	60.8[b] (29.8) *105*	54.8 (22.8) *105*
Carbohydrate (g/d)	440[a] (162) *105*	486[b] (147) *105*	484[b] (147) *105*
Gestational age (wks)	11.2 (2.0) *105*	23.3 (1.5) *105*	32.4 (1.4) *105*

For each variable separately, groups with different superscript are significantly different (Least significance difference test; $P < 0.05$)

Table 4.8 shows the frequencies of different food types which were consumed every day (at the first interview) by Thai pregnant subjects in this study. It can be seen that glutinous rice, pork, salted shrimp paste, vegetables and fruits were eaten often whereas sea foods were rarely eaten by these pregnant subjects.

Table 4.8: Percentage of Thai pregnant subjects eating selected foods as assessed by Food frequency questionnaire (consumed every day), at first interview

Foods (consumed every day)	The percentage of Thai pregnant subjects (n=344)
Sea foods	3.1 %
Rice	27.9 %
Beef	36.2 %
Chicken and duck	37.9 %
Egg	42.2 %
Fresh milk	45.0 %
Fruits	79.2 %
Glutinous rice	84.3 %
Pork	86.0 %
Salted shrimp paste	89.2 %
Vegetables	96.6 %

4.1.5 Urinary iodine excretion

During this study, casual urine samples were collected from pregnant subjects. The frequency of urine collection of each pregnant subject was dependent upon the number of visits at the centres. The range of the number of visits varied from one to five times. Urinary iodine and creatinine excretions in these casual urine samples were analyzed. Then the ratio of urinary iodine to urinary creatinine excretions was calculated. It was found that the distribution of urinary iodine excretion (both μg per cent and μg/g creatinine) was not normal. Therefore, urinary iodine excretion data were transformed to logarithm (base 10) as shown in **Table 4.9**.

Table 4.9: Urinary iodine excretion (UIE) expressed as μg per cent and μg/g creatinine (log form) from casual urine samples at 1 to 5 collections and gestational age (GA, wks)

Collections	UIE (log form)		GA
	μg %	μg/g creatinine	wks
	Mean (SD)	Mean (SD)	Mean (SD)
1st (n=361)	0.67 (0.41)	1.88 (0.32)	18.2 (6.2)
2nd (n=331)	0.87 (0.38)	1.97 (0.30)	26.2 (6.0)
3rd (n=254)	0.87 (0.42)	1.98 (0.26)	32.2 (3.4)
4th (n=155)	0.88 (0.38)	1.95 (0.27)	35.3 (1.6)
5th (n=18)	0.82 (0.35)	2.00 (0.34)	35.7 (0.8)

It can be seen from **Table 4.9** that the mean urinary iodine excretion (both in μg per cent and μg/g creatinine; log form) tended to increase from the first to the fifth collections. These casual urine samples were divided into three groups by the gestational age of collections. Group one was the first trimester (≤ 12 weeks); group two was the second trimester (more than 12 to 24 weeks) and group three was the third trimester (>24 weeks). As shown in **Table 4.10,** the mean urinary iodine excretion (μg per cent) of the third trimester was significantly higher than at the first and the second trimesters, but there was no significant difference in the mean of urinary iodine excretion (μg per cent) between the first and the second trimesters. However, considering urinary iodine excretion (μg/g creatinine; log form), It was found that urinary iodine excretion increased significantly through the trimesters, and unlike μg %, showed a continuous increase across each trimester.

Table 4.10: Urinary iodine excretion (UIE) expressed as μg per cent (log form) and μg/g creatinine (log form) from casual urine samples divided into first, second and third trimesters

Trimesters	UIE (μg %, log form)		F-ratio * (P-value)
	Mean (SD)	95 % CI	
1st (n=75)	0.70[a] (0.44)	0.61-0.79	20.1008 (0.0000)
2nd (n=315)	0.74[a] (0.42)	0.70-0.78	
3rd (n=329)	0.88[b] (0.38)	0.85-0.92	
Trimesters	UIE (μg/g creatinine, log form)		F-ratio * (P-value)
	Mean (SD)	95 % CI	
1st (n=75)	1.84[c] (0.32)	1.71-1.90	12.8651 (0.0000)
2nd (n=315)	1.91[d] (0.31)	1.88-1.94	
3rd (n=329)	1.98[e] (0.27)	1.96-2.00	

95% CI = 95 % confidence interval

* Oneway Analysis of Variance

Groups with different superscript are significantly different (Least significance difference test; P < 0.05).

4.1.6 Birth outcome

Birth outcome measurements were: birthweight (BWT), head circumferences (HC), length, the percentage of head circumference to length (RHL %) and chest circumference (CC) of infants who were delivered in this study. **Table 4.11** shows the mean results for these measurements. The mean birth weight was 3007 g (SD=403, n =361) and there were 32 infants (8.9 per cent) who had low birthweight (birth weight < 2,500 g). Head circumference and chest circumference were measured in 353 infants. The mean head circumference and chest circumference were 33.2 cm (SD=1.3) and 31.5 cm (SD=1.8) respectively. The length of 349

infants were measured. The infants' mean length was 49.4 cm (SD=2.1). Then the percentage of head circumference to length (RHL %) was calculated. The mean RHL % was 67.2 per cent (SD=3.0). The mean gestational age at birth was 39.0 weeks (SD=1.7, n =361). Twenty four infants (6.7 per cent) were preterm babies (delivered at gestational age < 37 weeks).

Table 4.11: The results of birth outcome in the study

Birth outcome	n	Mean (SD)	95 % CI
BWT (g)	361	3007 (403)	2965-3049
HC (cm)	353	33.2 (1.3)	33.1-33.3
Length (cm)	349	49.4 (2.1)	49.2-49.6
RHL %	349	67.2 (3.0)	66.9-67.5
CC (cm)	353	31.5 (1.8)	31.3-31.7
GA (wks)	361	39.0 (1.7)	38.8-39.2

BWT = Birthweight
HC = Head circuimference
RHL % = (Head circumference/length)*100
CC = Chest circumference
GA = Gestational age
95 % CI = 95 % confidence interval

4.2 THE VARIABLES WHICH AFFECTED URINARY IODINE EXCRETION AND BIRTH OUTCOME

From this study, the urinary iodine excretion (μg/g creatinine, log form) from casual urine samples of the first urine collection were used to investigate the interaction between birth outcome and dietary iodine intake.

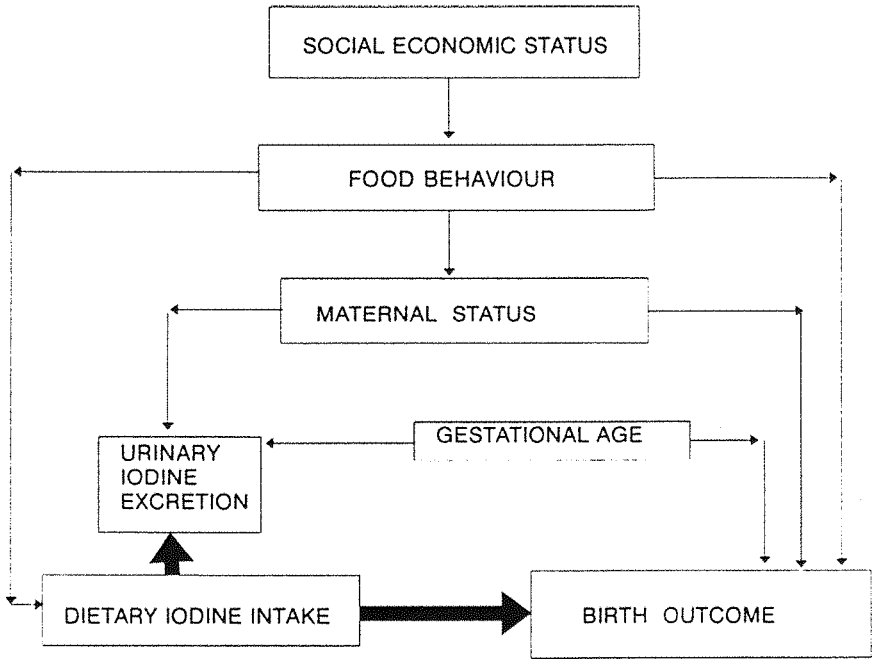
The reasons for this decision were: firstly, there was a positive relationship between urinary iodine excretion from casual urine samples of pregnant subjects and dietary iodine intake; secondly, the variation of concentration of urinary iodine excretion (μg per cent, log form) was higher than the urinary iodine excretion as μg/g creatinine (log form), see **Tables 4.10**, and finally, a casual urine sample was collected from every subject (n=361) at first urine collection.

Before the interaction between urinary iodine excretion (μg/g creatinine, log form) and birth outcome, there was a need to clarify whether other factors affect either urinary iodine excretion or birth outcome and may therefore affect the relationship between urinary iodine excretion and birth outcome.

Figure 4.1 shows the possibility of some factors affecting the urinary iodine excretion and birth outcome. Social economic status, for example, education, occupation and total income of pregnant subjects may affect food behaviour. Food behaviour, including nutrient intakes, food frequency and iodine supplementation, may have an effect on dietary iodine intake, maternal status and birth outcome. Maternal status may affect urinary iodine excretion and birth outcome. Gestational age at urine

samples collected may affect the level of urinary excretion. Finally, gestational at birth may affect the birth outcome.

Figure 4.1: Factors which may affect the urinary iodine excretion and birth outcome



Thus urinary iodine excretion of first collection (μg/g creatinine, log form) and birth outcome were stratified with those variables. The results were concluded in **Table 4.12**.

Table 4.12: Stratified urinary iodine excretion of the first collection (UIE, μg/g creatinine; log form) and the birth outcome with variables which were divided into either two groups or three groups by thirds

Variables	UIE	BWT	HC	LENGTH	RHL %	CC
Education						
Occupation*						
Total income (log)	●					
Energy intake#						
Protein intake#						
Fat intake#						
Carbohydrate intake#						
Glutinous rice (FFQ)#						
Rice (FFQ)#					●	
Pork (FFQ)#						
Chicken, duck (FFQ)#				●	●	
Beef (FFQ)#	●		●	●	●	
EGG (FFQ)#						
Fresh milk (FFQ)#	●					
Sea foods (FFQ)#	●		●			
Fermented fish, Kapi (FFQ)#						
Fruits#						
Vegetables#		●				
Age **					●	
Height **		●	●			
Weight **		●	●			●
Mid arm circumference **		●		●		●
Triceps skinfolds **		●	●	●		●

Variables	UIE	BWT	HC	LENGTH	RHL %	CC
Blood pressure Systolic **						●
Blood pressure Diastolic **						
Weight gain ***		●	●			
Gestational age at birth		●	●	●		●
Gender****		●	●	●		
Gestational at first urine collection	●					
UIE					●	
I_2 supplement	●			●	●	
Total I_2 supplement		●		●	●	●

```
#      = Nutrient intake and food frequency at first interview
*      = the occupation was divided into 2 groups; agriculture and not agriculture
**     = Maternal status at first admission
***    = Weight gain (kg)
       = the difference in pregnant body weight (before labour) and pregnant body weight (at the
                 first admission)
****   = Gender was divided into 2 groups; male and female.
●      = Oneway Analysis of Variance; significant difference at P < 0.05
Blank  = Non significant difference.
FFQ    = Food frequency
UIE    = Urinary iodine excretion at first collection
         (μg/g creatinine; log form)
I2 supplement (100 μg/d)
       = The length of time (days) of I2 supplement from admission until first collection of urine
          samples
Total I2 supplement (100 μg/d)
       = The length of time (days) of I2 supplement from admission until delivery.
Bwt    = Birthweight
HC     = Head circumference
RHL %  = (Head circumference/length)*100
CC     = Chest circumference
```

As shown in **Table 4.12**, the first column showed the variables which may affect the urinary iodine excretion (UIE, μg/g creatinine, log form; the second column) and birth outcome (from the third column to the seventh column).

Regarding the first column, the educational variable was divided into three groups by the length of education (yrs). They were ≤ 6 yrs, > 6 to 12 yrs and > 12 yrs, respectively. The occupational variable was divided into

two groups; agriculture and non agriculture. Food frequency of food items at first interview were divided into three groups, depending on how often they were consumed (every day; 1-4 times/week and 1-2 times/month). Some variables were divided into three groups by thirds. They were nutrients intake (energy, protein, fat and carbohydrate) at first interview; maternal status at first admission (age, height, weight, mid arm circumference, triceps skinfolds, systolic and diastolic blood pressure); weight gain; gestational age at birth; gestational age at first urine collection; and urinary iodine excretion at first collection (UIE, μg/g creatinine, log form). The iodine supplementation was divided into two categories. The first category was I_2 supplement (100 μg/d) which meant the length of time (days) of I_2 supplement from admission until first collection of urine samples. The length of time of I_2 supplement was divided into three groups (0 day, 1-30 days and $\geq$ 31 days). The second category was total I_2 supplement (100 μg/d) which meant the length of time (days) of I_2 supplement from admission until delivery. The length of time of total I_2 supplement was divided into three groups by thirds ($\leq$ 168 days, 169-199 days and $\geq$ 200 days).

Finally, the means of urinary iodine excretion and birth outcome among the groups of these variables were compared by using either Oneway Analysis of Variance or Chi Square test. It can be seen from **Table 4.12** that there were a number of factors (with symbol '●') which were associated with urinary iodine excretion and birth outcome. The results of the effects of these factors were shown in more detail in next sections.

4.2.1 Factors which were associated with urinary iodine excretion (μg/g creatinine, log form)

These were: total income, the frequency of foods consumed (beef, fresh milk, sea foods at first interview), gestational age at the first urine collection and I_2 supplement [100 μg/d; the length of time (days) of I_2 supplement from admission until first collection of urine samples]. The effect of these variables on urinary iodine excretion are shown in **Table 4.13**

Table 4.13: Stratified **urinary iodine excretion** (UIE, μg/g creatinine; log form) of the first collection by variables which were divided into three groups

Variables	n	UIE		95 % CI	F ratio (P-value)*
		Mean	(SD)		
Tot. income Baht/month					
≤2512	104	1.87	(0.30)	1.81-1.93	4.9716
>2512-3981	102	1.81[a]	(0.31)	1.75-1.87	(0.0075)
>3981	107	1.94[b]	(0.31)	1.88-2.00	
Beef #					
1-2 T/mo	51	1.80[a]	(0.29)	1.72-1.88	3.4671
1-2-4 T/wk	173	1.92[b]	(0.30)	1.88-1.97	(0.0325)
every day	127	1.86	(0.34)	1.80-1.92	
Fresh milk#					
1-2 T/mo	68	1.84[a]	(0.31)	1.76-1.92	3.4794
1-2-4 T/wk	125	1.85[a]	(0.28)	1.80-1.89	(0.0319)
every day	158	1.93[b]	(0.34)	1.88-1.98	
Sea foods#					
1-2 T/mo	66	1.84[a]	(0.27)	1.75-1.88	5.4715
1-2-4 T/wk	274	1.89[a]	(0.32)	1.85-1.93	(0.0046)
every day	11	2.14[b]	(0.32)	1.92-2.36	
Gestational age at 1st urine collection					
≤ 15 wks	125	1.81[a]	(0.33)	1.76-1.87	9.4498
>15-22 wks	122	1.84[a]	(0.32)	1.79-1.90	(0.0001)
>22 wks	114	1.98[b]	(0.28)	1.93-2.03	

Variables	n	UIE		95 % CI	F ratio (P-value)*
		Mean	(SD)		
I_2 supplement					
0 day	145	1.76[a]	(0.31)	1.71-1.81	17.6169
1-30 days	62	1.92[b]	(0.36)	1.83-2.01	(0.0000)
≥ 31 days	154	1.97[b]	(0.28)	1.92-2.01	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each individual variable, groups with different superscript are significantly different (Least significance difference test; P < 0.05).
T/wk = times/week; T/mo = times/month
= Nutrient intake and food frequency at first interview
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until first collection of urine samples

From **Table 4.13**, in this study, it can be concluded that:

- Pregnant subjects, who had incomes in the highest total income group, had the highest urinary iodine excretion.

- Pregnant subjects, who ate beef one to four times per week, had urinary iodine excretion higher than those who ate beef one to two times per month.

- Pregnant subjects, who drank fresh milk or ate sea foods every day, had the highest urinary iodine excretion.

- Regarding the gestational age at the first urine collection, the greater the gestational age, the higher the urinary iodine excretion.

- Pregnant subjects, who received iodine supplement had higher urinary iodine excretions than those who did not receive iodine supplement.In addition, the relationship between the gestational age at the first urine collection and the iodine supplement (the length of iodine supplement from the first admission until the first collection of urine samples) was investigated (see **Table 4.14**). Thai pregnant subjects were grouped into three groups by the gestational age in which the first urine samples were collected. It was found that in the first group (≤ 15 wks), 74.4 percent of pregnant subjects (n=125) did not

receive iodine supplement, whereas 25.6 per cent of them received iodine supplement for more than one day. In the second group (>15-22 wks), 38.5 per cent of pregnant subjects (n=122) did not receive iodine supplements whereas 61.5 per cent of them received iodine supplements for more than one day. In the third group (> 22 wks), only 4.4 per cent of pregnant subjects (n=114) did not receive iodine supplements, whereas 95.6 per cent of them received iodine supplements for more than one day. Therefore the higher urinary iodine excretion (when gestational age increased) might be due to an increase in the per cent of pregnant subjects who received iodine supplement.

Table 4.14: The association between gestational age at the first urine collection (GA, wks; 3 groups) and the length of iodine supplement from the first admission until the urine collection (days; 3 groups)

GA (wks)	Iodine supplement (days)			n
	0	1-30	≥ 31	
≤15.00	74.4 %	20.0 %	5.6 %	125
15.01-22.00	38.5 %	23.8 %	37.7 %	122
≥22.01	4.4 %	7.0 %	88.6 %	114

(likelihood ratio = 0.0000)

4.2.2 Factors which were associated with birthweight (BWT)

These were: the frequency of consuming vegetables, maternal status at first admission (height, weight and triceps skinfold, weight gain) gestational age at birth, gender and total I_2 supplement [100 μg/d; the length of time (days) of I_2 supplementation from admission until delivery]. **Table 4.15** shows the effect of these variables on birthweight.

Table 4.15: Stratified **birthweight** (BWT, g) by variables which were divided into three groups.

Variables	n	BWT (g)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Vegetables#					
1-2-4 T/wk	11	2698[a]	(518)	2349-3046	3.7208
every day	339	3026[b]	(388)	2985-3068	(0.0252)
Height (cm)**					
≤149.5	121	2932[a]	(374)	2865-3000	3.2384
>149.5-154.0	130	3056[b]	(441)	2980-3133	(0.0404)
>154.0	110	3028	(380)	2956-3100	
Weight (kg)**					
≤45.9	120	2884[a]	(413)	2809-2958	12.6647
>45.9-51.7	121	2998[b]	(379)	2930-3066	(0.0000)
>51.7	120	3137[c]	(379)	3069-3206	
Mid arm circumference (cm)**					
≤23.2	128	2913[a]	(422)	2839-2986	7.3013
>23.2-24.8	113	3009	(383)	2938-3080	(0.0008)
>24.8	119	3106[b]	(382)	3036-3175	
Triceps skinfold (mm)**					
≤16.0	132	2927[a]	(424)	2854-3000	5.0387
>16.0-21.0	112	3016	(409)	2939-3092	(0.0070)
>21.0	116	3088[b]	(360)	3022-3154	
Weight gain (kg)***					
≤8.233	72	2923[a]	(377)	2835-3011	5.9076
>8.233-11.500	77	3032	(400)	2942-3123	(0.0032)
>11.500	66	3150[b]	(384)	3055-3244	
Gestational age at birth					
≤38 wks	123	2818[a]	(412)	2744-2891	24.1312
>38-40 wks	181	3084[b]	(366)	3030-3137	(0.0000)
>40 wks	57	3168[b]	(347)	3076-3260	
Gender					
Male	200	3053[a]	(424)	2995-3113	6.2614
female	158	2947[b]	(368)	2889-3005	(0.0218)

Variables	n	BWT (g)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Total I_2 supplement					
≤168 days	122	2902[a]	(445)	2822-2982	7.2528
169-199 days	122	3026[b]	(402)	2953-3098	(0.0008)
≥200 days	117	3094[b]	(332)	3034-3156	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each variable separately, groups with different superscript are significantly different (Least significance difference test; $P < 0.05$).

\# = Nutrient intake and food frequency at first interview
** = Maternal status at admission
*** = Weight gain (kg)
= The difference in pregnant body weight (before labour) and pregnant body weight (at the first admission)
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.

From **Table 4.15**, in this study, it may be summarized that;

- The infant's mean birthweight of pregnant subjects, who ate vegetables every day, was heavier than that of pregnant subjects who ate vegetables one to four times per week.

- The infant's mean birthweight of pregnant subjects, who were between 149.6 to 154.0 cm, was heavier than that of pregnant subjects who were shorter or equal to 149.5 cm.

- The heavier pregnant subjects' weight (at first admission), the heavier infant's birthweight.

- The infant's mean birthweight of pregnant subjects, whose mid arm circumference was more than 24.8 cm, was heavier than that of pregnant subjects whose mid arm circumference was less than or equal to 23.2 cm.

- The infant's mean birthweight of pregnant subjects, who had triceps skinfold of more than 21.0 mm, was heavier than that of pregnant subjects who had triceps skinfolds less than or equal to 16.0 mm.

- The infant's mean birthweight of pregnant subjects, who gained more than 11.5 kg during pregnancy, was heavier than that of pregnant subjects who only gained 8.2 kg or less.

- The infant's mean birthweight who were delivered at a gestational age of more than 38 weeks was heavier than that who were delivered at a gestational age of less than or equal to 38 weeks.

- The male infants' mean birthweight was heavier than that of female infants' mean birthweight.

- The infant's mean birthweight of pregnant subjects, who received total iodine supplement (100 μg/d; from admission until delivery) for more than 168 days, was heavier than that of pregnant subjects who received total iodine supplement less than or equal to 168 days.

4.2.3 Factors which were associated with head circumference (HC)

These were: frequency of consumed foods (beef and sea foods), maternal status at first admission (height, weight and triceps skinfolds) and gestational age at birth.
The results were shown in **Table 4.16**

Table 4.16: Stratified **head circumference** (HC, cm) by variables which were divided into three groups

Variables	n	HC (cm)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Beef #					
1-2 T/mo	50	33.0[a]	(1.1)	32.7-33.3	4.1283
1-2-4 T/wk	169	33.1[a]	(1.2)	32.9-33.3	(0.0169)
every day	125	33.5[b]	(1.4)	33.2-33.7	
Sea foods#					
1-2 T/mo	64	33.6[a]	(1.4)	33.2-33.9	3.4847
1-2-4 T/wk	269	33.1[b]	(1.3)	33.0-33.3	(0.0318)
every day	11	33.3	(1.5)	32.3-34.3	
Height (cm)**					
≤149.5	117	32.8[a]	(1.3)	32.6-33.0	6.8460
>149.5-154.0	128	33.4[b]	(1.3)	33.1-33.6	(0.0012)
>154.0	108	33.4[b]	(1.3)	33.1-33.6	

Variables	n	HC (cm)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Weight (kg)**					
≤45.9	117	32.9[a]	(1.3)	32.6-33.1	5.9987
>45.9-51.7	120	33.2[b]	(1.4)	33.0-33.5	(0.0027)
>51.7	116	33.4[b]	(1.2)	33.2-33.7	
Triceps skinfold (mm)**					
≤16.0	129	32.9[a]	(1.4)	32.7-33.2	5.1392
<16.0-21.0	110	33.2	(1.3)	32.9-33.4	(0.0063)
>21.0	113	33.5[b]	(1.2)	33.2-33.7	
Weight gain (kg)***					
≤8.233	71	33.0[a]	(1.4)	32.6-33.3	3.1831
>8.233-11.500	76	33.3	(1.3)	33.0-33.6	(0.0435)
>11.500	66	33.5[b]	(1.4)	33.2-33.9	
Gender					
Male	197	33.4[a]	(1.4)	33.2-33.6	12.0495
female	156	32.9[b]	(1.2)	32.7-33.1	(0.0006)
Gestational age at birth					
≤38 wks	118	32.6[a]	(1.3)	32.3-32.8	21.7424
>38-40 wks	178	33.5[b]	(1.2)	33.3-33.7	(0.0000)
>40 wks	57	33.5[b]	(1.2)	33.2-33.8	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each variable separately, groups with different superscript are significantly different (Least significance difference test; P < 0.05).

T/wk = Times/week
T/mo = Times/month
= Nutrient intake and food frequency at first interview
** = Maternal status at admission
*** = Weight gain (kg)
= The difference in pregnant body weight (before labour) and pregnant body weight (at the first admission)

From **Table 4.16**, in this study, it can be seen that:

- The infant's mean head circumference of pregnant subjects who ate beef every day was larger than that of pregnant subjects who ate beef one to four times per week or one to two times per month.

- The infant's mean head circumference of pregnant subjects who ate sea foods one to four times per week was less than that of pregnant subjects who ate sea foods one to two times per month.

- The infant's mean head circumference of pregnant subjects, whose height (at first admission) was more than 149.5 cm, was larger than that of pregnant subjects whose height was less than or equal to 149.5 cm.

- The infant's mean head circumference of pregnant subjects, who weighed (at first admission) more than 45.9 kg, was larger than that of pregnant subjects who weighed less than or equal to 45.9 kg.

- The infant's mean head circumference of pregnant subjects, whose triceps skinfold (at first admission) was greater than 21.0 mm, was larger than that of pregnant subjects whose triceps skinfold was 16.0 mm or less.

- The infant's mean head circumference of pregnant subjects, who gained more than 11.5 kg during pregnancy, was larger than that of pregnant subjects who gained 8.2 kg or less.

- The mean of male infant's head circumference was longer than that of female infants' head circumference.

- The mean of infant's head circumference, who were delivered at a gestational age of more than 38 weeks, was larger than that of those who were delivered at a gestational age of less than or equal to 38 weeks.

4.2.4 Factors which were associated with length

These were: frequency of consuming foods (chicken, duck and beef), maternal status at first admission (mid arm circumference and triceps skin folds), gestational age at birth, I_2 supplement [100 μg/d; the length of time (days) of I_2 supplement from admission until first collection of urine samples] and total I_2 supplement [100 μg/d; the length of time (days) of supplement from admission until delivery].

The effect of these variables on infant's mean length are shown in **Table 4.17.**

Table 4.17: Stratified **length** (cm) by variables which were divided into three groups.

Variables	n	Length (cm)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Chicken and duck#					
1-2 T/mo	32	49.2[a]	(2.0)	48.4-49.9	15.5069
1-2-4 T/wk	176	50.0[b]	(2.2)	49.7-50.3	(0.0000)
every day	132	48.7[a]	(1.8)	48.4-49.0	
Beef #					
1-2 T/mo	49	49.6[a]	(2.1)	49.0-50.2	9.3325
1-2-4 T/wk	167	49.8[a]	(2.2)	49.5-50.2	(0.0001)
every day	124	48.8[b]	(1.8)	48.5-49.1	
Mid arm circumference (cm)**					
≤23.2	124	49.0[a]	(2.2)	48.6-49.4	5.2901
>23.2-24.8	112	49.5[b]	(2.1)	49.1-49.9	(0.0055)
>24.8	112	49.8[b]	(1.9)	49.5-50.2	
Triceps skinfold (mm)**					
≤16.0	127	49.2[a]	(2.3)	48.8-49.6	3.4721
>16.0-21.0	109	49.3	(2.1)	48.9-49.7	(0.0321)
>21.0	112	49.8[b]	(2.0)	49.5-50.2	

Variables	n	Length (cm)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Gestational age at birth					
≤38 wks	115	48.5[a]	(2.2)	48.1-48.9	17.0164
>38-40 wks	177	49.9[b]	(2.0)	49.6-50.2	(0.0000)
>40 wks	57	49.9[b]	(1.8)	49.4-50.4	
Gender					
Male	196	49.6[a]	(2.1)	49.4-49.9	4.5277
female	153	49.2[b]	(2.2)	48.8-49.5	(0.0341)
I_2 supplement					
0 day	140	49.1[a]	(2.2)	48.7-49.4	3.7792
1-30 days	61	49.4	(1.9)	49.0-49.9	(0.0238)
≥ 31 days	148	49.8[b]	(2.1)	49.4-50.1	
Total I_2 supplement					
≤168 days	117	49.1[a]	(2.3)	48 7-49.5	9.2898
169-199 days	119	49.1	(2.1)	48.7-49.5	(0.0001)
≥200 days	113	50.1[b]	(1.8)	49.8-50.4	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each variable separately, groups with different superscript are significantly different (Least significance difference test; P < 0.05).

T/wk = times/week; T/mo = times/month
= Nutrient intake and food frequency at first interview
** = Maternal status at admission
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until first collection of urine samples
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.

From **Table 4.17**, in this study, it may be inferred that:

- The infant's mean length of pregnant subjects, who ate chicken and duck every day, was the same as that of pregnant subjects who ate chicken and duck one to two times per month but it was shorter than that of pregnant subjects who ate chicken and duck one to four times per week.

- The infant's mean length of pregnant subjects, who ate beef every day, was shorter than that of pregnant subjects who ate beef either one to four times per week or one to two times per month.

- The infant's mean length of pregnant subjects, who had mid arm circumference of more than 23.2 cm, was longer than that of pregnant subjects who had mid arm circumference of less than or equal to 23.2 cm.

- The infant's mean length of pregnant subjects, who had triceps skinfold of more than 21.0 mm, was longer than that of pregnant subjects who had triceps skinfold of less than or equal to 16.0 mm.

- The infant's mean length, who were delivered at a gestational age of more than 38 weeks, was longer than that who were delivered at a gestational age of less than or equal to 38 weeks.

- The male infant's mean length was longer than that of female infant's mean length.

- The infant's mean length of pregnant subjects, who received iodine supplement (100 μg/d; from admission until the first collection of urine samples) for more than or equal to 31 days, was longer than that of pregnant subjects who received iodine supplement for less than or equal to 30 days.

- The infant's mean length of pregnant subjects, who received total iodine supplement (100 μg/d; from admission until delivery) for more than or equal to 200 days, was longer than that of pregnant subjects who received iodine supplement less than or equal to 199 days.

4.2.5 Factors which were associated with the percentage of head circumference to length (RHL %)

These were: frequency of consuming foods (rice, chicken, duck and beef), maternal status at first admission (age),

urinary iodine excretion at first collection (μg/g creatinine, log form), I_2 supplement [100 μg/d; the length of time (days) of I_2 supplement from admission until first collection of urine samples] and total I_2 supplement (100 μg/d; the length of time (days) of total I_2 supplement from admission until delivery). The effect of these variables on the percentage of head circumference to length are shown in **Table 4.18.**

Table 4.18: Stratified **percentage of head circumference to length** (RHL %) by variables which were divided into three groups

Variables	n	RHL %		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Rice#					
1-2 T/mo	88	66.5[a]	(2.8)	65.9-67.1	3.4074
1-2-4 T/wk	157	67.6[b]	(3.2)	67.0-68.1	(0.0343)
every day	95	67.4	(2.8)	66.8-68.0	
Chicken and duck#					
1-2 T/mo	32	67.3[a]	(2.8)	66.3-68.3	25.1163
1-2-4 T/wk	176	66.2[a]	(2.8)	65.8-66.7	(0.0000)
every day	132	68.6[b]	(2.9)	68.1-69.0	
Beef #					
1-2 T/mo	49	66.5[a]	(2.6)	65.8-67.3	22.0828
1-2-4 T/wk	167	66.4[a]	(2.9)	66.0-66.9	(0.0000)
every day	124	68.6[b]	(2.8)	68.1-69.1	
Age (yrs)**					
≤23	132	66.7[a]	(3.2)	66.1-67.2	4.3816
>23-26	106	67.2	(3.0)	66.6-66.7	(0.0132)
>26	111	67.8[b]	(3.7)	67.3-68.4	
UIE (μg/g Cr; log form)					
≤ 1.73	117	67.8[a]	(3.1)	67.2-68.4	3.3579
>1.73-1.99	115	66.8[b]	(2.9)	66.3-67.4	(0.0359)
>1.99	117	67.0[b]	(3.1)	66.4-67.5	
I_2 supplement					
0 day	140	67.7[a]	(3.2)	67.2-68.2	4.2247
1-30 days	61	67.3	(3.3)	66.4-68.1	0.0154
≥ 31 days	148	66.7[b]	(2.7)	66.2-67.1	

Variables	n	RHL %		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Total I_2 supplement					
≤168 days	117	67.4[a]	(3.2)	66.8-68.0	3.6371
169-199 days	119	67.6	(3.3)	67.0-68.2	(0.0273)
≥200 days	113	66.6[b]	(2.6)	66.1-67.1	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each outcome variable separately, groups with different superscript are significantly different (Least significance difference test; P < 0.05).

T/wk = times/week; T/mo = times/month
= Nutrient intake and food frequency at first interview
** = Maternal status at admission
UIE = Urinary iodine excretion at first collection (μg/g creatinine; log form)
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until first collection of urine samples
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.

From **Table 4.18**, in this study, it can be seen that:

- The infant's mean RHL % of pregnant subjects, who ate rice one to four times per week, was larger than that who ate rice one to two times per month.
- The infant's mean RHL % of pregnant subjects, who ate chicken and duck or beef every day, was larger than that who ate chicken and duck or beef either one to four times per week or one to two times per month.
- The infant's mean RHL % of pregnant subjects, who had age more than 26 years, was larger than that who had age less than or equal to 23 years.
- The infant's mean RHL % of pregnant subjects, who had urinary iodine excretion (μg/g creatinine, log form) of more than 1.73, was smaller than that who had urinary iodine excretion of less than or equal to 1.73.
- The infant's mean RHL % of pregnant subjects, who received iodine supplement (100 μg/d; from admission until the first collection of urine samples) of more than or equal to 31 days, was smaller than that of pregnant subjects who did not received iodine supplementation.

- The mean of infant's length of pregnant subjects, who received total iodine supplement (100 μg/d; from admission until delivery) of more than or equal to 200 days, was smaller than that of pregnant subjects who received iodine supplement less than or equal to 168 days.

4.2.6 Factors which were associated with chest circumference (CC)

These were: maternal status at first admission (weight, mid arm circumference, triceps skinfold and blood pressure:systolic), gestational age at birth and total I_2 supplement (100 μg/d; the length of time (days) of iodine supplement from admission until delivery). The results of these variables are shown in **Table 4.19**

Table 4.19: Stratified **chest circumference** (CC, cm) by variables which were divided into three groups

Variables	n	CC (cm)		95 % CI	F ratio * (P-value)
		Mean	(SD)		
Weight (kg)**					
≤45.9	117	31.1[a]	(1.9)	30.7-31.4	8.3268
>45.9-51.7	120	31.6[b]	(1.6)	31.3-31.8	(0.0003)
>51.7	116	32.0[b]	(1.6)	31.7-32.3	
Mid arm circumference (cm)**					
≤23.2	126	31.2[a]	(1.9)	30.8-31.5	5.3420
>23.2-24.8	112	31.6[b]	(1.6)	31.3-31.9	(0.0052)
>24.8	114	31.9[b]	(1.6)	31.6-32.2	
Triceps skinfold (mm)**					
≤16.0	129	31.2[a]	(1.9)	30.8-31.5	5.4646
<16.0-21.0	110	31.6	(1.8)	31.2-31.9	(0.0046)
>21.0	113	31.9[b]	(1.4)	31.7-32.2	

Variables	n	CC (cm)	95 % CI	F ratio * (P-value)
		Mean (SD)		
Blood pressure systolic (mmHg)**				
≤110	201	31.5 (1.8)	31.2-31.7	3.1463
>110-120	127	31.8[a] (1.6)	31.5-32.0	(0.0442)
>120	21	30.8[b] (2.0)	29.8-31.7	
Gestational age at birth				
≤38 wks	118	30.7[a] (2.0)	30.4-31.1	22.6195
>38-40 wks	178	31.9[b] (1.4)	31.7-32.1	(0.0000)
>40 wks	57	32.2[b] (1.6)	31.7-32.6	
Total I_2 supplement				
≤168 days	117	31.0[a] (2.0)	30.6-31.3	10.7149
169-199 days	122	31.7[b] (1.5)	31.4-32.0	(0.0000)
≥200 days	114	32.0[b] (1.5)	31.7-32.2	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each outcome variable separately, groups with different superscript are significantly different (Least significance difference test; P < 0.05).

** = Maternal status at admission
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.

From **Table 4.19**, in this study, it can be concluded that:

- The infant's mean chest circumference of pregnant subjects, who had weight (at first admission) of more than 45.9 kg, was longer than that who had weight of less than or equal to 45.9 kg.

- The infant's mean chest circumference of pregnant subjects, whose mid arm circumference (at first admission) was more than 23.2 cm, was larger than that whose mid arm circumference was less than or equal to 23.2 cm.

- The infant's mean chest circumference of pregnant subjects, who had triceps skinfold (at first admission) of more than 21.0 mm, was larger than that who had triceps skinfold of less than or equal to 16.0 cm.

- The infant's mean chest circumference of pregnant

subjects, who had blood pressure (systolic, at first admission) of more than 120.0 mmHg, was smaller than that who had blood pressure of less than or equal to 120.0 mmHg.

- The infant's mean chest circumference, who were delivered at a gestational age of more than 38 weeks, was larger than that who were delivered at a gestational age of less than or equal to 38 weeks.

- The infant's mean chest circumference of pregnant subjects, who received total iodine supplement (100 μg/d; from admission until delivery) for more than 168 days, was larger than that of pregnant subjects who received total iodine supplement less than or equal to 168 days.

The association between gestational age at birth and the length of total iodine supplement (from admission until delivery) was also investigated (see **Table 4.20**).

Table 4.20: The association between gestational age at birth (GABI, wks; 3 groups) and the length of total iodine supplementation from the admission until delivery (days; 3 groups)

GABI (wks)	Total iodine supplementation (days)			n
	≤ 168	169-199	≥ 200	
≤ 38	41.5 %	33.3 %	25.2 %	123
39-40	33.1 %	34.3 %	32.6 %	181
≥ 41	19.3 %	33.3 %	47.4 %	57

(Likelihood ratio = 0.0188)

Thai pregnant subjects were grouped into three groups by gestational age at birth. It was found that in the first group (≤ 38 wks), 41.5 percent of pregnant subjects (n=123) received iodine supplement for ≤ 168 days whereas 58.5 per cent of them received iodine supplement for more than 168 days. In the second group (39-40 wks), 33.1 per cent of pregnant subjects (n=181) received iodine supplement for ≤ 168 days whereas 66.9 per cent of them received iodine supplement for more than 168 days. In the third group (≥41 wks), 19.3 per cent of pregnant subjects (n=181) received iodine supplement for ≤ 168 days whereas 80.7 per cent of them received iodine supplement for more than 168 days.

4.3 THE CORRELATION BETWEEN URINARY IODINE EXCRETION (μg/g creatinine) AND BIRTH OUTCOME

The correlation between birth outcome and urinary iodine excretion (μg/g creatinine, log form) at the first to the fifth collections were investigated and the results are shown in **Table 4.21**. It was found that there was no correlation between urinary iodine excretion and birth outcome.

Table 4.21: The values of coefficient of correlation (r) between birth outcome and urinary iodine excretion (UIE, μg/g creatinine, log form) from the first to fifth collections

Birth outcome	Coefficient of correlation (r)				
	1stUIE n	2ndUIE n	3rdUIE n	4thUIE n	5thUIE n
BWT (g)	0.003 361	-0.063 331	-0.034 254	0.048 155	0.043 18
HC (cm)	-0.004 353	0.043 324	0.037 250	0.072 153	0.432 18
LENGTH (cm)	0.065 349	0.016 320	0.009 248	-0.014 153	0.205 18
RHL (%)	-0.063 353	0.032 320	0.025 248	0.082 153	0.399 18
CC (cm)	0.026 361	-0.044 324	0.019 250	0.092 153	-0.077 18
GABI (wks)	0.032 361	-0.066 331	0.132 254	0.100 155	-0.215 18

BWT = Birthweight
HC = Head circumference
RHL % = (Head circumference/length)*100
CC = Chest circumference
GABI = Gestational at birth
1stUIE, 2ndUIE, 3rdUIE, 4thUIE and 5thUIE = Urinary iodine excretion (μg/g creatinine, log form) at the first, second, third, fourth and fifth collections respectively.

Figures 4.2-4.6 illustrate the plot between birth outcome and urinary iodine excretion (μg/g creatinine, log form) at the first collection.

Figure 4.2: The correlation between urinary iodine excretion (μg/g creatinine, log form) at first collection and birthweight

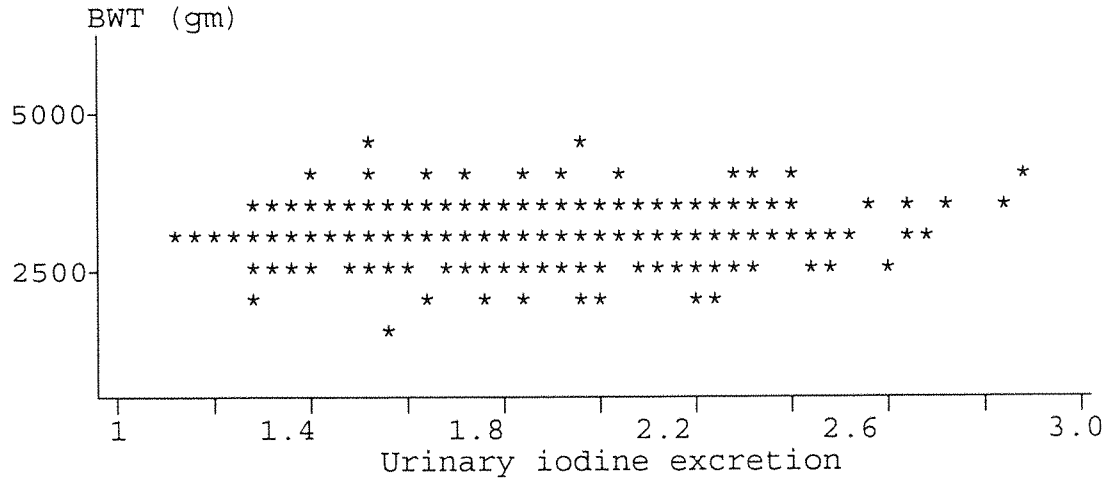


Figure 4.3: The correlation between urinary iodine excretion (μg/g creatinine, log form) at first collection and head circumference (HC, cm)

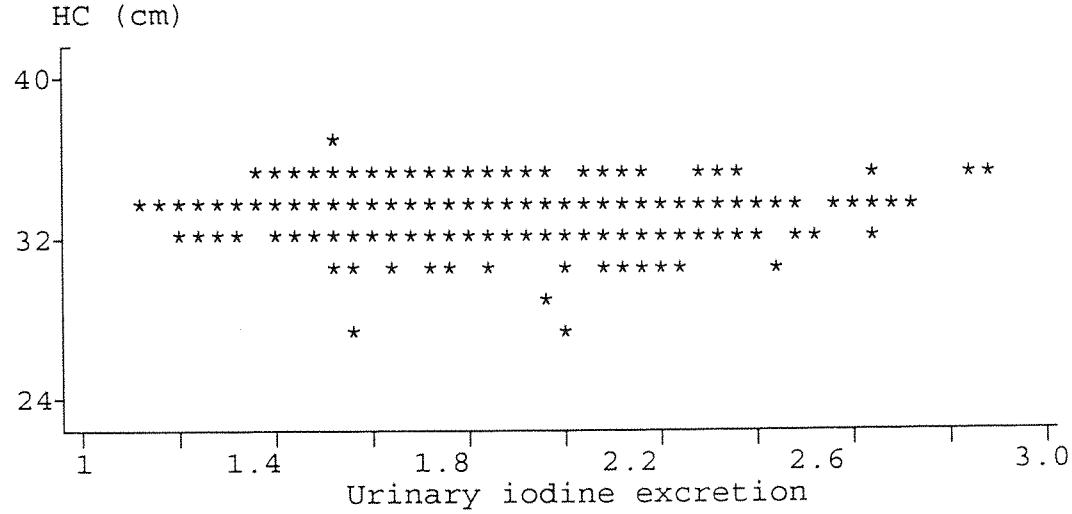


Figure 4.4: The correlation between urinary iodine excretion (μg/g creatinine, log form) at first collection and length (cm)

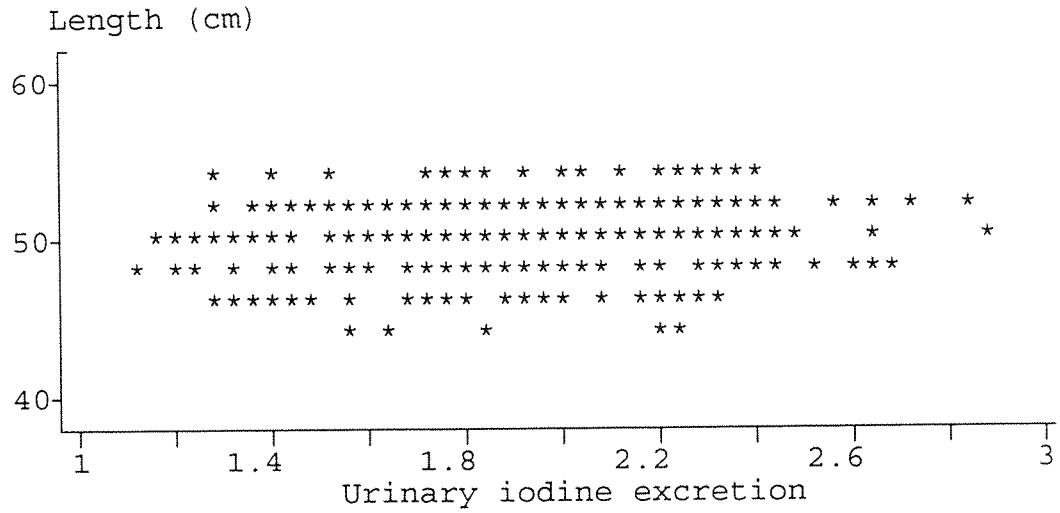


Figure 4.5: The correlation between urinary iodine excretion (μg/g creatinine, log form) at first collection and the percentage of head circumference to length (RHL %)

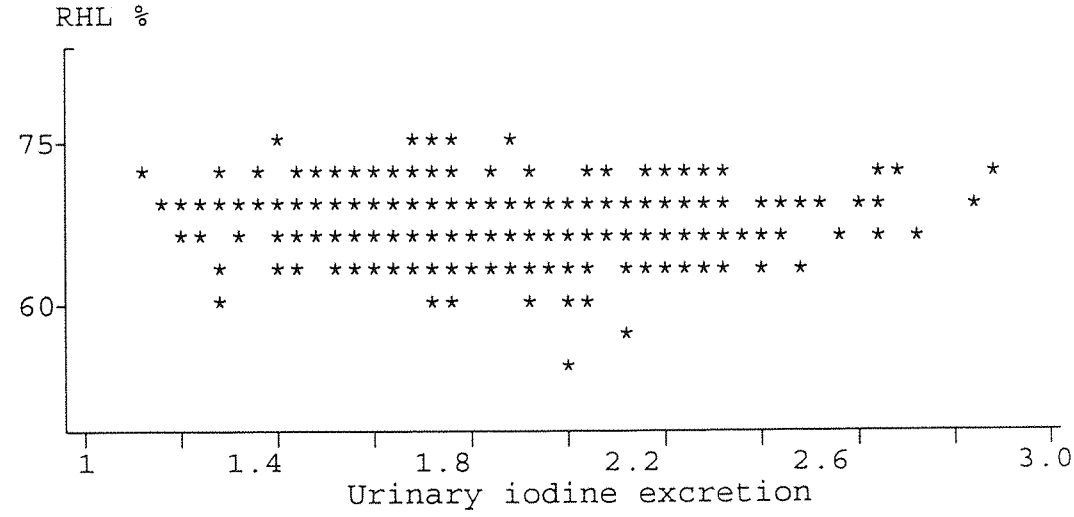
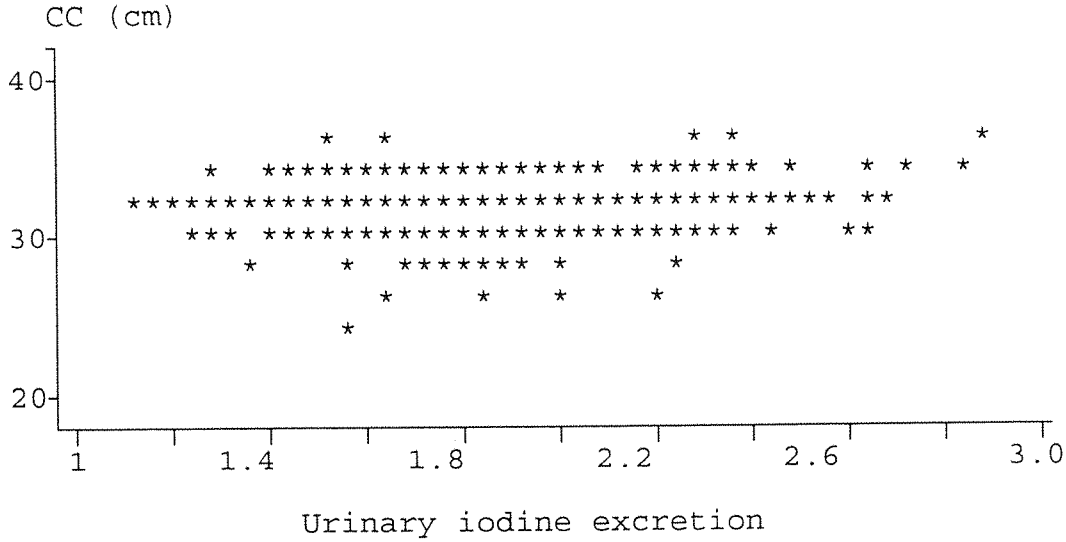


Figure 4.6: The correlation between urinary iodine excretion (μg/g creatinine, log form) at first collection and chest circumference (CC, cm)



4.4 THE INTERACTION BETWEEN URINARY IODINE EXCRETION (μg/g creatinine, log form) AT THE FIRST COLLECTION AND BIRTH OUTCOME

As shown in **Tables 4.12 and 4.18**, urinary iodine excretion at the first collection (gestational age varied from five to 37 weeks) showed an effect only on the percentage of head circumference to length (RHL %). The fetal growth rate (express as weight gain, g/d) up to 10 weeks of gestational age is relatively slow; subsequent development involves a large increase in mass during 11-27 weeks and the rate of fetal growth is maximal during 28-36 weeks of gestational age. Because the fetal thyroid gland can concentrate iodine and secrete thyroid hormones at about 11 weeks of gestational age (M.A. England, 1990). The effect of urinary iodine excretion on RHL % during 11 to 36 weeks and 11 to 27 weeks were investigated. It was found that urinary iodine excretion still had an effect on RHL %, as shown in **Table 4.22**.

Table 4.22: The effect of urinary iodine excretion on RHL% which were dependent upon the gestational age (wks) at urine samples collected

Gestational age at urine samples collected	n	RHL % Mean (SD)	95 % CI	F ratio * (P-value)
5-37 wks				
UIE (µg/g Cr; log form)				
≤ 1.73	117	67.8[a] (3.1)	67.2-68.4	3.3579
>1.73-1.99	115	66.8[b] (2.9)	66.3-67.4	(0.0359)
>1.99	117	67.0[b] (3.1)	66.4-67.5	
11-36 wks				
UIE (µg/g Cr; log form)				
≤ 1.74	104	67.9[a] (3.1)	67.3-68.5	3.4504
> 1.74-2.00	104	66.9[b] (2.8)	66.4-67.5	(0.0330)
> 2.00	101	67.0[b] (3.2)	66.4-67.6	
11-27 wks				
UIE (µg/g Cr; log form)				
≤1.73	99	68.0[a] (3.1)	67.4-68.6	3.2714
>1.73-1.98	96	66.9[b] (2.9)	66.4-67.5	(0.0394)
>1.98	95	67.1[b] (3.2)	66.5-67.8	

95% CI = 95 % confidence interval
* Oneway Analysis of Variance

For each interval of gestational age separately, groups with different superscript are significantly different (Least significance difference test; $P < 0.05$).

RHL % = (head circumference/length)*100
UIE = Urinary iodine excretion at first collection (µg/g creatinine; log form)

However, there were a number of the other variables which also effected the percentage of head circumference to length (RHL %), namely, the frequency of consuming: rice, chicken, duck and beef; age of pregnant subjects at the first admission, I_2 supplement and total I_2 supplement, see **Table 4.12**. These variables were considered as covariates and then the effect of urinary iodine excretion on the RHL % at each interval of gestational age was investigated. The results are shown in **Table 4.23**.

Table 4.23: The effect of urinary iodine excretion, at varied interval of gestational age, on the RHL % with covariates: the frequency of consuming rice, chicken, duck, and beef; age of pregnant subjects at the first admission; I_2 supplement and total I_2 supplement

Source of variation	P-VALUE		
	5-37 wks	11-36 wks	11-27 wks
COVARIATES			
Rice (FFQ)#	0.076	0.041	0.159
Chicken and duck (FFQ)#	0.522	0.485	0.554
Beef (FFQ)#	0.012	0.030	0.014
Age *	0.134	0.056	0.046
I_2 supplement	0.041	0.002	0.003
Total I_2 supplement	0.102	0.005	0.005
Length	0.000	0.000	0.000
MAIN EFFECTS			
UIE (log form)	0.264	0.233	0.287
N	340	301	285

\# = Nutrient intake and food frequency at first interview
* = Age of pregnant subjects (years) at first admission
FFQ = Food frequency
UIE = Urinary iodine excretion at first collection (μg/g creatinine; log form)
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until first collection of urine samples
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.
RHL % = (Head circumference/length)*100

As can be seen from **Table 4.23**, the length of the baby was also taken into account in the analysis because it was believed that it might lead to a bias of the association between exposure and outcome (because the RHL % might not be independent of the length of baby), for example, the smaller RHL % might be because of the longer baby and vice versa). The resulte showed that urinary iodine excretion at 5-37 weeks, at 11-36 weeks and at 11-27 weeks of gestational age did not effect the RHL %.

However, because fetal thyroid gland can start it's function at about 11 weeks of gestational age, and during 11-15 weeks of gestatinal age there is the most rapid growth in the length of the baby. Therefore, further analyses was done. The interval of gestational age of 11-27 wks was divided into three groups; the first group varied from 11-15 weeks of gestational age, the second group varied from 16-20 weeks of gestational age and the third group varied from 21-27 weeks of gestational age. Then the effect of urinary iodine excretion on the RHL % with those covariates in each group of gestational age was investigated. The results are shown in **Table 4.24**

Table 4.24: The effect of urinary iodine excretion on RHL% at varied intervals of gestational age with covariates: the frequency of consuming rice, chicken, duck, and beef; age of pregnant subjects at the first admission; I_2 supplement and total I_2 supplement

Source of variation	P-value		
	11-15 wks	16-20 wks	21-27 wks
COVARIATES			
Rice (FFQ)#	0.235	0.028	0.701
Chicken and duck (FFQ)#	0.853	0.219	0.016
Beef (FFQ)#	0.067	0.395	0.359
Age *	0.840	0.183	0.032
I_2 supplement	0.031	0.000	0.119
Total I_2 supplement	0.010	0.000	0.310
Length	0.000	0.000	0.000
MAIN EFFECTS			
UIE (log form)	0.019	0.986	0.778
N	80	96	109

\# = Nutrient intake and food frequency at first interview
* = Age of pregnant subjects (years) at first admission
FFQ = Food frequency
UIE = Urinary iodine excretion at first collection (μg/g creatinine; log form)
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until first collection of urine samples
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.
RHL % = (Head circumference/length)*100

It can be seen from **Table 4.24** that after adjusting for covariates the urinary iodine excretion at 11-15 weeks of gestational age had an effect on the RHL % whereas the urinary iodine excretion both at 16-20 weeks and at 21-27 weeks of gestational age did not affect the RHL %.

The mean of the RHL % (at 11-15 weeks of gestational age) of each urinary iodine excretion groups (low, medium and high), both unadjusted mean and adjusted mean, were shown in **Table 4.25**.

Table 4.25: Comparison of the RHL % by urinary iodine excretion between 11-15 weeks of gestational age; unadjusted and adjusted for covariates

UIE	N	Mean of RHL %	
		(unadjusted)	(adjusted)
≤ 1.668	25	69.2	68.9
1.669-1.924	27	67.9	68.2
>1.924	28	66.8	66.7

UIE = urinary iodine excretion (μg/g creatinine, log form).

Considering only term infants (gestational age at birth ≥37 weeks), the urinary iodine excretion (at 11-15 wks of gestational age) still effected the RHL %. The results are shown in **Tables 4.26-4.27**

Table 4.26: The effect of urinary iodine excretion, between 11-15 weeks of gestational age (only pregnant subjects who delivered term infants), on the RHL % with covariates: the frequency of consuming rice, chicken, duck, and beef; age of pregnant subjects at the first admission; I_2 supplement and total I_2 supplement

Source of variation	P-value
	11-15 wks
COVARIATES	
Rice (FFQ)#	0.226
Chicken and duck (FFQ)#	0.591
Beef (FFQ)#	0.043
Age *	0.875
I2 supplement	0.020
Total I2 supplement	0.005
Length	0.000
MAIN EFFECTS	
UIE (log form)	0.012
N	77

\# = Nutrient intake and food frequency at first interview
* = Age of pregnant subjects (years) at first admission
FFQ = Food frequency
UIE = Urinary iodine excretion at first collection (μg/g creatinine; log form)
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until first collection of urine samples
Total I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplement from admission until delivery.
RHL % = (Head circumference/length)*100

Table 4.27: Comparison of the RHL % by urinary iodine excretion, between 11-15 weeks of gestational age (only pregnant subjects who delivered term infants), unadjusted and adjusted for covariates

UIE	N	Mean of RHL % (%)	
		(unadjusted)	(adjusted)
≤ 1.668	25	69.2	69.0
1.669-1.924	25	67.8	68.0
>1.924	27	66.7	66.7

UIE = urinary iodine excretion (μg/g creatinine, log form).

Amongs these 77 pregnant subjects, there was no significant association between the groups of I_2 supplement (0 day, 1-30 days and ≥ 31 days) and the groups of urinary iodine excretion (UIE, μg/g. creatinine)(low, ≤1.668; medium 1.669-1.924 and high >1.924). On further investigation, it was found that 55 of the 77 pregnant subjects (71.4 %) had not received any iodine supplement. To compare the mean percentage of head circumference to length of babies (RHL %) delivered from pregnant subjects (without iodine supplement) with that of pregnant subjects (with iodine supplement), there was a trend that the mean infant's RHL % of pregnant subjects without iodine supplement (Mean± SD = 68.1±3.1, n=55) was bigger than that of pregnant subjects with iodine supplement (Mean± SD = 67.3±3.8 n=22) although the difference was not statistically significant. Since urinary iodine excretion in a casual urine sample was used as an indicator of iodine intake, I_2 supplement and total I_2 supplement might be excluded from those covariates. The effect of urinary iodine excretion, at 11-15 weeks of gestational age (only pregnant subjects who delivered term infants) was investigated. The results are shown in **Tables 4.28-4.29**.

Table 4.28: The effect of urinary iodine excretion, between 11-15 weeks of gestational age (only pregnant subjects who delivered term infants), on the RHL % with covariates: the frequency of consuming rice, chicken, duck, and beef; age of pregnant subjects at the first admission

Source of variation	P-value
	11-15 wks
COVARIATES	
Rice (FFQ)#	0.577
Chicken and duck (FFQ)#	0.339
Beef (FFQ)#	0.016
Age *	0.805
Length	0.000
MAIN EFFECTS	
UIE (log form)	0.012
N	77

= Nutrient intake and food frequency at first interview
* = Age of pregnant subjects (years) at first admission
FFQ = Food frequency
UIE = Urinary iodine excretion at first collection μg/g creatinine; log form)
RHL % = (Head circumference/length)*100

Table 4.29: Comparison of the RHL % by urinary iodine excretion, between 11-15 weeks of gestational age (only pregnant subjects who delivered term infants); unadjusted and adjusted for covariates (exclude, I_2 supplement and total I_2 supplement)

UIE	N	Mean of RHL %	
		(unadjusted)	(adjusted)
≤ 1.668	25	69.2	69.0
1.669-1.924	25	67.8	68.1
>1.924	27	66.7	66.6

UIE = urinary iodine excretion (μg/g creatinine, log form).

4.5 TOTAL IODINE SUPPLEMENT AND BIRTH OUTCOME

In this section, we considered only the iodine supplement whether it had an effect on birth outcome. Although every pregnant subject received a tablet of vitamin supplement per day (each tablet had 100 μg iodine) from the first day of admission until delivery, we had no record whether or not each pregnant subject ate the tablet everyday. We assumed that each pregnant subject took their tablets each day. Then the total I_2 supplement was calculated. Total I_2 supplement meant the length of time (days) of I_2 supplement (100 μg/d) from admission until delivery. It was divided into three groups by thirds: the first group, pregnant subjects received iodine supplements for $\leq$ 168 days; the second group, pregnant subjects received iodine from 169-199 days and the third group, pregnant subjects received iodine for $\geq$ 200 days.

It can be seen from **Table 4.12** that total I_2 supplement which was divided into three groups by thirds, affected birthweight (BWT), length, the percentage of head circumference to length (RHL %) and chest circumference (CC).

4.5.1 Total I_2 supplement and birthweight.

The effect of total I_2 on birthweight was investigated with the covariates (the frequency of vegetables consuming; maternal status at first admission: height, weight and triceps skinfold, weight gain; and gestational age at birth). According to the weight gain, the difference between gestational age at birth (GABI) and gestational age at first admission (GADIF) of each pregnant subject was not the same. Therefore, the GADIF was also considered as a covariate.

Table 4.30: The effect of total I_2 supplement on birthweight with the covariates

Source of variation	P-value
COVARIATES	
Vegetables (FFQ)#	0.073
Height *	0.240
Weight *	0.034
Mid arm circumference *	0.426
Triceps skinfold *	0.987
Weight gain *	0.015
GADIF	0.271
Gestatinal age at birth	0.000
Gender	0.000
MAIN EFFECTS	
Total I2 supplement	0.834
N	213

= Nutrient intake and food frequency at first interview
* = Maternal status at first admission
FFQ = Food frequency
GADIF = The difference between gestational age at birth (GABI) and gestational age at first admission

It can be seen from **Table 4.30** that total I_2 supplement did not affect the mean of birthweight, but the covariates; weight gain, gestational age at birth and gender still effected birthweight.

4.5.2 Total I_2 supplement and the infants' length

The effect of total I_2 supplement on the length was investigated with the covariates (the frequency of foods consumed: chicken, duck and beef; maternal status at first admission: mid arm circumference and triceps skin folds; gestational age at birth and I_2 supplement).

Table 4.31: The effect of total I_2 supplement on the infants' length with the covariates

Source of variation	P-value
COVARIATES	
Chicken and duck (FFQ)#	0.658
Beef (FFQ)#	0.056
Mid arm circumference *	0.224
Triceps skinfold *	0.614
I_2 supplement	0.005
Gestatinal age at birth	0.000
Gender	0.008
MAIN EFFECTS	
Total I_2 supplement	0.009
N	339

= Nutrient intake and food frequency at first interview
* = Maternal status at first admission
FFQ = Food frequency
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplementation from admission until first collection of urine samples)

As shown in **Table 4.31**, after adjusting for the covariates, total I_2 supplement still showed an effect on the infants' length, and the covariates: I_2 supplement, gestational age at birth and gender also affected the infants' length. The infants'length, both the unadjusted mean and the adjusted mean for the covariates, is shown in **Table 4.32.**

Table 4.32: Comparison of the infants'length by total I_2 supplement; unadjusted and adjusted for the covariates

Total I_2 supplement	N	Mean of length (cm)	
		(unadjusted)	(adjusted)
≤ 168 days	112	49.1	49.5
169-199 days	114	49.0	49.0
> 200 days	113	50.1	49.8

Considering only term infants (gestational age at birth ≥37 weeks), total I2 supplement still effected the infants' length. The results are shown in **Tables 4.33-4.34**.

Table 4.33: The effect of total I_2 supplement on the infants' length (only term babies, gestational age at birth ≥ 37 weeks) with the covariates

Source of variation	P-value
COVARIATES	
Chicken and duck (FFQ)#	0.680
Beef (FFQ)#	0.026
Mid arm circumference *	0.253
Triceps skinfold *	0.499
I_2 supplement	0.006
Gender	0.057
MAIN EFFECTS	
Total I_2 supplement	0.020
N	317

= Nutrient intake and food frequency at first interview
* = Maternal status at first admission
FFQ = Food frequency
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplementation from admission until first collection of urine samples)

Table 4.34: Comparison of the infants'length by total I_2 supplement; unadjusted and adjusted for the covariates (only term babies)

Total I_2 supplement	N	Mean of length (cm)	
		(unadjusted)	(adjusted)
≤ 168 days	99	49.4	49.5
169-199 days	108	49.2	49.3
> 200 days	110	50.1	50.0

4.5.3 Total I_2 supplement and the percentage of head circumference to length (RHL %)

The effect of total I_2 supplement on the RHL % was investigated with the covariates (the frequency of foods consuming: rice, chicken, duck and beef; age of pregnant subjects at first admission; and I_2 supplement).

Table 4.35: The effect of total I_2 supplement on the infants' RHL % with the covariates

Source of variation	P-value
COVARIATES	
Rice (FFQ)#	0.085
Chicken and duck (FFQ)#	0.593
Beef (FFQ)#	0.010
Age *	0.138
I_2 supplement	0.107
Length	0.000
MAIN EFFECTS	
Total I_2 supplement	0.624
N	340

\# = Nutrient intake and food frequency at first interview
* = Maternal status at first admission
FFQ = Food frequency
I_2 supplement (100 μg/d)
= The length of time (days) of I_2 supplementation from admission until first collection of urine samples

As shown in **Table 4.35**, after adjusting for the covariates, total I_2 supplement did not show an effect on the percentage of head circumference to length, but the covariates: the frequency of consuming beef, and the length of the baby I_2 supplement still affected the RHL %.

4.5.4 Total I_2 supplement and the chest circumference (CC)

The effect of total I_2 supplement on the chest circumference was investigated with the covariates (maternal status at first admission: weight, mid arm circumference, triceps skinfold and blood pressure (systolic); and gestational age at birth. As shown in **Table 4.36**, after adjusting for the covariates, total I_2 supplement still showed an effect on the chest circumference, and the covariate, gestational age at birth, also affected the chest circumference. The chest circumference, both the unadjusted mean and the adjusted mean for the covariates is shown in **Table 4.37.**

Table 4.36: The effect of total I_2 supplement on the chest circumference with the covariates

Source of variation	P-value
COVARIATES	
Weight *	0.342
Mid arm circumference *	0.661
Triceps skinfold *	0.242
Blood pressure (systolic)	0.628
Gestatinal age at birth	0.000
MAIN EFFECTS	
Total I_2 supplement	0.037
N	348

* = Maternal status at first admission

Table 4.37: Comparison of the chest circumference (CC) by total I_2 supplement; unadjusted and adjusted for the covariates

Total I_2 supplement	N	Mean of CC (cm)	
		(unadjusted)	(adjusted)
≤ 168 days	115	31.0	31.2
169-199 days	121	31.7	31.6
> 200 days	112	32.0	31.8

Considering only term infants (gestational age at birth ≥37 weeks), total I2 supplement still affected the chest circumference. The results are shown in **Tables 4.38-4.39**.

Table 4.38: The effect of total I_2 supplement on the chest circumference with the covariates (only term babies)

SOURCE OF VARIATION	P-VALUE
COVARIATES	
Weight *	0.812
Mid arm circumference *	0.978
Triceps skinfold *	0.241
Blood pressure (systolic)	0.636
MAIN EFFECTS	
Total I_2 supplement	0.004
N	324

* = Maternal status at first admission

Table 4.39: Comparison of the chest circumference (CC) by total I_2 supplement; unadjusted and adjusted for the covariates (only term babies)

Total I_2 supplement	**N**	**Mean of CC (cm)**	
		(unadjusted)	**(adjusted)**
≤ 168 days	101	31.4	31.3
169-199 days	114	31.8	31.8
> 200 days	109	32.0	32.0

4.6 THE CHANGE IN URINARY IODINE EXCRETION (dLICR; μg/g creatinine, log form) AND BIRTH OUTCOME

The change in urinary iodine excretion (dLICR) refers to the difference between the level of urinary iodine excretion of casual urine samples from the first and last collections. Then the change in urinary iodine excretion (dLICR) was divided into three groups by thirds, namely: low, medium and high changes. Since the gestational age at the first and the last urine sample collections of each pregnant subject may not be the same, the gestational age at the first urine sample collection (fGA); and the difference between the gestational age at the last and the first urine sample collections (dGA) were considered as covariates. As shown in **Table 4.40**, the changing of urinary iodine excretion (dLICR) did not show any effect on birth outcome. Adding centre to the model did not alter this interpretation (see **Table 4.41** and **Appendix 6**).

Table 4.40: The effect of the changing of urinary iodine excretion (dLICR, μg/g creatinine, log form) during pregnancy on birth outcome with covariates

SOURCE OF VARIATION	P-VALUE				
	BWT	HC	Length	RHL%	CC
COVARIATES					
fGA	0.125	0.149	0.748	0.275	0.163
dGA	0.021	0.081	0.500	0.023	0.022
MAIN EFFECT					
dLICR	0.863	0.411	0.994	0.456	0.804
N	331	324	320	320	324

Table 4.41: The effect of the changing of urinary iodine excretion (dLICR, μg/g creatinine, log form) during pregnancy on birth outcome with covariates

Source of variation	P-value				
	BWT	HC	Length	RHL%	CC
COVARIATES					
fGA	0.155	0.273	0.043	0.481	0.158
dGA	0.038	0.253	0.047	0.521	0.025
Centres	0.669	0.090	0.000	0.000	0.809
MAIN EFFECT					
dLICR	0.869	0.416	0.898	0.430	0.809
N	331	324	320	320	324

dLICR = The changing of urinary iodine excretion.
= The difference between the level of urinary iodine excretion of casual urine samples from the first collection and the level of urinary iodine excretion of casual urine samples from the last collection.
fGA = The gestational age at first urine samples collection
dGA = The difference between the gestational age at the last urine samples collection and at the first urine samples collection.
BWT = Birthweight
HC = Head circumference
RHL % = (Head circumference/length)*100
CC = Chest circumference

CHAPTER 5
DISCUSSION

5. DISCUSSION

The objective of this study was to investigate whether there was a positive relationship between dietary iodine intake during pregnancy and birth outcome.

The results from this study showed that urinary iodine excretion was related to the infant's RHL % (the percentage of head circumference to length) during 11 to 15 weeks of gestational age. These results suggest that growth of the baby, in relation to the head of the infant is affected by iodine intake during 11-15 weeks of gestational age.

Before considering what the biological implications might be, it is important to consider the way this research has been conducted and the effects any weakness in the study may have on the interpretation of the findings.

First, there is a discussion on how to measure dietary iodine intake during pregnancy. **Second**, there is a discussion about the use of urinary iodine excretion from a casual urine sample as a measure of the level of dietary iodine intake during pregnancy. **Third**, there is a discussion about the main study plan. **Fourth**, there is a discussion of the effects of measurement error. **Fifth**, there is a discussion on the relationship between urinary iodine excretion and birth outcome without considering other variables. **Sixth**, there is a discussion about the other variables and their effects on urinary iodine excretion and birth outcome. **Seventh**, there is a discussion about iodine requirements and the relation between fetal growth rate and fetal thyroid development. **Eighth**, there is a discussion on the relationship between urinary iodine excretion and birth outcome while considering the other variables as covariates.

Ninth, there is a discussion on the change in urinary iodine excretion and birth outcome. **Finally**, there is a discussion about effect of the total iodine supplement and birth outcome.

5.1 HOW TO MEASURE DIETARY IODINE INTAKE DURING PREGNANCY

We investigated how to measure dietary iodine intake during pregnancy. Generally, there are two methods for determining dietary iodine intake, a direct method and an indirect method. For the direct method all food items eaten are collected for the analysis of their iodine contents, the quantity of these food items is recorded and then the dietary iodine intake is calculated. This method is too difficult to do in field research. The indirect method uses urinary iodine excretion as a proxy of dietary iodine intake. It is assumed to be a reasonable measure of intake because it is known that iodine is excreted mainly in the urine (see **section 2.2.4.x**).

Generally, the 24-hour urinary iodine excretion may satisfactorily reflect the dietary iodine intake (Broadhead et al, 1965; Vough et al, 1964; Nelson et al, 1987; Brug et al, 1992). The main problem of this method is to be sure of the accuracy of the 24-hr collection. The excretion of iodine (μg/ g creatinine or μg %) in a single sample was considered to be an alternative method to evaluate the level of dietary iodine intake.

In this thesis, regarding the analysis of iodine in a urine sample, we used the method modified from Wayne's method (1964). We found that this method had good reproducibility (CV = 4.49 %) and a good percentage of recovery (varied from 94.4 % to 104.4 %). The urinary creatinine excretion of each urine sample was determined by using Jaffe's

reaction which was modified from Taussky's method (Bonsnes & Taussky, 1945; Henry, 1964). The method appeared to be satisfactory, in terms of both the reproducibility (CV = 3.57 %) and the percentage of recovery (varied from 95.5 % to 107.6 %).

5.2 HOW CAN WE BE CONFIDENT THAT USING URINARY IODINE EXCRETION FROM A CASUAL URINE SAMPLE (μg/g creatinine) WILL GIVE A RELIABLE MEASURE OF THE LEVEL OF DIETARY IODINE INTAKE DURING PREGNANCY ?

There have been a number of studies which have been undertaken to demonstrate that the I/Cr (μg/g creatinine) in a single urine specimen could be used as an indicator of dietary iodine intake (Follis *et al*, 1962; Vought *et al* 1963; Jolin & Escobar, 1965; Frey *et al*, 1973; Schmid *et al*, 1980; Nohr *et al*, 1993; Meng *et al*, 1994; Pedersen *et al*, 1995; Akanji *et al*, 1996).

A single supplement of a known amount of iodine was taken by mouth (**section 3.2.6**) to investigate how long it took for the iodine to be recovered in urine. It was concluded from this study that the majority of dietary iodine intake was excreted in urine on the day in which it was eaten. This finding implied that we could use 24-hour urinary iodine excretion to estimate the level of daily dietary iodine intake, at least in a healthy subject who may be considered to be in balance.

A pilot study (**section 3.3**) to investigate the relationship between urinary iodine excretion in a 24-hour urine collection and casual urine samples was conducted in Thai pregnant women (n=10). In this study, we analyzed the iodine content in all food items consumed and the 24-hour urinary iodine excretion. The method of determining the

iodine content in food samples was modified from Moxon's method (1980). The coefficient of variation was 8.30 per cent and the percentage of iodide recovered varied from 94.7-104.4 per cent. We found a positive and statistically significant relationship between total dietary iodine intake and 24-hour urinary iodine excretion. The correlation was r = 0.81 (**P**<0.05, n=10). We also found a positive significant correlation between the urinary iodine excretion in a casual urine sample (μg/g creatinine) of **the first voiding** (after 6 to 12 A.M.) and the 24-hr urinary iodine excretion (r = 0.68, **P**< 0.05, n=10). Although, we found that **the mean** urinary iodine excretion in casual urine samples (μg/g creatinine) during the period after 6 to 12 P.M. gave the best positive significant correlation with 24 hour urinary iodine excretion (r = 0.97, P<0.05, n=10), the variation of urinary iodine excretion during this period was the highest. Because, firstly, all subjects were followed up at the two centres during 8 to 12 A.M. and secondly, the variation of urinary iodine excretion during the first voiding (> 6-12 A.M.) was the lowest. We have used the casual urine sample from the first voiding for the present study.

We did not use the urinary iodine excretion (expressed μg %) to estimate the levels of dietary iodine intake during pregnancy because we found that the average of the coefficient of variation (% CV) in urinary iodine excretion from casual urine samples expressed as μg % (Mean ± SD = 55.22 ± 17.01, n=10) was higher than that expressed μg/g creatinine (Mean ± SD = 27.27 ± 12.11, n=10). The higher variation of urinary iodine excretion expressed μg % than expressed μg/g creatinine was confirmed by the study in **section 3.2.3.**

Thus, in this thesis, we used urinary iodine excretion from a casual urine sample (μg/g creatinine) after 6 to 12 A.M. to evaluate the level of dietary iodine intake during pregnancy.

However, it is important to bear in mind several factors when the urinary iodine excretion in a casual urine sample (μg/g creatinine) is used to estimate the level of dietary iodine intake during pregnancy. **First**, although there was a positive correlation between urinary iodine excretion in casual urine samples and 24-hour urine samples; and there was a positive correlation between total dietary iodine intake and 24-hour urinary iodine excretion, they were not perfectly correlated (r=1). This might lead to misclassification bias because either a subject with high urinary iodine excretion might be a person with low total dietary iodine intake or vice versa. Thus, it is still possible that there could be a distortion of the relationship between dietary iodine intake and birth outcome (discussed further in section 5.4). **Second**, the per cent CV of urinary iodine excretion (μg/g creatinine) was quite large and suggested within subject variation which may reduce the statistical power of the study. The number of subjects in a study should be considered with the most conservative estimate of the accuracy of the measures used. Therefore the number of subjects needed for collecting casual urine sample was calculated. It was found that the number of total subjects should be 273 if there were three groups of urinary iodine excretion (low, medium, and high) (see in **section 3.4.1**). The numbers of subjects in the present study was 361. **Third**, a number of factors should be considered if urinary iodine from a casual urine sample is used to evaluate the level of dietary iodine intake. For example, factors which can have an effect on urinary creatinine excretion; exercise,

emotional stress, menstrual cycle, infection, fever and trauma, renal diseases and diet (see in **section 2.3.2.iii.c**). Some of these factors were investigated in this thesis. In brief, a study in which a creatine supplement was given suggested that if a subject consumed the same level of iodine intake but consumed a different level of creatine, the 24-hour urinary iodine excretion expressed μg/g creatinine was not the same (see **section 3.2.4**). We also found that the lower the protein intake, the lower the 24-hour urinary iodine excretion. It might imply that on the one hand, a food which has a high protein content always has high iodine content, and on the other hand that the level of protein intake may affect iodine metabolism (see **section 3.2.5**).

Further explanation about measuring exposure and measurement error of exposure are discussed in **section 5.4**

5.3 STUDY DESIGN ISSUES

5.3.1 What was the basic study design?

The main study in this thesis used a prospective cohort design and derived some basic data from the Low Birthweight (LBW) project which was a cooperation between the Research Institute for Health Sciences and other faculties of Chiang Mai University. A cohort study means that exposure is assessed at baseline and subsequent health outcomes are observed. The prospective cohort design has been employed in the main study because; firstly, it enables more accurate information to be collected about the individuals before the onset of the disease being investigated, and secondly, the information obtained prospectively is not only more accurate but also less open to bias. Thirdly, a prospective cohort study is optimal where there is a

short latent period between exposure and subsequent outcome. Fourthly, it can yield information on multiple outcomes for a particular exposure. Finally it is the strongest observational design for establishing cause-and-effect relationships. However, there are some disadvantages of a prospective cohort study design. For example, a cohort study tends to be expensive; it is not suitable for a study in which the outcome is rare; and losses to follow-up may diminish validity.

5.3.2 From what population did the study subjects come and how were they selected? How many subjects were be selected? How long were subjects observed?

Since 1990, a project named 'THE LOW BIRTHWEIGHT PROJECT' has been conducting at Chiang Mai province (a province in the northern part of Thailand). The purpose of the project is to investigate the risk factors which may affect birthweight. The sample size was calculated (with 95 % cent power of the test and 0.05 % type 1 error rate), depending on the incidence of low birthweight (13 %), the risk factors needed to investigate (namely, family income, first prenatal visit, amount of food intake and age of mother), per cent drop out rate (30 %) and the percentage of abortions (10%). The sample size was about 2,156 pregnant subjects. There were two centres for recruiting pregnant subjects, namely, Maharaj Nakorn Chiang Mai (Centre 1) and Health Promotion, Region 5 (Centre 2). Under the criteria for inclusion (pregnant women attending antenatal clinics at the two centres and having gestational age less than or equal to 24 weeks) and exclusion (those pregnant women who have been recruited in the study but do not give birth at the two selected study centres), there were 2,623 pregnant subjects recruited at the two centres.

In centre 1, there were 1,415 pregnant subjects; 88.55 % of them lived in Chiang Mai province and 11.45 % of them lived in the other provinces next to Chiang Mai. In centre 2, there were 1,208 pregnant subjects; 86.67 % and 13.33 % of them lived in Chiang Mai province and in the other provinces next to Chiang Mai respectively.

The objective of this thesis is to investigate the relationship between dietary iodine intake (assessed by the level of urinary iodine excretion from casual urine samples; divided into three levels, low, medium and high) and birth outcome. Therefore, the sample size of casual urine samples required to compare the means of groups was calculated from the formula of Cole et al (1991)(assuming it needed to be able to detect a 10 % difference of the mean with 80 % power at five per cent significance). The result of the calculation showed that the sample size of casual urine samples should be 273 or it might be said that we needed to collect casual urine samples from 273 pregnant subjects. By the same formula, the sample size required for each group of birth outcome variables were calculated, we found that the total subjects should be 87 for birthweight, 9 for head circumference, 9 for length, 12 for percentage of head circumference to length and 18 for chest circumference. The numbers for birth outcome measures are small because of the accuracy of the measures. Assuming only a 5 % difference of the mean with 80 % power at five per cent significance, the total number subjects required for birth outcome measures increases four fold. These calculations were shown in detailed in **section 3.4.1.** Therefore the sample of 361 pregnant subjects should be large enough to be reasonably confident of detecting differences in birth outcome, and any relationship between urinary iodine and birth outcome, if one exists.

Generally, selection bias (including self selection bias, and referral bias) may happen during sampling. The major potential selection bias is loss to follow up. But, in this study, all subjects (total subjects=361; from centre 1 =192 and from centre 2=169) recruited were followed up and birth outcome data obtained. In addition, the distribution of the addresses of pregnant subjects in each centre of this study was similar to that of the LBW project (as shown in **Table 3.8**). Because we had two centres for recruiting subjects, to minimize the bias, the measures of exposure, outcome and the other variables were carefully standardization prior to the commencement of the study.

Of these pregnant subjects (n=361), casual urine samples, information about dietary intake and other factors which may affect birth outcome were collected throughout the pregnancy.

5.3.3 What data were collected (exposures, outcomes and other factors)? How? (Accuracy, precision) Why?

5.3.3.i Dietary iodine intake during pregnancy (exposure)

In this study, we estimated the level of dietary iodine intake during pregnancy by using urinary iodine excretion from a casual urine sample (μg/g creatinine). The positive relationship between dietary iodine intake during pregnancy and urinary iodine excretion from casual urine samples, the procedure to analyze iodine content in a casual urine sample, the accuracy and precision of the procedure were all discussed in **sections 5.1 and 5.2**. During the study, casual urine samples (9-12 a.m.) were collected from 361 pregnant subjects. There were statistically significant differences in the urinary iodine excretion **(UIE)** from trimesters 1 to 3 (see **Tables 4.10**). It was found that the

mean UIE (expressed as μg/g creatinine) in the third trimester was the highest and the mean UIE in the second trimester was significantly higher than that in the first trimester ($P<0.05$). There may be three reasons for this trend. Firstly, the renal iodine clearance of pregnant subjects increased during the trimesters (Aboul-Khair *et al.*, 1964). Secondly, during the second and the third trimesters, pregnant subjects ate more than before pregnancy whereas food intake during the first 12 weeks of gestation might decrease because of nausea and vomiting (Rosso, 1990). This was confirmed by our results, see **Tables 4.6-4.7**. Thirdly, these pregnant subjects received iodine supplements during the study from admission until delivery.

We also found that the mean UIE expressed as μg % (log form) in the third trimester was higher than those in the first and the second trimesters but there was no significant difference in the mean UIE between the first and the second trimester. This might be because the volume of urine was more variable than creatinine excretions.

Birth outcome was assessed in relation to the change in urinary iodine excretion during pregnancy.

5.3.3.ii Birth outcome

The outcome in this study was the birth outcome of these pregnant subjects. The size at birth of the infants (n=361; including, birthweight, length, head circumference, chest circumference) was recorded by specially trained nurses. To prevent information bias these nurses both in centres one and two were trained to measure birth outcome by an expert in anthropometric measurement and then their ability to assess birth outcome was standardized by

measuring the birth outcome of a number of infants. The results of the repeatability study showed no statistically significant differences between repeated measures in the same child by the interviewers. The methods and the apparatuses used for each measure of birth outcome were described in **section 3.4.4**. In addition, the percentage of head circumference to length (RHL %) of each infant was also calculated (Head circumference x 100/length).

The results (**Table 4.11**) showed that the mean BWT, HC, and CC were in the normal range but the mean length was slightly smaller when they were compared with the study of Siripoonya (1990). Siripoonya (1990) showed that Thai infants had a mean birthweight of 3,000 g; mean length of 50.0 cm; head circumference of 33-35 cm and chest circumference of 31-33 cm. Among 361 infants, there were 8.9 per cent of low birthweight (< 2,500g) infants (n=32) and 6.7 per cent was preterm (delivered before 37 weeks) babies (n=24). Among the 32 low birthweight infants nearly half were preterm.

5.3.3.iii The variables and their effects either on birth outcome or urinary iodine excretion

The other variables which may affect either birth outcome or urinary iodine excretion or both of them were recorded.

(a) Socio economic status

Socio economic status, for example, level of attained education may influence food beliefs and practices regarding food selection and preparation. Economic factors (for example, total income) determine the amount and to some extent the quality of food purchased. Maternal occupation may affect her physical activity and food consumption. The quality and quantity of food consumed,

and also the frequency of consuming these food items during pregnancy may effect the dietary iodine intake and urinary iodine excretion. This is dependent upon the iodine content in food stuffs, and may be influenced by the use of iodine supplements in vitamin tablets which subjects received during pregnancy.

In this study, pregnant subjects were interviewed about their total income (Baht/month), occupation (agriculture and not agriculture) and the length of education (≤ 6 yrs, >6-12 yrs and >12 yrs), as shown in **Table 4.2**.

These data were collected because there were a number of studies to show the effect of these factors on birth outcome, especially on birthweight. For example, Klinchom *et al.* (1981), in the study of factors affecting rural birthweight at Yala province (in the south part of Thailand), found that there was a statistically significant correlation between maternal education and birthweight (n=466). Behrman (1985) concluded that low socioeconomic status (SES) is associated with an increased risk of low birthweight and preterm delivery, but the effect of SES is probably the sum of a number of factors including smoking; low maternal weight gain and short stature; obstetrical complications; access, source, and utilization of prenatal care; and marital status. Lumley *et al* (1985) analyzed the data of all births in Tasmania from 1975 to 1983. The results showed that social class differences in low birthweight were almost entirely restricted to infants between 1,500 and 2,500 g weight at birth. There was a marginal increase in very low birthweight infants (<1,500 g) among women whose partners were unemployed or in unskilled work but extremely low birthweight infants (<1,000 g) were evenly distributed across the whole social spectrum. Sunakorn *et al.*(1988), in the study of

epidemiology and risk factors associated with low birth weight infants in Thailand (n=75,955), found that low socioeconomic status (family income less than 1,500 baht per month; ~38 baht = £1) and a low level of education of parents (primary school or lack of education) were risk factors associated with low birth weight (LBW) infants. Gould *et al* (1988) investigated the relationships between socioeconomic status, low birthweight, births to teenagers, and inadequate prenatal care among black and white infants (n=127,558). Socioeconomic status was estimated by the 1979 median family income of the census tract of maternal residence. For both racial groups the deterioration of residential area socioeconomic status was associated with a significant increase in the percentage of high-risk teenage mothers (<17 years of age), in the percentage of mothers with either no, only third trimester, or unknown prenatal care, and in the percentage of low birthweight infants. The rate of increase in the percentage of low birthweight in response to the socioeconomic deterioration of residential area was similar in black and in white groups. There was, however, a racial gap of 5% low birthweight that remained constant across all income areas. Parker *et al* (1994) compared associations of five indicators of socioeconomic status (maternal education, paternal education, maternal occupation, parental occupation, family income) and three reproductive outcomes (low birthweight, small for gestational age, preterm delivery) in a representative sample of US births. After controlling for parity , maternal height, marital status, maternal age, and separately by race, nearly all socioeconomic factors were associated with low birthweight among both black and white women. However, there was no consistent pattern between the socioeconomic indices and the other outcomes.

(b) Nutrient intakes and food frequency

Generally, in most laboratory animals food deficiency in pregnancy shows an effect on pregnancy outcome, for example, by reducing the size of the litter; the weight of the individual offspring and the survival rate can be increased (Ebrahim, 1983). In the human, we are not able to do experiments about the effects of dietary deficiency on the fetus. However, in the case of Western Europe, some information is available from experiments of nature such as war and famine (Ebrahim, 1983). For example, the siege of Leningrad during 1941 to 1943 resulted in a restriction of food and later a period of deficiency. The average birthweight decreased significantly. There was a period of famine in Holland in the winter of 1944-1945. Pregnant women were exposed to nutritional deprivation for varying lengths of time but none was exposed for the whole gestation period. Lowest median birthweight was reached when exposure to famine was in the second half of pregnancy, but exposure very early in pregnancy did not affect the birthweight. This suggested that an adequate food intake was important at a specific time during pregnancy.

In this study, we assessed nutrient intakes (energy, protein, fat and carbohydrate) in each pregnant subject by using 24-hour recall during the first (10-12 weeks), second (22-24 weeks) and the third (32-34 weeks) trimesters. We attempted to increase the accuracy of quantities of food consumed by giving pregnant subjects measuring cups or other devices to aid the estimation. We also assessed the habitual food in each pregnant subject by a food frequency questionnaire (FFQ). Data for both questionnaire forms of dietary survey (24-hour recall and FFQ) were collected by interviewers who had been specially trained at the Research

Institute for Health Sciences, Chiang Mai University, Thailand. As shown in **Tables 4.6-4.8**, there were statistically significant differences in nutrient intakes of subjects between the trimesters and the percentage of subjects eating selected foods (consumed every day) varied from 3.1 % to 96.6 %. These results suggested that nutrient intakes and the frequency of consuming some food items (as shown in **section 5.6**) should be considered as covariates when the relationship between dietary iodine intake during pregnancy and birth outcome was investigated.

(c) Maternal age

We recorded the age of pregnant subjects in this study. The mean age was 25.3 years (SD=4.5, n=361). Maternal age was recorded because a number of investigators have shown an association between birthweight and maternal age.

For example, Klinchom *et al.* (1981) and Sunakorn *et al.* (1988) demonstrated that maternal ages younger than 20 years and older than 35 years were risk factors associated with LBW infants. Behrman (1985) revealed that among females of reproductive age, the LBW rate is inversely related to age, with the highest rate occurring in those younger than 15 years. It falls throughout the teenage years, and reaches its lowest level between 25 and 29 years, thereafter rising slowly with increasing maternal age. Lee *et al* (1988) investigated the association between maternal age and incidence of low birth weight at term. A total of 184,567 singleton live births with gestational ages of 40 weeks were examined from 1980-1984 Illinois birth certificate data. The incidence is highest in mothers < 17 years of age (3.2%) and gradually declines with advancing maternal age to reach 1.3% in women aged 25 to 34 years. It increases to 1.7 % for those > 35 years of

age. Scholl *et al* (1988) studied the influence of young maternal age on term and preterm birthweight. Data on 4496 singleton births to young women (19 years or less) were analyzed. The results show that the risk of preterm low birthweight is increased with very young maternity (15 years or less). MacLeod *et al* (1988) also investigated the effects of maternal age on birthweight. New York city birth certificates for singletons (n= 36,056) were analyzed using multivariate regression techniques. They found that there was a significant progression of birthweight with advancing age.

(d) Maternal anthropometry and blood pressure

In this study, height of pregnant subjects was recorded at the first admission whereas weight, mid arm circumference, triceps skinfold, systolic blood pressure and diastolic blood pressure were measured at each visit throughout the pregnancy. The weight gain was also calculated by subtracting the pregnant body weight (before labour) from pregnant body weight (at the first admission). These measures were assessed using standard instruments and methods and measured by specially trained staff of the Research Institute for Health Sciences, Chiang Mai University, Thailand. These data were measured because there was evidence to show that they may affect birth outcome.

Height has been shown in a number of studies to be related to birth outcome. Studies in both industrialized and developing countries have shown that taller mothers have higher birthweight infants than shorter mothers (Thomson *et al*, 1968; Hytten and Leitch, 1971; Lechtig *et al*, 1979; Shah and Shah, 1972; Papiernik *et al*, 1981; National Institute of Nutrition, 1982; Tripathi *et al*, 1987;

Prentice *et al* 1987; Anderson 1989). Buckfield *et al* (1983), using a population of approximately 9,000 singleton deliveries collected over a 10-year period from a defined geographical area in New Zealand, examined the effect of maternal height on birthweight, length and head circumference. They found that each 5 cm increase in maternal height accounted for an increase of 85 g, 4.1 mm, and 1.5 mm respectively in these measurements in males; and 25 g, 1.1 mm and 0.5 mm in females. Naeye and Tafari (1985) found that maternal height affected birth length, but not birthweight of infants born to 60 Ethiopion women. Witter *et al* (1991) investigated the effect of maternal height on birthweight and birth length. This retrospective study (N= 10,976) examined the gestational age at which differences were observed between 24 and 42 weeks born to women 150-157 cm (59-62 inches) tall were compared to similar infants born to women 168-175 cm (66-69 inches) tall. Significant differences occurred in birthweight and birth length from 35 weeks onward. The infants of the shorter women were symmetrically smaller than the infants of the taller women.

Prepregnancy weight has been shown to be a significant determinant of birthweight in both industrialized and developing countries (Naeye, 1979; Kramer, 1987; Kardjati *et al*, 1988). Women with lower prepregnancy weights have lower birthweight babies. Prepregnancy weight has also been shown to have an important effect on birthweight independent from that of weight gain and other factors (Eastman and Jackson, 1968). U.S. women who weigh < 130 lbs (51.2 kg) prior to pregnancy were more than twice as likely to have LBW infants as those weighting ≥ 130 lbs (Taffel 1980). Brown *et al* (1990) demonstrated that the proportion of infants born with low birthweight declined as pregnancy weight status increased.

The effect of **weight gain** on intrauterine growth retardation (IUGR) has been shown in many studies to be greater for malnourished women and for women during times of acute nutritional stress (Naeye, 1979; Prentice *et al*, 1987; Kramer, 1987). Siedman *et al* (1989) studied the effect of maternal weight gain in pregnancy on birthweight in 14,121 term singleton birth. A significant positive influence of prenatal weight gain on birth weight was found. Abrams *et al* (1995) determined the relationship between maternal weight gain pattern and birth weight in 2,994 uncomplicated pregnancies. They concluded that specific patterns of maternal weight gain, particularly weight gain during the second trimester were related to fetal birthweight. Recommendations for weight gain in pregnancy are based on prepregnancy status which used body mass index (BMI) as an indicator. A total gestational weight gain of 12.5-18 kg is recommended for underweight women (BMI<19.8); of 11.5-16 kg, 7-11.5 kg, and at least 6 kg are the recommended weight gain for normal women (BMI of 19.8-26.0), overweight (BMI of 26.0-29.0) and obese (BMI>29.0) respectively (National Academic of Sciences, 1990).

A number of investigators demonstrated that **maternal arm circumference** can be used as tool during pregnancy to screen for risk of low birthweight and late fetal and infant mortality rate (Lechtig *et al*, 1979; Shah, 1982; Lechtig, 1988; Anderson, 1989; Krasovec, 1989). Arm circumference was found to be independent of gestational age because it was relatively stable during pregnancy (Lechtig *et al*, 1979; Hull, 1983; Husaini *et al*, 1986; Krasovec 1989). In Guatemala (the prevalence of LBW = 18 %), De Vaquera et al (1983) found that the relative risk of LBW was 1.5 times higher for women with arm circumferences < 22.5 cm (taken the first 14 days postpartum) compared to

those with larger arm circumference. In Chile (the prevalence of LBW about 9%), Atalah (1983) found that an arm circumference of < 24 cm had a relative risk of 2.6 for predicting small-for-date infants. In Brazil (the prevalence of LBW = 9.0 %), Lechtig (1988) showed that an arm circumference during pregnancy of 23.5 cm had a sensitivity of 77 % and specificity of 71 % for predicting LBW. In rural Bangladesh, Krasovec (1989) demonstrated that there was a much higher arm circumference (measurement of 22.5 cm taken at any time during pregnancy) than in Guatemala, Chile or Brazil. This much higher arm circumference was related to the higher prevalence of LBW (about 30 %).

Searching the literature from 1980-1996 did not show any studies of the association between triceps skinfolds during pregnancy and birth outcome. However, we tried to find out whether there was an association between triceps skinfold during pregnancy and birth outcome.

There is a little information on the relationship between **blood pressure** during pregnancy and birth outcome. Naeye (1981) studied the association between maternal blood pressure and fetal growth. Eleven thousand and eighty two term, singleton pregnancies were analyzed for clues as to how different levels of maternal blood pressure affect fetal growth. Naeye found that when maternal edema and proteinurea were absent, birthweights progressively increased with increasing pressures until the hypertensive range was reached. In other words, birthweight decreased when pressure reached the hypertensive range. Decreases in birthweight associated with hypertension were most severe when mothers were thin and had low pregnancy weight gain. In Sweden, Himmeleman *et al* (1994) investigated the relation of maternal blood pressure to birthweight. They

found a negative correlation between the highest recorded maternal blood pressure during pregnancy and birthweight.

(e) Gestational age

In this study, the gestational age of pregnant subjects was estimated clinically according to the Dubowitz scoring system (Dubowitz *et al*, 1970). Also Denver's intrauterine fetal growth curve was used as a standard measure (Lubckenco *et al*, 1963). There is a clear relationship between the length of gestational age and birth outcome. A premature baby (< 37 weeks) has a birthweight less than that of a term baby (≥ 37 weeks). Chalik *et al* (1982) demonstrated that there were more LBW infants among those born before 37 weeks compared with those born after 37 weeks. Showstack *et al* (1984) assessed the association between birthweight and the length of gestation. They found a nonlinear positive relationship between length of gestation and birthweight. A few studies have demonstrated that gestational age affected urinary iodine excretion. For example, Ziya *et al* (1996) evaluated the urinary iodine levels in pregnant women without goitre in Turkey. Ziya *et al* found that the urinary iodine levels among these pregnant women in the second and third trimesters were significantly higher than those of pregnant subjects in the first trimester (respectively $P< 0.01$, $P<0.05$).

Since these variables have shown an effect on birth outcome they were considered as covariates in the relationship between dietary iodine intake and birth outcome.

5.3.4 Statistical analysis

As mentioned in **section 3.4.6**, we used descriptive statistics to describe the data, for example, the

measurement of central tendency and the measurement of dispersion. We used Cross-tab tables listing cell frequencies for data classified by at least two variables and used Chi-square for evaluating whether there were associations between variables in Cross-tab tables. The Correlation techniques were used to study the relationships and finally, Analysis of Variances (ANOVA) was a statistical technique employed to analyze multigroup comparisons.

5.4 MEASURING EXPOSURE AND MEASUREMENT ERROR OF EXPOSURE AND IT'S EFFECT

Generally, a study is considered to be valid if the findings are a representation of the true situation.
The definition of validity can imply the measures used in a study give valid measures of exposure and outcome, and the relationships between exposure and outcome derived from a study give valid measures of effect. There are two types of the assessment of the validity, internal validity and external validity. There must be no bias in the way data are collected, analyzed and interpreted if a study is to be internally valid. To be externally valid, a study must be internally valid and the subjects studied must reflect the population from which they came. In a study design, neither exposure nor outcome nor any other factors can be measured without error. These measurement errors may arise because of a lack of accuracy and precision of the methods used or they may arise from some bias in the way data were collected. The effect of measurement error is to distort epidemiological findings which may be either to under-or overestimate of the true effect. The most frequently occurring distortion arises from exposure measurement error leading to an attenuation of effect. Thus, a good study should select the best way of: sampling

from the study population, data collection, and consider ways to reduce bias. There will always be some unmeasured or unknown potential bias which must be considered in the final interpretation of the results.

In this study, we used urinary iodine excretion (μg/g creatinine) as a biomarker to estimate the level of dietary iodine intake. The methods used for determining urinary iodine excretion and urinary creatinine excretion were good both in terms of reproducibility and percentage recovery (as mentioned in **section 5.1**). We did validation studies between the reference measure (24-hr urinary iodine excretion and the test measurement of exposure (urinary iodine excretion from a casual urine sample) We found a significant positive correlation between the reference and the test measures. That means that the test measure may be helpful to assess the average level of group outcome in discrete categories of exposure (e.g. average birthweight levels by thirds of urinary iodine excretion). We could not determine the sensitivity and the specificity of the method of measuring exposure because there were only 10 subjects in this pilot study (as shown in **section 3.3**). However, the correlations obtained were not perfect (r=1). As shown in **Table 3.6**, the correlation between 24 hour urinary iodine excretion and urinary iodine excretion from casual urine samples (of the first voiding, >6-12 A.M.) was r=0.68 (n=10, $P<0.05$); if it was converted to percent correctly classified (the percentage of individuals who could be classified in the correct third of the distribution of true exposure using the measured exposure), it was 67.9 %, indicating that 32.1 per cent of subjects were misclassified (Clayton and Gill, 1991). The effect of exposure misclassification upon observed relative risk was to underestimate the true relative risk. It implied that the strength of the association between exposure and

outcome was reduced. We also analyzed the correlation between total dietary iodine intake and urinary iodine excretion from casual urine sample (of the first voiding, > 6-12 A.M.). The similar correlation was achieved; it was r=0.69 (n=10, P< 0.05). Thus, once the association between the exposure and outcome was investigated it should be interpreted carefully; where we see no association this may be because of measurement error which may mask a true association. It was also found that there was variation of urinary iodine excretion from casual urine samples of the first voiding between subjects (%CV=24, n=10) and there was diurnal variation of urinary iodine excretion within subjects (Mean %CV =27, n=10). These variations might be due either to true subject variation for the measure or to the effect of observer (measurement) error, or differences in other characteristics of the subjects (e.g. the difference in gestational age at urine sample collected, the difference in the length of iodine supplementation and the frequency of ANC). These variations might increase subject misclassification. To minimize the effect of true subject variation, the optimal sample size of subjects was calculated. Because the larger the sample size, the more precise (less variability) the estimate of effect and the smaller the difference detectable between groups. In addition the variation might be reduced by collecting casual urine samples on two or three consecutive days from each subject. However, we did not collect repeat urine samples in this study because it was difficult for a subject coming to the centres two or three days consecutively and it was too complicated to ask a subject to collect her casual urine samples at her home. To reduce the effect of the variation from the difference in other characteristics of the subjects, these characteristics should be taken into account for analyzing the association between the exposure and outcome.

Considering bias (a systematic error in a study that leads to a distortion of the results), the most common classification divides bias into three categories: selection bias, information bias and confounding.
Regarding selection bias (including self selection bias, referral bias and diagnostic bias), self selection bias and referral bias were unlikely to affect the measurement of exposure in this study (a prospective cohort study). Diagnostic bias is due to the difference in using different diagnostic criteria in different centres. In this study there were two centres for recruiting pregnant subjects. The effect of this bias between the two centres was minimized by carefully standardizing the ways of collecting urine samples, interviewing subjects, and measuring outcomes prior to the commencement of the study.

Referring to information bias (including social desirability, recall bias and interviewer bias), it was unlikely to affect the measurement of exposure in this study. Subjects and interviewer (dietary survey) did not know the objective of the urine collection and when the analysis of iodine content in urine sample was done, the technician did not know the results of birth outcome.

Confounders are associated with both exposure and outcome. For a variable to be considered a potential confounder, it must satisfy two conditions: first, it must be a risk factor for outcome in the absence of exposure and second, it must be associated with the exposure but not as a consequence of the exposure. Food habits, nutrients intake during pregnancy and the length of gestational age were the example of confounders from this study. The presence of confounding is demonstrated by a change in the apparent strength of association between the exposure and outcome. More serious distortion of the relationship may be caused

by an inability to accurately measure strong confounders. Two forms of distortions may occur: residual confounding and exaggerated confounding (Margetts and Nelson, 1991). There are two methods to minimize the effect of confounders on the relationship between exposure and outcome. The first is to consider them in the design by matching on the potential confounder. The other method is to evaluate confounding in the analysis by stratification. In this study, we used the latter method.

In a cohort study, the major bias is loss to follow-up. If the lost subjects differ in their risk of the outcome of interest, biased estimates of risk may be obtained. In this study, we had no problem about lost follow-up. The data for all 361 subjects were collected for their casual urine samples and for their birth outcome.

However, there were some disadvantages of collecting urine samples and other variables. There was missing data because sometimes some subjects did not come to the centres at the time made for the appointment. In addition, the first and the last urine collections of different subjects might not be at the same time. This resulted in a reduced sample of subjects when the effect of changing urinary iodine excretion during pregnancy on birth outcome was investigated. This reduced the power of the study. However, we minimized the effect of the time at the first urine collection and the different length of time between the first and last collection by taking them into account for the analysis of the results.

External validity is the extent to which the results of a study are applicable to other populations. The study sample should be representative of the population from which it was drawn. Therefore, age, sex and other socio-

economic data would be compared between the sample and the population from which the sample was drawn. If they are similar, it would be considered reasonable to generalize from the sample to the population. However, this study was a sub-study of the LBW project. We only compared the distribution of the location of pregnant subjects in this study with that of pregnant subjects of LBW project. The similar location was found. Therefore, the association between exposure and outcome in this study might or might not be generalized to the other poulations.

In conclusion, it might be inferred that it was very difficult to measure an exposure without errors. As far as possible, this study has reduced possible sources of error and attempted to consider the impact of errors on the results obtained. The following sections consider the main results obtained in the study.

5.5 THE RELATIONSHIP BETWEEN URINARY IODINE EXCRETION AND BIRTH OUTCOME WITHOUT CONSIDERING THE EFFECTS OF THE OTHER VARIABLES

In this section, the relationship between urinary iodine excretion ($\mu g/g$ creatinine, log form) and birth outcome was investigated without considering the effects of other factors. First, there was an investigation of the correlation between urinary iodine excretion and birth outcome (as shown in **section 5.5.1**). Second, there was an investigation of the interaction between urinary iodine excretion from casual urine samples (at the first collection) and birth outcome (as shown in **section 5.5.2**)

5.5.1 The correlation between urinary iodine excretion and birth outcome

The data for urinary iodine excretion (μg/g creatinine, log form) from casual urine samples in the first to the fifth collections were used to find out whether there was a correlation between urinary iodine excretion and birth outcome. With reference to **Table 4.21** and **Figures 4.2 to 4.6**, we did not find any correlation between urinary iodine excretion (μg/g creatinine, log form) from casual urine samples in the first to the fifth collections and birth outcome (birthweight, head circumference, length, the percentage of head to length and chest circumference). Several reasons might explain this lack of correlation. First, in each collection of casual urine samples, the pregnant subjects did not have the same gestational age. So there were a number of variables which might affect the urinary iodine excretion, for example, the differences in the amounts of foods eaten and the duration of iodine supplementation among these pregnant subjects. Second, the results of birth outcome also depended on the other variables, for example, the gestational age at birth, prepregnancy weight, weight gain etc (as mentioned in **section 5.3.3**). Thus, in each collection of casual urine samples, no correlation was found between the urinary iodine excretion and birth outcome. Finally, a lack of association may be attributable to small differences which were not able to be measured sufficiently accurately as noted in **section 5.4**.

5.5.2 The interaction between urinary iodine excretion at the first collection and birth outcome

Only the data for urinary iodine excretion (μg/g creatinine, log form) from casual urine samples at the

first collection were used to investigate the interaction between urinary iodine excretion and birth outcome.

The urinary iodine excretion (μg/g creatinine, log form) was divided into three groups by thirds. The urinary iodine excretion was not related to the infant's mean head circumference or the infant's mean length alone but was related to the infant's mean RHL %. The RHL % in the second and the third iodine excretion thirds were significantly smaller than that in the first third of urinary iodine excretion ($P<0.05$). if a baby has a large head circumference but the length of that baby is too short; this should be an abnormal baby, and vice versa. A normal baby should have an optimal RHL %. To assess the normal shape of baby, the RHL % seems to be an indicator better than using head circumference or the length of baby alone.

This relation may be explained by the different stages of development during fetal growth which may be affected by limitations of iodine. Generally, prenatal development is divided into three periods: a preembryonic period (0-2 weeks); an embryonic period (>2-8 weeks); and a fetal period (>8-38 weeks). In addition, there are differences in the growth of fetal bone between the flat bone and the long bone, with the growth of a flat bone (for example, head) being faster than that of long bone (for example, length). A fetus, at a specific time of gestation, might firstly use dietary iodine for the growth of its head and then for the growth of its length. The variation in gestational age at the first collection of urine samples from five to 37 weeks raises the question of whether a specific time for receiving dietary iodine intake during pregnancy might be needed for fetal growth and development. This result is interpreted again in **section 5.8** by

adjusting for the gestational age (at the first collection of casual urine samples) and the other factors which were found to affect urinary iodine excretion and birth outcome in this main study (**as shown in section 5.6**).

5.6 THE EFFECT OF OTHER VARIABLES ON URINARY IODINE EXCRETION OR ON BIRTH OUTCOME IN THE MAIN STUDY

In the main study, we investigated whether there were other variables which had an effect on urinary iodine excretion (μg/g creatinine, log form) from casual urine samples at the first collection or on birth outcome. These variables are described below.

5.6.1 The variables which had an effect on Urinary iodine excretion at first collection (as shown in Table 4.13)

5.6.1.i Socio economic status:

The total income of pregnant subjects was associated with urinary iodine excretion from the casual urine samples at the first collection. Since the food items, which were rich sources of iodine, were likely to be more expensive (e.g, sea foods), pregnant subjects, who had more total income, had more chance to buy these kinds of food. Nevertheless, this association might be by chance because the mean urinary iodine excretion in the medium total income was significantly lower than that in the high total income, whereas the mean of urinary iodine excretion in the low total income was not different from that of high total income.

5.6.1.ii Food frequency (FFQ) at first interview:

The frequencies of food consumed (beef, fresh milk and

seafoods at first interview) had a positive effect on urinary iodine excretion. This could be because these food items had a high iodine content or a high meat/protein intake.

5.6.1.iii Gestational age at the first urine collection:

The gestational age groups (≤ 15.00 wks, 15.01-22.00 wks and ≥ 22.01 wks) at the first urine collection had a positive effect on urinary iodine excretion. This might be because of the effect of the length of iodine supplement. It could be seen from **Table 4.14** that 25.6 %, 61.5 % and 95.6 % of pregnant subjects in the first, second and third gestational age groups respectively, received iodine supplementation of 100 μg/day for more than or equal to one day.

5.6.2 The variables which had an effect on Birth outcome

5.6.2.i Food frequency (FFQ) at first interview:

Vegetable consumption had a positive effect on birthweight (Table 4.15). It might be because vegetables are a source of vitamins and minerals which help the growth and development of a fetus.

Rice consumption, source of carbohydrate, had a positive effect on the infant's mean RHL % (Table 4.18). It might imply that the greater the frequency of consuming carbohydrate, the lower the birth length. Recently, Godfrey *et al* (1996) found that mothers who had high carbohydrate intakes in early pregnancy had babies with lower placental and birthweights.

Beef, chicken and duck consumption showed a positive effect on the infant's mean RHL % (percentage of head circumference to length) (Table 4.18) whereas they showed a negative effect on the infant's mean length (Table 4.17). Beef consumption also had a positive effect on the infant's mean head circumference (Table 4.16). But seafood consumption, showed a negative effect on the infant's HC (Table 4.16). Beef, chicken, duck and seafoods are sources of proteins which are important for fetal growth. Burke *et al* (1943) demonstrated that infants born at term to a women receiving a diet low on protein intake were shorter and weighed less than those born to women whose protein intake was high. Godfrey *et al* (1996) found that low maternal intakes of dairy and meat protein in late pregnancy were associated with lower placenta and birthweight. However, from this study frequency of these kinds of food showed a different effect on birth outcome. It might be possible that there were other nutrients which could affect infants' head circumference, infants'length and infants'RHL % in beef, chicken, duck and seafoods or in food items using them as an ingredient.

In conclusion it might be implied that more rice and less meat consumption could affect birth outcome, but more research is required to clarify the nature of the reported relationships.

5.6.2.ii Maternal age at the first admission:

There was a positive effect of maternal age (≤23 wks, 23-26 wks and >26 wks) on the percentage of head circumference to length (RHL %). New born babies of younger mothers (≤ 23 years old) had a smaller percentage of head circumference to length (RHL %) than those of mothers over 26 years old (see **Table 4.18**). There are a number of studies which have

shown the association between low birthweight and maternal age, as mentioned in section 5.3.3.iii.c. But we did not find any effect of maternal age on birthweight in this study. It may be because we had fewer younger and older pregnant subjects; only 7.8 % and 1.9 % of the pregnant subjects were less than 20 years old and older than 35 years old respectively.

5.6.2.iii Maternal height at the first admission:

Maternal height (≤149.5 cm, >149.5-154.0 cm and >154.0 cm) influenced infants' mean birthweight, (see **Table 4.15**). Subjects, in the second group (149.6 to 154.0 cm), had infants whose mean birth weight was greater than those in the first group (≤149.5 cm). Siquera *et al* (1975) also found that Brazilian women whose height was ≤ 149 cm had the highest probability of having low weight infants at birth. However, in this study the infants'mean birthweight of pregnant subjects (in the third group, >154.0 cm) was not different from infants' mean birthweight of pregnant subjects in the first and second groups (≤149.5 cm and 149.6 to 154.0 cm). This suggested that there might be other variables which also affected birthweight, for example, maternal age, weight prior to or during pregnancy.

From Table 4.16, maternal height affected the infant's mean head circumference. The infant's mean head circumference of pregnant subjects whose height (at first admission) was more than 149.5 cm, was larger than that of pregnant subjects whose height was less than or equal to 149.5 cm. Buckfield *et al* (1983) also found a positive relationship between maternal height and head circumference.

5.6.2.iv Maternal weight at first admission

In this study, maternal weight (at first admission: mean

gestational age of 13 weeks) was divided into three maternal weight groups (≤45.9 kg; >45.9-51.7 kg; > 51.7 kg). **Maternal weight had an positive effect on birthweight, head circumference and chest circumference of newborn babies.** Regarding birthweight, it was found that the greater the maternal weight the greater the infant's mean birthweight (**Table 4.15**). The results of the present study are supported by other studies, as shown in section **5.3.3.iii**. Pregnant subjects who weighed more than 45.9 kg delivered infants whose mean head circumference and mean chest circumference were significantly larger than those whose mothers weighed less than or equal to 45.9 kg (as shown in **Tables 4.16 and 4.19**). This might be due to the fact there was a positive relationship between birthweight and head circumference ($r=0.64$, $P<0.001$, $n=353$) and between birthweight and chest circumference ($r=0.83$, $P<0.001$, $n=353$).

5.6.2.v Maternal weight gain

In this study, from **Table 4.3**, there were only 216 pregnant subjects whose weights were measured before labour. **The mean weight gained during pregnancy was 10.0 kg.** With regards to the maternal gestational age, the mean weight of pregnant subjects increased about 2.8 kg from the first to the second trimester and about 6.5 kg from the second to the third trimester (**Table 4.4**). Maternal weight gain was divided into three groups (≤ 8.23 kg; >8.23-11.50 kg; >11.50 kg). **Maternal weight gain showed a positive effect on birthweight and head circumference of newborn babies** (**Tables 4.15 and 4.16**). The infant's mean birthweight and infant's mean head circumference of pregnant subjects who gained more than 11.5 kg during pregnancy were heavier and larger than those of pregnant subjects who only gained 8.2 kg or less. Regarding birthweight, the results in the

present study corresponded with those from other studies (as shown in **section 5.3.3.iii**).

5.6.2.vi Maternal mid arm circumference at first admission:

Maternal mid arm circumference was divided into three groups (≤23.2 cm; >23.2-24.8 cm; >24.8 cm). **We found that the mid arm circumference of Thai pregnant subjects had a positive effect on birthweight, length and chest circumference of newborn babies (Tables 4.15, 4.17 and 4.19)**. Pregnant subjects who had mid arm circumference of ≤23.2 cm delivered newborn babies with the less of birthweight, length and chest circumference. There is some evidence to show only the effect of maternal mid arm circumference and birth weight, as mentioned in **5.3.3.iii**.

5.6.2.vii Maternal triceps skinfold at first admission:

Maternal triceps skinfold was divided into three groups (≤16.0 mm; >16.0-21.0 mm; >21.0 mm). **It was found that the triceps skinfold of Thai pregnant subjects had a positive effect on birthweight, head circumference, length and chest circumference of newborn babies (Tables 4.15, 4.16, 4.17 and 4.19)**. Pregnant subjects who had triceps skinfold of ≤16.0 mm delivered newborn babies with the lower birthweight, head circumference, length and chest circumference. The measurement of triceps skinfold thickness provides valuable information on the quantity of subcutaneous fat. Triceps skinfold measurements change little with pregnancy up to thirtieth week of gestation, then they tend to decline. However, the total change is 0.5-0.7 mm (Rosso, 1990).

5.6.2.viii Systolic and diastolic blood pressure

Maternal systolic blood pressure was divided into three

groups (≤110 mmHg; >110-120 mmHg; >120 mmHg). **We found only systolic blood pressure at first admission (13.1 wks of pregnancy) had a negative effect on chest circumference** (see **Table 4.19**). Pregnant subjects who had systolic blood pressure of >120 mmHg cm delivered newborn babies with the smallest chest circumference. There was no published information that could be found showing an effect of systolic and diastolic blood pressure on chest circumference of newborn babies. But there are some studies which have shown that blood pressure affects birthweight (as shown in section **5.3.3.iii**).

5.6.2.ix Gestational age at birth

Generally, it is known that the length of gestation has an effect on fetal growth and development since an infant who delivered before full term will have inadequate growth and development. Thus, it is found that premature babies are always smaller than fullterm babies.

In this study, the gestational age at birth was divided into three groups; ≤ 38 weeks, >38 to 40 weeks and > 40 weeks. **It was found that the length of gestation had a positive effect on birth outcome (birthweight, head circumference, length and chest circumference)** (**Tables 4.15, 4.16, 4.17 and 4.19**). Infants who were delivered at a gestational age of more than 38 weeks had greater mean birthweight, head circumference, length and chest circumference than those who were delivered at a gestational age of less than or equal to 38 weeks.

In conclusion, in this study, we demonstrated that there were a number of variables which affected urinary iodine excretion and birth outcome. Therefore these variables were considered as covariates when we investigated the

effect of dietary iodine intake during pregnancy on birth outcome.

5.7 IODINE REQUIREMENT AND THE RELATION BETWEEN A FETAL GROWTH RATE AND A FETAL THYROID DEVELOPMENT

The requirements of iodine can be determined by various methods, for example, by determinating the average daily urinary losses of iodine, balance studies and calculation of iodine requirements based on quantitative studies of iodine metabolism, (as mentioned in **section 2.2.6.**). The average daily urinary losses of iodine give an approximate iodine requirement (100-200 μg/day) because it excludes other iodine excretions, for example, faeces, sweat and breath. Balance studies take into account these other sources of iodine loss. The results of equilibrium or positive balance studies indicate the iodine requirement (44-162 μg/day). However, there are some disadvantages in a balance study, for example, the complicated procedures for analysing iodine excretions in sweat and breath. The calculation of iodine requirements based on quantitative studies of iodine metabolism is based on the definition of iodine requirement which is defined as the amount of iodine required to maintain a normal plasma inorganic iodide (PII) and so avoid the formation of an iodine-deficiency goitre. The calculation is dependent upon; the lower limit of the normal PII, a renal clearance of iodide (RCI), and faecal iodine excretion (FIE) of a person. Wayne *et al* (1964) suggested the figures of 0.15 per 100 ml, 55 ml per minute and 40 μg per day for PII, RCI and FIE respectively. Therefore, based on these estimates, it would give a desirable iodine intake of about 160 μg per day. However, all the iodine present in food may not be available in absorbable form in which case the iodine intake would have to be adjusted upwards. Wayne *et al* (1964) also suggested

that during pregnancy, the iodine intake may rise to 200 μg per day because of an increased renal clearance (58 ml per minute)(Aboul-khair et al, 1964)., and the additional demands of the fetal thyroid gland.

Regarding fetal growth expressed as weight gain per unit of time (g/day), Findlay (1984) demonstrated that fetal growth up to the 10th week of pregnancy is relatively slow. The major fetal organ systems differentiate during that time, and subsequent development involves a large increase in mass. Rate of growth is maximal between the 28th and 36th week, and then falls. But incremental growth rate (i.e. percentage increase in body weight per day between the eighth week and the 38th week of fetal life) falls from about 6 per cent per day during the first 12 weeks and falls to 1.3 per cent per day by the 38th week. Regarding the head and the body length (crown-rump) of the fetus, the head at week 9 is almost half of the fetus and the period of most rapid growth rate of crown-rump of the fetus is between weeks 11-15 (England, 1990)(as shown in **appendix 8**). Comparing fetal thyroid growth and fetal thyroid function, by week 7 the gland has reached its site in the neck. By the 11th week of intrauterine life, the fetal thyroid can concentrate iodine and secrete thyroid hormone; at the same time thyroid stimulating hormone (TSH) is detectable in fetal serum (Findlay, 1984; England, 1990). The ability of the fetus to concentrate iodides and release T4 is dependent on TSH. A correlation is demonstrated between serum TSH levels and the free thyroxine concentration which suggests that the fetal-pituitary-thyroid axis is functioning as early as 11 weeks of pregnancy (Fuchs et al, 1977). Since adequate fetal thyroxine levels are necessary for normal fetal development and the rapid growth of the fetus has been shown between 9-20 weeks of pregnancy, it is possible that adequate iodine

intake during pregnancy at a specific time may be needed for fetal growth and development. It is not clear whether the mother can provide adequate thyroid hormone to a fetus. There is evidence that the extent of thyroid hormone transfer is limited. However, the permeability of the placenta to thyroxine increases during the second half of pregnancy, so that the maternal thyroid hormone potentially becomes available to the fetus near term. Thus, a fetus should depend on the adequate function of its own thyroid gland during the first half of pregnancy. It may be inferred that a fetus should receive adequate iodine from a pregnant woman, especially after 10 weeks of pregnancy when its thyroid gland can start to function.

5.8 THE RELATIONSHIP BETWEEN URINARY IODINE EXCRETION AND BIRTH OUTCOME AFTER CONSIDERING OTHER FACTORS AS COVARIATES

Generally, there is the possibility that variables other than the exposure of interest have influenced the outcome. These factors may alter the estimate of the effect of the exposure on outcome. There are three types of other factors (Margetts and Nelson, 1996). First, **outcome modifiers** have an effect on the outcome variable but are independent of the exposure of interest. They do not lie in the causal pathway of interest and they do not change the relationship between the exposure of interest and outcome. Second, **confounders** are associated with both exposure and outcome. They can provide a true explanation for an apparent association (or lack of association) between the exposure of interest and the outcome. Third, **effect modifiers** help to account for the differences in the relationship between the exposure of interest and the outcome according to the level of the effect of the modifier. An effect modifier will lie in the causal

pathway which relates the exposure of interest to the outcome.

In this study, we have assumed that factors measured on pregnant subjects are outcome modifiers or confounders and not therefore effect modifiers and therefore it is appropriate to adjust for these factors in the analysis. We have to make this assumption because in many studies the important modifiers (both outcome modifier and effect modifier) and confounders may not be known. To reduce the effect of other factors it is very important at early design stage to have read the literature carefully both in relation to the epidemiology of the outcome in question and in relation to likely causal mechanisms. Then it requires careful and logical thought to identify those additional variables which need to be included amongst the study measurements. As mentioned in **section 5.5.2**, after urinary iodine excretion (μg/g creatinine, log form) at the first collection was divided into three groups by thirds, we found that the infant's mean RHL % of the second and the third groups were significantly smaller than that of the first group ($P<0.05$). The effect of urinary iodine excretion on the mean infant's RHL % was analyzed within gestational age groups. These gestational age groups were dependent upon the fetal growth rate (expressed as weight gain, g/d) which is relatively slow up to 10 weeks; it involves a large increase in mass during 11-27 weeks and the rate of the fetal growth is maximal during 28-36 weeks (England, 1990). The effect of urinary iodine excretion on the infant's RHL % during 5-37 weeks, 11-36 weeks and 11-27 weeks of gestational age were investigated (**Table 4.22**). Among these three gestational age periods, the mean infant's RHL % of the second and the third groups were still significantly larger than that of the first group ($P<0.05$). However, after the other variables (as mentioned

in **section 5.6**) were included in the analysis as covariates, the urinary iodine excretion did not show any effect on the mean infant's RHL % (**Table 4.23**). Among these variables, the frequency of consuming beef (at first interview), maternal age (at first admission), the duration of iodine supplement (from the first admission to the first collection of urine samples) and the duration of iodine supplement (from the first admission until delivery) still significantly affected the mean infant's RHL % ($P<0.05$). The length of the baby also showed a very srong effect on the mean infant's RHL % ($P=0.000$) among the gestational age period.

From the review of embryological development it appeared that the 11th to 15th weeks of gestation may be a critical period during which iodine may be rate limiting. Around that time the fetal thyroid gland begins to secrete thyroid hormones and this is the period of rapid increase in crown-rump length. The relationship between iodine excretion and the percentage of head circumference to length (RHL %, %) was investigated further. Gestational age was divided into 11-15 weeks (n=80), 16-20 weeks (n=96) and 21-27 weeks (n=109). It was found that only during 11-15 weeks of gestation was the mean infant's RHL % significantly associated with the urinary iodine excretion ($P=0.019$, n=80) (**Tables 4.24 and 4.25**). The duration of iodine supplement (both I_2 supplement and total I_2 supplement), and the baby length also showed an effect on the mean infant's RHL %. Since a premature baby always has a smaller body size than a term baby, three premature babies (gestational at birth less than 37 weeks) were excluded from the analysis; a higher negative effect of the urinary iodine excretion on the mean infant's RHL % was found ($P=0.012$, n=77) (**Tables 4.26 and 4.27**). After that, we considered the urinary iodine excretion (μg/g creatinine, log form) as an

indicator of iodine intake, the I_2 supplement and the total I_2 supplement may be excluded from those covariates. A similar result of a negative effect of the urinary iodine excretion on the mean infant's RHL % was found (P=0.012, n=77) (**Tables 4.28 and 4.29**).

Low iodine intake, particularly during 11-15 weeks of gestationa appears to influence the proportional growth of the length of the child relative to their head size. Sub-optimal iodine intake at a critical time in fetal development appears to have specific and long-lasting effects. However, there were only 80 subjects with urinary excretion data collected during 11-15 weeks. A bigger sample size may be needed to confirm this main finding.

As mentioned in **section 5.3.3**, the mean percentage of head circumference to length (RHL %) for a reference group of Thai infants (Siriponya, 1990) was 68.0 per cent. Based on this figure, with reference to Table 4.29, after adjusting for covariates, the mean infant's RHL % of the second and the third groups were similar to what was expected (68.1 % and 66.3 % respectively) whereas the mean infant's RHL % of the first group (69.3 %) was bigger. Since the urinary iodine excretion was > 61.7 to 97.7 μg/g creatinine in the second group and > 97.7 μg/g creatinine in the third group; and if the urinary creatinine excretion during pregnancy was one gram per day, it might be said that if a pregnant woman had urinary iodine excretion > 61.7 μg per day during 11-15 weeks of gestation, she should have a sufficient dietary iodine intake for her infant's RHL %. With regard to **Table 3.5** (in **section 3.3 in Chapter 3**), in Thai pregnant subjects (n=10), the mean±SD of per cent recovery iodine in urine (as per cent of total dietary iodine intake) was 93.65±40.26. It might be assumed that the minimum per cent recovery of iodine in urine was 54

percent, with the figure of urinary iodine excretion > 61.7 μg per day, a pregnancy should have dietary iodine intake > 115.0 μg per day during 11-15 weeks of gestation.

5.9 THE CHANGE IN URINARY IODINE EXCRETION (DLICR, μg/g creatinine, log form) AND BIRTH OUTCOME

In this study, urinary iodine excretion from a casual urine sample was used as a biomarker for evaluating the level of dietary iodine intake. Since it was found that the urinary iodine excretion tended to increase during pregnancy, the possible effect of this change on birth outcome was investigated. The change in urinary iodine excretion (DLICR) was defined as the difference between the first and the last urine collections in each pregnant subject. As shown in **Tables 4.40 and 4.41**, the effect of the change in urinary iodine excretion on birth outcome was investigated with taking into account the effects of the other covariates: the gestational age at the first urine sample collection; the difference between the gestational age at the last and the first urine sample collections; and the centres. After adjusting for these covariates the results showed that the change in urinary iodine excretion did not show any effect on birth outcome. It might be concluded that an increase in urinary iodine excretion (or total dietary iodine intake) during pregnancy was less important for birth outcome than the optimal iodine intake at a specific time of gestational age.

5.10 THE TOTAL IODINE SUPPLEMENT AND BIRTH OUTCOME

Generally, there are a number of methods of iodine supplementation, for example, iodized water, iodized salt, iodized tablet, and iodized oil. In developing countries, the addition of iodine to salt is the most favoured method for preventing iodine deficiency. However, it has been proved ineffective due to administrative difficulties in manufacturing the salt, in ensuring that iodized salt replaces locally produced salts and in distributing it to the remote areas where iodine deficiency is often most severe (Phillips et al 1988). The administration of iodized tablet or iodized oil (both oral and intramuscular iodized oil) can be used as means of prevention of iodine deficiency in populations where iodized salt is difficult to administer (Ermans, 1994). These methods are particularly useful if iodine prevention has to be given to specific, priority groups. An absolute priority is iodine deficiency in the pregnant women, because this may induce iodine deficiency disorders in the offspring, especially endemic cretinism.

As mentioned in **section 4.5** (**Chapter 4**), the total I_2 supplement meant the length of time (days) of iodine supplement (100 μg per day) from admission until delivery. This was divided into three groups by thirds: $\leq$ 168 days, 169 to 199 days and $\geq$ 200 days. The mean$\pm$SD gestational age at first admission of these three groups were 17.89$\pm$3.26 weeks, 12.56$\pm$1.86 weeks and 8.60$\pm$2.00 weeks respectively.

The results showed that the total I_2 supplement affected birthweight (BWT), length, the percentage of head circumference to length (RHL %) and chest circumference.

5.10.1 The total I_2 supplement and birthweight

It can be seen from **Table 4.15** the infant's mean BWT from women in the second and the third groups of total I_2 supplement were significantly heavier than those infants born from women in the first group (P=0.0008). However, after the effect of total I_2 supplement on birthweight was evaluated along with other variables (as mentioned in **section 4.5.1, Chapter 4**) as covariates, there was no significant difference in the mean birthweight among the three groups receiving total I_2 supplement (**Table 4.30**). This result suggests that first, iodine supplement (100 μg per day) starting from the first or the second trimesters might be not enough to increase infant's birthweight. Nevertheless, the mean infant's birthweight in each group was heavier than 2,500 g. Second, there were other variables which had more effect on birthwight than total I_2 supplement. These variables were maternal weight at first admission, weight gain during pregnancy, gestational age at birth and infant's gender (**Table 4.30**). There were a number of studies of the association among iodine supplement during pregnancy, maternal or neonatal thyroid functions and maternal or neonatal iodine status (Silva *et al*, 1981; Eber *et al*, 1990; Nohr *et al*, 1993 and 1994; Glinoer *et al*, 1995). But there were few studies investigating the effect of iodine supplement during pregnancy and birth weight. Thilly (1981), demonstrated that an iodized oil injection given in the latter half of pregnancy could improve the birthweight of infants. Chaouki *et al* (1994) showed that oral administration of iodized oil (lipiodol 0.5 ml= 240 mg iodine, coverage for six months) 1-3 months before conception or during the first trimester of pregnancy normalized thyroid function in new babies and mothers, increased placental and birth weight. From these two studies, it may be implied that

iodized oil given to a pregnant woman not later than the latter half of pregnancy can increase birthweight. In comparison with our study, we did not find an effect of iodine supplement on birth weight; it might be because of the dose and the form of iodine supplementation, or that all women received an iodine supplement and possibly raised iodine levels above a critical level although the shortest length of total iodine supplementation was ≤ 168 days or ≤ 24 weeks before delivery (equivalent to > 15 weeks of gestational age) .

5.10.2 The total I_2 supplement and the infant's length

As shown in **Table 4.17**, the mean length of infants from pregnant subjects in the third group of total I_2 supplement was significantly longer than those in the second and in the first groups (P=0.0001). Although the effect of total I_2 supplementation was investigated with the covariates, the total I_2 supplement still showed an effect on the infant's mean length and the covariates: I_2 supplement (from first admission until the first collection of casual urine samples), gestational age at birth and gender also affected the infant's length (**Tables 4.31-4.32**). Even if only the pregnant subjects who delivered term infants were considered, the total I_2 supplement and the covariate (the I_2 supplement) still affected the infant's length (**Tables 4.33-4.34**).

These results may have been caused by the fact that the pregnant subjects in the third group received iodine supplement at an earlier stage of pregnancy and the supplement covered the specific time when iodine was needed for the growth of infant's length. This finding was confirmed by the fact that the mean±SD gestational age at the first admission of the third group was 8.60±2.00 weeks

and is cover the period of rapid growth of body length (CR, crown-rump) during 11-15 weeks of gestation (see **section 5.7**).

5.10.3 Total I_2 supplement and the percentage of head circumference to length (RHL %)

As shown in **Table 4.18**, the infant's mean RHL % of pregnant subjects in the third group was smaller than that of pregnant subjects in the first and the second groups.

This might be because first, no significant difference was found in the infant's mean head circumference among the three groups of total I_2 supplement. The infant's mean±SD head circumference from women in the first to the third groups were 33.0±1.5 cm (n=117), 33.2±1.3 cm (n=122) and 33.3±1.1 cm (n=114) respectively. Second, the infant's mean length of women in the third group was longer than that of subjects in the second and the third groups (as mentioned in **section 5.10.2**). However, after the effect of the total I_2 supplement on the infant's mean RHL % was investigated with the other variables as covariates, no effect of Total I_2 supplement was found on the infant's mean RHL %. But the covariates: the frequency of consuming beef at first interview; and the baby length still affected the RHL % (**Table 4.35**). These results suggest that besides the total I_2 supplement, other sources of iodine intake (for example, iodine intake from foods) at specific times during pregnancy could be involved the infant's RHL %. Since it was found that the urinary iodine excretion from casual urine samples (as an indicator of the total dietary iodine intake) during 11 to 15 weeks of gestation showed an effect on the infant's mean RHL % (**Tables 4.28 to 4.29**).

5.10.4 The total I_2 supplement and the chest circumference (CC)

The infant's mean chest circumference from pregnant women in the second and the third groups was larger than that of pregnant subjects in the first group (**Table 4.19**). Although, the effect of total I_2 supplement on the infant's chest circumference was investigated with the other variables as covariates (as mentioned in **section 4.2.6**), the same result was found. Among these covariates, only the gestational age at birth showed a significant effect on the infant's mean chest circumference (**Tables 4.36 to 4.37**). After the preterm babies were excluded, the infant's mean chest circumference of pregnant subjects in the second and the third groups were still larger than those infants born from pregnant subject in the first groups (**Tables 4.38 to 4.39**). These results suggest that the iodine supplement (100 μg per day) starting at least from about 12 weeks of gestational age and supplementation for more than 168 days during pregnancy could improve an infant's mean chest circumference. This may be or may not be related to the fact that there is ossification spreading and some bone are already well outlined by 10-12 weeks of gestational age. It should be noted that the chest circumference of a fetus may be dependent upon the size of internal organs, for example, liver, lungs and heart.

5.11 SUMMARY OF THE MAIN FINDINGS IN THIS STUDY

We have summarized our main findings in relation to each hypothesis originally put forward.

GENERAL HYPOTHESIS: Low maternal iodine intake during pregnancy appears to adversely affect the size and shape (birth outcome) of the offspring.
FINDING: Low maternal iodine intake at a specific time during pregnancy adversely affects the size and shape (birth outcome) of the offspring.

SPECIFIC HYPOTHESIS 1: The lower the maternal dietary iodine intake during pregnancy the lower the birthweight (size) of the offspring.
FINDING 1: We did not find an association between dietary iodine intake during pregnancy and birthweight of the offspring.

SPECIFIC HYPOTHESIS 2: Lower maternal dietary iodine intake during pregnancy leads to specific effects on the growth of long bones, leading to shorter babies at birth.
FINDING 2: We didn't find any relationship between dietary iodine intake during pregnancy and the length of the baby.

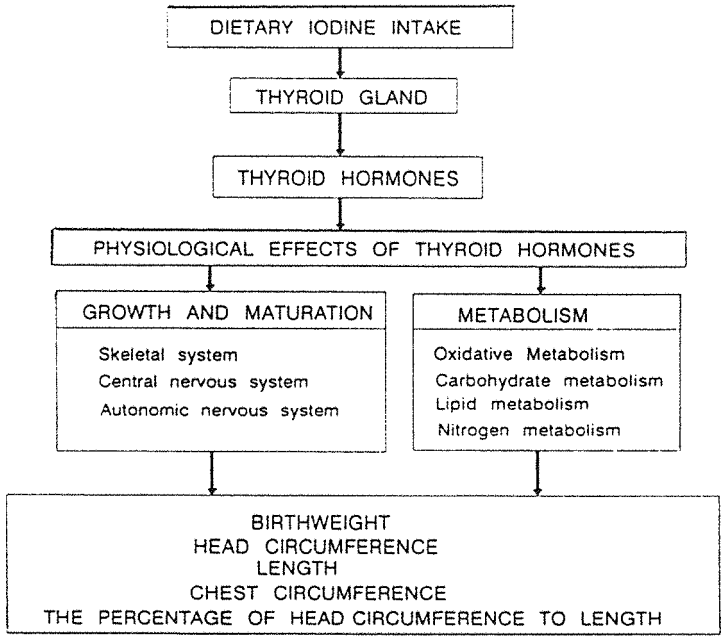
SPECIFIC HYPOTHESIS 3: Low maternal iodine intake during pregnancy leads to differential effects on specific aspects of fetal growth; head size relative to length.
FINDING 3: A low maternal dietary iodine intake during 11-15 weeks of gestational age was associated with a higher ratio of the percentage of head circumference to length.

The failure to detect the relationships for specific hypotheses 1 and 2 may have been related to limitations in

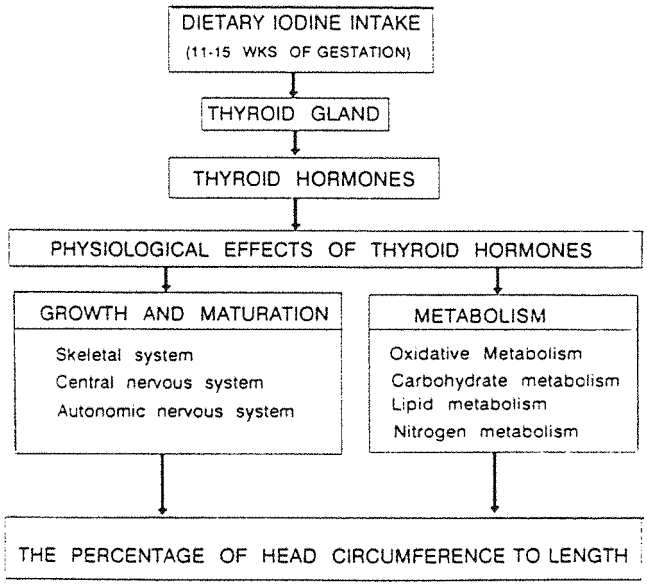
in the sample size, although the study was large enough to detect what were considered to be important biological differences.

The main findings may be concluded in diagram as follows:

HYPOTHESIS

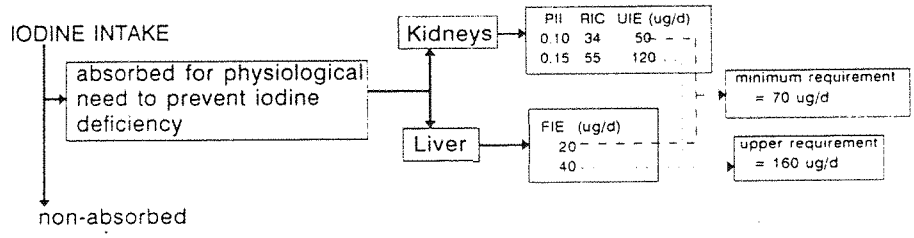


FINDING



5.12 PUBLIC HEALTH IMPLICATIONS

Generally, a requirement for a nutrient is that amount which an individual must consume to avoid deficiency as defined by clinical, physiological, biochemical or sociological criteria, and does of course vary from individual to individual. It has been assumed that the distribution of requirements in a group of individuals for a nutrient is normally distributed. This gives a notational mean requirement of Estimated Average Requirement (EAR). The Reference Nutrient intake (RNI) is two notional standard deviation (2SD) above the EAR (RNI = EAR + 2SD). In the United Kingdom, the Panel on Dietary Reference Values has set the RNI of iodine at 140 μg/d or 1.1 μmole/d. However, the distribution of requirements in a group of individuals for a nutrient may be not normally distributed. The requirement may be calculated from iodine metabolism and the difference between iodine intake and requirement may be shown by a diagram as follows:



PII = PLASMA INORGANIC IODINE (ug %)
RIC = RENAL IODIDE CLEARANCE (ml/min)
UIE = URINARY IODINE EXCRETION (ug/d)
FIE = FAECAL IODINE EXCRETION (ug/d)

Source: Adapted from Wayne *et al* (1964)

Therefore:

(1) For normal adult

Iodine intake

= requirement (70-160 μg/d) + non-absorbed.

(2) For pregnancy

Iodine intake

= requirement (70-160 μg/d) + for fetal growth and development + non-absorbed

(3) For lactation period

Iodine intake

= requirement (70-160 μg/d) + for infant growth and development (in breast milk)+ non-absorbed

(4) For areas where goitrogens may affect availability of iodine intake

Iodine intake

= iodine increment needs to add into (1), (2) and (3)

From this study, iodine requirement and iodine intake may be concluded in table as follows:

PREGNANCY DURING 11-15 WEEKS OF GESTATIONAL AGE	
Estimated urinary iodine excretion	= 62 μg/d
Estimated faecal iodine excretion	= ?
Roughly iodine requirement	= 62 μg/d
Minimum iodine recovered in urine (as percentage of iodine intake)	~ 54 %
Estimated iodine intake	= 115 μg/d
PREGNANCY AFTER 15 WEEKS OF GESTATIONAL AGE	
Iodine requirement for fetal growth and development=?	
Estimated iodine intake ~150 μg/d (=115 + ? μg/d)	
LACTATION PERIOD	
Breast milk iodine excretion (1st year) ~37 μg/d	
Estimated iodine intake ~150 μg/d (=115+37 μg/d)	

? = not known

However, in the present of goitrogens in the diet, intake may increase to 200-300 ug/day (Garrow *et al*, 1993). This higher intake should therefore apply to those areas where a high intake of goitrogen can be expected, e.g, in those population with a heavy reliance on cassava, maize, bamboo, shoots, sweet potatoes, lima bean and millets.

CHAPTER 6

CONCLUSION AND FUTURE WORK

6. CONCLUSION AND FUTURE WORK

In this chapter, the conclusions of this thesis and future work are stated, and there is a consideration of how the results of the study can be used in practice or how the findings can be generalized to other human populations-the public health relevance and generalizability.

6.1 CONCLUSION

From the main study, it might be inferred that:

(1) Three hundred sixty one healthy Thai pregnant subjects were recruited in the study. Casual urine samples were collected from pregnant subjects. The mean urinary iodine excretion (μg/g creatinine, log form) increased significantly through the trimesters. Birth outcome of their infants were recorded. The mean of birthweight (BWT), head circumference (HC), length, percentage of head circumference to length (RHL %), chest circumference and gestational age were 3,007 g, 33.2 cm 49.4 cm, 67.2 per cent, 31.5 cm and 39.0 weeks respectively.

(2) There were a number of variables affecting the urinary iodine excretion and birth outcome (as shown in Table 4.12 and section 5.6)

(3) The association between urinary iodine excretion and birth outcome was investigated after adjusting for the other variables which had an effect either on urinary iodine excretion or on birth outcome. It was found that, only during 11-15 weeks of gestation, the mean infant's percentage of head circumference to length was significantly affected by the urinary iodine excretion (P=0.004, n=77). These results suggested that 11-15 weeks

of gestational age might be the specific time when a fetus needs an optimal level of dietary iodine for appropriate proportional growth.

(4) There is a biologically plausible mechanism to show the relationship between fetal growth rate and fetal thyroid development. The fetal growth (g/day) up to the 10th week of pregnancy is relatively slow, subsequent development involves a large increase in mass. Rate of growth is maximal between the 28th and 36th week, and then falls (Findlay, 1984). The head at week 9 is almost half of the fetus and there is the most rapid growth of body length (crown-rump) during the 11-15 weeks of gestation (England, 1990). Comparing fetal thyroid growth and fetal thyroid function, by week 7 the gland has reached its site in the neck. By the 11th week of intrauterine life, the fetal thyroid can concentrate iodine and secrete thyroid hormone; at the same time thyroid stimulating hormone (TSH) is detectable in fetal serum (Findlay, 1984; England, 1990). Since adequate fetal thyroxine levels are necessary for normal fetal development and the rapid growth of the fetus has been shown between 9-20 weeks of pregnancy, it is possible that adequate iodine intake during pregnancy at specific times may be needed for fetal growth and development.

(5) Regarding the optimal level of dietary iodine intake, if a pregnant women had urinary iodine excretion > 61.7 μg/g creatinine per day during 11-15 weeks of gestation, she should have sufficient dietary iodine intake for optimal growth. If the minimum per cent recovery of dietary iodine intake in urine was 54 per cent and urinary creatinine excretion was one gram per day, a pregnant woman should receive 115 μg of iodine per day during this period. After correcting for iodide trapping by mammary glands and

for the further stage of fetal growth and development, iodine requirement during pregnancy may rise to be about 150 μg per day.

(6) The change in urinary iodine excretion during pregnancy did not show any effect on birth outcome. It might be concluded that an increase in urinary iodine excretion (or total dietary iodine intake) during pregnancy was less important for birth outcome than the optimal iodine intake at specific times during pregnancy.

(7) Total iodine supplement (100 μg/day) during pregnancy of more than 199 days (or >28.4 weeks before birth) affected infants' length whereas total iodine supplement (100 μg/day) during pregnancy more than 168 days (or >24 weeks before birth) affected infants' chest circumference. These results suggest that iodine supplement (100 μg per day) for more than 200 days during pregnancy (or we can say that pregnant subjects start receiving iodine supplement at 11 weeks of gestation) should be sufficient for normal growth of infant's length and infant's chest circumference at birth.

Finally, the findings in this study suggest that a pregnant woman should receive dietary iodine intake about 150 μg per day (especially during 11 to 15 weeks of gestation) from the early stage of pregnancy until delivery for normal fetal growth and development, and also during breastfeeding to ensure that there is an adequate quantity of iodine in breast milk for infant growth and development during the first year of life. Iodine supplementation later in pregnancy may be ineffective because it may miss the period of critical thyroid function.

6.2 FUTURE WORK

There are a number of further studies which should be investigated.

(1) It will be possible to explore the effects of the length of supplementation of iodine on birth outcome in the main project from which the present study was derived. Although urinary iodine was not measured in the large study, a crude measure of total iodine supplementation will be possible because the date of the first supplement is known.

(2) The results of this study need to be confirmed in another study with more subjects. Whether it is appropriate to consider on intervention study to supplement a group of women needs careful consideration. For an intervention study to be viable a control group is required who do not receive any supplement. The ethical issues involved need to be considered. However, without further investigation the results of the present study will not be confirmed. Although very suggestive the results of the present study are not sufficient to prove a causal link.

(3) Further research is required on thyroid function during the first trimester of pregnancy to confirm the effects of iodine on levels of T_3, T_4 and TSH

(4) While supplementation may be feasible to increase iodine intake, more research is required on the dietary sources of iodine and factors influencing availability of dietary iodine. It may be important to explore ways of increasing the iodine content of the staple foods eaten in iodine deficient areas. In Thailand this will be rice.

(5) There is little data available on the iodine content of breast milk and the effect this has on growth in the first year of life.

6.3 HOW THE MAIN FINDING OF THIS THESIS CAN BE USED IN PRACTICE OR CAN BE GENERALIZED TO OTHER HUMAN POPULATIONS?

If the findings of this study are true, it suggests that women who are planning to get pregnant should ensure that they have adequate dietary iodine intake. If 11-15 weeks of gestation is the critical period, most women will have already past this stage when they have their pregnancy confirmed. If iodine supplementation is only commenced at the first consultation, for many women this may be too late.

An education programme may be advisable to encourage women to eat iodine rich foods when they are planning a pregnancy. It is not clear at this stage whether iodine supplementation will be necessary to ensure adequate (say 150 μg/day) levels. If supplements are required, the best vehicle for this will need to be considered and modified to suit the local needs and resources.

In order judge whether a woman is likely to have sufficient iodine we recommend that an assessment of iodine status should be included in routine prepregnancy and pregnancy consultations.

APPENDICES

APPENDIX 1: IODINE CONTENT IN SOME CROPS, FRUITS AND VEGETABLES IN BANGKOK

CROPS/FRUITS/VEGETABLES	IODINE (µg/kg) wet	dry
Pak Boong		310.8
Takrai		182.4
Ho-ra-pa		1344.5
Hua Puek		1916.7
Man Tes	1500.0	
Prik Yuak		485.5
Bai Tam Lung		632.8
Kha		436.8
Bai Kana	449.0	
Bai Pak Kard Khao		355.5
Bai Kuy Chai		650.0
Prik Kee Noo		916.0
Bai Kalam Plee	260.0	353.0
Yod Fak		925.0
Kra-chai		441.0
Tang Kwa	290.0	335.0
Buab liam	338.0	461.0
Tua Fak Yao	294.0	448.0
Bai Hom	781.0	
Hua Hom		5564.0
Bai Pak Chee	468.0	643.0
Ma Krood		527.0
Tua Poo		490.0
Bai Tang On		948.0
Tua Kak	634.0	
Ma Kuea Tes	832.5	938.6
Ma Kuea Khao		740.0

CROPS/FRUITS/VEGETABLES	IODINE (μg/kg) wet	dry
Pak Chee Farang		400.0
Prik Yak		398.8
Tua Lan Tao	312.4	399.6
Ma Toom	1191.0	1456.0
Look Plub	2850.0	
Ma Done	1319.0	1762.0
Ma Kampiak	821.5	
Look Takob, ripe	111.0	
Himapan Seed	1006.5	
Kluay Hom	790.0	868.5
Kluay Hak Mook	1034.0	
Kluay Kai	764.0	
Kluay Nam Wah, ripe	211.0	
Mala-Koh, ripe	536.0	
Rice		
Polished rice (Bht2.25/L)		68.0
(Bht2.05/L)		61.2
Fragmentary rice (Bht1.35/L)		91.7
(Bht1.85/L)		70.0
(Bht1.20/L)		60.2

Source: Wanaratana, 1992.

APPENDIX 2: THE DETERMINATION OF URINARY CREATININE.

Appendix 2.1: Reagents.

(a) Picric acid (1.0 g %).

Ten grams reagent grade picric acid (50 % by weight of water has been added to ensure safety in transmit), made up to 500 ml with distilled water. Solution can be aided by heat. It is stable at room temperature but should be protected from sunlight.

(b) Sodium hydroxide (0.6 M).

Twelve grams of sodium hydroxide, make up to 500 ml with distilled water, store in a tightly closed polyethylene bottle.

(c) Oxalic acid, saturated solution.

Add about 90.0 grams oxalic acid to 500 ml water and shake until saturated.

(d) Creatinine standards.

Stock standard:

Dissolve 100 mg of pure creatinine and 0.8 ml of concentrated HCl in water, to make 1.0 dl. This solution contains 1000 μg/ 1.0 ml and is stable when kept in refrigerator.

Working standards

Pipette 1.0, 1.5, 2.0, 2.5, 3.0 and 4.0 of stock standard into 10 ml-volumetric flask then make to volume with distilled water. They contain 100, 150, 200, 250, 300 and

400 μg creatinine per 1.0 ml. These solution are not stable and should be prepared as needed.

Appendix 2.2: Apparatus.

1. Glass tube: 1 cm x 10 cm, Pyrex
2. Pipette: 5 ml, 10 ml
3. Cylindrical: 100 ml, 500 ml
4. Beaker: 50 ml, 100 ml, 250 ml, 500 ml
5. Volumetric flask: 10 ml, 100 ml, 500 ml
6. Autopipette: Gilson (0.0 -0.2 ml)
7. Hot plate with magnetic stirrer
8. Mixer: Gallenkamp, Spinmix, Ser. No. 60294
9. Centrifuge:Sorvall RT 6000 Refrigerated centrifuge
10. Spectrophotometer: LKB BIOCHROM, ULTROSPEC II + AUTOFILL II.

Appendix 2.3: Procedure.

(1) Add approximately 100 mg Lloyd's reagent to each tube.
(2) Set up in test tubes:
Blank: 100 μl distilled water+3.0 ml distilled water.
Stds: 100 μl working standards+3.0 ml distilled water.
Unknown: 20 μl (or 25, 50, 100 μl) of urine + 3.0 ml distilled water.
(3) Add 200 μl saturated aqueous oxalic acid to each tube.
(4) Mix 1 minute, stand for 10 minutes, centrifuge with speed 3000 rpm for 10 to 15 minutes.
(5) Discard supernatant and drain 2 hours.
(6) Add to each tube 3.0 ml distilled water and 1.0 ml alkaline picrate (freshly made equal volumes of picric acid solution and 0.6 M NaOH).
(7) Mix 1 minute and leave 20 minutes, mix again.
(8) Centrifuge with speed 3000 rpm 10 to 15 minutes.
(9) Read supernatant at 520 nm against reagent blank.

(10) Prepare a graph, showing creatinine concentration against absorbance (see **Figure II.1**).

(11) Calculation:

$$\mu g \text{ creatinine/tube} = \frac{OD\ (520\ nm)}{Slope}\ ;\ Slope = \frac{Y}{X}$$

$$g \text{ creatinine/100 ml urine} = \frac{(\mu g \text{ creatinine/tube})}{10{,}000 \times \text{assay urine (ml)}}$$

Figure II.1: Calibration graph of creatinine (10-40 μg/tube) which was used for the calculation of creatinine concentration in urine sample

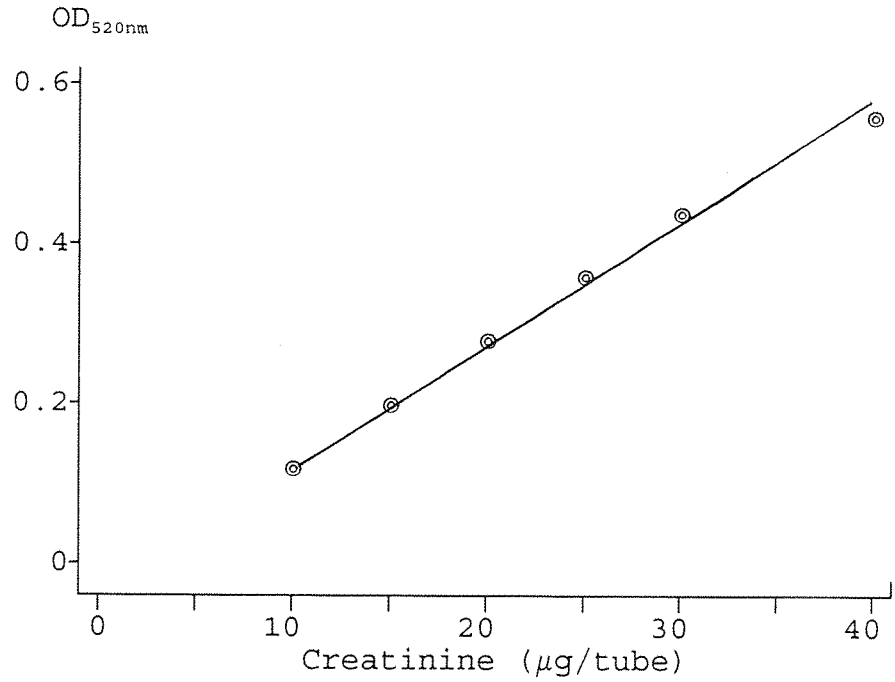


Table II.1: Recovery of added creatinine from urine

Creatinine added (μg/tube)	Creatinine found (μg/tube)	Creatinine recovered (%)
0	15.63	-
10	26.24	106.1
10	26.39	107.6
10	25.46	98.3
10	25.18	95.5

APPENDIX 3: THE DETERMINATION OF URINARY IODINE (using brucine sulphate to stop the reaction between Ce(4+)/AS(3+).

Appendix 3.1: Reagents.

All chemicals are analytical grade.

(a) Deionised water

Deionised water is obtained by passage of distilled water through an ion exchange cartridge.

(b) Chloric acid with chromate

A 2-litre flask equipped with a stirrer on a hot plate is partially filled with 250 g. of potassium chlorate, 100 mg. of sodium chromate and 500 ml of deionized water. The solution is constantly stirred and heated to a boiling temperature, until all of them are dissolved. Then, with heating discontinued, 185 ml of 72 % perchloric acid is slowly added. The addition of perchloric acid to the suspension is followed by the precipitation of potassium perchlorate from the mixture. Cool and place the covered beaker in the freezing compartment of a refrigerator for over night. Decant the supernatant liquid through a Whatman filter paper No. 541. The filtrate of chloric acid with chromate is stored in brown bottles in the cold for use for a 2-3 week period.

(c) Arsenious acid with sodium chloride

Six grams of arsenious oxide and four grams of sodium hydroxide are dissolved in 200 ml of deionized water. The mixture is warmed until a clear solution formed. The pH is adjusted to approximately seven with 10 % (v/v) sulphuric

acid and phenolphthalein is used as an indicator. Then 50 ml of 50 % (v/v) sulphuric acid are added. After that, 90.0 grams of sodium chloride are dissolved in the solution. A clear solution is obtained when the content is rinsed into 500-ml volumetric flask and dilute to volume. Working solution prepared fresh for each analysis by 1 in 10 dilution of stock with deionized water.

(d) Ceric sulphate solution

Dissolved 28.86 grams of ceric sulphate tetra hydrate into 10 % (v/v) sulphuric acid and then diluted further with 10 % (v/v) sulphuric acid to 500 ml.

(e) Brucine sulphate solution

Brucine sulphate (sigma B0378) 2.5 grams. is dissolved and diluted with 5 % (v/v) sulphuric acid to 500 ml.

(f) Standards:

Stock standard solution

Weigh out 168.6 mg of potassium iodate, dissolve and make to volume 100 ml with deionised water. It represents 1000 μg of iodine per one ml of solution. The solution should be preserved in brown bottle.

Intermediate standard solution

Dilute 0.1 ml of stock standard solution to 100 ml with deionised water. It represents 1.0 μg of iodine per 1.0 ml of solution.

Working standard solution

Dilute six ml of intermediate standard solution to 100 ml

with deionised water. The concentration of this solution will be 0.06 μg per 1.0 ml of solution.

Appexdix 3.2: Apparatus.

1. Glassware:
   - -Test tube (1.5 cm x 10 cm)
   - -Pipette (1 ml, 2 ml, 5 ml and 10 ml)
   - -Stirrer rod
   - -Beaker: 50 ml, 100 ml, 250 ml, 500 ml and 1000 ml
   - -Volumetric flask: 100 ml, 250 ml, 500 ml and 1000 ml
   - -Brown bottle: 100 ml and 500 ml
2. Auto pipette: Gilson, 0 - 0.2 ml
3. Hot plate with aluminum block: 4 blocks, 18 holes per block
4. Waterbath: thermostatically controlled
5. Spectrophotometer: Cescil
6. Thermometer: 100° C and 500° C
7. Mixer: Vortex.

Appendix 3.3: Procedure.

The method can be divided into two steps: 1) the digestion of organic matter (e.g protein) and oxidation of iodine to iodate; 2) colorimetric determination.

In brief, chloric acid in the presence of sodium chromate digest organic matter and also oxidise iodine in urine to iodate. Then the solution is evaporated to a small volume for removal of acid and organic matter, dilution of the residue, and colorimetric estimation of iodine by catalytic action on the ceric-arsenic system. The colour reduction is terminated by adding 0.5 per cent brucine sulphate in

five per cent sulphuric acid and then its absorbance was measured at 420 nm. Finally the results are compared with a standard iodine curve developed from the stock standard potassium iodate solutions.

(1) Preparation of blank, working standards and unknown in test tubes:

TUBES	Deionised H_2O (ml)	Working Std (ml)	Urine (ml)	I_2 (μg/tube)
Blk	0.40	0.00	-	0.000
Std1	0.35	0.05	-	0.003
Std2	0.30	0.10	-	0.006
Std3	0.20	0.20	-	0.012
Std4	0.10	0.30	-	0.018
Std5	0.00	0.40	-	0.024
Unk *	0.35	-	0.05	?

(* The volume of urine can be varied).

(2) Add chloric acid with chromate 1.0 ml to each tube, mix.
(3) Digest on hot plate with Dri-block (120°C-130°C) until remain approximately 0.10 ml (about 1.5-2.0 hours).
(4) Cool. At this step the liquid becomes colourless and deposits red crystals of chromium trioxide.
(5) Make volume to 2.0 ml with deionized water.
(6) Add 3.0 ml As^{3+} solution (prepare fresh for each analysis by 1 in 10 dilution of stock arsenious acid solution with deionized water) to the cooled tubes and mix until crystals dissolve.
(7) Place the tubes in a constant temperature water bath (32°C), and allow the contents to stabilize for 10 to 15 minutes.

(8) Add 0.25 ml of the Ce^{4+} solution into each tube at 30 seconds interval, mix 10 seconds and place it into water bath again.

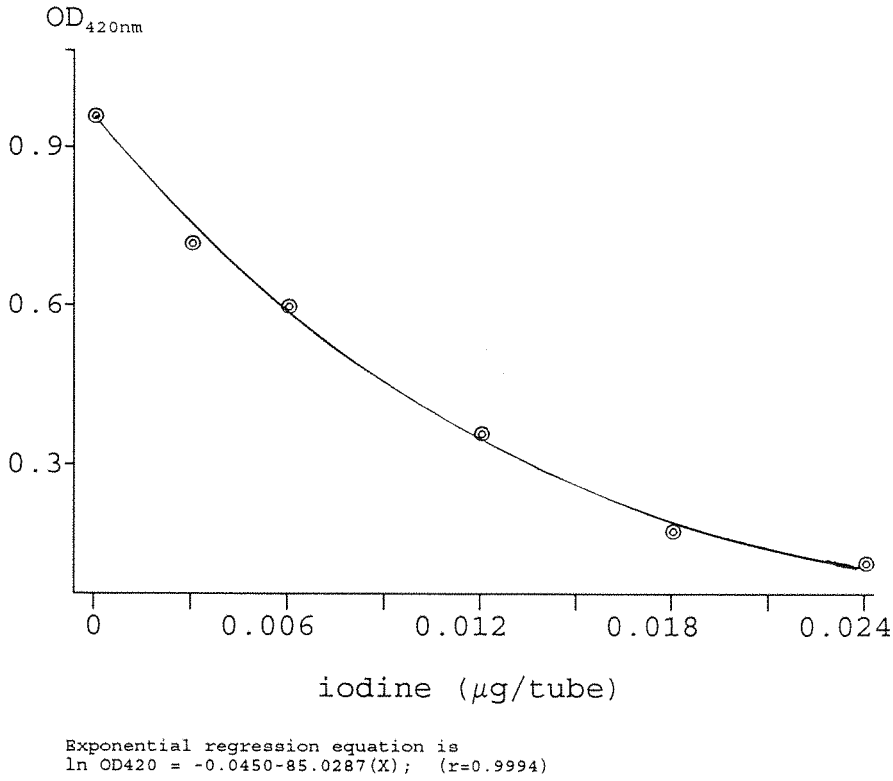
(9) After a certain time, equal to all tubes (36 minutes in practice), the colour reduction will be terminated by adding 0.25 ml brucine sulphate, mix.

(10) Remove the tubes from the water bath and allow to attain room temperature.

(11) Colorimeter readings at 420 nm are normally taken 2 hours after brucine addition.

(12) Plot a curve of iodine content against extinction both for the standards and blank. A fresh curve should be constructed for each batch of sample analyzed (see **Figure III.1**).

Figure III.1: The relationship between iodine concentrations and their absorbance at 420 nm, using brucine sulphate to stop the reaction.



(13) Calculation: The relationship between OD at 420 nm (colour intensity of Brucine-cer-complex and concentrations of iodine standard (μg/tube) will be an exponential regression (see **Figure II.1**). Therefore the concentration of iodine in urine sample can be calculated from the equation,

$$\ln OD420\ nm = \ln A + B(X)$$

where: OD 420 is the absorbance of unknown
X is the concentration of iodine in urine sample.

The table below shows an example of how to calculate the concentration of iodine in urine sample. Blank, standards and unknown were done in duplicate.

TUBES	I_2 (μg/tube)	OD420 nm (1)	OD420 nm (2)	OD420 nm average
Blk	0.000	0.951	0.961	0.956
Std1	0.003	0.729	0.713	0.721
Std2	0.006	0.572	0.578	0.574
Std3	0.012	0.354	0.370	0.362
Std4	0.018	0.212	0.200	0.206
Std5	0.024	0.116	0.128	0.122
Unk (0.1ml)	?	0.411	0.401	0.406

The relation between the concentrations of the iodine standard and their OD 420 nm gives the equation;

$$\ln OD420 = -0.0450-85.0287(X);\ (r=-0.9994).$$

Therefore, the concentration of iodine in urine sample (X) can be calculated by:

$$\ln 0.406 = -0.0450-85.0287(X);\ X= 0.0101\ \mu g/tube.$$

Since 0.1 ml of urine was used, the concentration of iodine in urine = 10.10 μg per cent. In general, this calculation can be done easily by using a simple calculator.

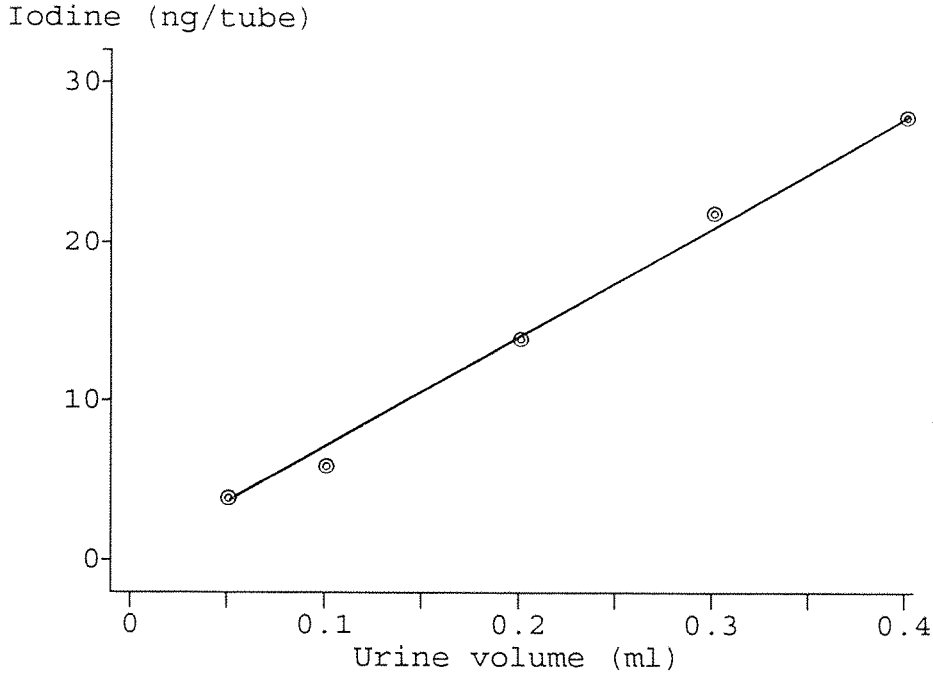
Table III.1: Recovery of added iodine from urine (with stopping the reaction between Ce^{4+}/As^{3+} by brucine sulphate)

	Iodine added (μg)	Iodine found (μg)	Iodine recovered (%)
(A)	0.000	0.0037	-
	0.006	0.0096	98.3
	0.009	0.0122	94.4
(B)	0.000	0.0074	-
	0.009	0.0168	104.4

Appendix 3.4: The effect of sample dilution.

The effect of sample dilution on the results of urinary iodine estimation was also studied by measuring the iodine concentrations in aliquots of 0.05, 0.10, 0.20, 0.30 and 0.40 ml from the same urine sample. A linear relation was found ($r = 0.9985$), see **Figure III.2**.

Figure III.2: Iodine concentrations in aliquots of 0.05, 0.10, 0.20, 0.30 and 0.40 ml from the same urine sample



In addition, the iodine content in 18 urine samples as analyzed in two laboratories (the Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand and the Research Institute for Health Sciences, Chiang Mai University, Chiang Mai, Thailand). The objective was to compare this method with Rama's method in which brucine sulphate was not used for stopping the reaction between Ce^{4+}/As^{3+}. It was found that there was no significant difference in urinary iodine between the laboratories, see **Table III.2**.

Table III.2: Comparison the results of iodine content in urine samples, (n = 18) given by RAMA (BKK) and those given by RIHES (CM) where the method, Brucine sulphate used

NUMBER	Iodine concentration	
	RAMA (BKK)	RIHES (CM)
1	1.04	1.14
2	1.53	1.85
3	2.32	2.37
4	3.36	3.09
5	5.24	5.05
6	7.72	9.63
7	10.31	10.33
8	12.07	12.13
9	15.62	15.03
10	14.70	16.50
11	15.20	17.00
12	17.36	17.16
13	20.22	17.33
14	20.71	18.93
15	24.59	25.65
16	29.67	29.29
17	28.65	30.40
18	55.09	50.63

Statistics: Paired test; Two tailed, p = 0.7904, n = 18

RAMA (BKK) = The Faculty of Medicine, Mahidol University, Ramathibodi hospital, Bangkok, Thailand.

RIHES (CM) = Research Institute for Health Sciences, Chiang Mai University Chiang Mai, Thailand

Iodine in urine may be determined without stopping the reaction Ce^{4+}/AS^{3+} but it needs an instrument which can

measure absorbance at specific times. The coefficient of variation (CV) of this procedure was 6.46 per cent (Mean±SD = 19.93±1.29 μg, n=20) and the range of percentage recovery of iodine from urine varied from 96 per cent to 106 per cent. However, for practical reasons, the stopping reaction method (brucine sulphated used) was selected because under field conditions in Thailand it would be easier to use.

APPENDIX 4: ESTIMATION OF 24-HOUR URINARY IODINE EXCRETION BY USING URINARY IODINE EXCRETION (UIE) FROM A CASUAL URINE SAMPLE.

Table IV.1: UIE (μg/g.creatinine)

Observed 24-hr UIE	Time (hrs)	UIE (μg/g.Cr)	Estimation UIE (μg/d)*	% Error
Day 1				
95.91	8 - 9	60.00	79.80	-16.7
(μg/d)	9 - 10	47.35	62.98	**-34.3**
or	10 - 11	51.23	68.13	-29.0
72.11	11 - 12	62.55	83.19	-13.3
(μg/g.Cr)	12 - 14	52.58	69.93	-27.1
	14 - 16	61.83	82.23	-14.3
	16 - 18	70.21	93.38	- 2.0
	18 - 20	108.12	143.80	**+49.9**
	20 - 22	60.77	80.82	-15.7
	22 - 24	84.52	112.41	+17.2
	24 - 6:30	89.56	119.11	+24.2
	6:30 - 8	57.89	76.99	-19.7
Day 8				
172.76	8 - 10	96.46	131.19	-24.1
(μg/d)	10 - 12	183.49	249.55	**+44.4**
or	12 - 14	96.28	130.94	-24.2
127.03	14 - 16	155.33	211.25	+22.3
(μg/g.Cr)	16 - 18	117.04	159.17	- 7.9
	18 - 20	125.44	170.60	- 1.2
	20 - 22	81.26	110.51	**-36.0**
	22 - 24	143.00	194.48	+12.6
	24 - 2	126.36	171.85	- 0.5
	2 - 4	112.11	152.47	-11.7
	4 - 6:30	151.28	205.74	+19.1
	6:30 - 8	122.47	166.56	- 3.6
Day 9				
168.14	8 - 10	139.55	188.39	+12.0
(μg/d)	10 - 12	158.51	213.99	+27.3
or	12 - 14	148.80	200.88	+19.5
124.55	14 - 16	81.50	110.20	-34.6
(μg/g.Cr)	16 - 18	110.38	149.01	-11.4
	18 - 20	111.92	151.09	-10.1
	20 - 22	167.14	225.64	+34.2
	22 - 24	70.20	94.77	**-43.6**
	24 - 2	110.00	148.50	-11.7
	2 - 4	117.12	158.11	- 6.0
	4 - 6:30	122.71	165.66	- 1.5
	6:30 - 8	194.10	258.18	**+53.6**

*Note
Estimation UIE (μg/d) of d1 = UIE (μg/g.Cr)x1.33 (g.Cr/d1)
Estimation UIE (μg/d) of d8 = UIE (μg/g.Cr)x1.36 (g.Cr/d8)
Estimation UIE (μg/d) of d9 = UIE (μg/g.Cr)x1.35 (g.Cr/d9)

Table IV.2: UIE (μg %)

Observed 24-hr UIE	Time (hrs)	Casual urine UIE (μg %)	% Error
Day 1			
10.37 (μg %)	8 - 9	20.50	+ 97.7
Total volume	9 - 10	13.67	+ 31.8
924 (ml/d)	10 - 11	9.74	- 6.1
	11 - 12	7.48	- 27.9
	12 - 14	8.53	- 17.7
	14 - 16	7.49	- 27.8
	16 - 18	7.07	- 31.8
	18 - 20	6.74	**- 35.0**
	20 - 22	12.74	+ 22.9
	22 - 24	23.29	+124.6
	24 - 6:30	12.19	+ 17.6
	6:30 - 8	26.04	**+151.1**
Day 8			
9.37 (μg %)	8 - 10	11.20	+ 19.5
Total volume	10 - 12	9.70	+ 3.5
1843 (ml/d)	12 - 14	5.20	- 44.5
	14 - 16	7.80	- 16.8
	16 - 18	13.60	+ 45.1
	18 - 20	13.60	+ 45.1
	20 - 22	5.20	- 44.5
	22 - 24	10.70	+ 14.2
	24 - 2	19.60	+109.2
	2 - 4	4.70	**- 49.8**
	4 - 6:30	12.90	+ 37.7
	6:30 - 8	26.80	**+186.0**
Day 9			
13.47 (μg %)	8 - 10	20.40	+ 51.5
Total volume	10 - 12	25.10	**+ 86.3**
1248 (ml/d)	12 - 14	20.00	+ 48.5
	14 - 16	18.70	+ 38.8
	16 - 18	18.40	+ 36.6
	18 - 20	13.70	+ 1.7
	20 - 22	10.70	- 20.6
	22 - 24	18.60	+ 38.1
	24 - 2	19.20	+ 42.5
	2 - 4	5.40	**- 59.9**
	4 - 6:30	8.20	- 39.1
	6:30 - 8	19.80	+ 47.0

APPENDIX 5: DETERMINATION OF IODINE CONTENT IN FOODS

Appendix 5.1: Reagents.:

All chemicals are analytical grade.

(a) Potasium carbonate solution (30 % m/v)

Dissolve 30 grams of potassium carbonate (K_2CO_3) in double distilled water (DD H_2O) and dilute to 100 ml.

(b) Zinc sulphate solution (10 % m/v)

Dissolve 10 grams of zinc sulphate ($ZnSO_4.7H_20$) in DD H_2O and dilute to 100 ml.

(c) Sodium chloride solution (20 % m/v)

Dissolve 20 grams of sodium chloride (NaCl) in DD H_2O and dilute to 100 ml.

(d) Potassium thiocyanate solution (0.023 % m/v)

Dissolve 0.23 grams of potassium thiocyanate (KCNS) in DD H_2O and dilute to 1,000 ml.

(e) Sodium nitrite solution (2.07 % m/v)

Dissolve 2.07 grams of sodium nitrite ($NaNO_2$) in DD H2O and dilute to 100 ml (stable for 1 day only).

(f) Ammonium iron (III) sulphate solution (7.7 % m/v)

Dissolve 77 grams of ammonium iron (III) sulphate [$NH_4Fe(SO_4)_2.12H_2O$] in approximately 400 ml of DD H_2O. Add 167 ml of concentrated nitric acid and warm until all

traces of the solid are dissolved. Cool the solution down to room temperature and dilute to 1000 mls with DD H_2O.

(g) Standard iodine solutions

Stock standard: concentration = 1,000 μg/ml

Dissolve 0.1683 gram of potassium iodate (KIO3) in DD H_2O and dilute the solution to 100 ml.

Intermediate standard: concentration = 200 ng/ml

Pipette stock standard (1,000 μg/ml) 0.10 ml, then dilute to 500 ml with DD H_2O and keep in the brown bottle. This solution is stable for 1 month in the refrigerator.

Working standard

Pipette 10, 8, 6, 4, 2, 0 ml of intermediate standard solution (200 ng/ml) into a series of 100 ml volumetric flask. To each volumetric flask add 1 ml of 30 % m/v potassium carbonate solution, then dilute to 100 mls with DD H_2O. Store the working standards in glass bottles away from light and prepare them in freshly at weekly intervals.

Appendix 5.2: Apparatus.

(1) Muffle furnace with thermostat:
Thermolyne type 1500, Fisher scientific Co.Ltd
(2) 10 mls glass beaker (Pyrex or Kimax brand).
(3) Glass slides (2.6x2.6 cm^2).
(4) Stainless rack.
(5) Centrifuge.
(6) Plastic centrifuge tubes of 50 mls capacity.
(7) Vortex mixer (Vortex-Genie).
(8) Hot plate

(9) Oven
(10) Incubator
(11) Stop watch
(12) Spectrophotometer.

Appendix 5.3: Procedure.

All the glasswares and plastic centrifuge tubes are soaked in 50 per cent HNO_3 overnight, then rinsed several times with DD H_2O before using. The water is demineralized distilled H_2O and all chemicals are analytical grade.

(1) Accurately weigh 0.1-0.3 g of lyophilized food samples into the glass beakers. The mass of used food samples depend on the content of iodine. The weight of milk powder used as a control is approximately 0.1 g.
(2) Add 1 ml of 30 per cent m/v potassium carbonate solution and then add 1 ml of zinc sulphate solution and make three beakers of reagent blanks.
(3) Slurry the mixture with a glass rod and wash any residue left on the rod back into the beaker with a jet of DD H_2O.
(4) Place the beakers in the stainless rack and place the rack in the oven at 95 ± 5°C over night.
(5) Cool the beakers down to room temperature and cover each beaker with the glass slides.
(6) After cooling down, place the beakers in the stainless rack and put in a muffle furnace at about 50°C. Then raise the temperature to 550°C in approximately 90 minutes and maintain this temperature for 1 hour.
(7) Remove the rack immediately and allow it to cool to room temperature.
(8) Add 1 ml of 10 per cent zinc sulphate solution and repeat steps (3), (4), (6) and (7).

(9) Transfer the cooled ash, normally white or grey in colour, to a centrifuge tube with 15 to 20 ml of DD H_2O, and spin the tubes at 2500 rpm for 5 minutes. Pipette out the supernatant and keep in the clean polyethylene tubes.

(10) Pipette 1 ml of each standard, sample, reagent blanks and control solution into the 17x100 mm test tubes. The volume of the solution can be varied to the maximum of 1.85 mls depending on the content of the iodine in the sample.

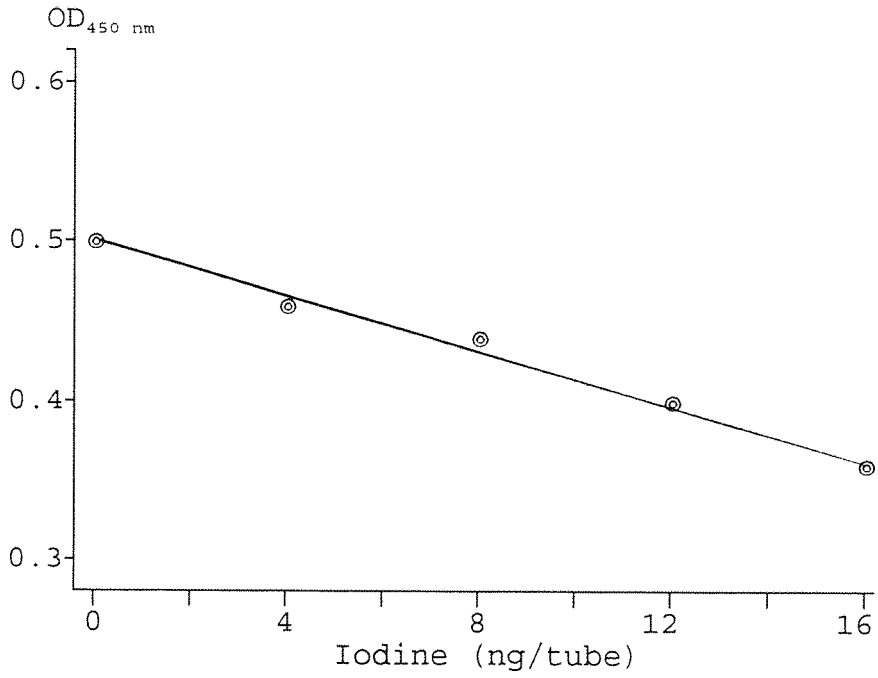
(11) To each tube add 0.85 ml of DD H_2O, 0.25 ml of 20 per cent m/v NaCl and 0.25 ml of 0.023 per cent m/v KCNS, mix well on a vortex mixer.

(12) One set consists of 20 tubes of a series of standard, reagent blank, control and sample solution, 0.5 ml ammonium iron (III) sulphate reagent is added into each tube. The total volume is 2.85 ml. Mix well on a vortex mixer then place in the incubator at 32°C for 15 minutes.

(13) At exactly 60 second intervals add 0.25 ml of 2.07 per cent m/v $NaNO_2$ solution and mix on a vortex mixer. After 20 minutes, measure the colour at a wavelength of 450 nm against DD H_2O as blank. It is essential that all solutions should be maintained at the same temperature.

(14) Calculation: By using a range of standards, prepare a calibration graph. From the absorbance values of the sample and blank solutions, determine the iodine content of the solutions, in ng/tube from the calibration graph (see **Figure V.1**).

Figure V.1: Calibration graph of iodine determination for calculation iodine content in foods



Calculate the iodine content of the sample in μg per 100 g dry weight by using the following equation:

$$\text{Iodine content } (\mu g/100\ g \text{ dry Wt.}) = \frac{[(AV/C)-B]X100}{W\ X\ 1000}$$

Where

A = the iodine content of the sample solution (ng/tube).

B = the iodine content of the reagent blank solution (ng/beaker).

C = the volume of sample solution used to form colour (ml).

V = the total volume of DD H_2O which is used in transferring the ash into the centrifuge tube (ml).

W = the amount of sample (g).

AV= is the iodine content of the sample solution (ng/beaker).

(15) If M is iodine content in μg/100 g dry weight. TD is the total dry weight of the sample in grams. TW is the total wet weight of the sample in grams. Iodine content in μg/100 g wet weight can be calculated using the following equation:

$$\text{Iodine content } (\mu g/100\ g \text{ wet Wt.}) = \frac{M \times TD \times 100}{TW \times 100}$$

Table V.1: Recovery of added iodine from milk sample

Iodine added (μg)	Iodine found (μg)	Iodine recovered (%)
0.00	128.20	-
81.53	205.43	94.7
81.53	213.13	104.4
81.53	210.56	101.0

APPENDIX 6: COMPARISON OF VARIABLES BETWEEN CENTRE 1 AND CENTRE 2

Table VI: Comparison of variables between centres 1&2

Variables	C1 > C2	C2 > C1
Education		
Occupation	●	
Total income		●
Energy intake#	●	
Protein intake#	●	
Fat intake#	●	
Carbohydrate intake#	●	
Sticky rice (FFQ)#	●	
Rice (FFQ)#		
Pork (FFQ)#	●	
Chicken, duck (FFQ)#	●	
Beef, Buffalo (FFQ)#	●	
EGG (FFQ)#	●	
Fresh milk (FFQ)#	●	
Sea foods (FFQ)#		
Fermented fish, Kapi (FFQ)#	●	
Fruits#	●	
Vegetables#		
Age *	●	
Height *		
Weight *		
Mid arm circumference *		●
Triceps skinfolds *		
Blood pressure Systolic *		
Blood pressure Diastolic *		
Gestational at birth		

Variables	C1 > C2	C2 > C1
Gestational at birth (PT, T)		
Gestational age at first interview of nutrient intake and FFQ		
Gestational at first urine collection		●
Urinary iodine excretion (UIE)		●
I_2 supplement		●
Total I_2 supplement		●
Birthweight		
Head Circumference	●	
Length		●
RHL %	●	
Chest circumference		

```
#      = Nutrient intake and food frequency at first interview
*      = Maternal status at admission
●      = Oneway Analysis of Variance; significant difference at P < 0.05
Blank  = Non significant difference.

FFQ    = Food frequency
PT     = Preterm
T      = Term
UIE    = urinary iodine excretion at first collection
         (µg/g.creatinine; log form)
I2 supplement
       = the length of time (days) of supplementation from
         admission until first collection of urine samples
Total I2 supplement
       = the length of time (days) of supplementation from
         admission until delivery.
RHL %  = (Head circumference/length)*100
```

Table VI.1: Comparison of the length of education (%) in Thai pregnant volunteers between centre 1 and centre 2.

VARIABLE	CENTRE 1	CENTRE 2
Length of education (yrs)		
≤ 6 yrs	74.1 %	73.0 %
> 6-12	15.7 %	18.9 %
> 12 yrs	10.2 %	8.1 %
n	166	148

(Likelihood ratio = 0.3840)

Table VI.2: Comparison of occupation in Thai pregnant volunteers between centre 1 and centre 2.

VARIABLE	CENTRE 1	CENTRE 2
Occupation		
Agriculture	27.7 %	10.8 %
Not agriculture	72.3 %	89.2 %
n	166	148

(Likelihood ratio = 0.0003)

Table VI.3: Comparison of total income (log form) in Thai pregnant volunteers between centre 1 and centre 2.

CENTRE	n	TOTAL INCOME		F ratio (P-value) *
		Mean (SD)	95 % CI	
Centre 1	165	3.47^{a} (0.30)	3.22-3.52	4.1236 (0.0431)
Centre 2	148	3.54^{b} (0.27)	3.49-3.56	

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

Groups with different superscript are significantly different (Least significance difference test; $P < 0.05$).

Table VI.4: Comparison of energy, protein, fat and carbohydrate (CHO) intakes and gestational age (GA) at first interview of Thai pregnant volunteers between centre 1 (C1) and centre 2 (C2).

NUTRIENT INTAKE	n	Mean (SD)	95 % CI	F ratio * (P-value)
ENERGY (kcal/d)	188 (C1) 156 (C2)	2816[a] (779) 2575[b] (831)	2704-2928 2444-2707	7.6948 (0.0060)
PROTEIN (g/d)	188 (C1) 156 (C2)	83.1[a] (27.9) 73.8[b] (25.1)	79.1-87.1 69.8-77.7	10.3960 (0.0014)
FAT (g/d)	188 (C1) 156 (C2)	60.1[a] (30.7) 52.2[b] (29.2)	55.7-64.5 47.5-56.8	5.9554 (0.0152)
CHO. (g/d)	188 (C1) 156 (C2)	489 (143) 457 (165)	468-509 430-483	3.7319 (0.0542)
GA. (wks)	188 (C1) 156 (C2)	19.0 (5.9) 18.7 (7.0)	18.2-19.9 17.6-19.8	0.1912 (0.6622)

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

For each variable separately, groups with different superscript are significantly different (Least significance difference test; $P < 0.05$).

Table VI.5: Comparison of food frequency of sticky rice, rice, pork, chicken and duck, beef and buffalo, egg, fresh milk, sea foods, fermented fish and kapi, fruits and vegetables which eaten by Thai pregnant volunteers between centre 1 and centre 2 at first interview.

VARIABLE	CENTRE 1	CENTRE 2	LR
STICKY RICE			
every day	86.8 %	81.4 %	0.0070
1-2-4 T/wk	10.5 %	8.1 %	
1-2 T/mo	2.6 %	10.6 %	
RICE			
every day	25.8 %	30.4 %	0.0794
1-2-4 T/wk	51.6 %	39.8 %	
1-2 T/mo	22.6 %	29.8 %	

VARIABLE	CENTRE 1	CENTRE 2	LR
PORK			
every day	90.5 %	80.7 %	0.0074
1-2-4 T/wk	9.5 %	17.4 %	
1-2 T/mo	0 %	1.9 %	
CHICKEN, DUCK			
every day	62.6 %	8.7 %	0.0000
1-2-4 T/wk	29.5 %	80.7 %	
1-2 T/mo	7.9 %	10.6 %	
BEEF, BUFFALO			
every day	57.9 %	10.6 %	0.0000
1-2-4 T/wk	31.6 %	70.2 %	
1-2 T/mo	10.5 %	19.3 %	
EGG			
every day	50.0 %	32.9 %	0.0042
1-2-4 T/wk	47.9 %	62.7 %	
1-2 T/mo	2.1 %	4.3 %	
FRESH MILK			
every day	46.3 %	43.5 %	0.2683
1-2-4 T/wk	32.1 %	39.8 %	
1-2 T/mo	21.6 %	16.8 %	
SEA FOODS			
every day	3.7 %	2.5 %	0.2564
1-2-4 T/wk	80.5 %	75.2 %	
1-2 T/mo	15.8 %	22.4	
FERMENTED FISH, KAPI			
every day	94.2 %	83.2 %	0.0036
1-2-4 T/wk	5.3 %	14.3 %	
1-2 T/mo	0.5 %	2.5 %	
FRUITS			
every day	84.7 %	72.7 %	0.0055
1-2-4 T/wk	15.3 %	27.3 %	
1-2 T/mo	0 %	0 %	
VEGETABLES			
every day	96.3 %	96.9 %	0.3736
1-2-4 T/wk	3.7 %	2.5 %	
1-2 T/mo	0 %	0.6 %	
n	190	161	-

(LR = likelihood ratio)

T/wk = times/wk, T/mo = times/month.

KAPI = a kind of Thai food made from fish, prawn, chile and onion.

Table VI.6: Comparison of age, height (Ht), weight (Wt), mid arm circumference (MAC), triceps skin fold (TSF), blood pressure systolic and diastolic (BPS, BPD) of Thai pregnant volunteers between centre 1 (C1) and centre 2 (C2) **at the first admission.**

VAR	n	Mean (SD)	95 % CI	F ratio * (P-value)
AGE (yrs)	192 (C1) 169 (C2)	26.1[a] (4.5) 24.2[b] (4.2)	25.4-26.7 23.6-24.8	16.6797 (0.0001)
Ht (cms)	192 (C1) 169 (C2)	152.2 (5.2) 151.6 (5.1)	151.4-152.9 150.8-152.4	1.1807 (0.2779)
Wt (kgs)	192 (C1) 169 (C2)	49.8 (5.9) 49.2 (8.7)	49.0-50.7 47.8-50.5	0.7889 (0.3750)
MAC (cms)	192 (C1) 168 (C2)	24.1[a] (2.3) 24.6[b] (2.8)	23.7-24.4 24.2-25.1	4.3899 (0.0369)
TSF (mms)	192 (C1) 168 (C2)	19.0 (5.1) 19.0 (6.3)	18.3-19.7 18.2-20.1	0.0794 (0.7782)
BPS (mmHg)	188 (C1) 169 (C2)	113.3 (8.8) 112.9 (9.9)	112.1-114.7 111.4-144.4	0.2589 (0.6112)
BPD (mmHg)	188 (C1) 169 (C2)	72.2 (6.9) 72.7 (7.3)	71.2-73.2 71.6-73.8	0.3268 (0.5679)

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

For each variable separately, groups with different superscript are significantly different (Least significance difference test; P < 0.05).

Ht = Height
Wt = Weight
MAC = Mid arm circumference
TSF = Triceps skinfold
BPS = Blood pressure (systolic)
BPD = Blood pressure (diastolic)

Table VI.7: Comparison of gestational age at birth (GABI; wks) of infants between centres 1 and 2

CENTRES	n	GABI	95 % CI	F ratio* (P-value)
		Mean (SD)		
Centre 1	192	39.1 (1.7)	38.8-39.3	0.9836 (0.3220)
Centre 2	169	38.9 (1.8)	38.6-39.2	

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

Table VI.8: Comparison of the percentage of preterm (PT) and term (T) babies between centres 1 and 2

CENTRES	n	PRETERM	TERM
Centre 1	192	6.8 %	93.2 %
Centre 2	169	6.5 %	93.5 %

(Likelihood ratio = 0.9205)

Table VI.9: comparison of gestational age of Thai pregnant women at first interview of nutrient intake and food frequency (Gafooda; wks) between centres 1 & 2

CENTRES	n	Gafooda	95 % CI	F ratio* (P-value)
		Mean (SD)		
Centre 1	188	19.0 (5.9)	18.2-19.9	0.1912 (0.6622)
Centre 2	156	18.7 (7.0)	17.6-19.8	

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

Table VI.10: comparison of gestational age of Thai pregnant women at first urine collection (GAI1; wks) between centre 1 and centre 2

CENTRES	n	GAI1	95 % CI	F ratio* (P-value)
		Mean (SD)		
Centre 1	192	17.0[a] (4.7)	16.4-17.7	17.2063 (0.0000)
Centre 2	169	19.6[b] (7.2)	18.6-20.8	

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

Groups with different superscript are significantly different (Least significance difference test; P < 0.05).

Table VI.11: Comparison of urinary iodine excretion (UIE, μg/g.creatinine: log form) from casual urine samples of Thai pregnant women at first urine collection between centres 1 and 2

CENTRES	n	UIE	95 % CI	F ratio* (P-value)
		Mean (SD)		
Centre 1	192	1.83[a] (0.34)	1.78-1.88	7.2611 (0.0074)
Centre 2	169	1.92[b] (0.28)	1.88-1.97	

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

Groups with different superscript are significantly different (Least significance difference test; P < 0.05).

Table V.12: Compare the number of pregnant subjects (%) among 3 groups of the length of time of iodine supplement from first admission until the urine sample were collected at the first collection between centre 1 and centre 2

CENTRES	IODINE SUPPLEMENTATION (days)			n
	0	1-30	≥ 31	
Centre 1	44.8 %	22.9 %	32.3 %	192
Centre 2	34.9 %	10.7 %	54.4 %	169

(Likelihood ratio = 0.0000)

Table VI.13: Compare the number of pregnant subjects (%) among 3 groups of the length of time of total iodine supplement from the first admission until birth between centre 1 and centre 2.

CENTRES	TOTAL IODINE SUPPLEMENTATION (days)			n
	≤ 168	169-199	≥ 200	
Centre 1	34.4 %	42.7 %	22.9 %	192
Centre 2	33.1 %	23.7 %	43.2 %	169

(Likelihood ratio = 0.0000)

Table VI.14: Stratified birth outcome by centres.

BIRTH OUTCOME	n	Mean (SD)	95 % CI	F ratio (P-value)*
BWT (gms)	192 (C1) 169 (C2)	3045 (390) 2963 (415)	2989-3100 2900-3025	3.7238 (0.0544)
HC (cms)	191 (C1) 162 (C2)	33.4[a] (1.4) 33.0[b] (1.2)	33.2-33.6 32.8-33.2	7.7031 (0.0058)
LENGTH (cms)	190 (C1) 159 (C2)	48.6[a] (1.7) 50.4[b] (2.1)	48.3-48.9 50.1-50.7	76.2762 (0.0000)
RHL (%)	191 (C1) 162 (C2)	68.7[a] (2.7) 65.4[b] (2.5)	68.3-69.1 65.1-65.8	134.1543 (0.0000)
CC (cms)	191 (C1) 162 (C2)	31.6 (1.8) 31.4 (1.7)	31.4-31.9 31.1-31.7	1.6229 (0.2035)

95 % CI = 95 % confidence interval
* Oneway Analysis of Variance

For each variable separately, groups with different superscript are significantly different.

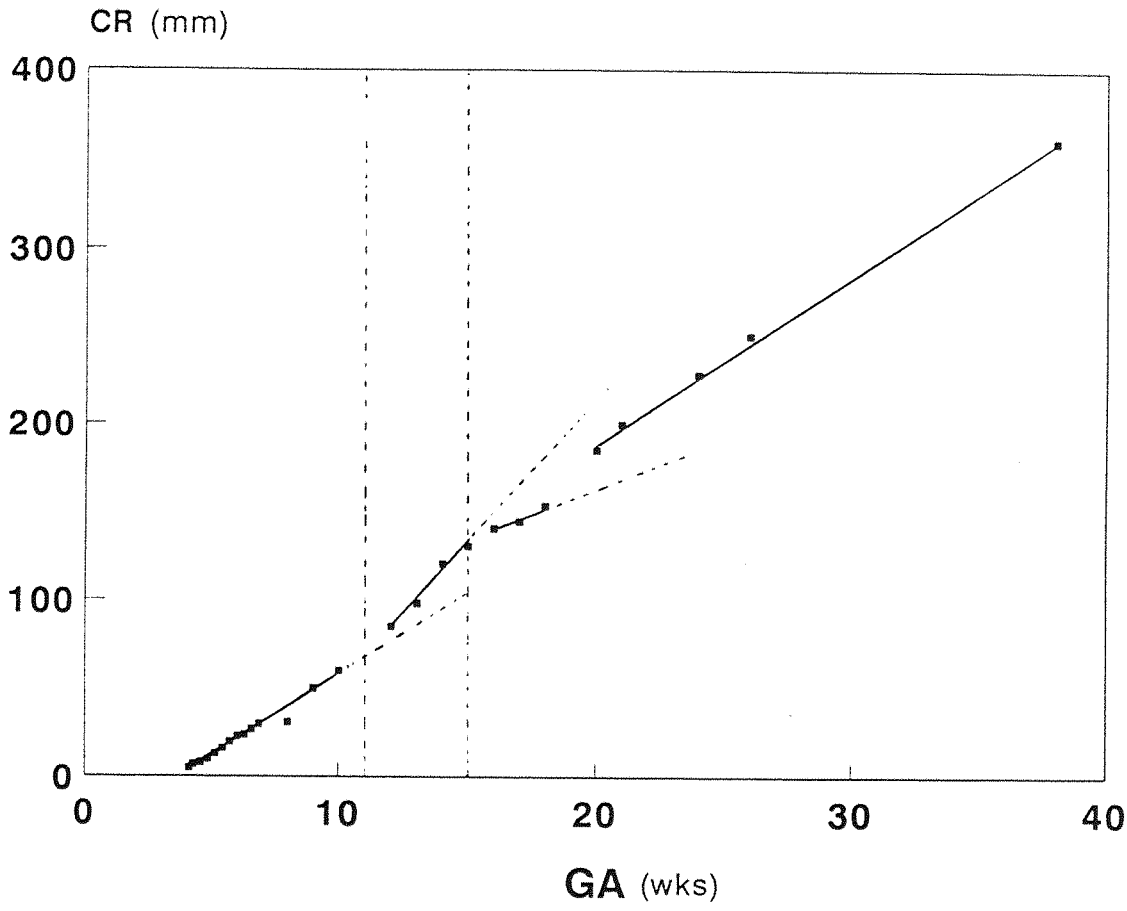
(C1) = Maharaj Nakorn Chiang Mai hospital (centre 1)
(C2) = Maternal Child Health Centre (centre 2)

BWT = Birthweight
HC = Head circumference
RHL % = (Head circumference/length)*100
CC = Chest circumference

APPENDIX 7: SUMMARY OF QUESTIONNAIRE FORM

No	FORM	LIST OF VARIABLES
1	ADMISSION FORM	(1) Socio demographic data of pregnancy (2) Past medical history (3) Habit (e.g, smoking, medicine) (4) Past and present obstetric history (5) Family planning (6) Anthropometric measurement (7) Clinical examination
2	FOLLOW-UP FORM	(1) Anthropometric measurement (2) Clinical examination (3) Habit (e.g, smoking, medicine)
3	DELIVERY FORM	(1) Information of pregnant subjects at labour (2) Delivery record
4	INFANT FORM	(1) Birth outcome (birthweight, length, head and chest circumference, gender) (2) Gestational age of newborn
5	24 HOUR RECALL FORM	(1) Gestational age at each interview (2) Quantity of daily of food intake
6	FOOD FREQUENCY FORM	(1) Gestational age at each interview (2) Frequency of food intake every day 1-2-4 times/week 1-2 times/month
7	END OF STUDY FORM	(1) Frequency of follow-up (2) Complete or incomplete of studying (3) Date at the end of study

APPENDIX 8: TREND IN FAETAL LENGTH DURING PREGNANCY



Source: Adapted from England, 1990.

The figure above shows the growth of faetal length during pregnancy (Crown-rump: CR, mm) by gestational age (GA, wks). This figure was plotted by using the data from England (1990). There is no information about the sample size of measuring crown-rump in each week of gestational age in this data set. Thus we can show neither the standard deviation nor standard error in each plot. However, this graph, at least shows the trend that there is the most rapid growth of faetal length during the 11th to 15th of gestational age.

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