IODINE DEFICIENCY

IN THE

NORTHERN PUNJAB OF PAKISTAN

by
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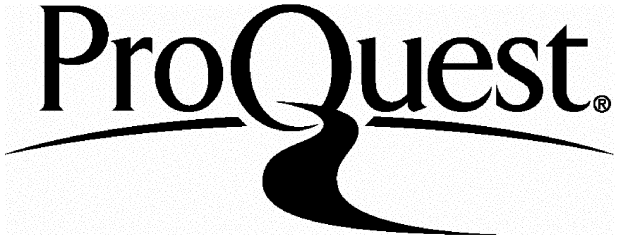

ABSTRACT

A study was conducted in a remote, mountainous area of the northern Punjab of Pakistan, where women's access to health care is particularly difficult. Against a background of iodine deficiency, supplementation camps were held in 16 villages and almost 1500 women were given oral iodised poppy seed oil. Baseline blood samples, taken at the camps, revealed a high degree of biochemical hypothyroidism, with low serum thyroxine (T_4) and/or high serum thyrotropin (TSH), although serum triiodothyronine (T_3) was generally maintained at normal levels.

The changes in thyroid hormone levels, usually associated with pregnancy, were modified in this population, as pregnancy exerted a further stress on the thyroid gland, already functioning under conditions of iodine deficiency. Iodine deficiency poses a particular threat to the developing foetus and interviews with women indicated high rates of foetal wastage and infant mortality, although it was not possible to establish a causal link with iodine deficiency or thyroid hormone aberrations.

Following supplementation, blood-sampling of non-pregnant women, attending follow-up clinics, indicated that serum T_4 levels had increased while serum TSH levels decreased, with little effect on serum T_3 levels. The proportion of women experiencing biochemical hypothyroidism thus decreased. Although some women experienced short-term biochemical hyperthyroidism, there was no clinical evidence of thyrotoxicosis.

This study highlights the urgent need for promotion and marketing of iodised salt in this area, with particular emphasis on influencing the male household heads and community leaders. In addition, it is suggested that baseline assessment of iodine deficiency and monitoring of control programmes, including the introduction of iodised salt, is best done through measurement of a combination of T_4 and TSH.

For the Women of Pakistan

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CHAPTER 1 - INTRODUCTION AND LITERATURE REVIEW

1.1. The Biology and Physiological Function of Iodine

Approximately 15-20 mg of iodine is present in the human body, 75% of it in the thyroid gland, and 100-150 μg is required daily for normal physiological function (Hetzel, 1989; Orr and Leitch, 1929).

1.1.1. Iodide Uptake

Dietary iodide (chemically bound iodine) is absorbed through the gut and about a third is actively taken up by the thyroid gland of the neck, which can trap about 60 μg of iodine per day, through a very efficient mechanism which maintains a gradient of 100:1 between the thyroid cell and the extracellular fluid. In iodine deficiency, this gradient may be as high as 400:1 (Wolff, 1964). The remaining two-thirds are excreted by the kidneys. The salivary glands, gastric mucosa and mammary glands are also capable of concentrating iodide. The iodide is rapidly converted to iodine. Uptake is blocked by thiocyanate and perchlorate.

1.1.2. Thyroid Hormone Synthesis

Tyrosine residues in thyroglobulin, a large glycoprotein, are iodinated to form mono- and diiodotryosine (MIT and DIT). This step can be inhibited by a number of goitrogens, including carbimazole and propylthiouracil. The iodotyrosines are coupled to form thyroxine, 3,5,3',5'-tetraiodothyronine (T_4) (DIT and DIT) and 3,5,3'-triiodothyronine (T_3) (DIT and MIT), shown below in figure 1.1.2.

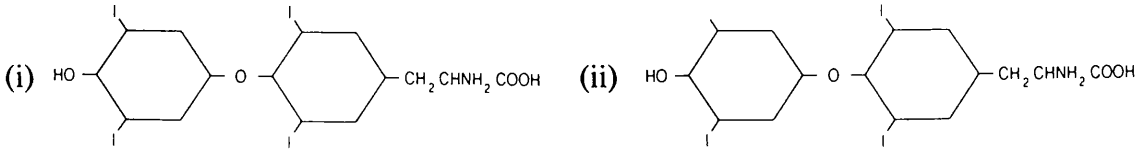


Figure 1.1.2. Chemical formulae of (i) thyroxine and (ii) triiodothyronine. Source: Hetzel, 1989.

Normally there is far more T_4 than T_3 formed but the T_3 to T_4 ratio in the gland increases if there is an inadequate supply of iodine. The thyroid hormones, still incorporated in thyroglobulin, are stored in the colloid of the thyroid follicles.

1.1.3. Thyroid Hormone Secretion

Thyroglobulin is taken up, from the colloid, into the follicular cells where and T_3 and T_4 are released from it, by proteolytic enzymes, and secreted into the bloodstream. The hormones are immediately bound to plasma proteins, mainly to thyroxine-binding globulin (TBG), an α-globulin, and, to a lesser extent, to albumin and pre-albumin. Changes in the levels of binding proteins alter total T_3 and T_4, but not free hormone concentrations. It is the free hormones FT_3 and FT_4 which are physiologically active. DIT and MIT, released at this stage, are deiodinated and the iodine reutilized.

1.1.4. Thyroid Hormone Conversion

In the peripheral tissues, particularly in the liver and kidneys, some of the circulating T_4 is enzymically deiodinated to form T_3. Deiodination of the outer (ß) ring produces about 80% of the circulating T_3 (the other 20% being secreted directly from the thyroid gland). Deiodination of the inner (α) ring produces the (probably) physiologically inactive reverse T_3 (rT_3).

This conversion of T_4 to T_3 is reduced by systemic illness, prolonged fasting, drugs such as propanolol and amiodarone and, transiently, by some radiocontrast media. It is increased by drugs which induce hepatic enzymes, such as phenytoin. Plasma T_3 concentration is thus a poor indicator of thyroid function because it is influenced by so many non-thyroidal factors (Zilva, Pannel and Mayne, 1989).

1.1.5. Action of Thyroid Hormones

Thyroid hormones influence the functions of most organs in many species, from fish to man, with the general effect of speeding up processes such as the metabolism of carbohydrates, lipids and proteins and so increasing the basal metabolic rate (Hoch, 1974).

In addition, thyroid hormones increase the level of specific hormones, such as growth hormone (Hervas, Morreale de Escabor and Escabor del Rey, 1975), and alter the responsiveness to other hormones by influencing the number of receptors in the target tissues. In this way, they increase the sensitivity of the cardiovascular and central nervous systems to catecholamines and so influence cardiac output and heart rate (Zilva, Pannel and Mayne, 1989). Similarly, the level of glucagon receptors in adipocytes is increased by thyroid hormones (Nistrup-Madsen and Sonne, 1976).

Thyroid hormones also influence cell replication and development and so are also essential for normal growth, mental development and sexual maturation. Their role in brain development has long been recognised (Eayrs and Taylor, 1951) and deficiency, during the crucial stages of development, can lead to severe psychomotor impairment and mental retardation.

Many of these actions are thought to be mediated by T_3 binding to specific receptors in cell nuclei and altering gene expression (Oppenheimer, 1979; Oppenheimer and Samuels, 1983; Sterling, 1979). A schematic representation of thyroid hormone action is shown in figure 1.1.5. In addition, some actions may be mediated through extranuclear events but there have been considerable discrepancies surrounding reports of such events.

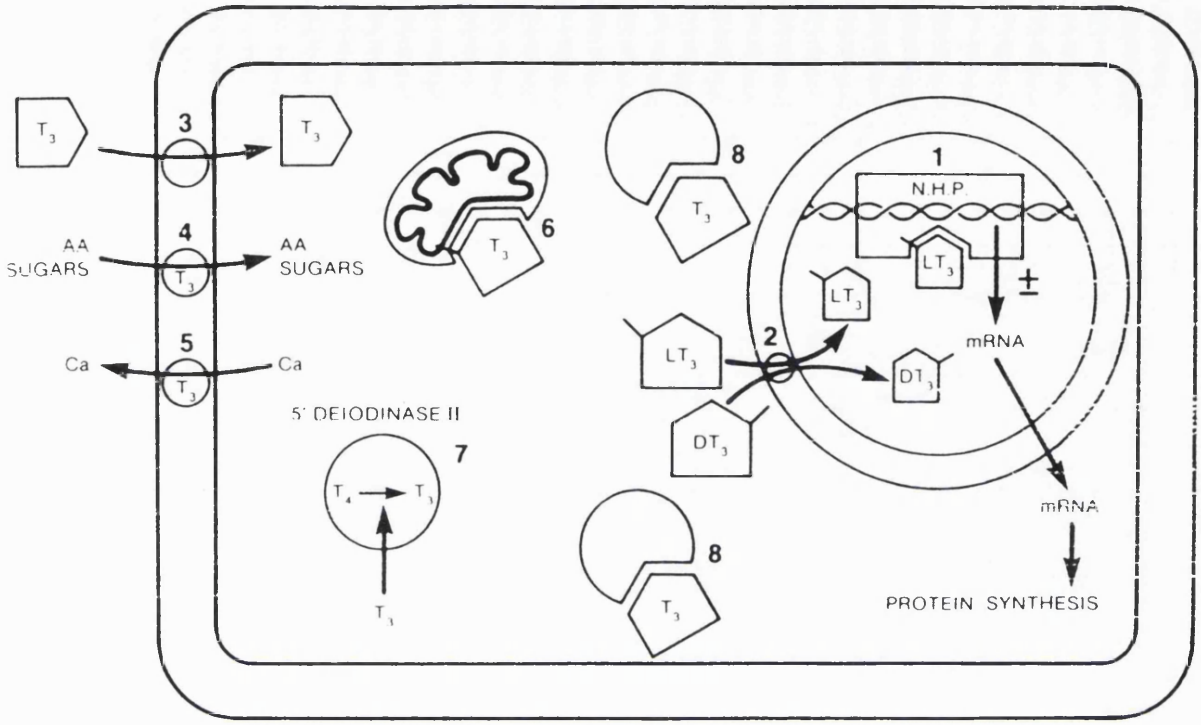


Figure 1.1.5. Thyroid hormone action. Source: Dillman, 1985.

1. T_3 binds to a nuclear receptor protein, non-histone protein (NHP), and leads to an changes: increase (+) and decrease (-) in the levels of specific mRNAs. 2. The entry of thyroid hormone analogues into the nucleus appears to be regulated by a stereospecific transport system in the nuclear membrane. The transport system distinguishes between L-T_3 and D-T_3 in hepatocytes. 3. A specific transport system in the membrane is in part responsible for T_3 entry into hepatocytes. 4 T_3 stimulates the entry of amino acids (AA) and sugars into the cell via an active transport mechanism which is independent of nuclear T_3 effects. 5. T_3 stimulates scarcolemmal Ca^{2}-ATPase leading to Ca^{2+} extrusion from cardiac myocytes. 6. A putative mitochondrial T_3 receptor has been described which is reported to mediate nuclear independent effects of T_3 onto mitochondria. 7. T_3 and r-T_3 influence the 5'deiodinase type II activity in the brain. The influence appears independent of nuclear T_3 action. 8. Cytoplasmic binding proteins which have low-affinity, high-capacity-binding characteristics bind T_3 in the cytosol. These cytosol-binding proteins are not needed to transport T^3 into the nucleus. T_3 enters into the nucleus in the free form.

1.2. Thyroid Hormone Regulation

The production of thyroid hormones is regulated by the thyroid, via iodide uptake; the pituitary, via thyroid-stimulating hormone (TSH) secretion; and the hypothalamus, via thyrotropin-releasing hormone (TRH) secretion, as shown in figure 1.2.

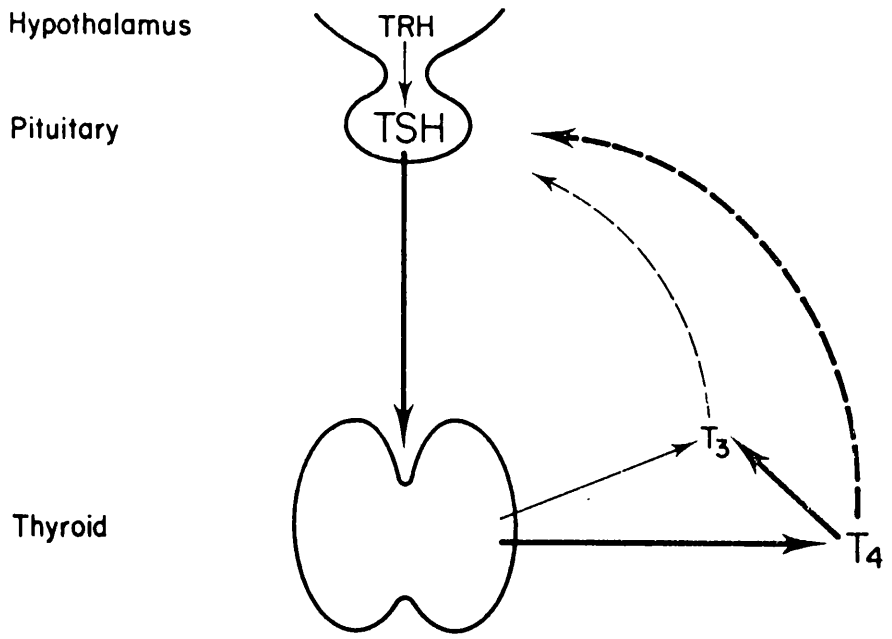


Figure 1.2. Regulation of the secretion of thyroid hormones.
Source: Zilva, Pannel and Mayne, 1989.

Solid lines = secretion and metabolism of hormones
Dotted lines = negative feedback mechanism

1.2.1. Iodide Uptake

When iodide uptake is low, either through a deficient intake or because of an impairment of the iodide-concentrating mechanism of the thyroid, the tyrosine residues of thyroglobulin are insufficiently iodinated and increased amounts of MIT and T_3 are secreted, compared to DIT and T_4. Increased T_3 secretion may minimise the clinical effects of low T_4 levels, but plasma TSH secretion may be increased by the negative feedback response to the low levels of T_4 (Zilva, Pannel and Mayne, 1989).

1.2.2. TSH

TSH stimulates the follicular cells of the thyroid to release T_3 and T_4 into the bloodstream and is itself controlled by negative feedback from the thyroid hormones. This negative feedback, mediated by T_3 binding to pituitary nuclear receptors, tends to counteract thyroid hormone fluctuations and maintain euthyroidism ("normal" thyroid function). It also amplifies the TSH response, so that a minor change in thyroid hormone concentration, particularly of FT_4, from which most pituitary T_3 is derived, produces an approximately 10 times greater inverse change in pituitary TSH release (Bayer, 1991).

1.2.3. TRH

TRH has a stimulatory effect on TSH secretion and thus on the secretion of T_3 and T_4. Increased levels of TRH will lead to increased thyroid hormone secretion, although the stimulatory effect can be overridden by abnormally high levels of circulating FT_4.

1.3. The Ecology of Iodine

1.3.1. The Iodine Cycle

There is a natural iodine cycle, shown below, in which iodine, mainly present in the sea, evaporates and falls to earth with the rain. Large amounts of iodine have been leached from the soil by glaciation, rain and snow and, in general, the older an exposed soil surface, the more likely it is to have been leached of iodine. Thus, the most severely iodine-deficient areas are mountainous - particularly the Himalayas, Alps, Andes and the vast mountains of China. Coastal regions are rarely iodine-deficient, although other factors such as the consumption if food imported from iodine-deficient regions can lead to the occurrence of iodine deficiency disorders (IDD) in areas where the soil is not deficient.

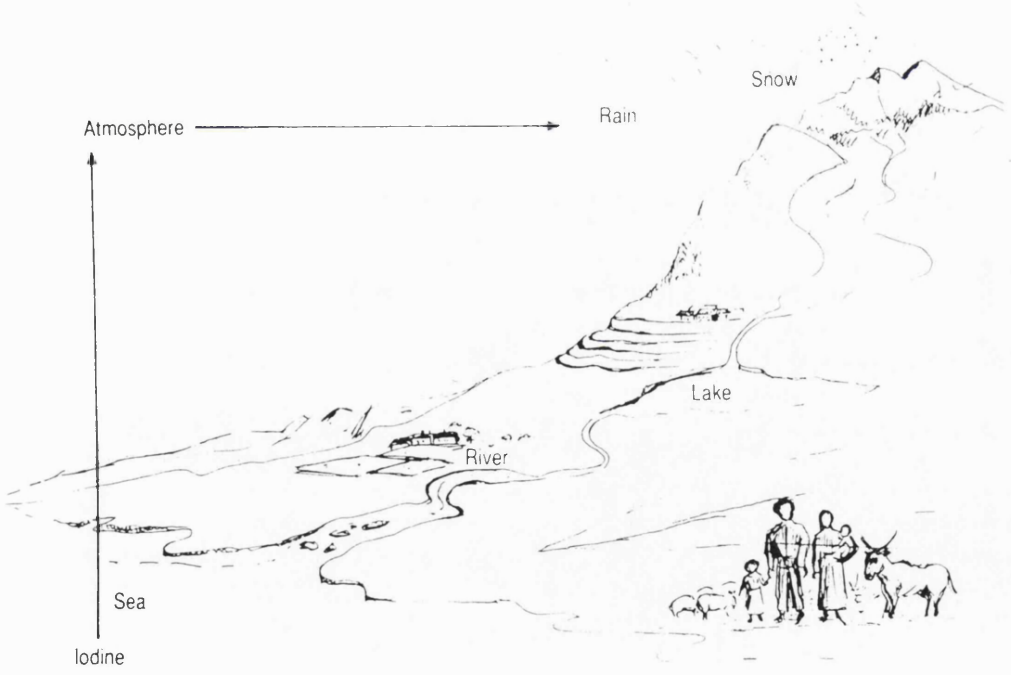


Figure 1.3.1. The natural iodine cycle. Source: Hetzel, 1989.

1.3.2. Worldwide Distribution of IDD

An estimated one billion people are at risk of IDD, by virtue of living in an iodine-deficient environment. Of these, 211 million are believed to suffer from goitre, nearly six million overt cretins and millions more have some intellectual or motor impairment (WHO, 1990.) Table 1.3.2. shows the estimated numbers at risk in different regions of the world and figure 1.3.2. the global distribution of iodine-deficient areas.

Region	At Risk	Goitre	Overt Cretinism
South-east Asia	280	100	4.0
Asia (other countries)	400	30	0.9
Africa	227	39	0.5
Latin America	60	30	0.3
Eastern Mediterranean	33	12	-
Total	**1,000**	**211**	**5.7**

Table 1.3.2. Prevalence of IDD in developing countries (in millions) Source: Hetzel and Pandav, 1994.

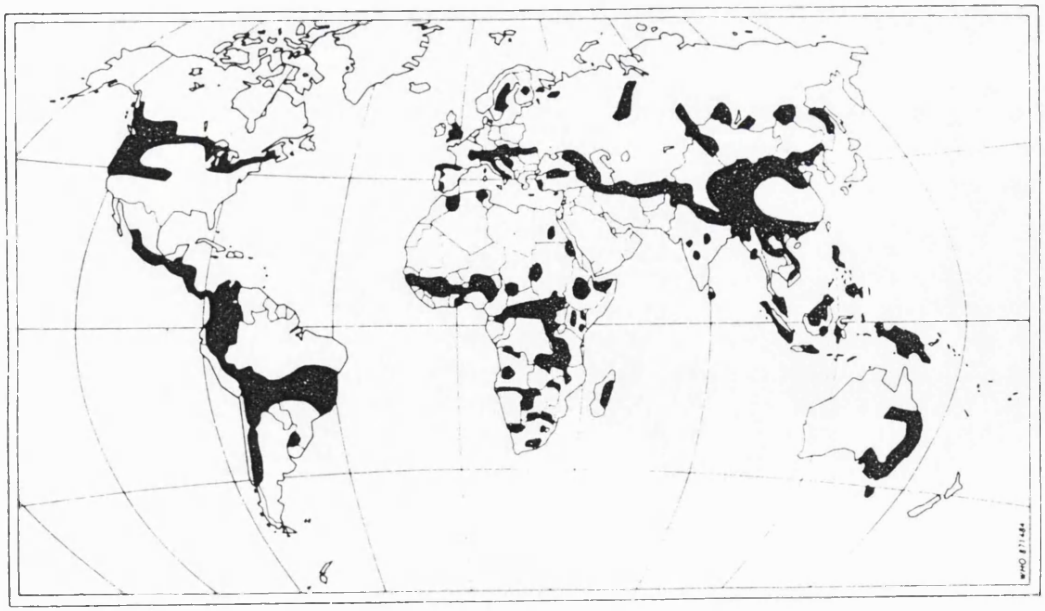


Figure 1.3.2. Global distribution of IDD. Source: Hetzel and Pandav, 1994.

1.4. Consequences of Iodine Deficiency

The term "Iodine Deficiency Disorders" (IDD) was introduced in 1983 by Hetzel (1983) to emphasize the wide spectrum of the effects of iodine deficiency on growth and development of individuals and on the socioeconomic advancement of societies. Previously the term "goitre" had been used, as this was the most obvious and familiar feature of iodine deficiency, but a new term was needed to include the other effects of iodine deficiency.

1.4.1. Endemic Goitre

In a condition of iodine deficiency, blood levels of T_4 decrease, leading to increased TSH output from the pituitary, increased iodide uptake and increased turnover associated with hyperplasia of the thyroid follicle cells. This mechanism is shown schematically below. The size of the gland increases and enlargement is considered significant when the size of the lateral lobes of the thyroid is greater than the terminal phalanx of the thumb of the person being examined.

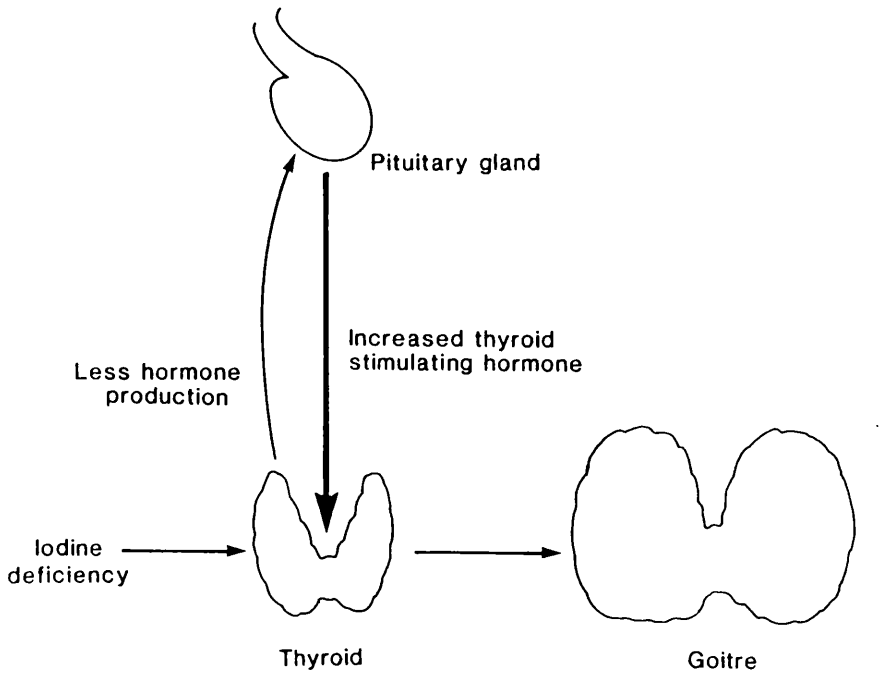


Figure 1.4. Low iodine intake leading to endemic goitre. Source: Phillips, 1989

Generalities about the size of a thyroid constituting a goitre cannot, therefore, easily be made but in adults, a thyroid gland weighing less than 20 g with a volume under 20 ml will probably be considered normal (Hetzel, 1989.) Various grades of goitre have been defined. The most widely-used grading system has been based on the Pan American Health Organisation/ World Health Organisation (PAHO/WHO) classifications (Delange, Bastani, Benmiloud *et al.*, 1986), as shown below.

Stage	Description
0	No Goitre
1-a	Goitre detectable only by palpation and not visible even when the neck is fully extended.
1-b	Goitre palpable but visible only when the neck is fully extended. (This stage includes nodular glands, even if not goitrous.)
2	Goitre visible with neck in normal position; palpation is not needed for diagnosis.
3	Very large goitre which can be recognised at a considerable distance.

Table 1.4.1. Estimation of goitre size by palpation. Source: Stanbury, 1987

A joint WHO/UNICEF/ICCIDD consultation has recently recommended a simplified classification, with grades 1a and 1b combined to form a simple grade 1 category and grades 2 and 3 combined into a grade 2 category (WHO/UNICEF/ICCIDD, 1994).

Chronic, severe iodine deficiency is associated with gross thyroid hyperplasia and goitre prevalence increases with the severity of the deficiency, becoming almost universal in a population with a daily iodine intake of less than 10 μg. Goitre is defined as endemic when the prevalence in a specified community exceeds 10%, this figure being chosen because a prevalence below this level is not uncommon even when all known environmental factors have been taken into consideration (Hetzel, Potter and Dulberg, 1990.)

1.4.2. Endemic Cretinism

A precise description of an endemic cretin or cretinoid is very difficult, however there are three main features associated with these terms:

(i) Endemic cretinism is **associated with severe iodine deficiency and severe endemic goitre.** Since the 16th century, the association of severe endemic goitre with the birth of deaf-mute and imbecile inhabitants, known as cretins, has been recognised, long before it was known that a goitre was an enlarged thyroid gland (Koenig, 1981). Indeed, Diderot's Encyclopedie of 1754 described a cretin as "an imbecile with a goitre hanging down to the waist". It was not until the 1930s, however, that iodine deficiency was recognised in the etiology of endemic cretinism.

Several workers have shown that both mental and motor development in children are retarded in areas of iodine deficiency, compared to children in non-iodine deficient areas, and particularly extensive work has been done in China (Bleichrodt, Garcia, Rubio *et al.*, 1987; Boyages, Collins, Maberly *et al.*, 1989; Ma, Wang, Chen, *et al.*, 1989; Vermiglio, Sidoti, Finocchiar *et al.*, 1990).

(ii) There are **two main clinical manifestations** of endemic cretinism, both associated with mental deficiency, which were first described in the Karakoram mountains of what is now northern Pakistan (McCarrison, 1908):

1.4.2.1. Neurological Cretinism

This consists of defects of hearing and speech, most severely deaf-mutism, and of stance and gait (ataxia and spasticity.) Height is often less than age-group peers but regarded as normal. Some subjects are severely affected and may be entirely vegetative whilst others are able to perform simple tasks such as herding sheep or gathering crops. Thyroid hormones rarely indicate hypothyroidism and postnatal treatment with thyroxine or iodine does not reverse the condition.

1.4.2.2. Myxoedematous Cretinism

This is characterised by classic signs of hypothyroidism, including elevated TSH and low T_4, coarse, dry skin and hair, a prominent tongue, dwarfism and delayed (or absent) sexual maturation. Most signs of motor impairment are less striking and hearing is generally preserved. If treated early, this condition is, at least partly, reversible.

1.4.2.3. Distribution

In some regions one type predominates; in Zaire, myxoedematous cretinism is almost exclusively observed, whereas Andean cretins are more often of the neurological type, but both types can be found in each region (Stanbury, 1987.) In other areas a mixture of both types is seen, as in China (Wang, Ma, Li *et al.*, 1983; Halpern, Morris, Boyages *et al.*, 1989). Costa (1989) has suggested that the myxoedematous type is more common in women and the neurological in men.

1.4.2.4. Etiology

The different manifestations may be due to different etiologies, perhaps indicating iodine deficiency during different "critical periods" of brain development and these will be more fully discussed below. Maternal iodine deficiency before and during pregnancy may be associated with neurological cretinism whereas peri- and post-natal iodine deficiency is believed to be associated with the myxoedematous form (Pharoah, Buttfield and Hetzel, 1972). Thus, the latter is potentially reversible while the former is not.

It has also been suggested that factors other than iodine deficiency may influence the type and degree of cretinism, for example, in the severely-endemic regions of Zaire, consumption of cassava is high and the goitrogens present in this staple food crop are thought to favour the development of myxoedematous, rather than neurological cretinism (Delange, Ermans, Vis *et al.*, 1972).

(iii) Endemic cretinism can be **prevented by adequate correction of iodine deficiency**, as has occurred in Papua New Guinea (PNG) (Pharoah, Buttfield and Hetzel, 1972) and South America (Querido, Delange, Dunn *et al.*, 1974). This refers to the pre-conceptual or first trimester correction of the deficiency, as later supplementation is not thought to be so effective in preventing brain damage (Pharoah, Buttfield and Hetzel, 1971). There have been some anxieties expressed that supplementation in pregnancy may be detrimental to foetal development as a result of maternal thyroid inhibition from the Wolff-Chaikoff effect (Kochupillai, 1991) but other workers have found no long-term neurological damage in the offspring of supplemented pregnant women (Pharoah and Connolly, 1991). A review of supplementation studies in pregnancy is presented in section 1.18.

Cretinoidism refers to lesser degrees of mental and motor impairment and may be present in large sectors of iodine-deficient populations. It is more difficult to diagnose but should not be ignored in assessing the severity of IDD in a community. These milder manifestations of mental and motor impairment have also been prevented by correction of the iodine deficiency, as studies of psychomotor development of children whose mothers were supplemented with iodized oil in pregnancy have shown (Bautista, Barker, Dunn *et al.*, 1982; Fierro-Benitez, Cazar, Stanbury *et al.*, 1988; Pharoah, Buttfield and Hetzel, 1972; Connolly, Pharoah and Hetzel, 1979; Pharoah and Connolly, 1991). The role of iodine in brain development is discussed in section 1.5.

1.4.3. Iodine Deficiency in the Foetus

Animal studies have shown that during the first trimester, before development of a functional foetal thyroid, maternal hypothyroidism due to iodine deficiency leads to foetal thyroid hormone deficiency (Held, Cruz and Moncayo, 1990; Obregon, Mallol, Pastor *et al.*, 1984; Thilly, Delange, Lagasse *et al.*, 1978) and that the only source of thyroxine for the foetus is maternal thyroxine (Ekins, Sinha and Woods, 1986; Morreale de Escobar, Escobar del Rey, Pastor *et al.*, 1986).

By extrapolation to human development in iodine-deficient areas, these results provide a plausible explanation for the mechanisms involved in foetal iodine deficiency disorders such as reproductive losses, congenital disorders, cretinism and high perinatal and neonatal mortalities.

1.4.4. Iodine Deficiency in the Neonate and Infant

Neonatal hypothyroidism is a well-known cause of mental defect. This is because brain development, which is only one third complete at birth and continues into the second year of life, requires an adequate supply of thyroxine (Dobbing, 1974.) A normal level of thyroxine is therefore important in pregnancy and lactation to maintain normal thyroxine levels in the foetus and breast fed infant.

In most developed countries and in India and China, all newborns are screened for hypothyroidism, usually by assay of TSH levels from a heel prick at 4-10 days, and those who prove positive are further tested and given replacement thyroxine therapy immediately upon confirmation of hypothyroidism. The incidence of hypothyroidism in the developed world is about 1:3500 live births and is generally due to the absence of a thyroid, ectopia or a biosynthetic defect of thyroxine synthesis (Hetzel, 1989.)

In iodine-deficient environments, tests on umbilical vein blood have revealed much higher rates of neonatal hypothyroidism; 5-10% in Northern India, Bhutan and Nepal and up to 10% in Zaire (Kochupillai, Godbole, Pandav *et al.*, 1984; Kochupillai and Pandav, 1987; Belchetz, 1987.) Neonatal goitre is also commonly seen in iodine-deficient areas.

1.4.5. Iodine Deficiency in Childhood and Adolescence

This is characteristically associated with endemic goitre, hypothyroidism and impaired mental function (Hetzel, 1983), including poor school performance (Kochupillai, 1989), although mental functions are difficult to assess, being confounded by other factors such as socio-economic status, location and general nutritional status (Stanbury, 1987.)

In almost every area studied the incidence of goitre is higher is females than in males and this difference becomes maximal at the age of puberty. The acceleration of growth during the pre-puberty period may determine the high frequency of goitre at this age (Orr and Leitch, 1929) The activity of the thyroid and hence the body's iodine requirement are increased during pregnancy - another reason why the incidence of goitre is higher in women than in men. A typical pattern of goitre frequency among different ages and sexes in an iodine-deficient community is shown below.

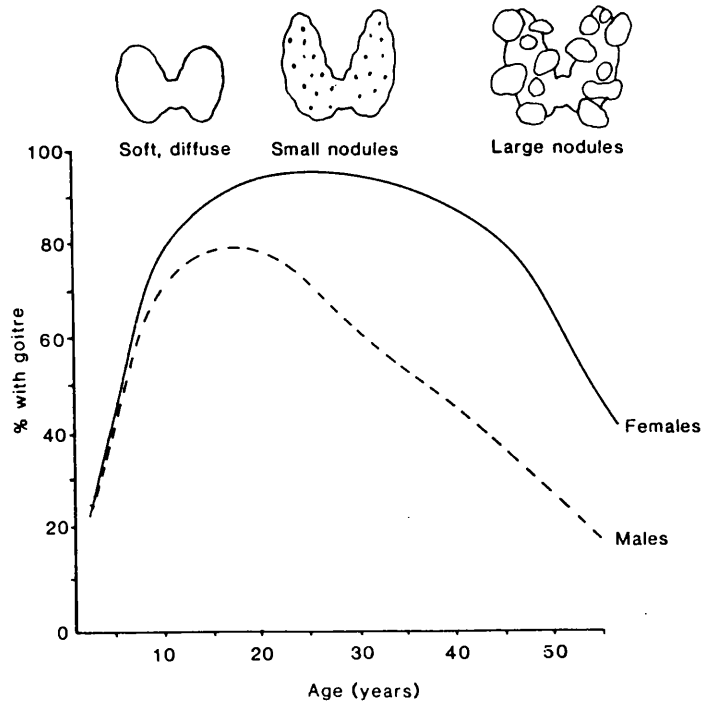


Figure 1.4.5. Goitre frequency and changes, by age and sex. Source: Phillips, 1989.

1.4.6. Iodine Deficiency in Adults

Chronic iodine deficiency is associated with goitre, often of considerable size and with nodule formation - the incidence, size and number of nodules increasing with age. Although rarely life-threatening, a goitre can obstruct the wind-pipe, leading to difficulties in breathing. There is often some discomfort and many societies dislike the cosmetic appearance of a goitre, although some actually prize them; for example, in Nepal they are believed to contain gold - the larger the goitre, the greater the store of gold (M.Marlow, personal communication.)

There is usually an absence of classical clinical hypothyroidism although an altered pattern of thyroid hormones is often observed (Beckers and Delange, 1980), with low levels of T4 and high levels of TSH. T_3 is normal or slightly raised.

1.4.7. Iodine Deficiency and Socio-economic Retardation

Domestic animals will also suffer from the effects of iodine deficiency in similar ways to humans. Thus animals are seen with goitre in iodine-deficient environments (Stantham and Bray, 1975), there is an increased prevalence of reproductive losses and sterility, animals are smaller, producing less meat, eggs and wool and this can contribute to impoverishment of the community (Hetzel and Maberly, 1985.)

A high degree of apathy has been noted in some populations living in iodine-deficient areas (Dunn and van der Haar, 1990) - affecting even domestic animals. Reduced mental function in such communities affects their work capacity and ability to take initiatives and make decisions, thus blocking the social development of the community. In addition, handicapped individuals divert the community's resources and are dependent on others. Correction of the iodine deficiency can be a major contribution to the socioeconomic development of the community, as in the classic case study in Jixan in Northern China (Li, He and Wang, 1987.)

1.5. Iodine and Brain Development

The most important consequence of iodine deficiency is the impairment of nervous system development and function. It has been estimated that up to a third of those living in an iodine-deficient environment may have some hypothyroidism affecting brain function (Hetzel, 1989).

Indeed, in many developing countries, iodine deficiency is possibly the most common and widespread preventable cause of impaired neurological development and mental function (DeLong, 1987). In some developed countries, interuterine iodine deficiency is still sufficiently prevalent to cause concern about congenital goitre, bone-growth retardation and impaired motor performance during childhood, indicating morphological and functional damage (Stubbe, Schulte and Heidemann, 1986).

1.5.1. The Timescale of Brain Development

1.5.1.1. Human Studies

Examination of the chronology of events involved in foetal brain development is essential in understanding the effects of iodine deficiency during pregnancy. Figure 1.5.1.1. shows the developmental events in the human forebrain as increments in DNA or weight over five-week periods. Peak neuroblast replication occurs at 18-20 weeks of gestation, with glial cell replication peaking after birth, at around 4-5 months of age. Cerebellar events are retarded and most of the growth spurt there occurs after birth.

Clearly, then, brain development occurs over a fairly wide time span and the possible insults to development, due to iodine deficiency, need to be considered with reference to the specific **actions** of thyroid hormones in controlling this development and to their **sources**, whether foetal or maternal.

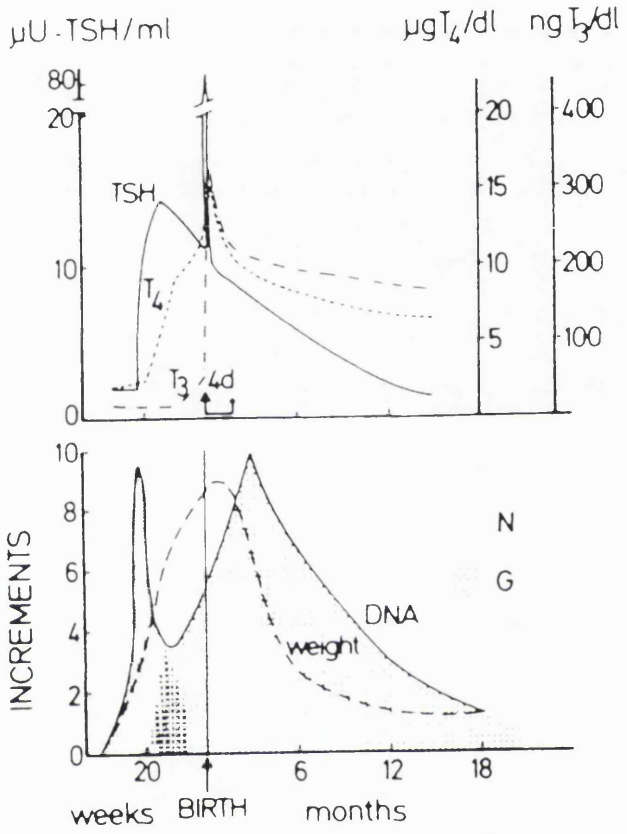


Figure 1.5.1.1. Developmental events in the brain. Source: Morreale de Escobar, Obregon and Escobar del Rey, 1987.

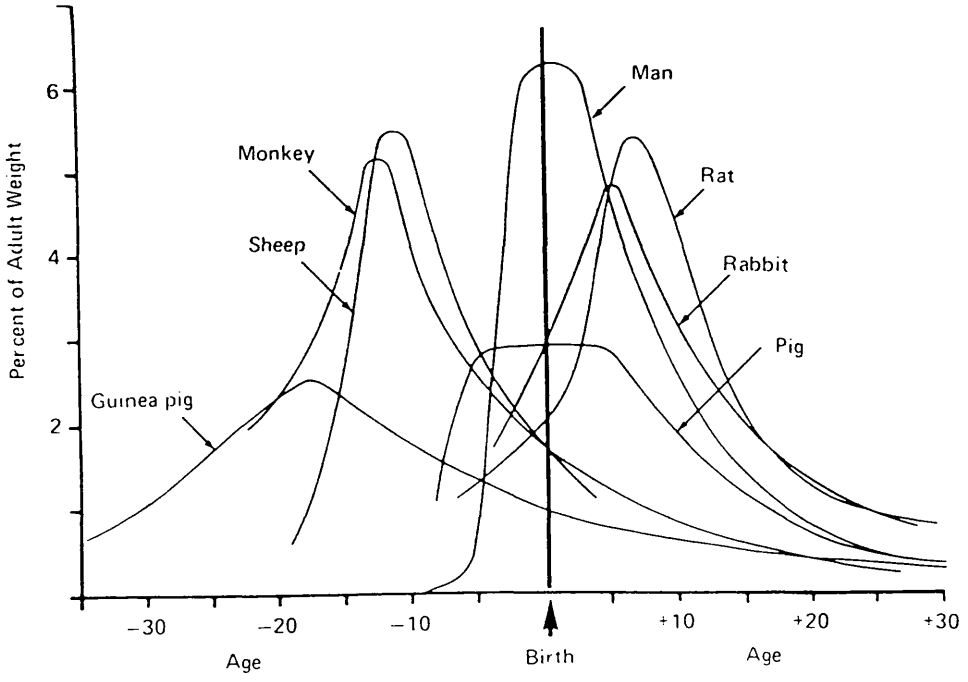
N= phase of neuroblast replication, G= phase of glial cell replication

- - - represents overall brain growth.

1.5.1.2. Comparison with Animal Models

Much of the work on brain development has been done in rat or sheep models, both of which have similar developmental patterns to that of humans although the three species differ in the stage of development which is reached by the time of birth. This means that if comparisons between species are to be made, developmental **stages**, rather than **ages** should be compared.

In the rat (and also the rabbit), the phase of active neuroblast multiplication, which in man (and the pig) takes place at mid-gestation, occurs in the last few days preceding birth and the spurt of brain growth peaks in the second to third post-natal weeks, compared with the perinatal period in man. Thus the new-born rat is comparable to the mid-gestation human foetus and the neonatal rat to the new-born baby. In the sheep (and also marmoset monkey), maximal brain growth occurs prenatally so that a third trimester sheep foetus is comparable to a newborn human baby (Fisher, 1989; Hetzel, Chavedej and Potter, 1987). Figure 1.5.1.2. compares the brain development of several animal models with that of man.



Weight gain as a percentage of adult brain weight per unit of time.
Units of time are as follows: guinea pig and rat=days, rabbit=2 days, rhesus monkey=4 days, sheep=5 days, pig=weeks, man=months.

Figure 1.5.1.2. Brain growth spurts Source: Dobbing, 1974.

1.5.2. Thyroid Hormone Control of Brain Development

1.5.2.1. Thyroid Hormone Transport from Blood into Brain Cells

Endothelial cells in central nervous system (CNS) capillaries are surrounded by tight junctions which form the blood-brain barrier and limit the passage of macromolecules and hydrophillic molecules from the capillaries into the brain cells. Thyroid hormones are both small and lipophilic but their transport into the cells of the brain does not seem to be by diffusion, as might be expected, but is active, saturable and stereospecific.

Most of the T_4 present in the brain is delivered by the more readily-dissociated albumin-T_4 carrier protein complex, rather than by TBG or pre-albumin. Some T_4 is also thought to be transported via choroid plexus and cerebro-spinal fluid (CSF) (Robbins, Goncalves, Laksmanan *et al.*, 1989).

1.5.2.2. Local Regulation of Thyroid Hormones

Almost all the nuclear T_3 in the brain is derived locally from deiodination of T_4 by an important brain enzyme - a type II 5'-deiodinase. In hypothyroidism, the activity of this enzyme increases five-fold within a few days of the induction of the hypothyroxinaemia, whereas in hyperthyroidism its activity decreases. In rat brain, the activity of this enzyme is highest during brain development, indicating an increased requirement for thyroid hormone during this period, and falls off to adult levels as brain development slows down (Larsen, 1989).

Thus, T_4 deficiency may be critical for brain defects leading to cretinism, as this hormone, taken-up by the brain, is the main source of nuclear T_3 in the organ. Other tissues which are able to take-up and utilise T_3 directly, may not suffer from hypothyroidism if T_4 alone is reduced but T_3 levels remain constant.

1.5.2.3. Thyroid Hormone Receptors in the Brain

T_3 receptors first appear in developing brain at 10 weeks gestation, in low concentration, but by 16 weeks there is a sharp increase in concentration. The change in maximum binding capacity (MBC) varies between different regions of the brain, the increase being only two-fold in the cerebellum but ten-fold in the cerebrum. There are many more receptors in neuron nuclei than in glial nuclei in all areas of the CNS.

The surges in receptor numbers (and, in some regions, affinity) coincide with critical periods in brain development such as neuroblast differentiation and synaptogenesis, suggesting that thyroid hormones regulate the expression of genes important in these phases of development and so influence the organisation of the neuronal networks. In hypothyroidism, which may be due to iodine deficiency, synchronisation of cell division, migration and differentiation may be compromised, leading to impaired brain development.

The differences in MBC are reflected in the wide variation in concentrations of receptors in adult tissues, with the highest concentration being in the cells of the anterior pituitary. Rat studies have shown that there is a peak in receptor concentration in the first few days after birth with a decrease towards adulthood (Dussault, 1989; DeNayer and Dozin, 1989).

1.5.2.4. Neuroanatomic Effects of Hypothyroidism

Appropriate levels of thyroid hormones are needed for all phases of neural ontogeny and both insufficiency and excess cause disturbances in cell proliferation, migration and outgrowth, particularly in the cerebellum and hippocampus.

Hypothyroidism tends to slow cell proliferation and migration, reduce the length of parallel fibres of cerebellar and hippocampic granule cells and retard synapse formation. Hyperthyroidism has opposite effects on cell growth, although synapse formation is also decreased because development stops prematurely (Lauder, 1989).

Hypothyroidism also reduces the number of spines and dendritic density in the distal

parts of the apical dendrites of cerebral cortical neurons but does not affect basal neurons (Ruiz-Marcos, 1989). The impairment of dendritic and axonal outgrowth may be explained by a delay in the maturation of microtubule - the major linear structures of these cells - formation (Nunez, Couchie and Brion, 1989).

In rat studies, the critical period for these effects appears to be before 21 days after birth, roughly corresponding to the time immediately before birth in humans. Thyroid hormone defects in this period result in permanent impairment of neuronal connectivity with serious implications for functional and behavioural consequences (Lauder, 1989; Dussault, 1989).

It is known that thyroid hormones have their most critical influences on brain development during late foetal and early postnatal periods and that deficiency during these times can lead to permanent brain damage. Myelination, a later process in brain development, which occurs around this time, may also be dependent on thyroid hormone control and thus impaired by a deficiency of the hormones (Sarlievè, Besnard, Labourdette *et al.*, 1989). This would provide an explanation for the effects of later iodine deficiency on brain development.

1.5.2.5. Control of Neurotransmission

The onset of electrical activity, in rat brain, is correlated with a ten-fold increase in the activity of Na,K-ATPase and changes in the ionic composition of the brain. These changes begin a few days before birth and adult levels of activity are reached by 30 days. These changes may be comparable to those occurring in a human foetus in the last trimester of pregnancy.

Brain T_3 receptors and plasma T_3 concentrations rise rapidly after birth, suggesting that the changes in Na,K-ATPase activity are regulated by thyroid hormone, either directly or indirectly by increasing the fluxes of ionic substrates through the sodium channels. In adults, T_3 regulates Na,K-ATPase in other tissues but not in brain. Thus, there appears to be a critical period of brain development in which T_3 is important (McDonough and Schmitt, 1989).

In addition, thyroid hormone interacts with nerve growth factor, which stimulates choline acetyltransferase (CAT) synthesis in neurons of the forebrain, and thus has a role in cholinergic neurotransmitter systems. Changes in CAT activity, due to thyroid hormone deficiency are generally reversible on correction of the hypothyroidism, except in the forebrain where they appear to be permanent (Patel, Hunt and Kiss, 1989).

T_3 itself may act as a neurotransmitter at synapses in the brain, a hypothesis supported by studies which show high T_3 concentrations, deiodinase activity and T_3 receptors at synaptosomes. Synaptosomal uptake of T_3 is absent at birth but increases during development and it is possible that thyroid hormones, together with other growth-regulating compounds, such as serotonin, may influence developmental activity at different times (Dratman, Crutchfield and Gordon, 1989).

1.5.3. Development of Foetal Thyroid Function

Several animal studies have shown that during the first trimester, before development of a functional foetal thyroid, T_3 receptors are present in foetal brain. The source of T_3 for the foetal brain can only be locally-deiodinated maternal T_4. Thus, maternal transmission of thyroid hormone is important for foetal brain development and in areas of iodine deficiency, where maternal T_4 is low, even if maternal T_3 concentration is preserved, the fetal brain will be hypothyroid and developmental damage may result (Morreale de Escobar, Obregon and Escobar del Rey, 1987; Obregon, Mallol, Pastor *et al.*, 1984; Ekins, Sinha and Woods, 1986; Morreale de Escobar, Escobar del Rey, Pastor *et al.*, 1986; Morreale de Escobar, Ruiz de Ona, Obregon *et al.*, 1989).

Maternal hypothyroidism in humans has a similar effect on foetal thyroid hormone status, as several studies of iodine-deficiency in early pregnancy have shown (Held, Cruz and Moncayo, 1990; Thilly, Delange, Lagasse *et al.*, 1978). Work on the human foetus, in China, has shown that there is reduced brain weight at all stages of gestation and increasing hyperplasia of the foetal thyroid, as it develops (Jia-Liu, Zhong-Jei, Zhon-Fu *et al.*, 1989). In addition, psychomotor performance of a group of 10-year old children, in Papua New Guinea, was found to correlate well with maternal T_4 levels in pregnancy but not with T_3 levels (Connolly and Pharoah, 1989).

The levels of free T_3 and free T_4 in foetal serum are lower than in maternal serum, indicating that there is some placental regulation of thyroid hormone passage from mother to foetus. Additionally, deiodination of T_4 to rT_3 and to MIT and DIT takes place in the placenta and the amniotic fluid thus has a much higher concentration of these inert compounds than does the foetal serum. Iodine, TRH and TSH-blocking antibodies cross the placenta but TSH does not (Braverman, 1989b).

The foetal thyroid follicular cells develop fairly early in pregnancy and begin functioning at 70-80 days gestation in a human foetus, trapping iodide and secreting hormones, although thyroglobulin is produced from about 29 days. With increasing gestational age there is an increased ability to respond to TSH, to produce thyroid hormones and to protect against excessive iodide by decreasing iodide trapping.

This last attribute is present in the adult but absent in the foetus, until the last month of term pregnancy, thus making the foetus very vulnerable to iodide overload, such as may happen following administration of iodine either therapeutically or prophylactically. Iodine-induced hypothyroidism is more common in preterms, possible due to their higher skin permeability (Braverman, 1989a; Fisher, 1989). Table 1.5.3.1. shows the events in foetal thyroid development for man and the two most-commonly used animal models.

Thus, a deficiency in maternal thyroid function, due to environmental iodine deficiency can have a profound effect on foetal growth, particularly in terms of brain development and function.

Event in Thyroid Development	Time of Appearance (*)		
	Man	Sheep	Rat
TSH Responsiveness	0.5	0.3	0.5
Iodide Trapping	0.2	0.3	0.3
Thyroglobulin Synthesis	0.1	---	0.3
T_4 Synthesis	0.2	0.3	0.3
T_4-T_3 Conversion	---	<0.1	---
Iodide Inhibition of Iodide Trapping	1.0	---	1.0

Table 1.5.3.1. Maturation of foetal thyroid function. Source: Fisher, 1989.

Hypothalamic control of TSH release begins in the first trimester in man but TRH is formed by the placenta and pancreas prior to its formation by the hypothalamus and there is thus considerable independence of the foeto-placental thyroid function from that of the mother and the foetal brain may be protected against maternal hypothyroxinaemia at this stage (Fisher, 1989). Table 1.5.3.2. shows the events in the maturation of hypothalamic control of TSH release.

Event in the Maturation of Hypothalamic Control of TSH Release	Time of Appearance (*)		
	Man	Sheep	Rat
TRH Synthesis	<0.3	<0.3	<0.4
TRH Stimulation of TSH	0.5	0.5	0.5
T_3 Inhibition of TRH Synthesis	<0.6	<0.8	1.0
TSH Response to Cold	---	<0.6	0.4
Somatostatin Inhibition of TSH Secretion	---	<0.1	>1.0
Dopamine Inhibition of TSH Secretion	>1.0	---	---

(*=Fractional proportion of maturation time:

1.0 = 10 months in man, = 5 1/2 months in sheep, = 50 days in rat.)

Table 1.5.3.2. Maturation of hypothalamic control. Source: Fisher, 1989.

1.6. Clinical Assessment of IDD

1.6.1. Goitre Survey

This is the simplest, most commonly used technique and involves thyroid palpation. Although little clinical training is needed, attention must be paid to technique, to avoid error, and to sampling procedures to avoid bias. In some societies, it may be culturally inappropriate to expose and palpate the neck, particularly for women in mixed-sex situations. Figure 1.6.1. shows the position of the thyroid in the neck.

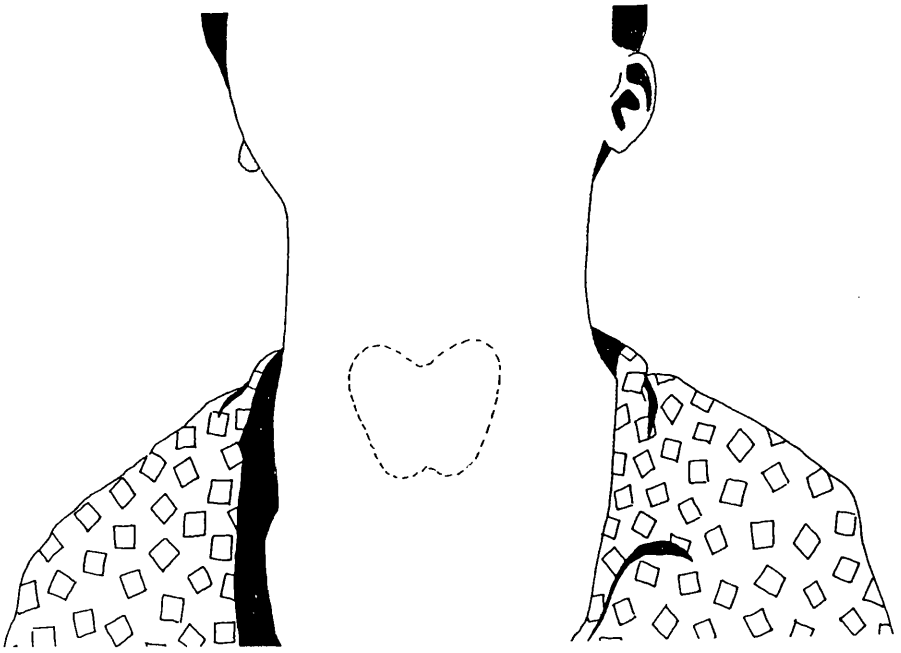


Figure 1.6.1. The position of the thyroid gland in the neck. Source: Dunn and van der Haar, 1990.

Subjects are generally primary school children because of their accessibility, representativeness of the state of nutrition in the community and status as a target group for intervention (Hetzel, Potter and Dulberg, 1990; Follis, 1964). Severely affected children, including cretins, are likely be precluded from this group, thus artificially lowering the goitre rate.

The visible goitre rate (VGR), which includes goitres of grades 2-3, and total goitre rate (TGR), including goitres of grades 1a and 1b, are computed. IDD is defined as a Public Health problem when the TGR in primary school children is over 10%. The problem is considered severe when TGR is greater than 60% and this is often accompanied by a VGR of over 10% (Hetzel, 1989.)

This gives a fairly crude estimate of the level of iodine deficiency in a community, marginal iodine deficiency may not be detected from a simple goitre survey and, to some extent the presence of goitre in a community is a measure of past iodine status and may not accurately reflect the current situation.

1.6.2. Cretinism Prevalence Survey

Goitre surveys may also include the noting of cretins but considerable clinical training and experience is needed for the diagnosis. Culturally-appropriate tests for sitting, walking, hearing defects, clear intellectual limitations and sensory neuro-defects are used. Sporadic cretinism occurs in about 1:4000 live births so a prevalence of 0.5% represents 25 times the expected rate. Mental deficiency not related to iodine deficiency has a prevalence of about 2.5% so a cretin rate of about 3% is generally taken as the criterion for intervention (Hetzel, 1989).

1.7. Biochemical Assessment of IDD

1.7.1. Urinary Iodine Excretion

Normal urinary iodine excretion (UIE) varies between individuals, regions and from day to day, according to dietary iodine intake (Wayne, Koutras and Alexander, 1964.) When a person is in iodine balance, UIE provides a good estimate of dietary intake, assuming that excretion equals intake (Vought, London, Loutwak *et al.*, 1963.)

Renal iodide clearance is, however, affected by several factors, increasing with high glomerular filtration rate, puberty, pregnancy, hyperthyroidism and goitrogens and decreasing with renal impairment, energy- and salt-restricted diets and Protein-Energy Malnutrition (PEM) (Wayne, Koutras and Alexander, 1964) making interpretation of levels problematic. Methods for estimating urinary iodine are described in section 1.9.

1.7.2. Thyroid Hormone Analysis

Iodine **intake** can be assessed by determination of urinary iodine excretion, as previously discussed, but iodine **deficiency**, as defined by certain criteria, is a purely **descriptive** state, giving information only about an individual's, or a community's, iodine status and revealing nothing of the **functional** impairment which may accompany such a deficiency. To elucidate the **functional** effects of iodine deficiency, it is necessary to measure parameters which reflect the **functional** role of iodine in the body.

Such a functional estimate of iodine nutrition may be provided by measuring serum T_3, T_4 and TSH. Over the past 25 years, new, sensitive, precise laboratory methods for measuring these hormones have become available, particularly radio- and fluorescence-enhanced immunoassay techniques, which can provide clear hormone profiles for goitre-endemic areas (Kochupillai, 1989.) In general, low T_4, normal or slightly raised T_3 and high TSH values are found in goitrous subjects (Pharoah, Lawton, Ellis *et al.*, 1973.)

These methods are, however, costly and labour-intensive and are more useful for research purposes than routine screening in developing countries. They are used in screening for neonatal hypothyroidism in most developed countries and in India and China. They are described, in detail, in section 1.10.

1.8. Other Methods of Assessing IDD

1.8.1. Radioiodine Uptake by the Thyroid

This method was first used in the Andes (Stanbury, Brownell, Riggs *et al.*, 1954) and involves oral or intravenous dosing with radio-actively labelled iodine (^{131}I), followed by measurement of radioactivity in the thyroid gland, using a counter over the neck.

It is a rapid and accurate method of measuring the uptake and hence the affinity of the thyroid gland for iodine, a factor influenced by the degree of iodine deficiency of the thyroid. This method does, however, have its limitations; it cannot be used on pregnant women or children because of the radioactivity and is expensive, requiring sophisticated equipment and highly-trained personnel. It is therefore inappropriate for surveying community prevalence of IDD in developing countries but a useful research tool.

1.8.2. Ultrasonography

This is a fairly new technique, used to measure more accurately the size of the thyroid. It is impractical for use in routine surveys due to its expense and sophistication but has been useful for research purposes (Wachter, Pickardt, Gutekunst *et al.*, 1987.)

1.8.3. Environmental Analysis

This is not usually necessary for diagnostic purposes but can provide useful supporting data, concerning the level of iodine in drinking water and soils.

1.9. Estimation of Urinary Iodine

1.9.1. Degrees of Iodine Deficiency

Ideally, 24 hour urine specimens are collected and UIE expressed as microgrammes of iodine per gramme of creatinine (μg I/g Cr). This method was first proposed by Follis (1964) and has been extensively used in field studies over the past 20 years (Bourdoux, 1988.) Average UIE levels for a community indicate levels of iodine deficiency in three categories (Hetzel, 1987):

(i) **50-100 μg I/g Cr: mild** iodine deficiency

(ii) **25-50 μg I/g Cr: moderate** iodine deficiency (with endemic goitre present)

(iii) **< 25 μg I/g Cr: severe** endemic goitre, complicated by cretinism.

There are, however, many problems associated with measurement of the iodine/creatinine ratio. Creatinine excretion depends on an individual's age, sex and protein intake and can lead to erroneous estimates of habitual iodine excretion. In particular, unusually low creatinine excretion, which has been reported in some endemic areas for iodine deficiency such as parts of Spain, Papua New Guinea and Kivu, Zaire, can mask a low urinary iodine excretion by presenting a normal iodine/creatinine ratio (Bourdoux, 1988; Bourdoux, Delange, Filetti *et al.*, 1984).

Problems also arise due to the unreliability of urinary creatinine as an index of the completeness of a 24 hour collection (Dworkin, Chandler and Beierwalts, 1965; Patterson, 1967) but also to the high variability in coefficients of variation for urinary creatinine among individuals (Bourdoux, 1988). In addition, 24 hour urine samples are difficult to collect in the field and a number of casual samples (usually 50-100) are taken instead, for surveys.

An alternative approach is to measure iodine concentration as μg/dl (or μmol/l) of urine -a value independent of the urine concentration. If sufficient samples are taken (50-100), variation in urine concentration between individuals is evened out (Bourdoux, 1988.) The simplicity and reliability of this method makes it the preferred one amongst most researchers (Hetzel, 1989; Dunn and van der Haar, 1990). The different methods available for measuring urinary iodine are outlined below.

(i) **5.0-9.9 μg I/dl** indicates **mild iodine deficiency**
(0.40-0.78 μmol/l) typical goitre prevalence 10-30%

(ii) **2.0-4.9 μg I/dl** indicates **moderate iodine deficiency**
(0.16-0.39 μmol/l) typical goitre prevalence 20-50%

(ii) **< 2.0 μg I/dl** indicates **severe iodine deficiency**
(<0.16 μmol/l) typical goitre prevalence 30-100%

1.9.2. Reporting of Results

Expressing urinary iodine as a mean or median in a population can be misleading if the group is heterogeneous in iodine intake. Recognition of iodine deficiency in part of the population may be obscured by higher intakes in other parts of the population. It is therefore recommended that data is grouped to show the percentages of samples in each of the above groups, to provide a more complete picture of the extent of iodine deficiency and the need for corrective measures.

1.9.3. Methods for Measurement of Urinary Iodine

1.9.3.1. Titration by Thiosulphate

This is the classical way of measuring iodine concentration but is useful only for relatively high iodine levels and lacks the sensitivity to detect the very low levels found in urine (Dunn, Crutchfield, Gutekunst *et al.*, 1992).

1.9.3.2. Sandell-Kolthoff Reaction

The catalytic effect of iodide on the reduction of Ce^{4+} by As^{3+} in acidic medium was first reported in 1937 (Sandell and Kolthoff, 1937). The reaction is extremely slow in the absence of iodide and the rate is critically dependent on and directly proportional to the concentration of iodide present in the reaction mixture. The following scheme has been proposed for the reaction, which is first order.

$$2Ce^{4+} + 2I^- \text{----}> 2Ce^{3+} + I_2$$
$$2Ce^{4+} + I_2 \text{----}> 2Ce^{3+} + 2I^+$$
$$As^{3+} + 2I^+ \text{----}> As^{5+} + I_2$$
$$As^{3+} + I_2 \text{----}> As^{5+} + 2I^-$$

The ceric ion (Ce^{4+}) is yellow and the cerous ion (Ce^{3+}) is colourless. Thus the course of the reaction may be followed by the disappearance of the yellow colour of the ceric ion, as it is reduced to cerous. Iodide levels down to less than 1μg/dl can be detected using this method, which is highly specific for iodide, the catalytic effect of iodate, chloride and bromide being minimal (Bourdoux, 1988).

The rate of reaction is dependent on the temperature, with a one degree increase in temperature, at ambient room temperature, causing an decrease in reaction rate of about 5%. The concentrations of sulphuric acid and chloride, present in the reaction mixture, also influence the rate of reaction: a doubling of sulphuric acid concentration from 1.5 M to 3.0 M slightly raises the reaction rate and an increase in sodium chloride concentration decreases the rate but stabilizes the reaction by inhibiting the oxidation of iodide to iodate (Bourdoux, 1988; Dunn, Crutchfield, Gutekunst *et al.*, 1992).

Some substances interfere with the reaction by oxidizing or reducing the arsenite or ceric reactants, thus altering the reaction velocity and artifactually raising or lowering the apparent iodide concentration in the Sandell-Kolthoff reaction (May, Wu, Eastman *et al.*, 1990). Such substances include the reducing nitrite, thiocyanate and ferrous ions and the oxidising bromate and permanganate ions.

Thiocyanate can be a particular problem in the determination of urinary iodine because it is found in several foods consumed in iodine-deficient areas, including cassava, the staple food in many areas of sub-Saharan Africa, where IDD is endemic. It is also produced by cigarette smoking and high levels of thiocyanate are found in the urine of heavy smokers. Thiocyanate elevates the apparent iodide level in the urine, thus masking the true extent of iodine deficiency as measured by urinary iodine. Goitrogens are discussed in section 12.

1.9.3.3. Neutron Activation Analysis

This is a sophisticated technique, requiring expensive instrumentation and is therefore not suitable for use in developing countries. In addition, this method cannot be used for large numbers of samples in a routine manner. It is, however, sensitive and specific, once chlorine and bromine have been removed from the sample, and results compare well with those obtained from Sandell-Kolthoff-type determinations (Hellstern, Keller and Weinheimer, 1980; Holzbecher and Ryan, 1980).

1.9.3.4. Iodide-selective Ion Electrode

This method, though fairly simple, is not sensitive enough to detect the low levels of iodide found in iodine-deficient areas (Bourdoux, 1988) but it has been successfully used to measure iodine-overload in patients on iodinated drugs such as amiodarone (Mura, Piriou, Guillard *et al.*, 1986).

1.9.3.5. Other Methods

Normal pulse polarography (Turner, 1975), complex formation with thiocyanate (Clarke and Skoog, 1975), neutron activation, coupled to mass spectrometry (Rook, Suddueth and Becker, 1975), HPLC (Hurst, Stefovic and White, 1984) and Gas-liquid chromatography have been proposed as methods for measuring iodide but they require sophisticated, expensive equipment and cannot handle large numbers of specimens. Their use is restricted to research.

1.10. Measurement of Serum Thyroid Hormones

1.10.1. TSH

Measurement of serum thyroid-stimulating hormone levels provides a good estimate of thyroid status for two reasons:

1. TSH synthesis and secretion are under the control of negative feedback by thyroid hormones so that serum TSH directly reflects the action of thyroid hormones in the pituitary thyrotrophs. If this action is paralleled in other organs, TSH should reflect overall thyroid status (Bayer, 1991).

2. The amplification of the TSH response, described in section 2.2., makes TSH a sensitive indicator of thyroid status (Bigos, Ridgeway, Kourides *et al.*, 1978).

For these reasons, serum TSH level is the first choice biochemical test for assessing thyroid status, although it does not always reflect the momentary thyroid status of a patient who has started therapy for hypo- or hyperthroidism, as resetting of pituitary TSH can take several weeks to reach a new steady state between T_4 and TSH.

1.10.1.1. Radioimmunoassays

Serum TSH concentrations have been measured by competitive binding radioimmunoassays (RIAs) over the past 20 years, with concentrations expressed in bioassayable International Units (IU) compared to a reference standard (Kaplan, 1985). These could measure TSH levels corresponding to hypothyroid and lower euthyroid states but were too insensitive to measure the low levels of TSH found in hyperthyroidism and discriminate between these and normal levels. In addition, the specificity of the RIAs was rather low, and cross-reactivity with luteinizing hormone (LH), follicle-stimulating hormone (FSH) and human chorionic gonadotropin (hCG) was often considerable.

1.10.1.2. Immunometric Assays

In the early 1980s more sensitive immunometric assays (IMAs) appeared, in which TSH was determined by the use of two different monoclonal antibodies which recognize different epitopes on the TSH molecule. One of the antibodies is specific to the ß-subunit of the TSH molecule and one is immobilised on a solid surface, or has a magnetic particle attached to facilitate separation in a magnetic field.

Most methods are two-step, invlolving initial binding of TSH to the solid phase antibody, separation of the solid phase and quantification of bound TSH by using a second antibody, labelled with either I^{125}, an enzyme, or a fluorescent of chemiluminescent tag (Weeks and Woodhead, 1984; Toft, 1988; Woodhead and Weeks, 1985; Libeer, Simonet and Gillet, 1989).

Sensitivity has been greatly enhanced and cross-reactivity with LH, FSH and HCG reduced and these newer tests discriminate between hyperthyroid and euthyroid patients better than any previous test. Over 30 commercial IMA kits for determination of TSH are available so standardization between assays and laboratories is needed, if a single TSH result is to be reliable (Bayer, 1991).

The normal range, in a Western population, for TSH is about **0.5-6 mIU/l**, depending on the particular assay used.

1.10.2. Thyroxine (T_4)

More than 99% of plasma T_4 is protein-bound so results of assays of total T_4 (TT_4) therefore reflect the protein-bound, rather than the free, physiologically active, thyroxine (FT_4). In the absence of binding protein abnormalities, changes in thyroid secretion produce parallel changes in bound (or total) and free thyroxine. When these inherited or acquired, temporary abnormalities are present, total T_4 levels can vary drastically, with no observed thyroid dysfunction. Total T_4 should therefore not be used as a reliable indicator of individual thyroid status (Larsen, Alexander, Chopra *et al.*, 1986), although it can be useful at a population level, where the proportion of people with binding protein abnormalities will be small.

1.10.2.1. TT_4 Assays

Total T_4 is usually measured by radioimmunoassay, competitive protein-binding assay or enzyme-, fluorescent- or chemiluminescent-linked immunoassays, as described for TSH. Determination of TT_4 has, however, recently been superceeded by measurements of free T_4 as first-line tests for assessing thyroid function (Larsen, Alexander, Chopra *et al.*, 1986).

The normal range, in Western populations, for TT_4 is approximately **60-140 nmol/l**, depending on method.

Several methods exist for measuring FT_4 but not all are suitable for routine clinical testing. Amongst those methods which fall into this category are "indirect" tracer equilibrium dialysis (ED) (Sterling and Brener, 1966), "direct" equilibrium dialysis, in which FT_4 is measured directly in the dialysate by a sensitive RIA (Nelson and Tomei, 1988) and ultrafiltration. ED is the reference method for FT_4, against which other methods are evaluated.

Common to all the FT_4 immunoassays (IAs) is the premise that the assay does not disturb the equilibrium between bound and free T_4. To achieve this, a very low concentration of high-affinity anti-T_4 antibody is used (Ekins, 1985). There are two approaches used in the many kits, commercially available:

1.10.2.2. FT_4 Immunoassays

two-step immunoassays: FT_4 is first immunoextracted, in a concentration-dependent manner, by incubation of serum with a solid-phase T_4 antibody, often a coated tube, or with an antibody which is attached to a magnetic particle. The unbound serum is removed and a T_4 tracer added to quantitate the unoccupied sites on the T_4 antibody. The tracer may be linked to I^{125}, an enzyme or a fluorescent or chemiluminescent particle, as described for TSH and the FT_4 concentration is inversely related to the tracer bound to the antibody. In these IAs, tracer is added after removal of the serum binding proteins, so that interactions between the two are excluded. These assays are thus more accurate than:-

one-step, analog immunoassays: The tracer, a labelled T_4 derivative (analog), and serum FT_4 compete directly for antibody, which is added to the serum simultaneously with the tracer. Although simple in concept, the assay is difficult to realize in practice, because it is extremely important that the T_4 analog does not interact with the serum binding proteins, but does bind to the anti-T_4 antibodies. In fact, evaluations of FT_4 IAs indicate that some tracer analogues interact significantly with serum binding proteins and FT_4 results in some patient groups can be strongly method-dependent (Csako, Zweig and Glickman, 1989).

In patients with simple thyroid disease, such as hypothyroidism due to iodine deficiency, which is not complicated with other factors, almost all FT_4 IAs have a diagnostic accuracy of 90-100% but in patients with altered thyroid binding proteins, FT_4 IAs often produce conflicting results (Bayer, 1991).

1.10.2.3. FT_4 Index

FT_4 index is an estimate of FT_4, calculated from two test results: TT_4 concentration (by IA) and thyroid hormone binding ratio (THBR), a new term for T_3 or T_4 uptake (Larsen, Alexander, Chopra *et al.*, 1986), also known as thyroid hormone binding index (THBI). THBR estimates FT_4 (or FT_3) indirectly, by measuring how much labelled T_4 or T_3, added to serum, remains free for binding to a matrix, such as talc, ion-exchange resin or charcoal, or to albumin, anti-T_3 or anti-T_4 linked to a solid surface (Bayer, 1991).

Older THBR tests use I^{125} or enzyme labels but newer fluorescence polarization IAs (FPIAs) measure thyroxine binding capacity (TBC) of serum more directly, by means of a fluorescein-labelled T_4 analog that changes its signal when bound to serum proteins. These tests were designed to correct for binding protein abnormalities, so are more useful in a clinical situation, for managing patients with these conditions, than for assessment of thyroid function in a population.

The normal range, in Western populations, for FT_4 is approximately **10-25 pmol/l**, depending on method.

1.10.3. Tri-iodothyronine (T_3)

T_3 is approximately three times more metabolically active than T_4, mediating the majority of thyroid hormone actions at a molecular level, as described in section 1.1.5. It is also less expensive, in terms of iodine atoms, containing only 3 iodine atoms, compared with the 4 in a thyroxine molecule. Determination of T_3, however, should not be used to diagnose hypothyroidism because euthyroid T_3 levels are maintained, at the expense of T_4 secretion, by a failing thyroid gland, under the stimulation of increased TSH. Peripheral T_3 levels are thus insensitive indicators of hypothyroidism.

Measurement of T_3 concentration is valuable for diagnosing T_3 toxicosis and less common forms of hyperthyroidism but the same care over interpretation of free/bound T_3 levels apply as for T_4. Binding abnormalities are fewer than for T_4 (Bayer, 1991).

Serum reverse T_3 (rT_3) is usually elevated in hyperthyroidism and low in hypothyroidism, although hypothyroid and normal ranges overlap considerably. Its measurement may be useful in discriminating between truly hypothyroid patients and those who have low FT_4 levels due to nonthyroidal illness (Chopra, Solomon, Hepner *et al.*, 1979).

Methods for total T_3 are similar to those for total T_4. Little evaluation of FT_3 methods has been done and it is not clear whether or not FT_3 is clinically superior to TT_3 (Bayer, 1991), although many service laboratories are routinely measuring free, rather than total, hormones.

In western populations:
The normal range for TT_3 is approximately **1.0-3.2 nmol/l.**
The normal range for FT_3 is approximately **4.0-7.5 pmol/l.**

1.10.4. TRH-Test

Abnormalities of TSH secretion can be magnified by administration of synthetic TRH (protirelin) (Hershman, 1974). Serum TSH is measured before and 30 minutes after injection of TRH. A sharp rise in TSH is seen in hypothyroidism which due to thyroid damage, whereas the TSH rise in hypothyroidism due to pituitary or hypothalmic disease may be absent, blunted, delayed or prolonged (Kaplan, 1985). Accurate determination of serum TSH is crucial to the interpretation of the TRH-test.

1.10.5. Other Tests

Serum thyroglobulin levels may be elevated in a variety of conditions, including neoplasms and goitre, but because of the non-specific nature of the elevation interpretation is problematic. It is most useful as a marker for residual, recurrent or metastatic disease in patients with a known thyroid carcinoma (Van Hearle and Uller, 1975).

Thyroid antibodies and autoantibodies may also be useful in the diagnoses of Hashimoto's thyroiditis, Grave's disease and some other conditions which are difficult to distinguish on results of more common hormone tests.

1.11. Other Causes of IDD - Goitrogens

According to Greer (1962), 96% of human goitre is caused by dietary insufficiency of iodine but it may also be precipitated by other factors, such as consumption of contaminated drinking water or dietary goitrogens, PEM or vitamin A deficiency. Therefore, before deciding on intervention measures suggested by an assessment of the extent of IDD, it is necessary to ask if there are any special factors which influence the severity and extent of IDD in a particular community.

1.11.1. Water Sources

High mineral contents, particularly of lithium, magnesium or calcium salts (Spaulding, 1989; Koutras, 1980; Langer, 1960) have been implicated as goitrogenic factors in drinking water (although the evidence regarding the goitrogenic effects of the latter two are somewhat conflicting) as have many compounds found in rocks of organic origin, such as coals and shales. Contaminants in these rocks include phenols, sulphurated organic compounds, pyridines and halogenated or polycyclic aromatics (Gaitan, 1986a,b; Gaitan, 1987; Gaitan, 1990b).

In addition, some goitrogenic action has been observed in water contaminated with certain micro-organisms (Orr and Leitch, 1929; Gaitan, 1973; Vought, Brown and Sibinovic, 1974; Gaitan, Medina, DeRouen *et al.*, 1980) In particular, *E. coli* and *Yersinia enterocolitica* are thought to induce the production of antibodies which are thyroid-growth promoting and therefore goitrogenic (Weiss, Kasper and Ingbar, 1982). In contrast, the concentration of *K. pneumoniae* was found to be inversely related to goitre prevalence in one study, suggesting a biodegredation of organic contaminants which cause goitre (Gaitan, Medina and DeRoen, 1980).

1.11.2. Dietary Goitrogens

Certain substances found in some foods can interfere with thyroid hormone synthesis or release and induce goitre, although the consumption of the food alone is unlikely to be the sole cause of the goitre (Tookey, Van Etten, Daxenbichler, 1980). In individuals who have a low or marginal iodine status, consumption of such foods can precipitate clinical manifestations of IDD such as goitre and hypothyroidism.

Some goitrogenic agents act directly on the thyroid by inhibiting iodine-uptake, iodine organification to form thyroid hormones or release of thyroid hormones and their breakdown. Other goitrogenic agents act indirectly by interfering with thyroid hormone binding or the peripheral action of the thyroid hormones (Gaitan, 1986b).

A common example of a dietary goitrogen is cassava, which is extensively cultivated in many developing countries and contains cyanogenic glucosides which are hydrolysed to liberate cyanide. This, in turn, is converted to thiocyanate which can isomerise to isothiocyanate, a blocker of thyroid hormone synthesis, causing increased kidney excretion of iodide (Greer, Stolt and Milne, 1966.)

Other goitrogenic foods include many members of the brassica genus (*Cruciferae* family) such as turnips, cabbage, kale, broccoli and cauliflower, and also millet (Gaitan, 1990a; Gaitan, Lindsay, Reichert *et al.*, 1989.)

1.11.3. PEM and Vitamin A Deficiency

There is evidence that PEM can contribute to IDD by altering thyroid gland morphology and function (Gaitan, Cooksey and Lindsey, 1986; Medeiros-Neto,1989). In this way, thyroidal exchangeable iodine, a measure of iodine stores, and urinary iodine excretion are both reduced, indicating a depletion of iodine stores, although hormone secretion may be adequate.

Protein-deficient rats, with impaired thyroid function associated with defective iodine transport, have had their thyroid abnormalities reversed on administration of sufficient protein to remove their deficiency (Gaitan, Mayoral and Gaitan, 1983).

Studies in Senegal have shown that vitamin A deficiency increases the severity of IDD and similar work in Tanzania has supported these findings (Inglebleek and De Visscher, 1979.) The reduced levels of retinol may impair glycosylation of thyroglobulin and its subsequent iodination to form thyroid hormones.

1.12. Methods of Controlling IDD

In view of the wide spectrum of disorders induced by iodine deficiency, the following table has been suggested as a guide to the necessity of action.

Stage	Goitre	Hypothy -roidism	Cretinism	TGR (%)	UIE μg I/dl	Need for Action
I Mild	+	0	0	10-30	3.5-5.0	Important
II Moderate	++	+	0	20-50	2.0-3.5	Urgent
III Severe	+++	+++	++	30-100	<2.0	Critical

Table 1.12. IDD severity and need for action. Source: Dunn and van der Haar, 1990.

1.12.1. Early Cures for Goitre

A relationship between dietary iodine deficiency and goitre was first suggested in 1820, by Coindet, but early attempts to reduce goitre prevalence and size were not very successful, owing to the very high amounts of iodine used which led to toxic side-effects, such as "**Jodbasedow Syndrome**", in which excessive sweating, heart disturbances, tremors and wasting were noted. In 1831, Boussingault reported the use of Guaca sea-salt as a cure for goitre, prevalent in the Andes, noting that the salt was rich in iodine. Boussingault suggested the use of iodized salt in preventing goitre but his attempts to use it also met with opposition because of the side-effects mentioned above.

1.12.2. Supplementation Trials

Baumann's discovery of iodine in the thyroid, in 1895, led to renewed interest in the relationship between iodine and goitre and to controlled trials with schoolchildren. In these, children provided with a daily drop of tincture of iodine experienced a reduction in goitre size and were observed to have accelerated growth rate and mental development, compared to those not receiving the iodine (Orr and Leitch, 1929).

The classic experiment by Marine and Kimball (1921), in Akron, Ohio, involving supplementation with sodium iodide, daily for 10 days, twice a year, confirmed the earlier results and showed a reduction in goitre size and prevalence in those schoolchildren receiving the supplement, compared to those who did not. At about the same time, certain water plants, including watercress, were shown to be rich in iodine and effective in treating goitre (Orr and Leitch, 1929.)

Controlled trials and supplementation programmes, reviewed in section 19, have shown that IDD can be largely prevented and controlled by correction of the iodine deficiency (Lamberg, 1991; Tonglet, Bourdoux, Minga *et al.*, 1992) and socioeconomic advancement, which often leads to the diversification of diet to foods produced outside the iodine-deficient area (Thilly and Hetzel, 1980.)

Today, iodine intake is artificially increased by several methods: consumption of iodized salt, administration of iodinated oil and fortification of water, bread or condiments with iodine. A number of strategies for controlling IDD are being used and these are reviewed below, with particular emphasis on the use of iodinated oil. Most of the iodine used in fortification and supplementation programmes is produced from brine wells in the USA, Japan and Chile (Hetzel and Maberly, 1985.)

1.13. Iodized salt

This was first used on a large scale in Switzerland in the 1920s (Hetzel, 1989), where it helped to eradicate endemic cretinism. It is now widely used throughout the world, particularly in developed countries but now increasingly in the developing world. Some dramatic results have been reported in China and India (Hetzel, 1987; Sooch, Deo, Karmarkar *et al.*, 1973) amongst other countries.

Salt is a suitable vehicle for fortification because of its universal consumption (Mannar, 1987) and because an individual's daily consumption is fairly constant, unlike many other foods. Intake varies from country to country, however, so fortification levels need to be adjusted accordingly. In general, the fortification level is such that the daily requirement is provided - at least 100-150 μg per day - and this is achieved through the addition of 20-60 mg iodine /kg salt (Lamberg, 1991.)

1.13.1. Iodization Process

Two sources of salt are commonly used:

Potassium iodide (KI) is cheap but less stable.

Potassium iodate (KIO_3) is more expensive but more stable in heat and humidity and more resistant to evaporation.

Strictly speaking, salt which is fortified with potassium iodate is called "iodated" but in practice, both types are referred to as "iodized".

The iodide or iodate can be added to salt on a conveyor-belt in a variety of processes, including dry-mixing, drip-feed (a liquid solution is dripped on at a constant rate), spray method (a fine spray is applied at a constant pressure) and submersion (the salt passes through a saturated solution of the iodide/iodate and is then dried.) Drip-feeding and submersion are cheaper but less reliable, giving uneven distribution of iodine.

It is relatively cheap to introduce iodine into the salt trade when the product is refined, comes from a few production sites and has an extensive, effective distribution network. It is more difficult to introduce iodized salt when there are many sources of salt, for

example, salt mines, rock salt, evaporation from seawater or inland lakes, and communications are poor.

1.13.2. Advantages of Iodized Salt

1. Salt is a dietary requirement.
2. Sources are often limited and therefore easily controlled.
3. The technology required is simple.
1.13. The intervention can be long-term.
5. It is the most cost-effective choice of prophylaxis where. there is a well-developed salt industry.

1.13.3. Potential Problems of Iodized Salt

1. Salt needs to be intercepted at some stage in its path to the consumer and this may be difficult to regulate where there are many sources.
2. Impurities in the salt may affect the iodization process.
3. Distribution networks need to be efficient, delivery of salt to remote areas can be problematic.
1.13. The stability of the iodide or iodate is affected by climatic conditions such as humidity, heat and sunlight, so storage conditions and times need to be controlled.
5. Packaging must be designed to minimise such storage losses.
6. Consumer preference may be for coarse, non-iodized salt which can also be used for animals.
7. Cultural habits such as washing salt before use may lead to further loss of iodide/iodate. (Mannar, 1988)

Inefficient iodized salt programmes also fail to ensure that the person at risk from iodine deficiency is receiving sufficient iodine in the salt they consume. When the iodization of salt cannot be implemented quickly or where iodine deficiency is particularly severe, more immediate measures such as iodinated oil may be necessary.

1.14. Iodinated Oil

This is a major alternative to prophylaxis with iodized salt which is particularly useful for communities in geographical isolation to which delivery of iodized salt is problematic (Ibbertson, 1979; Filetti, Squatrito and Vigneri, 1985; Dunn, 1987)

Iodine can be covalently attached to the unsaturated carbon atoms of fatty acids in a simple chemical reaction. Poppy-seed, soy bean, walnut and recently peanut oils have all been successfully used. The oil is stable and requires no refrigeration - an important property, as there is no cold-chain in many of the remote areas where it is distributed.

The most widely used commercial preparation is Lipiodol®, a poppy-seed oil containing 38% iodine by weight, made by Laboratoire Guerbert in Paris, France. 1 ml of this product contains 480 mg of iodine - approximately 30 times the amount of iodine stored in the body. It is marketed in 10 ml vials, suitable for intramuscular injection or oral administration or in capsules containing 200 mg iodine each. The Fourth Pharmaceutical Company of Wuhan in China manufactures another commercial preparation using soybean or walnut oil, containing 24-28% iodine by weight, which is also available in 200 mg capsules. The International Council for Control of Iodine Deficiency Diseases (ICCIDD) has made efforts to encourage the introduction of cheaper products onto the market (Dunn, 1987).

In the late 1950s Clarke, McCullagh and Winikoff (1960) pioneered work on intramuscular injections of iodinated oil in PNG, using 4 ml injections of Lipiodol and showed it to be an effective prophylaxis against goitre (McCullagh, 1963.) Similar studies in Africa, Asia and South America supported these findings, although a variety of doses were used (Delange, 1974; Dunn, 1987.)

Since then, over 20 million people have received injections of iodinated oil and a further estimated 56 million people have received prophylaxis by oral iodinated oil (Dunn, 1990.) Iodinated oil has been found to be highly effective in correcting iodine deficiency in many populations, although the value of giving it to people over the age

of 45 or to individuals with nodular goitre is uncertain (Dunn, 1987.) Despite its widespread popularity, there is little objective evidence of the prevalence of side effects, biochemical or clinical, or duration of protection.

1.14.1 Intramuscular Administration of Iodinated Oil

A single, intramuscular injection of iodinated oil of 0.5-1.0 ml protects from iodine deficiency for 3-5 years and possibly for longer at higher doses (Dunn, Thilly and Pretell, 1986; Hetzel, Thilly, Fierro-Benitez *et al.*, 1980.)

1.14.1.1. Metabolism

Iodine is slowly released from the injection site, usually the muscles of the upper arm, into the blood, where it is deiodinated and taken up by the thyroid, stored in the adipose tissue or excreted as inorganic iodide in the urine. Pretell (1972) used radioactively-labelled Lipiodol in a study which showed that 87% of the injected oil was still at the site of injection 23 days later. Animal studies of the metabolism of iodinated oil, following different delivery modes have supported this finding (Wei and Li, 1985.)

1.14.1.2. Advantages of Iodinated Oil Injections

1. One injection lasts 3-5 years so there is a good, sustained duration effect, which may be important for remote communities which are unlikely to be revisited within 3 years.
2. Administration can be implemented quickly and by-pass the delays which affect the introduction of iodized salt.
3. Direct contact is made with the populations at risk of IDD, unlike iodized salt.

1.14.1.3. Potential Problems of Iodinated Oil Injections

1. Personnel need to be trained in administering the injections, sterile precautions and proper handling and disposal of materials, particularly needles.
2. Appropriate disposal of needles must be rigorously controlled to combat the risk of AIDS and Hepatitis B
3. There is immediate discomfort at the injection site and the risk of infection and abscesses developing.

1.13. Direct contact must be made with each subject by the injection team - tracing non-attenders can be very time-consuming.

5. The cost of importation of oil, syringes and needles, and the training of staff are relatively high.

1.14.2. Oral Dosing with Iodinated Oil

Iodinated oil, given by mouth in a dose of 400 mg (two capsules) is thought to protect against IDD for 1-2 years, about half the time of an equivalent intramuscular dose (Watanabe, Moran, El Tamer *et al.*, 1974; Kywe, Tin, Khin *et al.*, 1978; Lu and Ma, 1985, Eltom, Karlsson, Kamal *et al.*, 1985.) This reduced coverage time means that distribution of the supplement must be more frequent than if it is given by injections and it is therefore not suitable for prophylaxis in extremely remote communities.

1.14.2.1. Metabolism

The oil is absorbed from the gut into the circulation where it is promptly deiodinated to release inorganic iodide. The iodide is then excreted in the urine or stored in the thyroid and adipose tissue. The body's use of oral iodinated oil is not as efficient as that of intramuscular oil, mainly due to incomplete absorption and an absence of a slowly-released store of iodine in the muscle.

In normal individuals, absorption of the iodinated oil is high at over 90% (Groen, 1948) but factors which limit absorption such as diarrhoea, malabsorption syndromes, PEM and the presence of intestinal parasites (particularly prevalent in most developing countries) may limit the uptake of iodine from the gut (Ingenbleek and Beckers, 1973.) There is some evidence that absorption is enhanced when the subject is in a fasting state at the time of capsule administration (Chiwona, 1991.)

Once absorbed, much of the iodine is excreted as inorganic iodide in the urine. Animal studies by Wei and Li (1985) showed that 83% of orally administered iodine was lost in the urine in the first few days, compared with only 8% from intramuscular iodine. Human studies have shown higher UIE from oral administration at first but lower levels

later, when compared to UIE levels from intramuscular administration (Lu and Ma, 1986; Eltom, Karlsson, Kamal *et al.*, 1985; Boudiba, Bachtarzi and Benmiloud, 1985.) There are, however, wide variations between individuals during the first few days (Eltom, Karlsson, Kamal *et al.*, 1985.)

The state of the storage tissues (the thyroid and adipose tissue) will also affect the efficiency of iodine utilisation. Adipose tissue has a much higher turnover than muscle, so iodine is more easily lost when its main store outside the thyroid is fat, as in oral administration, rather than the muscle at the site of injection.

In particular, individuals in negative energy balance, who are losing adipose tissue may retain less absorbed iodine than those in positive energy balance, who are laying down adipose tissue. Alterations to thyroid gland structure and function which increase turnover may also reduce the effective retention time. This is known to occur in PEM (Ingenbleek and Beckers, 1973; Ingenbleek and De Vischer, 1979; Gaitan, Mayoral and Gaitan, 1983.) Certain febrile conditions which induce catabolic states may also reduce the effective retention time, as iodine-containing proteins are more rapidly broken down than usual.

1.14.2.2. Advantages of Oral Iodized Oil

In addition to advantages 2 and 3 mentioned for intramuscular oil above, oral administration has some additional advantages over intramuscular injections:

1. Little staff training is required, Primary Health Care services, local teachers and village leaders have been successfully used in distribution programmes.
2. There is no risk of AIDS or Hepatitis infection from contaminated needles.
3. Direct, invasive injection is avoided and with it the risks of discomfort, abscesses and infection.

1.13. Direct contact does not need to be made with each subject - capsules can be distributed when convenient to the subjects.

5. The cost is considerably less than for intramuscular injections since fewer syringes are required if oil is being given by drops (direct contact with the subject is not made,

so the syringes can be reused) and not at all if by capsule. Needles are not required and staff costs are much lower.

1.14.2.3. Potential Problems of Oral Administration

1. Coverage is only effective for 1-2 years, necessitating more frequent contact with the communities at risk of IDD, so may be impracticable for very remote areas.
2. The cost is still considerably higher than iodization of salt.
3. There is the possibility of transient biochemical hypothyroidism.

1.15. Other Methods of Control

In some circumstances other control measures have been used but they do not, in general, have the wide applicability of the techniques described above.

1.15.1. Inorganic Iodide

The controlled trial in Ohio, conducted by Marine and Kimball (1921) used sodium iodide tablets and in Tasmania, Clements, Gibson and Howeler-Coy (1968) used weekly 10 mg tablets of potassium iodide, distributed through schools and child health centres. Although some improvement was seen in the prevalence of goitre, co-operation at some of the centres was poor and an alternative strategy of iodizing bread was tried with better success (Clements, Gibson and Howeler-Coy, 1970.) Wei and Li's animal studies (1985) showed that very little of the ingested iodide was retained (97% excreted in the first few days) so frequent dosing is required using this method of prophylaxis.

1.15.2. Lugol's Solution

This saturated solution of iodine in potassium iodide is commonly used as an antiseptic in rural hospitals in developing countries. It can be useful in treating patients after thyroidectomies but is stored only by thyroid recycling, so has a very short duration of effect and needs to be administered frequently, generally daily or several times a week. It has to be given in carefully controlled doses and therefore needs to be distributed by responsible persons in the community. A small programme in Bolivia has used teachers and household heads to distribute the solution (Dunn and van der Haar, 1990.)

1.15.3. Water Iodization

Iodine can be added directly to drinking water to correct iodine deficiency (Maberly, Eastman and Corcoran, 1981.) Sometimes this is done by adding a measured amount of iodine, potassium iodide or iodate to a jar of drinking water in the home, to supply about 150 ug of iodine a day. This method is used in northern Thailand.

Alternatively a public supply of drinking water may be iodized by diverting some of the water through a canister, containing crystals of iodine, and reintroducing this to the main water flow (Dunn and van der Haar, 1990.) This method has been successfully implemented in parts of Indonesia, Thailand and Sicily (Hetzel, 1989; Squatrito, Vigneri, Runello *et al.*, 1986.)

It is a suitable method for prophylaxis, only when a specific source of drinking-water can be identified, as less than one percent of a general water supply is used for drinking purposes. There is the additional benefit of antiseptic action on the water.

1.15.1.13. Iodized Bread

This has been used in Holland and Tasmania, with some success (Stewart, Vidor, Buttfield *et al.*, 1971; Clements, Gibson and Howeler-Coy, 1970), but is no longer needed due to a much greater diversity of dietary iodine sources being available. In particular, the iodophors used in the dairy industry have greatly increased the levels of iodine in milk consumed in most developed countries (Hetzel, 1989.)

1.15.5. Iodized Condiments

In parts of Southeast Asia and China, iodized fish and soy sauces have been useful in reducing the prevalence of goitre (Hetzel and Maberly, 1985).

1.16. Changes in Thyroid Function During Pregnancy

The hormonal changes and metabolic demands of pregnancy affect many aspects of thyroid hormone economy with the result that complex changes in thyroid function occur in order to maintain a clinically "euthyroid state" throughout the pregnancy and meet the increased demands for iodine, due to foetal development. Conversely, thyroid disorders can compromise reproductive function, contributing to menstrual disorders, infertility and recurrent pregnancy losses.

Before examining the pathology of thyroid disorders in relation to pregnancy, particularly in areas of low iodine intake, an understanding of normal thyroid physiology in a "euthyroid" pregnancy is necessary. Several longitudinal studies have shaped our understanding of the changes in thyroid function which accompany pregnancy, although not all findings are in agreement (Yamomoto, Amino, Tanizawa *et al.*, 1979; Guillaume, Schussler, Goldman *et al.*, 1985; Thomas and Reid, 1987; Price, Griffiths and Morris, 1989; Berghout, Endert, Ross *et al.*, 1994).

Many of the reported changes in thyroid function during pregnancy, although significant, are within the normal range for non-pregnant women. Some of these changes are shown in figure 1.16.

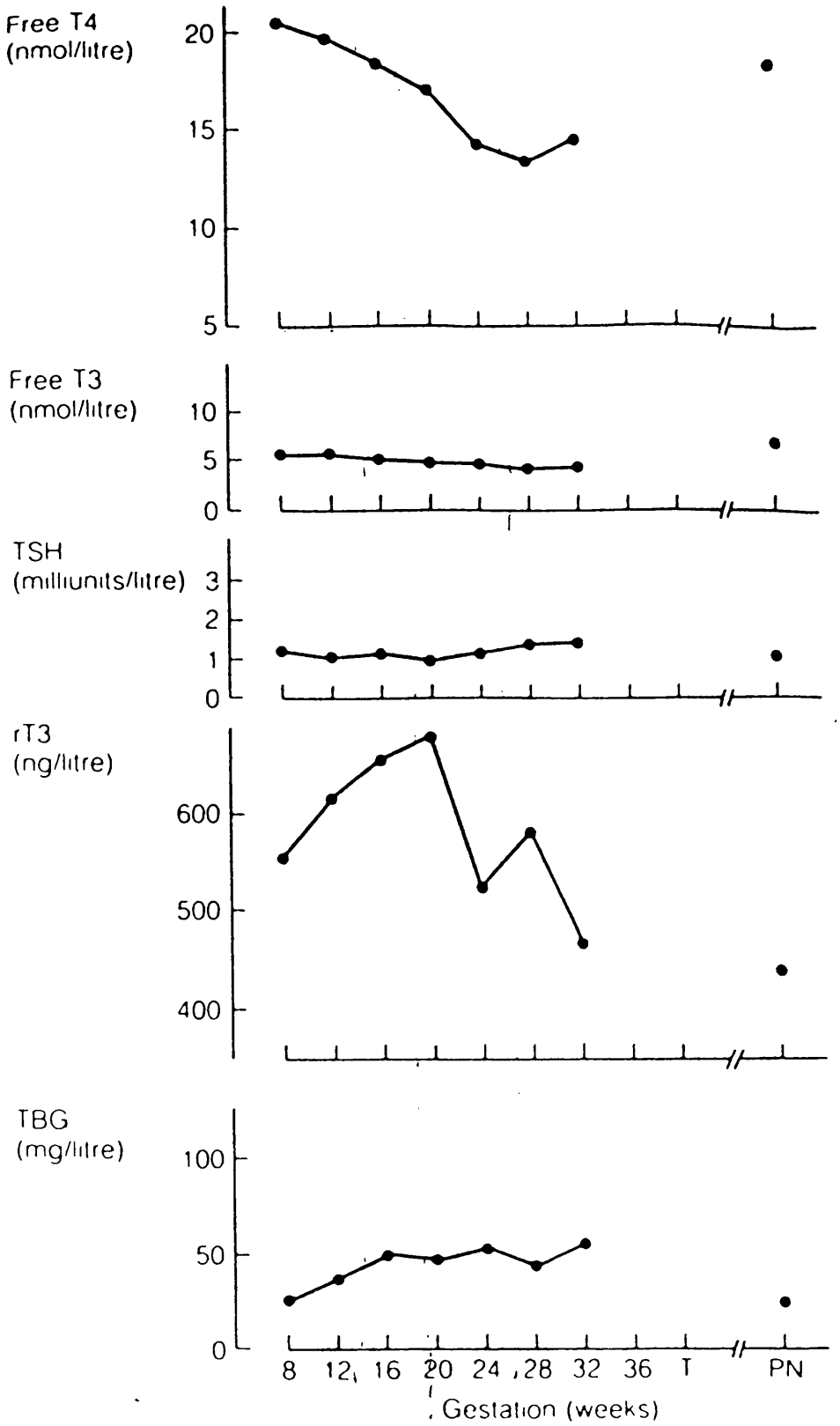


Figure 1.16. Alterations in thyroid function tests during pregnancy. Source: Adapted from Rodin and Rodin, 1989.

1.16.1 Thyroid Histology

The thyroid gland continues active synthesis and secretion of hormones with well developed follicles and abundant colloid. Papillary hyperplasia, columnar epithelium and increased blood supply may be found (Stoffer, Koencke, Chesky *et al.*, 1957) with enlargement of the gland (Glinoer, De Nayer, Bourdoux *et al.*, 1990; Berghout, Endert, Ross *et al.*, 1994). Serum thyroglobulin (TG) has also been reported to increase in pregnancy (Rasmussen, Hornnes, Hegedus *et al.*, 1989; Glinoer, De Nayer, Bourdoux *et al.*, 1995) and if the circulating iodide pool is reduced, iodine uptake by the thyroid gland increases (Burrow, 1989).

1.16.2. Goitre Development

In Ancient Egypt, goitre, assessed by the snapping of a reed tied around a woman's neck, was viewed as a positive indication of pregnancy and as recently as 1964, up to 70% of pregnant women in Scotland and Ireland were reported to have a visible or palpable goitre (Crooks, Tulloch, Turnbull *et al.*, 1964). Some authors have disputed the notion that thyroid size increases during normal pregnancy, as applicable only to areas of sub-optimal iodine intake (Berghout, Endert, Ross *et al.*, 1994; Levy, Newman, Rejali *et al.*, 1980). In areas of marginal iodine deficiency, development or enlargement of goitre is often seen in pregnancy, despite seemingly normal thyroid function tests and may be only partially reversible postpartum (Rasmussen, Hornnes and Hegedus, 1989; Glinoer and Lemone, 1992; Glinoer, Lemone, Bourdoux *et al.*, 1992). In iodine-sufficient areas, most of the goitres of pregnancy are related to autoimmune thyroid disease, non-toxic goitre and subacute thyroiditis (Long, Felice and Hollingsworth, 1985).

1.16.3. Binding Proteins

The concentration of circulating thyroxine-binding globulin (TBG) rises markedly in early pregnancy, to approximately double its non-pregnant value, with maintenance of a plateau, after the first half of gestation. This increase in serum TBG concentration may be attributed to oestrogen-enhanced TBG sialylation in the liver, leading to an increased half-life of TBG in the serum. The serum levels of the two other major binding proteins, albumin and TBPA, fall during pregnancy (Ain, Mori and Refetoff, 1987; Ain and Refetoff, 1988; Ball, Freeman, Holmes *et al.*, 1989).

1.16.4. T_4, T_3 and rT_3

Increases in TBG are accompanied by increases in total T_4, T_3 and rT_3 following adjustment of the thyroid machinery to maintain homeostasis of free hormone levels. This is not mediated through the normal feedback mechanism, involving TSH, but by human chorionic gonadotropin (hCG). This hormone is produced in large quantities during early pregnancy by the placenta, and acts as a weak thyrotropic hormone, directly stimulating the maternal thyroid gland to increase iodide uptake (Hersham, Lee, Sugawara *et al.*, 1988; Yoshikawa, Nishikawa, Horimoto *et al.*, 1989). Thus, free T_4 and free T_3 levels are generally normal or elevated in the first trimester and gradually fall to low-normal or low by the third trimester, returning to normal levels by 6 weeks post-partum (Yamamoto, Amino, Tanizawa, *et al.*, 1979; Guillaume, Schussler, Goldman *et al.*, 1985; Price, Griffiths and Morris, 1989; Ballabio, Poshyachinda and Ekins, 1991; Glinoer, De Nayer, Bourdoux *et al.*, 1995).

1.16.5. TSH

That TSH is not the main agent in maintaining free thyroid hormone levels in early pregnancy is shown by its normal or low serum concentration in the first trimester. During the second and third trimesters, TSH levels rise within the normal range, as free thyroid hormone levels fall and hCG exerts a reduced effect on thyroid hormone production (Guillaume, Schussler, Goldman *et al.*, 1985; Chan and Swaminathan, 1988; Ball, Freeman, Holmes *et al.*, 1989; Price, Griffiths and Morris, 1989). The TSH response to TRH is increased, relative to the non-pregnant state, and this may be an oestrogen effect as it is seen in women taking oral contraceptives (Burrow, 1985).

1.16.6. Peripheral Metabolism of T_4

Some studies indicate that normal T_4 turnover of 80μg per day (or 10% of the circulating T_4 pool) appears to be maintained in pregnancy (Burrow, 1989) but there is increased deiodinating activity in the placenta and acceleration of the peripheral metabolism of thyroxine may increase hormonal needs (Roti, Gnudi and Braverman, 1981; Yoshikawa, Nishikawa, Horimoto *et al.*, 1989).

1.16.7. Renal Iodide Clearance

This is increased in pregnancy and may be accompanied by a fall in serum inorganic iodide (Abdoul-Khair, Crooks, Turnbull *et al.*, 1964). Urinary iodine excretion is, thus, expected to increase during pregnancy.

1.16.8. Basal Metabolic Rate (BMR)

BMR rises slowly after the first trimester and increases by 15-30%, due to the increase in body mass and the work of conceptus and uterus (Mussey, 1938; Burwell, 1954). BMR is a good reflection of clinical assessment of thyroid status but is cumbersome, prone to inaccuracies and rarely measured today.

1.17. Thyroid Disorders and Reproductive Dysfunction

A wide variety of reproductive problems may be consequent upon thyroid disorders but correction of the thyroid disorder generally results in normalisation of reproductive function (Thomas and Reid, 1987). Indeed, Greenman *et al.* observed that "The ability of a woman with a thyroid disorder to become pregnant, to carry a pregnancy to term and to produce an infant with adequate endowment for good development is diminished unless the disease is well controlled." (Greenman, Gabrielson, Howard-Flanders *et al.*, 1962)

1.17.1 Infertility and Anovulation

Chronic, severe primary hypothyroidism is often accompanied by amenorrhea and anovulation (Goldsmith, Sturgis, Lerman *et al.*, 1952; Thomas and Reid, 1987) and may be associated with hyperprolactinemia (Honbo, Van Herle and Kellet, 1978), galactorrhoea (Kleinberg, Noel and Frantz, 1977), enlarged sella turcica and high levels

of luteinizing hormone (LH) (Yamada, Tsuki, Ikerjiri *et al.*, 1976) via a hypothalamic dopamine turnover defect (Scanlon, Chan, Heath *et al.*, 1981). Milder hypothyroidism, accompanied by mild hyperprolactinemia, may be associated with infertility, via a luteal phase defect, and menorrhagia (DelPozo, Wyss, Tolis *et al.*, 1979; Bohnet, Fieldlerk and Leidenberger, 1981).

Early treatment of infertility in hypothyroidism with extracts of thyroid proved effective in restoring normal reproductive function (Litzenberg, 1926; Litzenberg, 1929) and today the defects mentioned above are reversed with thyroxine replacement therapy. Where the hypothyroidism is due to iodine deficiency, appropriate correction of the deficiency may restore normal reproductive function.

Mild to moderate thyrotoxicosis, which can be transiently induced after correction of iodine deficiency by supplementation with IPSO, does not appear to have any impact on fertility but severe hyperthyroidism may be associated with menstrual irregularities, including amenorrhea (Thomas and Reid, 1987). Control of thyrotoxicosis generally restores normal menstrual function and fertility.

1.17.2. Spontaneous Abortion

Several studies have shown that clinical hypothyroidism in pregnancy is associated with high rates of spontaneous abortion and stillbirth, up to twice the rate in euthyroid pregnancies, and may be linked with recurrent miscarriage. Pregnancy outcome in these hypothyroid cases was improved with thyroxine replacement therapy (Greenman, Gabrielson, Howard-Flanders *et al.*, 1962; Jones and Man, 1969; Niswander and Gordon, 1972; Winikoff and Malinek, 1975). Two mechanisms have been suggested to account for the increased risk of pregnancy failure associated with hypothyroidism (McMichael, Potter and Hetzel, 1980).

1) Prior to fetal thyroid ontogenesis, maternal thyroid hormone deficiency results in decreased availability of thyroxine to the fetus, possibly to below critical levels for fetal development.

2) Low maternal thyroid hormone levels may produce a less favourable environment for the fetus by reducing the passage of other hormones, essential for fetal development, or by failure of the Na^+K^+-ATPase component of the glucose membrane transport system .

1.17.3. Stillbirth, Perinatal, Neonatal and Infant Mortality

Studies in Papua New Guinea, Zaire and Tasmania support the observation that in iodine-deficient areas, there is increased incidence of abortion and stillbirth (Giroud, 1968) and demonstrate that correction of the iodine deficiency leads to a decrease in these reproductive losses and in perinatal deaths (Pharoah, Buttfield and Hetzel, 1971; Pharoah, Ellis, Ekins *et al.*, 1976; McMichael and Hetzel, 1977). Infant mortality due to congenital anomalies (discussed below) may also be increased in iodine deficiency (McMichael and Hetzel, 1977).

1.17.4. Congenital Anomalies

Early studies in maternal hypothyroidism reported high incidences of perinatal mortality and congenital anomalies, among women who were hypothyroid during pregnancy, compared to euthyroid pregnancies (Greenman, Gabrielson, Howard-Flanders *et al.*, 1962; Niswander and Gordon, 1972) but these studies were poorly characterised and may have overestimated the extent of the problem. Other data which suggest a link between maternal iodine deficiency and fetal malformations come from comparisons, over time, of deaths due to congenital anomalies in Western countries with and without iodisation programmes (McMichael, Potter and Hetzel, 1980), with a particular focus on neural tube defects (Horrowitz and McDonald, 1969). The role of iodine in CNS development is discussed in section 6.

Maternal antithyroid antibodies, present in auto-immune thyrotoxicosis, are implicated in some congenital anomalies associated with hyperthyroidism (Southerland, Esselborn, Burket *et al.*, 1960; Blizzard, Landing, Chandler *et al.*, 1960).

1.18. Iodine Supplementation in Pregnancy

The effectiveness of iodine in preventing goitre had been shown in Marine and Kimball's classic trial in Ohio (Marine and Kimball, 1921) and the geographical association between endemic goitre and cretinism had been recognised for centuries but, until the 1960s, no studies investigating a possible causative role for iodine deficiency in the development of cretinism had been conducted.

To test for the existence of such an etiological relationship, studies involving the supplementation of pregnant women in iodine-deficient areas and the observation of the subsequent neuromotor development of their infants were carried out in a series of supplementation trials. The most well-defined studies are reviewed below.

1.18.1. Papua New Guinea

A trial was started in 1966, in the remote and poorly-accessible Jimi Valley, in the PNG highlands. This was an area with a high endemicity for goitre and the "neurological" form of cretinism.

27 villages with a population of 16,500 were enrolled in the study and alternate families injected with either iodinated oil or saline. All the children born since the beginning of the trial in 16 of the 27 villages (population 8,000) were later examined for evidence of motor retardation (defined as the absence of certain "milestones of development") and neurological deficits (squint and/or deafness) (Pharoah, Buttfield and Hetzel, 1971; Pharoah, Buttfield and Hetzel, 1972;).

A summary of the results are shown in table 1.18.1. and show that pre-conceptual iodine supplementation effectively prevented neurological endemic cretinism. In addition, foetal and infant deaths in the saline (control) group were significantly higher than in the iodinated oil (treatment) group (Pharoah and Connolly, 1991).

	Injected before conception		Injected after conception	
	Iodinated oil	Saline	Iodinated oil	Saline
Number of births	593	597	95	90
Number of cretins	1	26	5	5
Number of cretins per 1000 births	1.7	43.5	52.6	55.6

Table 1.18.1. Iodine supplementation in PNG. Source: Pharoah, 1993.

3-4 years after supplementation, Mothers in the control group who gave birth to cretins had very low levels of protein-bound iodine (PBI), an early measure of thyroid function, whereas those treated mothers who gave birth to normal infants had normal levels of PBI (Pharoah, Buttfield and Hetzel, 1971).

The children born in the trial were further tested for motor and cognitive performance at ages 6-12 and 10-16 (i.e. 12 and 16 years after supplementation of their mothers). In those tests where differences were noted between the treatment and control groups, children born to mothers who had received iodinated oil performed significantly better than those whose mothers had received saline (Connolly, Pharoah and Hetzel, 1979; Pharoah and Connolly, 1991).

1.18.2. Ecuador

In 1966, 2 Andean villages were enrolled in a supplementation trial. All 960 persons in the "test" village received intramuscular injections of iodinated oil, whereas the population of 2,500 in the "control" village did not. Three years later, there were 3 cases of severe mental deficiency, including one typical cretin, reported in the control village but none in the treated village (Fierro-Benitez, Ramirez, Estrella *et al.*, 1969).

At three years of age and above, cognitive testing of children born after the start of the trial confirmed this finding for those children whose mothers had been supplemented preconceptually but indicated that supplementation at 4-9 months of gestation resulted in the same prevalence of severe mental deficiency as was found in the control group

(Fierro-Benitez, Ramirez, Estrella *et al.*, 1974), suggesting that late pregnancy supplementation was not effective in preventing cretinism.

At ages 8-13, the treated group performed better on school achievement but did worse on tests of cognitive function. At ages 14-21, the treated group had advanced further in education, had migrated more to urban centres and performed better in tests of neuro-motor function but had lower levels of income than the control group (Fierro-Benitez, Cazar, Stanbury *et al.*, 1988). These results may reflect the small numbers in the study and the difficulty of measuring indicators of socio-economic status.

There were reports of sporadic cases of transient hyperthyroidism, which was restricted to older villagers with large, pre-existing goitres, but no measures of thyroid function were conducted (Fierro-Benitez, Ramirez, Estrella *et al.*, 1969).

1.18.3. Peru

In 1966, all children under 18 years old age and females under 45 years of age, in 3 villages in the Peruvian Andes, were allocated to receive either iodinated oil (treatment) or non-iodinated oil (placebo) by injection. Infants born after the start of the trial were studied for thyroid function and, later, for cognitive function. Cord blood samples taken from neonates of the unsupplemented women, had much lower levels of T_4 and much higher levels of TSH than samples taken from neonates of the treated women, which were comparable to levels in controls from a non-iodine deficient area (Pretell, Palacios, Tello *et al.*, 1974). A few cases of sporadic hyperthyroidism were reported (Pretell, Moncloa, Salinas *et al.*, 1969) but no adverse side-effects were observed in either the mothers or neonates.

1.18.4. Zaire

Several detailed studies of iodised oil given during pregnancy have been conducted in an area of severe iodine deficiency and endemic goitre complicated with cretinism, in Zaire. In the first trial, 300 pregnant women were randomised to either receive 1 ml of iodised oil (480 mg I) by IM injection or to remain untreated. Of those infants followed-up, 4/45 born to women in the untreated group were cretins, compared with

only 1/44 in the treated group, where the single case had received supplementation very late in pregnancy (Delange, Thilly, Camus *et al.*, 1976).

A larger, placebo-controlled trial, involving nearly 1,000 women, supplemented during the second and third trimesters, included detailed biochemical determinations of thyroid status in mothers and infants. In the absence of supplementation, many women had high TSH and low T_4 levels, with some elevation in T_3 levels. Thyroid function was normalised, however, following supplementation, such that women in the treatment group had TSH, T_4 and T_3 levels comparable with those from iodine-sufficient areas (Thilly, Delange, Lagasse *et al.*, 1978).

In the unsupplemented group, thyroid function was severely impaired for many neonates, with the deviation from normal values in neonates being more severe than in mothers and directly related to the severity of the hypothyroidism present in the mother. There were higher incidences of spontaneous abortions, prematurity and stillbirths in the untreated group, whereas birth weights were somewhat higher in the treated group (Thilly, Swennen, Moreno-Reyes *et al.*, 1994). Table 1.18.4. summarises the results from these studies.

	Mothers		Neonates	
	Untreated	Treated	Untreated	Treated
Urinary Iodine (μg/dl)	3.63	56.6	1.5-3.6	15.5
Ur I < 5 μg/dl (%)	65	8		
Ur I < 2 μg/dl (%)	25	0		
Ur I > 1000 μg/dl (%)	0	5		
Serum TSH (mIU/l)	6.08	2.37	18.45	7.19
Cord TSH > 10 mIU/l (%)			46-49	5
Serum T_4 μg/dl	9.1	14.2	8.2	11.2
Serum T_3 ng/dl	187	154	86	62
Cretinism (%)			8.3	2.6

Table 1.18.4. Supplementation studies in Zaire. Source: adapted from Delange, 1996.

Evaluation of urinary iodine and serum hormone concentrations, following supplementation, has shown that the iodine status of the children deteriorates progressively with age, such that the same degree of deficiency is found in children at 2-3 years of age born to supplemented and unsupplemented mothers.

Following supplementation only a few cases of "iodine overload" (indicated by urinary iodine levels over 1000 μg/dl) were detected but there has been no clinical or biochemical evidence of hyperthyroidism in the treatment group of women or in their children, over more than 7 years of follow-up.

Measurement of cognitive function, at 3 years of age, indicated that the treated group performed significantly better than the controls (Thilly, Roger, Lagasse *et al.*, 1980).

1.18.5. Algeria

Following the detection of an increased incidence of transient neonatal hypothyroidism, during a pilot screening programme (Chaouki, Delange, Maoui *et al.*, 1986), a study was initiated to investigate the effects of maternal supplementation, with 0.5 ml IPSO (240 mg I), preconceptually and in the first trimester, on maternal and neonatal thyroid function and pregnancy.

When compared with a control group, who had not received iodine, supplementation 1-3 months before, 1 month after or 3 months after conception, resulted in significantly lower goitre prevalence and serum TSH concentrations, higher urinary and breast milk iodine and serum T_4 concentrations, in the mothers, a reduction in rates of spontaneous abortion, prematurity and stillbirth, increased placental and birth weights, increased neonatal T_4 and decreased neonatal TSH, as determined from cord blood and complete elimination of neonatal hypothyroidism (Chaouki and Benmiloud, 1994).

Two cases of transient neonatal hypothyroidism were reported in the control group but no deleterious effects associated with excess iodine administration were noted in any of the treatment groups. Duration of protection was estimated at one year, which is shorter than that afforded by intra-muscular injection, although no measurements were

taken beyond 6 months post-supplementation. The authors suggest that the side-effects reported from larger doses of iodine could be avoided by yearly administration with this low dose of oral iodine, until iodised salt can be introduced.

1.19. Thyroid Function in Iodine Excess

The effects, on thyroid function, of exposure to supraphysiological amounts of iodine, depend on the extent and duration of the excess. An understanding of the etiology and progression of these effects is necessary before considering the changes in thyroid function which may be induced by administration of supplementary iodine to a deficient population.

1.19.1. Iodide Uptake

Small to moderate amounts of stable iodide, administered acutely, do not influence the percentage uptake of concomitantly-administered radio-labelled I^*, indicating that iodide transport is not affected. The absolute iodide uptake, with small increases in iodide dose, is thus increased (Nagataki, 1976; Wolff, 1980). Iodide uptake is greatly enhanced in iodine deficiency (Wolff, 1964) and administration of supplemental iodine, whether in the form of IPSO or iodised salt, will initially result in large amounts of iodine being trapped by the cell of the gland.

Continued administration of moderate to large doses of iodine, however, decreases iodide transport activity, in an autoregulatory response (Larsen and Ingbar, 1992). Both thyroid/serum iodide concentration ratios and iodide transport maxima are reduced by administration of supplemental iodine but this inhibition of iodide transport is abolished if propylthyrouracil, which inhibits binding of iodide to tyrosine, is administered concomitantly. This suggests that the autoregulatory influence is exerted by organic, rather than inorganic iodide (Larsen and Ingbar, 1992).

1.19.2. Thyroid Hormone Synthesis

If iodide transport is not compromised, administration of excess iodide results in an increased amount of iodide available for organification in the thyroid follicles and hence an increased rate of thyroid hormone synthesis, at least for a time. After administration of small, acute amounts of iodine, there is little change in the fraction of accumulated iodine which has undergone organification or in the proportions of iodinated amino acids formed. Thus, both T_3 and T_4 synthesis are increased in the same proportion. In iodine deficiency, this means that T_3 production will continue at a higher rate than normal.

With progressively larger, acute doses of iodide there is a biphasic response in the amount of iodine which undergoes organification. At first, the fraction of organified iodine increases with the dose of iodine but it then decreases as a result of relative blocking of organic binding. This decrease in organic iodine, in response to increasing doses of iodine is termed the acute **Wolff-Chaikoff** effect, after the authors who first reported the phenomenon (Wolff and Chaikoff, 1948). Below is a schematic representation of this effect. This effect has been demonstrated in repeated administration with KI (Emerson, Anderson, Howard *et al.*, 1975; Robuschi, Manfredi, Salvi *et al.*, 1986) and Lugol's solution (Tan, Morat, Ng *et al.*, 1989).

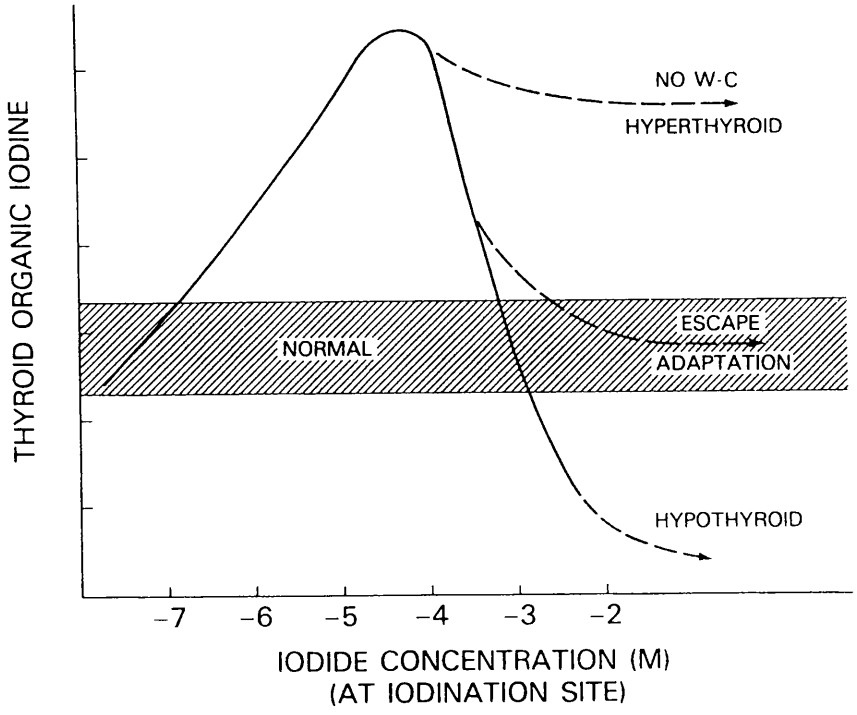


Fig 1.19.2. The Wolff-Chaikoff Effect. (Source Larsen and Ingbar, 1992).

The mechanism of the effect is uncertain but depends on the establishment of a high concentration of inorganic iodide within the thyroid. Oxidative mechanisms generate the reactive form of iodine and this may complex with iodide to form a species which is relatively inefficient in iodinating tyrosine (Larsen and Ingbar, 1992). The progressive accumulation of iodine in the thyroid is also prevented by a reduction in the re-utilisation of the iodine derived from the intrathyroidal deiodination of iodothyronines (Nagataki, 1976).

Along with this quantitative decrease in organic binding there may also be qualitative changes in hormone synthesis. Organic iodinations are probably not completely inhibited but synthesis of hormonally-active iodothyronines is almost abolished. The organically-bound iodine is mainly incorporated in MIT, with smaller amounts appearing as DIT and virtually none as T_3 or T_4 (Woeber, 1991). This is in common with other situations where organic binding is decreased, as in polypropyluracil administration.

Such an effect in subjects with underlying iodine deficiency would be expected to result in large decreases in T_3 concentrations, from the high-normal or high levels to sub-normal levels, with smaller decreases in the already low T_4 levels.

Susceptibility to this effect is increased either by stimulation of the iodide-trapping mechanism, as occurs in patients with Graves disease or after TSH administration or stimulation or by impairment of organic iodide formation, as may occur after radioiodine therapy, during polypropyluracil treatment, or in patients with Hashimoto disease (Larsen and Ingbar, 1992). In these cases, prolonged administration of iodides may result in goitre or hypothyroidism.

Pituitary TSH secretion is stimulated in iodine deficiency by low levels of circulating T_4 in a negative feedback mechanism. Iodine deficiency thus represents a state of enhanced iodide trapping which may be particularly susceptible to the Wolff-Chaikoff effect. It has been suggested that supplementation of iodine-deficient populations may induce the effect, at least transiently, in susceptible individuals (Wolff, 1969; Mantovinovic, 1980; Fradkin and Wolff, 1983).

The autoregulatory response to repeated administration of moderate to large amounts of iodine, mentioned above, provides a means of "escape" or "adaptation" to the Wolff-Chaikoff effect (Wolff, Chaikoff, Goldberg *et al.*, 1949). The decrease in iodide transport activity reduces the thyroid iodide concentration to a level insufficient to maintain the full effect and relative inhibition of organic binding is thus reduced. Synthesis of iodothyronines resumes and development of goitrous hypothyroidism (iodide myxoedema) is thus prevented.

The quantity of iodide accumulated and organified is still in excess of normal but the thyroid synthesises and releases the non-hormonally active, non-calorigenic forms of iodine, including iodide. This iodide leak varies directly with the dietary iodine intake in normal individuals but may be reduced in iodine deficiency. Occasionally, adaptation does not occur and synthesis of hormone is chronically inhibited, leading to goitrous hypothyroidism (Larsen and Ingbar, 1992).

1.19.3. Thyroid Hormone Release

Excess iodine inhibits hormone release and thus rapidly lowers the serum T_4 concentration. The mechanism by which iodine affects the hormone release is not known, although it is mediated at the thyroid level, rather than through an action on TSH, because it occurs in Graves disease and other conditions where thyroid function is independent of TSH stimulation (Wolff, Chaikoff, Goldberg *et al.*, 1949; Green and Ingbar, 1962; Nagataki, 1976).

Iodine may also play a role in blocking proteolysis of thyroglobulin, because iodine inhibits iodide leak (Bagghi, Brown, Shivers *et al.*,1977). In iodine deficiency, reduction of the already low levels of T_4, in susceptible individuals, could potentially result in subclinical hypothyroidism becoming frank but reports of such cases are extremely rare (Delange, 1996).

This effect on hormone release is seen in the alleviation of thyrotoxicity, by the administration of pharmacological doses of iodine, in the patient with diffuse toxic goitre (Silva, 1985). This decrease in serum T_4 concentrations cannot be attributed to

persistence of the acute Wolff-Chaikoff effect, with inhibition of T_4 synthesis, because it is much more rapid than that produced by other antithyroid agents which inhibit hormone synthesis (Larsen and Ingbar, 1992). The decrease is also unlikely to be caused by any effect of iodine on the peripheral metabolism or metabolic effectiveness of T_4 because no such effect can be demonstrated.

In normal individuals, inhibition of hormone release leads to an increase in TSH secretion which counteracts the effects of iodine by stimulating the production and release of thyroid hormones. Mild TSH elevation, in the absence of hypothyroidism, in subjects exposed to long-term iodide excess, may indicate a degree of compensation, rather than escape from the blocking effect on hormone secretion (Cavalieri and Pitt-Rivers, 1981; Becker, Pineda, Silva *et al.*, 1982; Silva, 1983).

In iodine-deficient populations, TSH secretion is often already supranormal and may result in goitre formation, as described below, or enlargement with enhanced T_3 secretion.

1.19.4. Thyroid Morphology and Histology

The effect of iodine on thyroid size and appearance depends largely on the underlying functional state of the gland. The diffuse, toxic goitre of Graves disease is characterised by hypervascularity and hyperplasia which are reduced by administration of iodine (Larsen and Ingbar, 1992). Iodine-induced hypothyroidism, however, is characterised by thyroid hyperplasia which increases with iodine dose .

In iodine deficiency, there is often hypertrophy and hyperplasia of the epithelial cells lining the follicles and as the height and number of cells increase, they protrude into the follicular lumen, forming papillary projections and decreasing the internal size of the follicles and hence the colloid content. Vascularity increases and a diffuse, hyperplastic goitre, usually seen in children in endemic areas, is formed (Larsen and Ingbar, 1992).

With an increase in iodine intake, this hypertrophy and hyperplasia disappears and colloid reaccumulates in the follicles. If the hyperplasia is of relatively short duration,

the gland returns to normal size but if it has been present for years, a diffuse, colloid goitre probably results. Repeated cycles of hyperplasia and involution eventually lead to formation of nodules of involuted tissue and so to multinodular goitre (Larsen and Ingbar, 1992).

Administration of excess iodine to subjects with fairly long-standing diffuse goitre may thus induce nodularity and further compromise thyroid function. Patients who already have nodular goitre are more susceptible to iodine-induced thyrotoxicosis (Fradkin and Wolff, 1982).

1.19.5. Effect on Foetal Thyroid Function

The foetal thyroid is very sensitive to iodine inhibition, although it is not known if this is an inherent hypersensitivity or due to placental concentration of iodine (Larsen and Ingbar, 1992). Exposure to excess iodine, e.g. large amounts of iodine-containing drugs, such as some anti-arhythmics, or X-ray contrast media, during pregnancy is frequently accompanied by the development of foetal goitre which can cause obstetric difficulties, including neonatal asphyxia and death (Silva, 1985). It is therefore not recommended that pregnant women in non-goitrous areas, particularly those with underlying hyperthyroid function, should be given iodine-containing preparations. There have also been some fears that supplementation of sub-clinically hypothyroid pregnant women with iodine may cause adverse side-effects in the foetus but these are largely unsubstantiated (Delange, 1996).

1.19.6. Supplementation Trials and Iodine Excess

In view of the potential dangers of suddenly increasing the iodine intake in individuals suffering from iodine deficiency and the consequent effect on policies for the correction of IDD in communities of such individuals, it is important to examine the evidence from studies involving iodine supplementation, particularly in pregnant populations. A recent review (Delange, 1996) of programmes involving supplementation in pregnancy has concluded that the benefits of supplementation are well-documented and substantiated by several studies and that the fear of side-effects has been greatly exaggerated and is not supported by the evidence.

1.20. Introduction to IDD in Pakistan

1.20.1. Historical Context

The Republic of Pakistan contains the remote villages in the Karakoram mountains where Robert McCarrison made his famous "Observations on endemic cretinism in the Chitral and Gilgit valleys" which appeared in the Lancet of 1908 (McCarrison, 1908). He described the two types of cretinism he found, "nervous" and "myxedematous", in some detail and conducted a thorough goitre survey in several villages.

Today, the north of Pakistan, consisting of the Northern Areas (NA) (which include the villages where McCarrison made his observations), North-West Frontier Province (NWFP), the northern part of Punjab Province and Azad Jammu and Kashmir (AJK) remain among the worst-affected regions of the world for iodine deficiency, despite two decades of control measures. Below is a map of Pakistan.

Figure 1.20.1. Map of Pakistan. Source: adapted from GOP, 1988.

1.20.2. Situation Analysis

A 1990 report by the Planning Commission of the Government of Pakistan (Khan, Ayub and Abbas, 1990) estimated that 7 million people lived in goitre endemic areas in the north of the country with 50-70% affected by IDD in some way. A further 3 million people were estimated to be affected in the Punjab plains, Baluchistan and Sindh, areas of the country not traditionally associated with IDD. These 10 million represented 10% of the national population at the time of the report and was said to include 2-300,000 affected with moderate to severe cretinism, over 600,000 with mild mental impairment and to result in 4-5,000 stillbirths and 4,000 neonatal deaths per year.

A UNICEF situation analysis in 1992 (UNICEF and GOP, 1992) put the estimated number of people at risk of IDD at 15 million, partly due to the increase in population (to 115.6 million in 1991) but possibly also reflecting the increasing awareness of IDD as a problem of national proportions, requiring immediate policy action. A later UNICEF document, concerning mid-decade goals for children and development (UNICEF, 1994a), estimated that the number of people at risk of IDD in northern Pakistan had risen to 8 million by 1994.

1.21. A History of Goitre Surveys

1.21.1. Early 1900s

The earliest goitre survey in what is now Pakistan was carried out by McCarrison in the Gilgit and Chitral valleys, between 1906 and 1910, the results of which are shown in table A.1.21.1., appendix 1.21. (McCarrison, 1908). He found that 497/2192 (22.9%) of adults had a visible goitre. McCarrison commented on the remote nature of the villages (which, no doubt, contributed to the length of time he took to complete his investigations) and the remarkable number of cretins there were in the region. He described two kinds of cretins: those with neurological impairment, often involving deaf-mutism and spastic diplegia with mental defects, and those with characteristics of severe biochemical hypothyroidism, including dry, swollen skin and tongue and mental deficiency.

1.21.2. 1960s

A WHO survey of primary school children (aged 6-16) in 1960 (WHO, 1960) selected towns throughout the northern Punjab, NWFP and NA and determined goitre rates by sex and by severity of goitre. The results of this survey are shown in table A.1.21.2., appendix 1.21., and show that the prevalence and severity of goitre were highest in NA.

The Nutrition Survey of West Pakistan (at a time when Bangladesh was known as East Pakistan), carried out by the Health Division of the Planning Commission of the Government of Pakistan (GOP), in 1965-8, found a much lower prevalence of goitre in the country as a whole but reported high rates for pregnant and lactating women (GOP, 1968). Data on individual provinces or areas was not available.

In view of the high prevalence of goitre and cretinism in the north of the country, IDD control measures were targeted there, with interventions beginning in the 1970s. These interventions and associated follow-up surveys are discussed in some detail below.

1.21.3. Recent Studies

The Nutrition Division of the National Institute of Health (NIH) conducted a goitre survey among children in and around Islamabad, the federal capital, in 1994 and found a total goitre rate of about 40% in 8-10 year old school attenders. The rate was higher in the rural schools and there was some variation according to the region of parental origin (Islamabad attracts migrant workers from the whole country) but these were not statistically significant. Urinary iodine measurements indicated a moderate degree of deficiency (Rajput, Mothi-ur-Rehman, Saleem *et al.*, 1994).

In 1994, a study of cord blood TSH levels, from newborns in 4 major cities, revealed a high proportion of infants with "high TSH levels", although it has not been possible to ascertain the cut-off level. Results were used for advocacy purposes, to urge immediate government action on salt iodisation (GOP, 1994). In view of the marked TSH surge during and immediately after birth, in all populations, leading to neonatal screening for hypothyroidism being recommended a few days after birth, the results are very difficult to interpret but served the purpose of raising awareness of IDD.

1.22. Salt Iodisation

1.22.1. Skardu

A small salt iodisation plant, imported from Germany, was opened in 1971 at Skardu, in the Northern Areas (NA). It had the capacity to produce 2 tonnes of iodised salt per day, against an estimated requirement of 10 tonnes of salt per day for the population in the area, but remained largely inoperative for a variety of reasons (Khan, Ayub and Abbas, 1990). No information concerning the level of iodisation (in ppm) or the size of the target population was available but at an average individual salt intake of 5 kg/year (Mannar, 1987) this represents a population of 7-800,000.

A Nutrition Cell was established by the Planning and Development Division (PDD) of the Planning Commission and given the responsibility of conducting a micronutrient survey of the country and implementing some intervention programmes, including the supply of iodised salt to the Northern Areas. The 1975-6 Micronutrient Survey (GOP, 1976) yielded similar results to that of 1965-8, mentioned above, highlighting particular concern over the goitre rates in pregnant women.

The Skardu plant was re-opened in 1974 but continued to function below capacity and a full time officer from the Nutrition Cell was appointed in 1977. In addition to technical and managerial assistance, the Nutrition Cell provided packing materials, covered the cost of maintenance and repairs and made up processing losses but production remained low. Surveys in and around Skardu in 1974 (Univ. of Agric., 1974), 1978 (GOP, 1978) and 1982 (Siraj-al-Haq, 1982) indicate that the iodised salt had very little effect in lowering goitre rates and it is not known if there was any quality control checking of iodisation levels at the plant.

1.22.2. Rawalpindi

In 1977 the Health Board of the Aga Khan Foundation and Industrial Promotional Service, Pakistan Ltd. jointly opened an iodisation plant in Rawalpindi, in the northern Punjab, producing iodised salt for marketing in the Gilgit area. The salt is obtained from the nearby Pakistan Mineral Development Corporation (PMDC) Kalabagh Salt Mines and iodised at a maximum rate of 3 tonnes per day. Again, the target iodisation

level in ppm was not available. Average annual production is 550 tonnes but it is not clear how much of the target population this is able to cover or if the level of iodisation was subject to quality control checking.

Surveys in the Gilgit area in 1972 (Chapman, Grant, Taylor *et al.*, 1972) and 1982 (Siraj-al-Haq, 1982) suggest a small reduction in goitre prevalence, although it is unclear whether the same methodology was used in both surveys. More rigorous surveys in 1978 and 1988 revealed a marked decreased in goitre rates and a widespread use of iodised salt, over a period of five years or more, although some remote villages remained badly affected by IDD and continued to use uniodised rock salt (Charania, Malik, Bhojani *et al.*, 1988).

1.22.3. Peshawar

With assistance from UNICEF, the Sarhad Development Authority (SDA) opened a larger iodisation plant in 1980, in Peshawar, to supply iodised salt to the NWFP. The plant had a capacity to iodate nearly 6,500 tonnes per year, which is insufficient for the province's demand, and in the first two years, production of iodised salt was only just above half capacity. Production further fell in the next decade with frequent closures due to management and marketing problems (GOP, 1990).

1.22.4. Utility Stores

In view of the continuing problems of providing iodised salt to some of the more remote areas affected by IDD and the production difficulties encountered at some of the plants, an interim strategy of providing iodised poppy seed oil (IPSO) to the population in the worst affected areas was proposed and is discussed below.

At the same time, measures for facilitating the production and delivery of iodised salt were investigated and new policies formed. These were incorporated into the 7th 5-year plan (1988-93) strategy for health (GOP, 1987) and a Salt Iodisation Subsidy of 40 million Rupees (US$ 2 million), over 5 years, was earmarked for use in production of iodised salt and promotion of its use, by a target population of 7 million, in endemic IDD regions, in the north of the country.

The government has been working with the Utility Stores Corporation (USC), a semi-public, profit-making organization that deals with government-subsidised food sales. USC were responsible for marketing and distributing iodised salt, as directed by the Prime Minister, and the project was monitored by the Nutrition Cell of the PDD for availability, quality control, price etc. It was anticipated that about 500 tonnes of iodised salt would reach the Northern Areas by the beginning of 1990 but, in fact, total supply of iodised salt through USC was only 475 tonnes for the whole country in 1989 (GOP, 1990; UNICEF, 1994a).

Initially, USC sold both iodised and non-iodised salt, the iodised salt being subsidised by the government, through a UNICEF grant, so that its price was the same as the non-iodised. The iodised salt was consistently promoted and non-iodised salt was withdrawn from sale in USC stores. USC stores are, however, mainly located in urban areas and serve the middle socioeconomic classes and a 1993 World Bank consultancy report (Van der Haar, 1993) estimated that coverage of the target areas was only 5-10% of the total salt market, with USC supplying 3,370 tonnes of iodised salt in 1993 (UNICEF, 1994a). It is not clear how the coverage figure was calculated.

UNICEF co-operated by ordering and importing the potassium iodate which was then given to the PDD to supply to manufacturers. The iodate was, theoretically, available to all producers, who could meet certain criteria laid down by the PDD, but there was no external, systematic checking of iodine content in salt, either in markets or at household level, and no legislation was in place to control the production or sale of iodised salt. The PDD recommended an iodate level of 70 ppm.

1.22.5. Legislation

Since 1993, the government has worked with UNICEF to introduce legislation, covering the production and sale of iodised salt, but the federal organisation of government within Pakistan means that there are some complications in drafting and passing legislation which will be applicable and enforceable at both provincial and federal level (UNICEF, 1994b). Such legislation would ensure that all salt produced in the country for human and livestock consumption is adequately iodised at factory, retail and household levels.

1.22.6. Iodised Salt Support Facility (ISSF)

In 1994, an ISSF was established in Islamabad, as an integral part of the UNICEF-supported GOP Programme for the Elimination of IDD, "to encourage, support, promote and sustain the universal access to iodised salt in Pakistan" (UNICEF, 1994c). This involved working with mainly smaller salt producers and government towards some specific objectives, outlined below, under national policy, in collaboration with the national IDD Co-ordination Committee (IDDCC).

The ISSF has a role in mobilising resources to aid installation of iodisation equipment and to ensure access to lowest-cost potassium iodate. It is also responsible for developing quality control procedures, including production-site monitoring by the producers and for supporting sustainable methods of financing the extra costs involved in iodisation. In view of the very large number of small salt producers in Pakistan, the ISSF is helping small producers to form co-operatives which may then be able to install and use a centrally-based iodisation plant, thus reducing costs. The ISSF works in partnership with producers to raise the quality and quantity of iodised salt available and enables them to prepare their processes for meeting new legislation (UNICEF, 1994c).

1.23. Iodised Oil

1.23.1. Initiation Using Injections

The 6th 5-year plan for health (GOP, 1982) proposed a large scale goitre programme, including the administration of Lipiodol iodised oil injections to a target population of 4½ million, made up of women of childbearing age and children under 20, in the worst affected areas of NA, AJK and northern districts of NWFP. The programme was approved in 1986 but, following recruitment and management problems, operations did not begin until late 1987 (GOP, 1990).

Mobile teams of health workers were formed in Swat (NWFP) and parts of Gilgit (NA) and AJK to administer supplements in special campaigns. In parts of Chitral and Hunza (NA) the Aga Khan Foundation assisted the government through its health services.

The aim was to cover the entire population in a period of about 2-3 years, with a repeat round of supplementation beginning if iodised salt supply was still problematic. The programme started rather slowly, with only 76,000 people supplemented in the first year, but by 1990 450,000 injections had been administered (approximately one tenth of the target population). It has not been possible to locate systematic follow-up surveys to chart the reduction in goitre rate.

1.23.2. Transition to Oral Supplementation

A revised strategy, using capsules rather than injections, was introduced in late 1990 and services extended to include fixed health institutions and private outlets in 1991. Coverage rates are now reported to be in the region of 70-80% in most of NA and about 40% in Gilgit and NWFP (Van der Haar, 1993) but each province decides its own device for reaching adequate coverage so the situation in communities may not be systematically reported by informants at federal level. Central co-ordination of reports is by the Nutrition Cell, PDD, GOP.

1.23.3. Limitations

There have been some problems in procuring an adequate supply of injectable and oral IPSO and in communication and co-ordination between provincial and federal levels. In particular, central management of a massive dosing campaign is complex and may be difficult to sustain in view of the nature of the remote target areas and the demands on health personnel there.

The oral capsules are thought to be effective for 1-2 years but coverage rates refer to cumulative supplementation over 3 years. The effective coverage of the population may thus be lower than suggested by the reported coverage. Following questions about the possible toxicity of large doses of iodine (discussed later, in some detail), oral supplementation with IPSO is being phased out and efforts are being concentrated on public awareness campaigns and iodised salt legislation. Health centres continue to stock IPSO capsules but mobile supplementation teams are being deactivated. There may be the need for continuation of iodised oil supplementation programmes in very remote areas, until the iodised salt becomes universally available and affordable

1.24. Social Mobilisation

1.24.1. Awareness Education

In the initial phase of the salt iodisation component, the IDD programme supported the salt sector with promotional material for distribution in IDD endemic areas. Health workers promoted iodised salt through education, prescription and free samples and USC erected hoardings to advertise iodised salt along many trunk roads. National and local radio stations were used to advertise iodised salt, often incorporating information about IDD into popular "soap operas".

The Nutrition Unit of the National Institute of Health (NIH) has been active in promoting awareness of IDD, devoting 3 pages of the 1989 newsletter to an article on IDD control (NIH, 1989) and conducting a small, qualitative survey concerning people's perceptions of IDD in NWFP (Godwin, Mothi-ur-Rehman, Anis *et al.*, 1989). With UNICEF financing it has also developed and distributed materials, to aid communication and understanding about IDD, for health workers, teachers etc.

1.24.2. Media Coverage

There has recently been much media interest in IDD, with newspaper and magazine articles, radio and television programmes and public meetings and workshops, highlighting the problems of IDD and urging people to consume iodised salt. Reports have focused on the intellectual impairments caused by IDD and the consequent educational deficits in the country, which are said to contribute to general underdevelopment and "backwardness"

1.25. Political Commitment

1.25.1. International Declarations

Pakistan was among the 159 nations which signed The World Declaration on the Survival, Protection and Development of Children, in September 1990, at the UN's Summit for Children, in New York. This declaration and the accompanying Plan of Action set goals for the 1990s which include the elimination of IDD by the year 2,000.

The country was also an active participant in the Policy Conference on Hidden Hunger, in November 1991, in Montreal. This conference addressed the problems of micronutrient deficiencies and strengthened the commitment of participating nations towards achieving the goals set in New York. This resolve was further confirmed in the Declaration on Nutrition which was passed at the International Conference on Nutrition in December 1992, in Rome.

In the political arena of South Asia, the goals of these international declarations are reflected in the September 1992 Colombo Resolution of the South Asian Association for Regional Cooperation (SAARC) which expressed the resolve for universal iodised salt availability by 1995. The World Bank is helping countries in the region to achieve this target by supporting national policy for implementation of these resolutions and declarations.

1.25.2. National Policy

Pakistan has a history of applying significant resources for assessment studies, surveys and control measures for IDD and the health components of recent 5-year plans (GOP, 1982; GOP, 1997; GOP, 1992) have included commitments to control and soon eliminate IDD. In particular, the most recent (8th) 5-year plan (1993-8) contained a strong commitment to eradicating IDD in the National Programme of Action (NAP) within the Social Action Programme (SAP) of the Plan (GOP, 1991).

1.25.3. Current Situation

The political upheavals of 1993 and the subsequent shifts in responsibilities for planning and implementation towards provincial levels created complications and some confusion in the management of the IDD control programme. The new government, however, strongly committed itself to the control of IDD and its virtual elimination by the year 2,000. The Prime Minister has, herself, taken a keen interest in improving the health status of women and children in the context of community and has been particularly concerned to increase awareness of IDD and encourage measures for its control (Van der Haar, 1993).

1.26. IDD in the Study Area

1.26.1. Introduction

The geography and demographics of the study area, a remote, hilly region about 50 km from Rawalpindi, will be discussed later, in section 35, but it is helpful to look at the historical context of IDD in this area, before describing the current study design.

1.26.2. Previous Studies

A survey by the Health Division of the Planning Commission, in collaboration with NIH, in 1968-9, revealed a high prevalence of goitre in an area not traditionally identified as very iodine deficient and a relatively short distance from the large, industrial and commercial city of Rawalpindi and the site chosen for the new Federal capital, Islamabad (GOP, 1969). The results of this survey are shown in table 1.26.2.

AGE GROUP (years)	SEX	NO	% GOITRE - BY GRADE/VISIBILITY				
			I	II	III	VISIBLE	TOTAL
0-5	male female	46 37	2.1 ---	2.1 ---	--- ---	2.1 ---	4.2 ---
6-14	male female	310 129	20.9 13.9	9.6 10.1	1.9 1.5	11.5 11.6	32.4 25.5
15 plus	male female	136 182	25.7 29.6	5.7 16.4	2.2 4.9	7.9 21.3	33.6 50.9
Pregnant and lactating women		27	22.2	18.5	3.7	22.2	44.4

Table 1.26.2. Prevalence of goitre in Lehtrar. Source: adapted from GOP, 1969.

1.26.3. Recent Work

A small-scale goitre survey by members of the Department of Psychiatry, Rawalpindi General Hospital (RGH), in 1990, amongst school children in the same Thesil (administrative subdistrict) of Kotli Sattian, revealed a very high prevalence of goitre (almost 100%) and low urinary iodine excretion, strongly suggesting that IDD might be a problem in this community (Zia, 1990). In addition, anecdotal evidence indicated widespread and almost universal goitre, particularly among women.

The village of Lehtrar and the surrounding area were originally chosen as the study site because the Department of Psychiatry, Rawalpindi General Hospital had been doing some community mental health work and had good relations with the medical personnel in the area. Preliminary talks with the doctor at the Rural Health Centre (RHC) in Lehtrar, however, revealed that iodised oil capsules had recently been administered to any women presenting at the health centre with goitre (Baksh, 1993.). This admirable effort to tackle the problem of IDD within the community meant that Lehtrar village was no longer suitable as a study site because of iodine "contamination".

For this reason, 4 villages in the surrounding area, not served by the health centre at Lehtrar were chosen as the study site. This necessitated working in more remote Union Councils (subdivisions of the Thesil) to ensure that the population had not been exposed to prior iodine supplementation. The villages in the study site and organisation of the programme are described in detail in section 28.

CHAPTER 2 - METHODOLOGY

2.1. Study Design

2.1.1 Original Study Design

The original aim of the study was to compare the effects on foetal and infant growth and development of supplementation of iodine-deficient pregnant women with oral Iodised Poppy Seed Oil (IPSO) preconceptually, early and later in pregnancy. Details of this study design are set out in appendix 2.1., including a sample size calculation. This project was to be carried out in conjunction with research into the effect of iodine supplementation on cognitive function of schoolchildren and a projected timetable of events for these two projects is also given in appendix 2.1.

2.1.2. Modified Study Design

Initial discussions with collaborating institutions in Pakistan and with community leaders were very encouraging and the study commenced in August 1993 with only minor changes to the original design (the inclusion of a greater number of villages, as described in section 2.2.). It soon became clear that it would not be possible to achieve the original aim of the study, for reasons described below, and the aim of the study was revised. The working aims of the study became:

1. a cross-sectional study of the thyroid biochemistry of iodine-deficient non-pregnant and pregnant women in the study area,

2. a follow-up study of the thyroid biochemistry of iodine-deficient non-pregnant women before and 10-14 weeks after supplementation with iodine in the study area,

A description of the pattern of reproductive histories in the area and of health care practices in the study area are also described in appendix 3.

The constraints and design changes to the original study are described below, as they occurred during implementation.

2.2. Implementation - Constraints and Design Changes

2.2.1. Target Population

Following discussions between local community leaders, in the study area, and collaborating investigators in the Department of Psychiatry, Rawalpindi General Hospital, it was felt that expecting 60-100% of the women in the target group to attend iodine camps was unrealistic but at this stage of discussion, the widespread observation that women do not like to leave their homes to walk far, especially during pregnancy, was not raised by either the community leaders or the local investigators at RGH.

The decision was taken to extend the study site to include more villages, so that a lower camp attendance of the possible target group would still provide a sufficiently large sample size. The original 4 study villages, in Lehtrar Union Council, were chosen as the centres from which to launch the work but a further 12 villages, in neighbouring Union Councils, were identified for inclusion in the study. A sketch-map of the study area is shown in figure 2.2.1.

The extended area was reported to have a total population of 80-100,000, according to statistics kept in the RHC in Lehtrar, giving rise to 12-16,000 married women of child-bearing age (Lehtrar, 1991). On further discussion, community leaders estimated that 30% of the target group would attend. ie 4-5,000 women, or an average of 250-300 in each camp.

2.2.2. Local Co-operation

Health committees were set up in each village, consisting of local political and administrative officials, school-teachers, traditional health providers and highly-respected "elders" in the community. All members of the health committees were male and efforts to include some of the more experienced women in the village, such as school-teachers and traditional birth attendants, met with considerable opposition, both from the health committees and from local investigators.

The major problem: that women in this community were so subjected to male authority, made it impossible to utilise the TBAs to help with data collection at home, particularly

in recording birth weights and lengths, or in taking blood samples immediately post-delivery. This meant that the study objectives concerning growth and development of the foetus, as assessed at birth, and thyroid hormone levels in mothers at the time of delivery, had to be abandoned. At the same time, contact with the women had to be established through male networks. Had women been included in the committees, it is doubtful that they would have been able to take part in any decision-making process.

The camps were launched at a time of major political instability in Pakistan. Following the dissolution of the Punjab Provincial Assembly, by the President, on the grounds that the province had become ungovernable, and the subsequent re-instatement of the Assembly by the Supreme Court, who declared the dissolution to be unconstitutional, the Federal Government had resigned and a general election campaign was being fiercely (and sometimes violently) contested.

At the time that the health committees were set up, there was no suggestion that there would be intense social and political rivalry between different groups, but, being dominated by local political leaders, the health committees became fora for settling old scores and canvassing for support in the elections. There was mistrust between the different political factions, with suspicions concerning motives in holding camps in particular members' homes, so that women of one family or political grouping were sometimes prevented from attending camps arranged by another faction.

The siting of a camp within a particular village, was often based on local political considerations, rather than being the most central or accessible building in the village, and there were some communication problems between the rival groups, so that information concerning the camps was not always disseminated to the whole community. Some health committees were completely dysfunctional by the time the camps were launched and others disintegrated as the work progressed. It became clear that the factionalism between families and groups had only occurred as a result of heightened political awareness due to the elections, which were not planned at the start of the study.

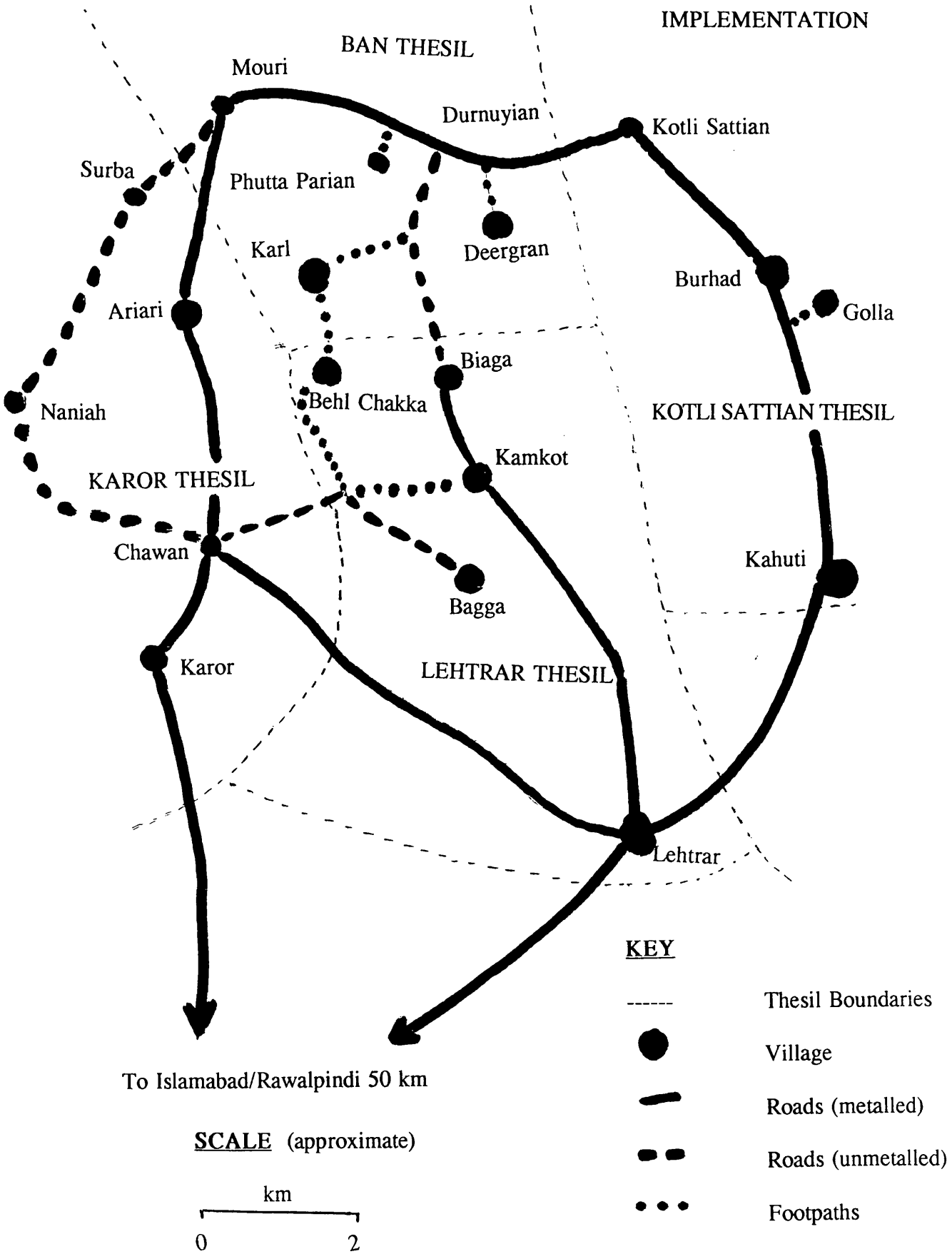


Figure 2.2.1. Sketch-map of study area

2.2.3. Accessibility

The village nearest to Rawalpindi was a 1½ hour drive away and the most remote village was reached following a 2½ hour drive and one hour walk. Each village was at least an half hour drive or one hour walk from the next. In view of the distances to be covered, it was decided that the field team should work for 4 days a week in the study area, staying overnight in the villages for the intervening 3 nights.

The villages themselves were not nuclear communities, but collections of households spread over the hill sides, so that access to most of the camps entailed walking long distances, in difficult terrain. This caused some difficulties for the team, in transporting equipment, drugs, etc. but the siting of clinics seemed eminently "sensible" from the investigator's perspective and "suitable" from the community leaders' perspective. For the women, however, particularly those who formed our target population - pregnant women and those carrying infants - the siting of clinics was problematic.

2.2.4. Schedule

There were considerable delays in recruitment of staff, due to the reluctance of female health workers to travel to the rural areas. This resulted in nursing staff being engaged in early August, rather later than originally intended, but discussion between the local investigators and the village health committees suggested that postponement of the camps would result in loss of good will amongst the community.

The 16 camps had been programmed to take place in a 4-week period in mid-August to mid-September, 1993. Several unannounced, religious holidays fell during this period, resulting in poorly-attended or cancelled camps, and subsequent rescheduling of activities. In addition, the unusually heavy monsoon rains in 1993 sometimes prevented the team from reaching the camps.

At first, the team stayed overnight in the villages but after a few nights elected to travel back to Rawalpindi in the evenings and travel daily to the field area. Delays in departure from Rawalpindi, in the mornings, frequently resulted in late arrival for a camp and long waiting times for those attending. This often led to women leaving the

camp before they had been registered and supplemented and so reduced the initial contact sample size.

In addition, the local hospitality code often over-rode the work schedule as team members had to leave their tasks to take formal, sometimes lengthy, tea or lunch-breaks, during which women left the camp before they had been seen by the team.

2.3. Recruitment - Iodine Camps

2.3.1. Consent

Community leaders and health committee members in the village explained the reason for the study and the need to take blood samples so that the health of the whole community could be improved and monitored. Individual consent was obtained from every woman, on a separate "Consent Form", shown in translation in appendix 2.3.1., and confirmed with a thumb-print.

It was difficult to be sure if explanation of the study was always thorough, as there were some misconceptions about the purpose in taking blood samples. A common view expressed was that the team were selling the blood for transfusions, as there had been some recent publicity on the radio, concerning the lack of blood donors in the city. There were some rumours circulating that the iodine capsules were a contraceptive, or led to infertility, and, in at least one village, this led to widespread non-attendance at the camp.

2.3.2. Registration

A general "Camp Questionnaire", shown in appendix 2.3.2., was completed for each woman by medical students, assisting at the camps, or by a doctor from the department of psychiatry, detailing the camp location and date, the woman's name, age, marital status and husband/father's name. Further health-related questions were completed during the consultations and procedures described below.

2.3.3. Medical Consultation

Each woman saw a doctor who briefly assessed her health and prescribed any necessary essential drugs. Despite recognition of the need of women in this strict Muslim area to be examined by female doctors, and promises that such doctors would be available, it proved impossible to recruit a female doctor to the project team, nor were female doctors in the department of psychiatry willing or able to travel out of the city to do community work.

All the doctors and medical students at the iodine camps were therefore male and there were understandable communication problems between the women and the doctors. This undermined the communities' confidence in the programme to address the perceived medical needs of the women, and may have influenced attendance at follow-up clinics. Seeing that they would not receive the service they wanted and be seen by a female doctor, some women left the camps early, often at their accompanying male relative's insistence.

There was a high expectation that some pills would be prescribed, so all women were supplied with a few low-dose multi-vitamin tablets. The essential drugs and vitamins were dispensed as the woman left the camp, after blood sampling had taken place. The doctors performed a visual assessment of goitre grade, as it was not culturally-appropriate to palpate the thyroid, and noted any very obvious clinical signs of hypo- or hyper-thyroidism.

2.3.4. Sample Collection

Women were individually seen by a nurse, supervised by the senior investigator, and asked about their pregnancy status and date of last menstrual period. They were given containers and asked to provide a urine specimen, before a 5 ml venous blood sample was taken. Haemoglobin was measured on a HemoCue® portable haemoglobinometer and the results discussed with the woman. Women with a haemoglobin below 80 g/l were given a short course of ferrous sulphate tablets (14 tablets of 60 mg each) and dietary advice. Women with a haemoglobin between 80 and 110 g/l were given dietary advice.

2.3.5. Iodine Administration

All women were shown IPSO capsules and asked if they had previously taken iodine capsules like those and if so, when. Very few women (2%) had ever received iodine and none within the last year. Women who had been noted as having gross or nodular goitre or exhibiting signs of hyperthyroidism were not given iodine (4%) but their condition was explained to them and some (2%) were referred to the General Hospital in Rawalpindi for further investigations.

Women who did not have nodular goitre or signs of hyperthyroidism were given two capsules of IPSO (total dose 400 mg iodine) and a glass of water and observed in taking the capsules. These women were encouraged to return for follow-up clinics and to bring their infants with them on the next visit.

2.3.6. Summary of Contacts Made

16 sites were visited, one per day, and 1432 women registered and supplemented. 115 pregnant women were identified by self-reporting. A summary of the camps is shown in table A.2.3.6, appendix 2.3.3.

2.4. Follow-up Clinics - I

2.4.1. Schedule

Following identification of some logistical problems, the number of field days was reduced to 3 per week, with a return to Rawalpindi each evening. This allowed more time for planning, ordering supplies and managing data but reduced the frequency of contacts in the study area.

Follow-up clinics were due to begin in late September but it was not possible to do any work in the month before the general election so clinics began in late October, 1993. Attendance at some clinics was extremely low, partly due to the long time interval between the camp and clinic, so that community enthusiasm had waned, and partly due to the continuing local political resentments.

A second round of clinics in December 1993 - February 1994 proved to be even less well-attended and, after further consideration of the problems, a revised plan was implemented. This is described in section 2.5.

2.4.2. Consent

Community leaders again explained the importance of continuing the study and the need for further samples to be taken. Initially, there was some reluctance to further blood sampling but in villages where the relationship with community leaders was particularly strong, women agreed to a follow-up blood sample.

Community leaders also encouraged women to bring their babies for health check-ups, growth measurements and blood samples and explained the importance of the work to the health and well-being of the whole community. In this area, there was no culture of preventive health care, such as ante-natal check-ups or growth monitoring for children, so that the concept of attending a clinic when not actually ill, needed some explanation for most women. A separate consent form was filled out for women who agreed to let their babies be measured and to have blood samples taken but women often withdrew their consent during the actual blood-taking process, as described below.

2.4.3 Registration

New attenders at the clinic were registered on the "Camp Questionnaires", as described above in section 2.3.2., and went through the same process as at the camps. Records for women who had been registered at a camp and were attending the clinic as follow-up subjects were identified and details checked. Women attending for follow-up, without an infant, completed a second "Camp Questionnaire".

Mothers attending with infants were registered on a separate form entitled "Mother - General Information", which included questions on education and occupation, husband's education and occupation, consanguinity and some details of the birth. A brief reproductive history was also included on this form, which is shown in appendix 2.4.1. These mothers also completed a second form entitled "Mother - Clinic Sheet", similar in structure to the "Camp Questionnaire", shown in appendix 2.4.2.

Infants were registered on separate forms, entitled "Baby - Clinic Sheet", shown in appendix 2.4.3., containing details of clinic location and date, name, date of birth and food source. Further details of health status, anthropometry and iodine status were recorded during the stages described below.

2.4.4. Medical Consultation

This was similar to the consultation given at the camps but the lower numbers of women attending gave more time for discussion of medical problems with the doctor. In view of the cultural constraints surrounding male/female interactions, the nurses and senior investigator often acted as filters for medical complaints and opinions between the patient and doctor.

Infants were also seen and their general health assessed at this point. Pregnant women were given tetanus toxoid and infants were immunised, according to the schedule recommended by the Ministry of Health in Pakistan.

2.4.4. Anthropometry

Women had height and weight measured and infants had length, weight, head circumference and chest circumference measured, where possible, as described in appendix 2.1. These measurements were recorded on the appropriate forms.

Women were reluctant to allow anthropometry to be carried out on their infants and were particularly concerned that clothes were not removed. It was thus difficult to measure chest circumference and impossible to obtain nude weight. So few anthropometric measurements were taken (less than 20 women and less than 30 infants) that anthropometric results have not been analysed and are, therefore, not presented in the results section.

2.4.5. Sample Collection

Urine and venous blood samples were taken from the women, as previously described, but many women (>50%), including all pregnant women, withdrew consent at this point. Women who refused to allow a venous sample to be taken were also reluctant

to allow a finger-prick capillary sample. There was a widespread belief that each drop of blood required a mouthful of food to replace it so there was an understandable reluctance to allowing a process which was perceived as necessitating extra work and expense. There was also a misconception about the purpose of the blood-taking, many women believed that the research team was selling the blood for commercial gain.

A heel-prick capillary sample was obtained from infants whose mothers' gave permission but during the sample-taking, many mothers became distressed at their babies' cries and sample-taking often had to be abandoned before completion of the process. Where sampling was possible, 4 drops were collected on a Guthrie Card and one drop was used for haemoglobin measurement on the Hemocue.

It was not possible to obtain urine samples from babies in the field situation.

2.5. Follow-up Clinics - II

Following a disappointing response to the follow-up clinics, particularly in the number of babies seen, a change in strategy was decided in March 1994. Some of the major constraints and problems were identified and the 5 villages where follow-up had been most successful, mainly due to very good community relations with local leaders, were chosen and intensive efforts put into improving attendance at follow-up clinics.

2.5.1. Staffing Difficulties

The collaborating investigator, a doctor from the department of psychiatry, who was well-liked and respected in the villages, had done an excellent job in motivating the community, arranging the health committees and explaining the project but had been unable to participate in many of the camps. People in the study area put great store by personal contacts and his withdrawal from the camps and clinics had led to great disappointment in the villages and weakened support for the project.

In addition, it had not been possible for one doctor from the department of psychiatry to be seconded to the project on a full time basis, so junior doctors accompanied the team on a rota basis. Continuity was thus difficult to maintain and the village

communities did not appreciate seeing a different face each time they came to the clinic - familiarity being an important cultural consideration.

Towards the end of the first year, a female doctor was recruited to the project but she had to leave after a month, at her mother-in-law's insistence. The nurses continued to be the main channel for women to access health care, particularly for gynaecological advice and treatment. They received training in the obstetrics department and were able to perform simple antenatal checks.

2.5.2. Access Difficulties

Women who wanted to attend the clinic were not always able to do so, being reliant on older female or male relatives to give them permission to attend and to accompany them to the clinics. Some of the clinics were very remotely sited, in relation to the main concentration of households and these were discontinued.

In April, local *dais* (TBAs) were identified by the health committees and recruited to help in the community liaison. These women were active in encouraging women to attend the clinics and were able to accompany those women who had no chaperon to take them to the clinic.

Baseline blood samples, from all the women in these villages who had attended the iodine camps, were assayed for TSH and women with high TSH (ie $>$ 4.5 mIU/l) were identified. The *dais* were asked to bring these women to the next clinics, held in May to August 1994.

Birth registers in the local administrative offices were examined and any babies born within the study period were identified, together with their fathers' names (mothers' names are not recorded in Pakistan) and the *dais* asked to bring these babies and their mothers to the clinic. The records were incomplete and there was little overlap with the infants we had already identified. In addition, the *dais* were asked to encourage attendance by any pregnant women or women who had infants.

2.5.3. Local Events

Frequent weddings and funerals disrupted clinic schedules and the total lack of communications to the area meant that many visits resulted in postponed or cancelled clinics, demoralising the clinic team and further eroding community relations.

Security threats, such as *dacoits* (bandits) in the surrounding area, reduced clinic attendance and made travel to the study area more problematic. During one phase of the work, it was necessary to travel in convoy with other vehicles, under police protection, and some ex-Army members of the team carried firearms for protection.

In one village, the TBA associated with the project was discredited when her son was arrested and imprisoned for the theft of a neighbour's vehicle, thus discouraging contact with the clinic team. In another village, the murder of a woman who worked with us, by her two brothers, on suspicion that she had brought "dishonour" on the family, disrupted attendance for the rest of the year.

2.5.4. Climate and Geography

The weather disrupted clinics throughout the Monsoon period and in Winter, as expected, but the most severe rains in 40 years caused extensive flooding and landslides which blocked the roads for several weeks and prohibited entry to the study area for the project team.

2.5.5. Registration

New forms were designed, similar in content to those used earlier, but recording more information and these were used at subsequent clinics. Clinic sheets for mother and baby were essentially similar to previously but were easier to complete.

Every married woman completed a "Family Information" form, shown in appendix 2.5., with names and dates of birth (or approximate ages) for herself, their husband and their infant, and educational, occupational and consanguinity information. Reproductive losses were also discussed and recorded on a "Brief Pregnancy History" form, shown in appendix 2.5.

2.5.6. Blood Samples

All blood samples taken in this second phase were capillary -finger-pricks for the mothers and heel-pricks for the babies - and were collected on Guthrie Cards. Attendance continued to be poor and very few samples were collected. It was hoped that collection of dried blood-spots would allow longer-term follow-up of TSH and TT_4 levels, thus providing information on the duration of protection from iodine deficiency afforded by supplementation with IPSO but the number of samples collected was disappointingly small (4 for non-pregnant women and 7 for pregnant women).

There were some problems with the analysis of the blood-spots; many samples failing to elute from the filter-paper, leading to undetectable levels of TSH or TT_4 in the assay. The cause of this problem is not known. None of the 4 non-pregnant follow-up samples and only 2 of the 7 pregnant follow-up samples eluted satisfactorily. Both of these samples were collected 7½ months after supplementation, following delivery, and had TSH concentrations within the normal range for the analysis. Unfortunately, there was insufficient blood to analyse these samples for TT_4. Accordingly, these samples are not discussed in the results sections.

2.6. Laboratory Methods

2.6.1. Sample Handling

2.6.1.1. Urine

Urine samples were collected in plastic, wide-mouthed, 100 ml containers and transferred to smaller, 20 ml plastic screw-capped bottles in the field. Each sample was labelled with a unique number, matching the blood sample number for the same woman. Urine wase transported from the field, daily and kept in a refrigerator at 4°C, at NIH.

At a time when the senior investigator was on leave, the samples were removed to the "Cold Store" - essentially a storage room containing an air-conditioner - where they deteriorated in the extreme heat when the electricity was frequently off and where some were even consumed by rats.

2.6.1.2. Serum Samples

Venous blood was drawn into plain, 5 ml disposable syringes and transferred into glass test-tubes to clot and separate. In many villages there was no electricity supply so the bloods were left to separate for a minimum of 3 hours. In the few villages where electricity was both supplied and functioning, a portable centrifuge was used to speed up blood separation. Later in the study, a small generator was purchased, facilitating use of the portable centrifuge, until this was stolen.

The serum was drawn off the separated sample, using a disposable plastic pastette, and transferred into two 1.5 ml bullet tubes, labelled with the same unique number as the woman's urine sample. Serum samples were stored in labelled bags, containing 15-20 samples each, in a cool-box containing ice-packs, until transfer to a freezer at -20°C in the hospital.

On occasions where the team returned to Rawalpindi in the evening, samples were transferred to the freezer within 8 hours of collection. When the team stayed overnight in the village, the samples were kept in the cool-box for 24 hours and transferred to the freezer within 32 hours of collection.

2.6.1.3. Dried Blood Spots

Finger and heel-prick, capillary samples were collected on Neonatal Screening Guthrie cards, air-dried and kept in the dark, at room temperature, under dry conditions. They were analysed in London within 6 months.

2.6.2. Determination of Iron/Vitamin A Status

2.6.2.1. Haemoglobin

Haemoglobin was measured at the iodine camps and follow-up clinics using a portable HemoCue® haemoglobinometer (HemoCue AB, Ängelholm, Sweden). The assay cuvette was dipped into the freshly-collected whole blood and blotted on a tissue before measuring. After every 15-20 blood samples, the reference cell was measured. At the end of each week, control whole blood, at three levels of Hb were measured on the HemoCue® in the laboratory.

2.6.2.2. Ferritin

Ferritin was determined on duplicate serum samples, using an immunoradiometric assay kit from Diagnostic Products Corporation (DPC, Los Angeles CA, USA). In-house controls were used.

2.6.2.3. Vitamin A

Vitamin A was determined on duplicate serum samples, using an in-house hplc method described in Filteau, Roberts, Abbott *et al.*, 1993. In-house controls were used.

2.6.3. Determination of Thyroid Parameters

2.6.3.1. TSH

TSH was determined on duplicate serum samples, using the TSH "Serozyme" kit, an immunometric assay with a magnetic solid phase, from Serono Diagnostics (Geneva). Absorbances were measured and concentrations calculated on the "Serozyme I" analyser. The mean concentration and CV between duplicate pairs were calculated by the analyser. Any duplicates with CV higher than 15% were reanalysed. Commercially available controls (Biorad "Lyphocheck"), at three different levels of TSH concentration, were also assayed in duplicate with each batch of samples.

2.6.3.2. FT_4

FT_4 was determined on duplicate serum samples, using the Serono FT_4 "Serozyme" kit, an enzyme immunoassay with a magnetic solid phase. As with TSH, absorbances were measured and concentrations calculated on the "Serozyme I" analyser. Duplicates with CV higher than 15% were reanalysed. Biorad "Lyphocheck" controls, at three levels of FT_4 concentration, were also assayed in duplicate with each batch of samples.

2.6.3.3. TT_4

TT_4 was determined on singlicate serum samples, using a direct radioimmunoassay kit from Nicholls Institute Diagnostics (San Juan Capistrano, CA, USA). Radioactivity was measured and concentrations of TT_4 calculated. Any samples with TT_4 concentration far outside the "normal range" given on the kit (see section 34.2.3.) were reanalysed. In-house controls were assayed at two levels of TT_4 concentration.

2.6.3.4. TBG

TBG was also determined on singlicate serum samples, using a Nicholls Institute Diagnostics direct radioimmunoassay kit. Radioactivity was measured and concentrations of TBG were calculated, as for TT_4. In-house controls were assayed at two levels of TBG concentration.

2.6.3.5. FT_3

FT_3 was determined on singlicate serum samples, using the "Coat-a-count" solid-phase radioimmunoassay kit, from DPC. Radioactivity was measured as above and FT_3 concentrations calculated. Two in-house controls were used as above and samples with unusually high or low FT_3 concentrations were reanalysed.

2.6.3.6. TT_3

TT_3 was determined in a similar fashion, using the DPC "Coat-a-count" TT_3 kit.

2.6.3.7. TG

TG was determined using the DPC TG kit, a double antibody radioimmunoassay, with in-house controls and reanalysis conditions as above.

2.7. Data Analysis

Data from approximately half (732/1432) of the women contacted at the iodine camps has been analysed. These 732 women included:

1. All women who returned for follow-up blood samples,
2. All women from the 5 best follow-up clinic sites,
3. All pregnant women,
4. All women who attended a camp with an infant,
5. All women who completed a "Family History Questionnaire" (FHQ).

In general, results are presented separately for pregnant and non-pregnant individuals and comment is made on any differences arising from analyses of groups 1 and 2 individually or combined.

It was initially expected that restricting analysis to all women from the 5 key village sites might remove any bias due to the self-selecting nature of follow-up in other villages, but the make-up of these 5 villages may not have been representative of the whole area either. The population characteristics, therefore, refer to these groups and may not necessarily be extrapolated to the whole population.

2.7.1. Population Characteristics

2.7.1.1 Subject Selection

Data on age, haemoglobin and marital status was taken from the initial camp questionnaires (CQ) of all the women detailed above (groups 1 to 5), i.e. 732. The data is presented separately for non-pregnant and pregnant women, with discussion, in the text, of differences between the various groups above.

Data on consanguinity, education, occupation and husband's age was taken from FHQ completed by married women at follow-up clinics (group 5). Just over half (53/102) of these women allowed a blood sample to be taken.

Ferritin and Vitamin A determinations were performed on a random selection of samples which had already been tested for some thyroid functions (from groups 1 and 2). There is little discussion of these results, as they form part of the background to the study

population, rather than being the main focus of the investigations.

2.7.1.2. Presentation of Results

Results are presented in diagrammatic form, with some summary statistics in the text, or as tables with **mean**, **standard deviation (SD)**, **standard error (SE)** and **95% confidence interval (CI)**, **median** and **mode** values (where appropriate) for **continuous variables**, e.g. maternal age, haemoglobin; and as **proportions (%)** of the total, with **95% CI (±)**, for **categorical variables**, e.g. occupation. For variables which were normally distributed, arithmetic means etc. are presented. Where a transformation was necessary to yield normal data, explanation is given in the text.

2.7.2. Thyroid Function in Pregnant and Non-pregnant Women

2.7.2.1. Subject Selection

Biochemical analysis of blood samples was initiated during the early stages of the field-work, when growth monitoring of infants was still thought to be achievable. For this reason, samples selected and analysed during this early stage of analysis included all (self-reported) pregnant women (group 3) and those attending with infants (group 4) who were expected to form part of a pre-supplementation comparison group (i.e. infants born to mothers who had not been supplemented during pregnancy).

During the first round of follow-up clinics, when it became apparent that attendance was lower than expected, particularly amongst the target study group of mothers with infants, targeted recall was attempted, with TSH screening of all samples taken at iodine camps in the 5 key villages (group 2) - those with a particularly good response to the first round of follow-up or with a particularly large number of pregnant women contacted at the camps.

In addition, pre- and post-supplementation sample pairs were analysed for all women who returned for the first follow-up clinic and allowed a second blood sample to be taken (group 1), not only for those who attended with an infant, as had been originally planned.

These four groups of samples (1,2,3,4) were assayed for TSH and/or FT_4 at the NIH laboratories in Islamabad, using the methods described in section 33.2. FT_3, TT_4, TT_3, TBG and TG were assayed in London, as described in section 33.2., following completion of the field-work.

In all, 509 pre-supplementation samples were analysed for TSH, of which 140 were analysed for at least one other parameter. The number of samples analysed for each parameter differs, according to the volume of serum available. Not all samples were analysed for all parameters, due to inadequate sample volume, sample deterioration or sample loss between different assays. All 7 biochemical thyroid parameters analsyed (TSH, FT_4, TT_4, FT_3, TT_3, TBG and TG) were measured in 97 samples.

2.7.2.2. Presentation of Results

Results are presented in summary tables as:

arithmetic mean, with **SD**, **SE** and **95% CI**, for **continuous variables** which had an **arithmetic** distribution (TT_4, TBG)

geometric mean, with **GSD**, **GSE** and **95% CI**, for **continuous variables** which had a **geometric** distribution (TSH, TG, FT_4, FT_3, TT_3, FT_3/FT_4 ratio, TT_3/TT_4 ratio)
proportions (%) of the total, with **95% CI (±)**, for **categorical variables.**

2.7.2.3. Thyroid Category

In order to compare the usefulness of various measures of thyroid status in this iodine-deficient community, the samples were allocated to one of three categories, "**hypothyroid**", "**euthyroid**" and "**hyperthyroid**", as defined by a range appropriate for the particular method used to measure each parameter. In most cases this was the "**normal range**" given with the method instructions for each kit used (i.e. the range within which 95% of "**normal**" subjects assayed by the kit manufacturer fell). "Normal ranges" were not always available for pregnancy and explanations of ranges used are given for individual parameters below.

2.7.2.4. Normal Ranges

The question of what constitutes a **"normal range"** is fundamental to any classification of a sample as **"normal"** or **"abnormal"**. It should, properly, refer to the particular method used to assay the parameter and the particular **"normal"** population from which patients are drawn. Hospital laboratories in UK often construct their own **"normal range"** for each particular test, based on assays of **"normal subjects"** - generally hospital patients not suffering from a clinical condition related to that particular test.

For this study, a similar, Pakistani population, living in a non-iodine deficient area might be considered a suitable control population from which to construct a **"normal range"**. From enquiries at local hospitals and at the National Institute of Health (where some of the assays were performed) it was clear that such Pakistani normal ranges were not in use and that the practice was to use the reference values supplied with assay kits. It was not possible, within the framework of the study, to construct normal ranges specifically for this study and the reference ranges supplied with the assay kits were used to assign thyroid status, with some exceptions explained below.

In scattergrams, normal ranges are marked with boundary lines and the area representing the normal range for both axes is shades yellow.

2.7.2.4.1. TSH

The euthyroid range supplied with the assay kit, based on the 2.5 and 97.5 percentiles of **"euthyroid subjects"**, was 0.3-4.5 mIU/l, with a median of 1.68 mIU/l and this range was used to define thyroid categories. This may have slightly overestimated the proportions of women classified as TSH-**"hypothyroid"** and TSH-**"hyperthyroid"**, who were in the extreme limit of the "euthyroid" range.

Clinically **"hypothyroid"** subjects tend to have TSH over 6.0 mIU/l (Lazarus, 1996) but several other studies have used lower cut-offs, as shown in table 38.4. Recall in the 5 key villages was based on a TSH level above 4.5 mIU/l, in order that women who might be mildly "hypothyroid" could be further investigated.

Clinically **"hyperthyroid"** subjects rarely have TSH over 0.1 mIU/l, as determined by modern, sensitive methods (Wilkinson, Rae, Thomson *et al.*, 1993), but some earlier studies used higher cut-offs, as shown in table 38.4.2. For consistency across parameters and ease of comparative analysis, this **95% central range** was used to define the categories as below:

TSH-"hypothyroid": > 4.5 mIU/l

TSH-"euthyroid": 0.3-4.5 mIU/l

TSH-"hyperthyroid": < 0.3 mIU/l

There were no ranges for pregnancy given with the kit and comparison with the reference text (Teitz, 1995) indicated that the ranges for each trimester of pregnancy were similar to that for non-pregnant women. Accordingly, the results have not been analysed by trimester-specific ranges.

2.7.2.4.2. TG

The reference range given with the kit included an absolute range of undetectable to 53 ng/ml with a **"central 95% range"** of "up to 21 ng/ml" for "subjects with no known thyroid disease". TG is raised in both **"hypothyroid"** and **"hyperthyroid"** conditions, as well as a variety of other thyroid disorders (Teitz, 1995). Samples were classified according to this **"95% central range"** as below:

TG-"normal": < 21 μg/l

TG-"abnormal": > 21 μg/l

No specific ranges were given for pregnancy.

2.7.2.4.3. FT_4

The reference range given with the kit was based on the **2.5 and 97.5 centiles** of a FT_4 distribution of samples from **"normal healthy subjects"**. The **"normal range"** so-defined was 9.3-22.1 pmol/l with a median of 13.1 pmol/l. The **95% central range** was used to define the categories as below:

FT_4-"hypothyroid": < 9.3 pmol/l

FT_4-"euthyroid": 9.3-22.1 pmol/l

FT_4-"hyperthyroid": > 22.1 pmol/l

For pregnancy, the **"normal ranges"** given in the kit were based on the central 95% of the FT_4 distributions for **"healthy pregnant women"** in the second and third trimesters. No normal range was given for the first trimester, but comparison with other kits and method descriptions (Teitz, 1995) suggested that the range was similar to that for non-pregnant women. The **"normal ranges"** quoted with the kit were:

9.3-22.1 pmol/l for **non-pregnant** women,

no range given for **first trimester**

8.9-23.0 pmol/l for the **second trimester**,

8.2-17.4 pmol/l for the **third trimester.**

2.7.2.4.4. TT_4

The **"normal range"** provided with the kit was based on the **"central 95% range"** of the distribution of TT_4 concentrations found in **"normal subjects"** and used cut-offs of 73 and 146 nmol/l to define the **"normal range"**. This kit provided sex-specific **"95% normal ranges"** as well as the **"absolute range of observed values"** for **"hypothyroid"** (1.3-62 nmol/l) and **"hyperthyroid"** (163-362 nmol/l) subjects. For non-pregnant women the following categories were defined:

TT_4-"hypothyroid": < 73 nmol/l

TT_4-"euthyroid": 73-146 nmol/l

TT_4-"hyperthyroid": > 146 nmol/l

For pregnancy, the **"normal range"** given in the kit was based on the **"absolute range of observed values"** for a group of 21 women in the last 5 months of pregnancy and was 124-213 nmol/l. There was no range given for early pregnancy.

2.7.2.4.5. TBG

The **"normal range"** provided with the kit was 14-30 mg/l for women aged 20-50 but the **absolute ranges** of TBG values for both **"hypothyroid"** and **"hyperthyroid"** subjects fell within these limits (8-27 and 9-29 mg/l, respectively) and overlapped with one another. TBG is dependent of many factors other than thyroid status (Teitz, 1995) and therefore should not be used alone, as a basis for classification of thyroid status. Comment is made on the TBG concentrations in relation to the given normal range but no thyroid categories are defined.

For pregnancy, the **"normal range"** given for the kit referred to the **"absolute range of observed values"** in a group of 21 healthy, pregnant women with no known thyroid disease, and was 34-69 mg/l for the last 5 months of pregnancy (the same group as in TT_4 above). No range was given for early pregnancy, when TBG levels are known to increase rapidly. As with non-pregnant subjects, no attempt was made to put samples into TBG-thyroid categories, but comment is made on the number of samples outside this range.

2.7.2.4.6. FT_3

The reference range provided with the kit was 2.2-6.8 pmol/l for **"euthyroid"** subjects and represented the **"95% central range"** of the FT_3 distribution for **"normal adult"** subjects, with a median value of 4.3 pmol/l. In addition, the **absolute ranges** of values measured in **"hypothyroid"** and **"hyperthyroid"** patients were reported as 0.5-4.8 and 8.0-35 pmol/l, respectively, with corresponding medians of 0.9 and 14 pmol/l. The **"95% central range"** for euthyroid subjects was used to define the categories as below:

FT_3-"hypothyroid": < 2.2 pmol/l

FT_3-"euthyroid": 2.2-6.8 pmol/l

FT_3-"hyperthyroid": > 6.8 pmol/l

The kit used to measure FT_3 gave an **"absolute range of observed values"** for an unspecified number of pregnant women in the first and third trimesters but no value for second trimester. The reference text (Teitz, 1995) suggested that the range for the second trimester was similar to that for the first trimester. The ranges given with the kit were:

3.2-7.2 pmol/l for the **first trimester**,

no range for the **second trimester**,

2.6-7.1 pmol/l for the **third trimester**.

2.7.2.4.7. TT_3

The reference range provided with the kit was 1.3-2.9 nmol/l and represented the **"95% central range"** of the TT_3 distribution for **"normal adults"**, with a median value of 2.0 nmol/l. This **"95% central range"** was used to define the categories as below:

TT_3-"hypothyroid": < 1.3 nmol/l

TT_3-"euthyroid": 1.3-2.9 nmol/l

TT_3-"hyperthyroid": > 2.9 nmol/l

The kit used to measure TT_3 gave no "normal range" for pregnancy but the ranges in the reference text (Teitz, 1993) suggested that the range for the last 5 months of pregnancy would be approximately 0.7 nmol/l higher, compared to the reference range for non-pregnant women. Thus, the range would be approximately 2.0 to 3.8 nmol/l.

2.7.2.5. Associations Between Variables

Associations between the different measures of thyroid status were investigated by three methods. Where significant associations were found these are discussed in the text.

Firstly, **correlations** between the different parameters were investigated and appropriate **regression equations** calculated. The correlation testing required data to be normally distributed, so for some measures of thyroid status, the appropriate data for correlation testing was the concentration of the particular parameter itself (TT_4, TBG), and for others, it was the log-transformed concentration (TSH, TG, FT_4, FT_4 and TT_3,). TT_4 might be expected to follow a skewed distribution similar to the other thyroid hormones but it was more normally distributed, due to it's strong relationship with TBG concentration, a normally distributed parameter.

Secondly, one way analysis of variance (**one-way ANOVA**) was performed to test for **differences in the (appropriate) mean** value of one parameter, according to thyroid status category assigned by use of a second parameter. Again, normally-distributed data were used, which meant that the appropriate measurement was sometimes the arithmetic mean (for thyroid parameters which were normally distributed - TT_4 and TBG) and sometimes the geometric mean (for thyroid parameters which were normally-distributed following a natural log transformation - TSH, TG, FT_4, FT_4 and TT_3).

Thirdly, a **X^2 test** was performed on a **cross-tabulation** of the thyroid categories assigned by reference to the two parameters. In general, this was a **X^2 test on a 3x3 table.**

2.7.2.6. Practical Limitations of Goitre Grading

At the iodine camps, goitre grade was assessed by the doctors, during the medical consultation with the women, and recorded on the "camp questionnaires". There were, however, some problems associated with the assessment:

1. It is widely accepted that there is considerable inter-observer variability in assessing goitre and that, in the ideal situation, one observer should do all the goitre grading (Dunn and Van de Haar, 1990). The investigator had expected to do the grading herself but this proved to be impractical in the field situation, given her other responsibilities e.g. supervision of blood sampling, labelling and separation of samples, interviewing women. Doctors involved in the camps were briefed by the investigator on the WHO goitre grading definitions (see section 7.1.) and asked to assess the goitre grade for the women.

A large number of doctors took part in the camps, with up to 4 doctors in any one site responsible for seeing patients and a changing team of doctors each day (the monthly rota of doctors for the camps included 5 medical officers, 4 house officers and 6 medical students). Some of the doctors were not familiar with the WHO goitre classification and it was not always possible to give a demonstration in the camp situation, so that uniformity of assessment was not possible to achieve.

2. As mentioned previously, all the doctors in the initial camps were male and it was not culturally appropriate for them to palpate a woman's thyroid to assess goitre grade, in the absence of an obviously visible goitre. Without exception, women wore head-coverings and sometimes declined to remove these so that even assessment by eye was not always possible. There was, however, a reluctance, on the part of the assessors, to leave the goitre grade blank on the questionnaire, so it was not always possible to determine which women had actually been graded and which had been guessed at.

3. Cross-checking of goitre grade assessment was sometimes possible, during some of the less well-attended camps, and there were considerable inter-observer differences in recorded goitre grade. In particular, many women who had goitre grade recorded as 1b (visible only when the neck is in the extended position), when examined by the investigator, before blood sampling, were reassessed as having a clearly visible grade 2 goitre. It was not, however, possible to systematically reassess goitre grade at the camps, due to the large number of women attending and the time constraints in the field.

Due to the variability of goitre grading at baseline, and continuation of the problems outlined above, determination of goitre grade at follow-up was not carried out.

2.7.3. Thyroid Function in Pregnancy

Many thyroid parameters change during pregnancy (see section 17) so it was appropriate to analyse data according to **stage of pregnancy** and data were therefore grouped by **trimester**, according to the self-reported **number of completed months** of pregnancy. One way analysis of variance (**one way ANOVA**) was performed on the (appropriate) mean concentrations of each parameter, according to trimester, and comments are made on any trends or significant differences observed between trimesters.

Due to the small number of pregnant women recruited, it has not been possible to perform such extensive analysis as was performed on the samples from non-pregnant women.

2.7.4. Follow-up Thyroid Function in Non-pregnant women

The results presented in section 42 refer only to **baseline-follow-up sample-pairs**, i.e. women for whom both pre- and post-supplementation samples were available. For this reason, pre-supplementation data may vary slightly from that presented in the baseline results chapter, although the differences are small and are mainly seen in higher SDs and wider 95% CI of means and proportions.

e.g. pre-supplementation geometric mean TSH concentration for:

all samples: 1.91 mIU/l (GSD 0.65, GSE 0.04, 95% CI 1.76-2.08, n=509),

paired samples: 2.11 mIU/l (GSD 1.04, GSE 0.10 95% CI 1.72-2.60, n=101).

Results are presented in a summary table, using the same measures of distribution as for baseline results alone. A comparison of pre- and post-supplementation samples was made by comparing (appropriate) mean values and proportions in the different thyroid categories.

For parameters which were normally distributed, an **arithmetic paired difference** was calculated by subtracting the pre-supplementation concentration from the post-supplementation concentration, for each pair of data, and an **arithmetic mean paired difference** calculated and tested for significance using a **paired t-test.**

For parameters which were normally distributed, following a natural log-transformation, a **geometric paired difference** was calculated by subtracting the natural log-transformed pre-supplementation concentration from the natural log-transformed post-supplementation concentration, for each pair of data, and a **geometric mean paired difference** was calculated and tested for significance using a **paired t-test.** This represents the mean **ratio** of the pre- and post-supplementation concentrations. i.e. the mean of post-supplementation concentrations divided by pre-supplementation concentrations. Further explanation is given in the text for each parameter.

The **paired t-test** is valid only for parameters which are **normally distributed** and, as the data were not perfectly normal in this study, the **non-parametric Wilcoxon Matched-pairs Signed-ranks test**, was also used to test for differences in pre- and post-supplementation concentrations of the parameters. The differences between the results of applying these two tests were small, because most parameters (or their natural log-transformed values) were distributed fairly normally.

2.7.4.1. Individual Changes in Thyroid Function

The change in mean value of a thyroid parameter or the change in proportions of subjects in different thyroid categories can hide widely differing, individual responses to the supplementation. Pre- and post-supplementation values for individual samples are plotted against one another. The thyroid profiles of subjects who responded differently to the iodine supplementation, as revealed by changes in their thyroid status categories, for each appropriate thyroid parameter, are discussed.

2.7.5. Comparison with Other Studies

The results from this study are discussed in the light of previous work, although it has not always been possible to find directly comparable measurements in earlier studies. In particular, few studies exist where the sample population was exclusively non-pregnant women and individual studies vary widely in the biochemical parameters measured, the particular assay methods used and the reference ranges (or control populations) quoted. Studies chosen for comparison purposes are those which contained measurement of parameters most nearly comparable to those made in this study.

2.7.6. Reproductive Data

This data is presented in appendix 3.1.4.

2.7.6.1. Subject Selection

Data was analysed for all (102) women who completed the FHQ (group 5). Subjects were thus self-selecting, in that any woman who completed a FHQ did so after presenting at a follow-up clinic. These women may have been those who were particularly careful about their health care and seek preventive medical care more readily than others, those who received permission to attend or those who were not well and so came to the clinic. Such women may have had a greater or a smaller risk of experiencing reproductive losses than the general population in this area and may not have been representative of the childbearing community as a whole. Results are thus presented as interesting background information, rather than as the outcome variable initially envisaged.

2.7.6.2. Presentation of Results

The number of reproductive events was summed across all women who completed an FHQ and a rate calculated per 1000 pregnancies, total births or live births, as appropriate.

Information concerning the woman's age at, or length of, marriage was not collected so an estimate of "reproductive exposure years" (REY) was made by subtracting 16 (the reported, average age of marriage in this community) from the reported age of the woman, to give the number of years that a women may be regarded as being "at risk of pregnancy".

This crude estimate of the length of time that a woman may be at risk of pregnancy, allowed calculation of an average "reproductive profile" of a typical woman in this community, although it should be remembered that the REY estimate is itself based on an estimated age and the assumption that the average age of marriage is 16 and that women are at risk of pregnancy from this age onwards.

In practice, the age of marriage may vary considerably, as may an individual woman's risk of pregnancy but, in the absence of more detailed information, an estimated REY does allow some general comment of childbearing patterns in this community and agreed with the annecdotally observed patterns (Baksh, 1994, pers.comm.).

It had, initially, been hoped that reproductive histories could be obtained from all married women attending the iodine camps, and that these could be analysed in relation to a woman's thyroid status before supplementation but this proved to be not possible for a variety of reasons. Given the staff and time available in the iodine camps, reproductive histories were not recorded at the time of initial contact but it was planned that all pregnant women, identified at the camps, should have a reproductive history taken when they attended for follow-up.

As explained in sections 2.4. and 2.5., fewer pregnant women than expected were identified at the camps and the response to follow-up was poorer than anticipated. Thus, few of the pregnant women returned and pregnancy questionnaires were collected on any women who attended a clinic, and had experienced at least one pregnancy, mainly during the second phase of follow-up. During this phase, very few blood samples were taken, and those that were obtained were capillary blood-spot samples.

Thyroid status was, thus, available for only half the women interviewed concerning reproductive losses and it has not been possible to show any relationship between thyroid status and reproductive losses, due to the small sample size. The data was analysed for any associations between thyroid status, as defined by the various categories of thyroid parameter and the different loss rates but no significant associations were found, probably due to the small number of women on whom data was available.

CHAPTER 3 - RESULTS

3.1.Background Population Characteristics

3.1.1. Location

3.1.1.1. Topography and Climate

The study site was Lehtrar Union Council, in Kotli Sattian Thesil (an administrative sub-district), Rawalpindi Division, Punjab Province. This is a remote area, about 50 km north-east of Rawalpindi, at an elevation of 1,000 - 1,200 m, in the Murree hills which rise from the Potohar Plateau of the northern Punjab. The soil is thin but supports some subsistence farming, along with coniferous forestry. There are very few metalled roads serving this area. A sketch map of the area is shown in figure 2.2.1.

Rainfall is in the range 2.0-2.5 m and falls in the Summer Monsoon and Winter rains. The temperature varies between an average of 10°C in Winter and 30°C in Summer. Springs, which are abundant and often protected in this area, are the main source of water and in some villages these are capped and water piped down the hill to the houses. There are also some streams and wells which are used for drinking water.

3.1.1.2. Economic Status

The main occupation in the area is Army Service, so many of the men are away on duty, leaving the households headed by an older male, usually himself retired from the army. There is little other paid employment in the area, although some men work as commercial transport drivers, shopkeepers or school teachers. Commercial activity is centred on the few roads and consists of a few flour and saw mills, chicken farms and local shops. The area is described as "economically backwards" by local officials and political leaders, although there are plans to bring electricity and therefore greater commercial development to the area.

Educational data were not available for this particular area but school attendance rates are likely to be lower than national rates, which include both urban and more remote areas. Nationally, average primary school enrolment, between 1986 and 1992, was 54% for boys and 30% for girls, with only 48% of enrolled school children reaching grade 5 (UNICEF,1995). Adult literacy was estimated, nationally, at 47% for males

and 21% for females, although very few women in this area reported **ever** attending school (see section 3.2.4.).

3.1.1.3. Culture

The area has a conservative, traditional Muslim culture, with village life centred around the mosque. All commercial activity is controlled by men and it is rare to see women outside their homesteads. People live in extended families, in grouped homesteads, surrounded by their land, which is often terraced. Women perform most of the agricultural work, while men undertake paid employment, usually outside the village. The language spoken is *Potowari,* a dialect of *Punjabi*, but *Urdu*, the national language of Pakistan is widely understood. All schools in the area are Urdu-medium and few people speak English.

3.1.2. Discussions in Focus Groups

3.1.2.1. Perceptions of Goitre

Goitre was recognised as an "abnormal" condition, despite its widespread distribution in the community. It was considered unattractive, so always covered by a woman's "*dupatta*" (scarf), and was blamed for tiredness, weakness, aches and shortness of breath but no link was made between goitre and reproductive losses. Goitre was recognised as beginning in childhood, around 5-6 years of age, and growing in the teenage years, particularly in girls. No mention was made of goitre in the mother being linked to mental disability in children.

It was thought to be caused by some agent in the water -some people reported it as an excess of calcium or magnesium, others as a deficiency. At the beginning of the study, no-one identified the causative agent as a lack of iodine, but extensive media coverage over the timescale of the project meant that many community leaders (though still very few women) knew that goitre was linked to iodine deficiency by the end of the study. Interestingly, after the study, although iodised salt was cited as a cure for goitre, only one community leader had made a special effort to obtain it from Rawalpindi and reported using it in his family.

3.1.2.2. Perceptions of Disability

The types of disability recognised in the community were sensory (blindness, deafness), motor (lameness, weakness of limbs) and mental (slowness, stupidity, madness) and in each village where focus group discussions were held, some people were identified as being disabled. Some disabilities were recognised from birth, e.g when a TBA noticed something different about the baby, but others were not apparent until later, e.g. a child who did not learn to speak. No cretins were observed or reported in the community.

The cause of disability was identified as "*Inshallah*" (God's will), which allowed some people to be born disabled and others to experience disabling accidents. There was, therefore, seen to be no point in seeking to treat or manage a condition which had been ordained by God. Medical advice or help was rarely sought for disability and it was generally believed that there were no means to prevent disability, either before or after birth. Disabled children were thought to be specially blessed by God and valued as "saints".

3.1.2.3. Existing Health Care Provision

In each Union Council there was one Government-built Basic Health Unit (BHU), intended to serve a population of about 25, 000, but these were either not completed, or not staffed, or under-equipped and were not functioning as intended. Health care in the area was therefore on a rather *ad hoc* basis and access to allopathic medicine was extremely limited.

3.1.2.4. Health-seeking Behaviour

Informal interviews and guided focus-group discussions revealed a number of channels for accessing informal health care, at the family and village level, and the widespread use of traditional medicines and remedies for conditions ranging from "*kamzor*" (weakness) to aches and pains, fevers and broken bones.

3.1.2.5. Health Care Providers

Women live in extended families, in their husband's home and reported that their initial requests for health care were often made to their mothers-in-law. Other, older women

in the village, such as the traditional birth attendants (TBAs) were also felt to be useful sources of health advice and local "*devaii*" (medicine).

Some villages had people who were identified as being particularly skilled in preparing remedies or who were faith-healers. Others had "private clinics" where male nurses, medical aides or dispensers (often retired army personnel, calling themselves "doctor") could be consulted. These practitioners dispensed commercially available drugs and preparations and often offered "*tikka*" (injections) as a matter of course. Syringes and needles were reused, often with no cleaning at all.

3.1.2.6. Treatments Available

For weakness, aches, pains and fevers, locally-brewed "*kahawa*" (herbal teas) were made from fenugreek, mint and various grasses. Vitamin pills and aspirin were very popular, particularly for cases of "weakness", although injections were generally felt to be the most effective in treating disease and "weakness". Many women reported having injections of vitamin B-complex "for strength". Local remedies were cheap or free but many were prepared to pay for pills and injections.

3.1.3. KAP Study of Childbearing

A Knowledge, Attitudes and Practice Study of Childbearing in presented in appendix 3.1.3.

3.1.4. Reproductive Data

This is presented in appendix 3.1.4.

3.2. Maternal characteristics

3.2.1. Maternal Age

The mean age of all women attending the iodine camps, pregnant and non-pregnant combined, for whom data was analysed, was 25.8 years (±0.6, n=710). Table 3.2.1., details the mean, median and mode ages, under various analysis conditions, but it should be remembered that all ages were self-reported and there was often considerable uncertainty as to a woman's age. When asked to give her age, women would often confer with friends and relatives, or estimate the number of years since marriage and add to the supposed age on marriage. There was, thus, considerable "nesting" of ages, around 22, 25, 30, 35 and 40, as shown by the modes in the table below.

The youngest women supplemented were 15, with the exception of two 12-year-old girls who were related to one of the camp organisers, and the oldest, reported age was 45.

Women included in analysis	n	mean age in yrs (95%CI)	SD	SE	median	mode
All	710	25.8 (±0.6)	8.2	0.31	25	15
All pregnant	101	25.4 (±1.0)	5.1	0.51	25	25
All non-pregnant	573	25.8 (±0.7)	8.8	0.37	25	15
All returners	136	27.7 (±1.4)	8.2	0.70	27.5	30
All in 5 key sites	547	25.6 (±0.7)	8.6	0.37	25	15
Pregnant in 5 key sites	38	25.6 (±1.6)	4.8	0.78	25	22
Non-preg. in 5 key sites	476	25.6 (±0.8)	9.0	0.41	25	15
Returners in 5 key sites	66	28.4 (±2.2)	8.9	1.10	27.5	40
All in 4 key sites	427	26.7 (±0.8)	8.4	0.41	25	35
Pregnant in 4 key sites	33	25.0 (±1.6)	4.6	0.81	25	22
Non-preg. in 4 key sites	369	26.8 (±0.9)	8.8	0.46	26	35
Returners in 4 key sites	63	28.5 (±2.3)	9.1	1.15	27	40

N.B. Data for the 4 key sites excludes Naniah from the analysis

Table 3.2.1. Age of women seen at iodine camps.

In one of the key villages, Naniah, a local female school teacher, who was related to the camp host, brought along all the 15 and 16 year old girls from her school. This admirable public health strategy, in terms of protecting girls who could expect to be married soon, from iodine deficiency, artificially altered the age distribution in that camp, lowering the mean age.

Women who returned for follow-up had slightly higher mean ages, whether analysis was done for all women who returned or those from the 5 key sites. This may reflect the fact that follow-up was targeted at women with infants and therefore selected against younger women who were unmarried or had no children.

3.2.2. Marital Status

The marital status of all women for whom data was analysed is shown below. Analysis by individual village, for the 5 key sites, revealed differing patterns of attenders, with regard to marital status, in each village.

Marital Status	All	Biaga	Behl Chakka	Durnuyian	Naniah	Golla
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
Single	206 (28.1)	44 (25.3)	28 (25.7)	42 (32.3)	71 (57.7)	3 (9.7)
Engaged	11 (1.5)	6 (3.4)	1 (0.9)	1 (0.8)	0 (0)	0 (0)
Married	500 (68.3)	118 (67.8)	78 (71.6)	85 (65.4)	50 (40.7)	28 (90.3)
Widowed	10 (1.4)	5 (2.9)	1 (0.9)	1 (0.8)	1 (0.8)	0 (0)
Divorced	2 (0.3)	0 (0)	1 (0.9)	0 (0)	1 (0.8)	0 (0)
Missing Data	3 (0.4)	1 (0.6)	0 (0)	1 (0.8)	0 (0)	0 (0)
Total	732 (100)	174 (100)	109 (100)	130 (100)	123 (100)	31 (100)

Table 3.2.2. Marital Status of women at the camps.

3.2.3. Consanguinity

In common with many areas of Pakistan, there was a high degree of intra-family marriages. Figure 3.2.3. shows the familial relationships between husbands and wives. 50% of women were married to their first cousins - i.e. shared one set of grandparents with their husband. In many cases, the relationships were more complicated and couples were related to each other through several family branches, as there was much inbreeding in these village communities.

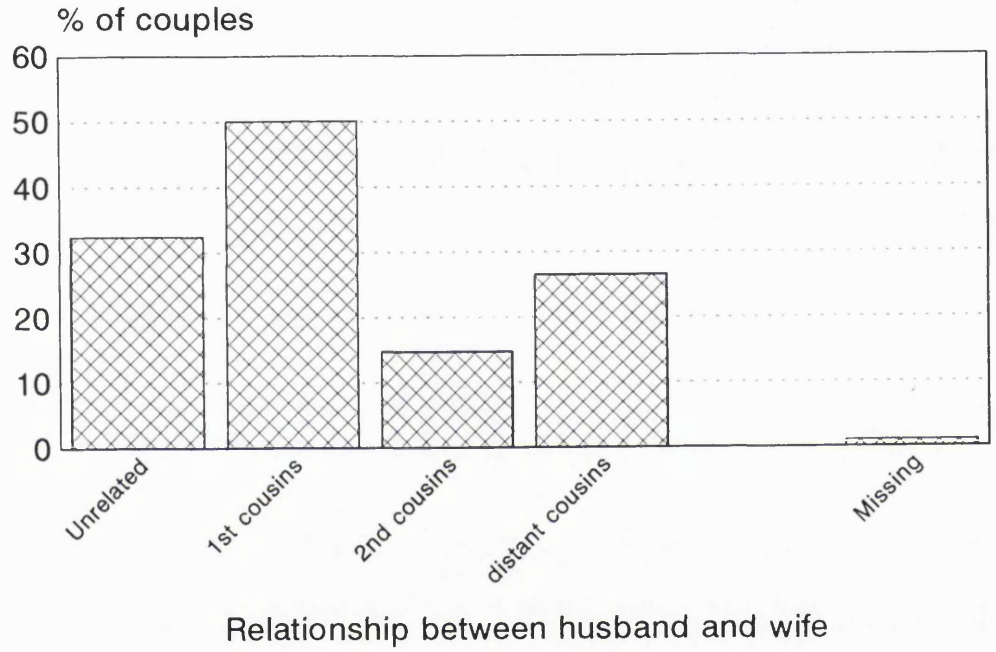


Figure 3.2.3. Consanguinity in the study area

3.2.4. Maternal Education

Almost two-thirds (63%) of those interviewed had received no formal education. One quarter had completed primary school (grade 4, about age 12) and very few had completed middle (grade 6, about age 14) or secondary (grade 10, about age 18) school (6% and 2%, respectively). The education level reached is shown in figure 3.2.4.

3.2.5. Maternal Occupation

Only 2 women reported any occupation, other than being a "housewife", and these were both schoolteachers who were involved in the school-child study.

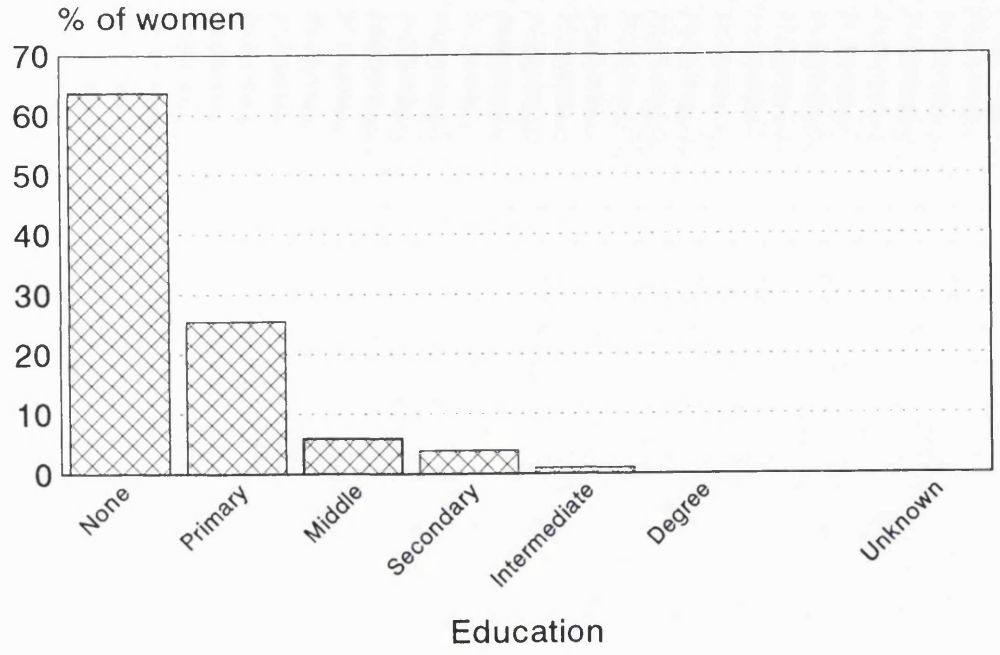


Figure 3.2.4. Maternal Education (n=102)

3.2.6. Haemoglobin (Hb)

3.2.6.1. Community Response

There was widespread appreciation of the immediate feedback of haemoglobin values to the women, together with an explanation of the implications for their health, dietary advice and prescribing of iron supplements where this was necessary. Measuring haemoglobin in the camp meant that the women could see a use for the blood-collection and were less reluctant to allow sampling, once they saw that the blood was being tested and results given to them immediately.

3.2.6.2. Hb Distribution

Haemoglobin values followed a typical, negatively-skewed distribution, shown in figures 3.2.6.2.1. and 3.2.6.2.2. for non-pregnant and pregnant women, respectively, with median Hb concentrations of 124 g/l and 114 g/l. A squared transformation normalised the data and the mean concentrations were:

121.7 g/l (95% CI 120.1-123.3, n=520) for **non-pregnant** women,

112.5 g/l (95% CI 109.0-115.0, n=92) for **all pregnant** women.

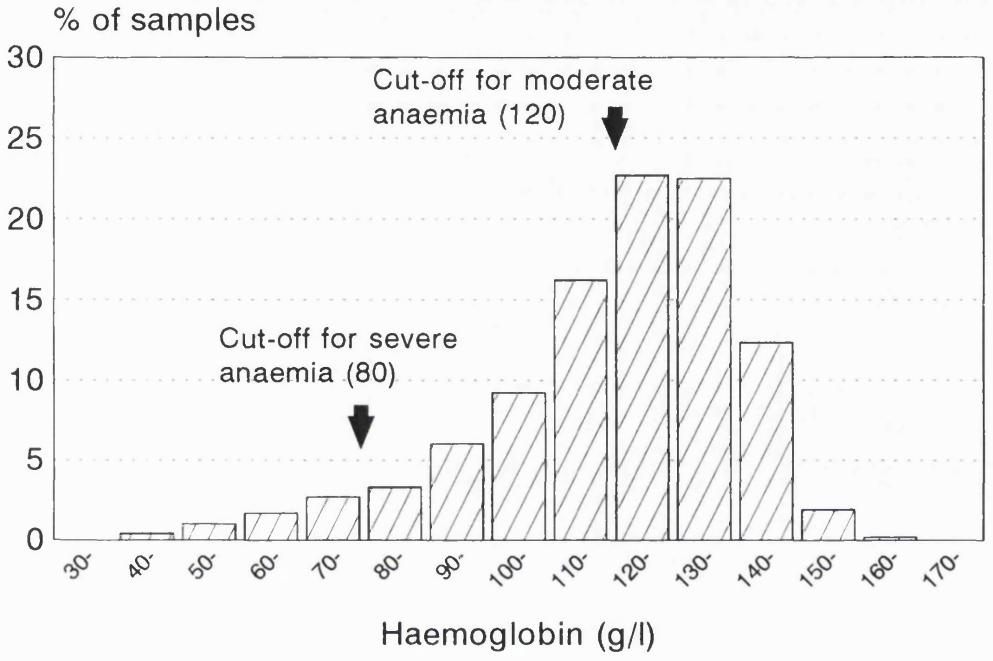


Figure 3.2.6.2.1. Hb distribution for non-pregnant women (n=520)

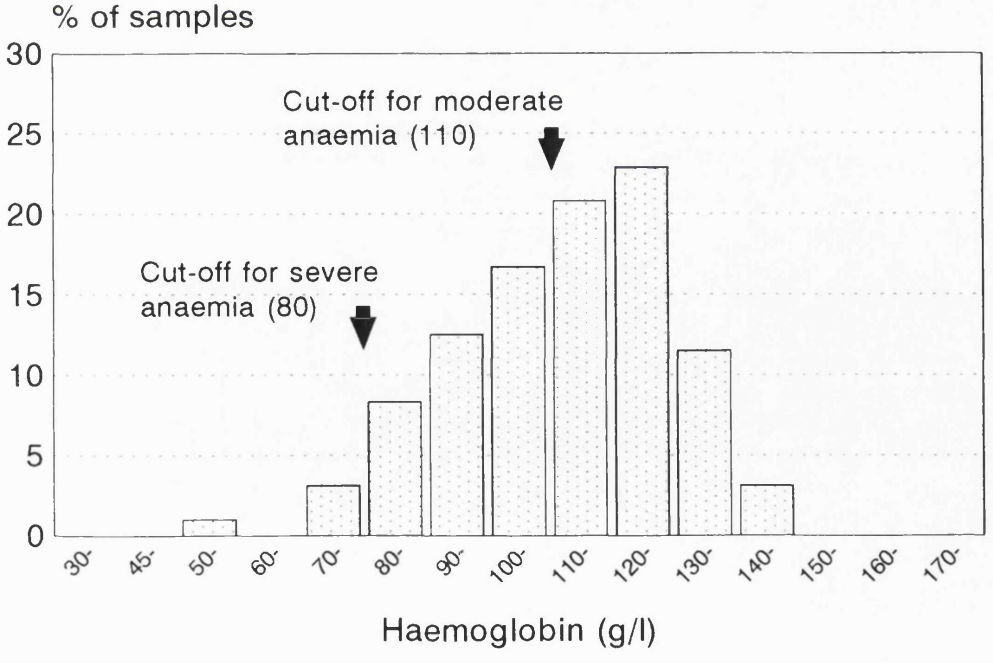


Figure 3.2.6.2.2. Hb distribution for pregnant women (n=96)

3.2.6.3. Hb Category (Anaemia Status)

The cut-off values for intervention in the camps and clinics (Hb < 80 g/l for iron supplementation, 80 ≤ Hb < 110 g/l for dietary advice) were chosen after consultation with local colleagues, for ease of use by field staff. This meant that there would not be different cut-offs for intervention, according to (self-reported) pregnancy status. Analysis was performed using the cut-offs recommended by WHO in defining anaemia (WHO, 1968), according to pregnancy status (< 110 g/l for pregnant women, < 120 g/l for non-pregnant women). In addition, samples with Hb < 80 g/l were classified as "severely anaemic" and further analysis was performed. Using these cut-offs, "moderate anaemia" was found in:

180/520 (34.6% ± 4.1) **non-pregnant** women (Hb 80-120 g/l),

36/96 (41.7% ± 9.7) **pregnant** women (Hb 80-110 g/l).

[96/520 (18.5% ± 3.3) non-pregnant women had Hb 80-110 g/l]

"severe anaemia" was found in:

30/520 (5.8% ± 2.0) **non-pregnant** women (Hb < 80 g/l),

4/96 (4.2% ±4.1) **pregnant** women were (Hb < 80 g/l).

3.2.6.4. Hb Concentration During Pregnancy

The appropriate measurement for comparison was the transformed mean Hb concentration. Figure 3.2.6.4.1. shows the (transformed) mean Hb for each trimester of pregnancy, compared with that for non-pregnant women.

There was a trend towards decreasing mean Hb concentration as pregnancy progressed, although this was not significant. The (transformed) mean Hb concentrations were:

117.9 g/l (95% CI 112.4-123.2, n=26) in the **first trimester**,

112.0 g/l (95% CI 107.0-116.8, n=37) in the **second trimester**,

108.0 g/l (95% CI 99.8-115.5, n=29) in the **third trimester**.

These results suggest that there was a considerable degree of iron-deficiency in this area.

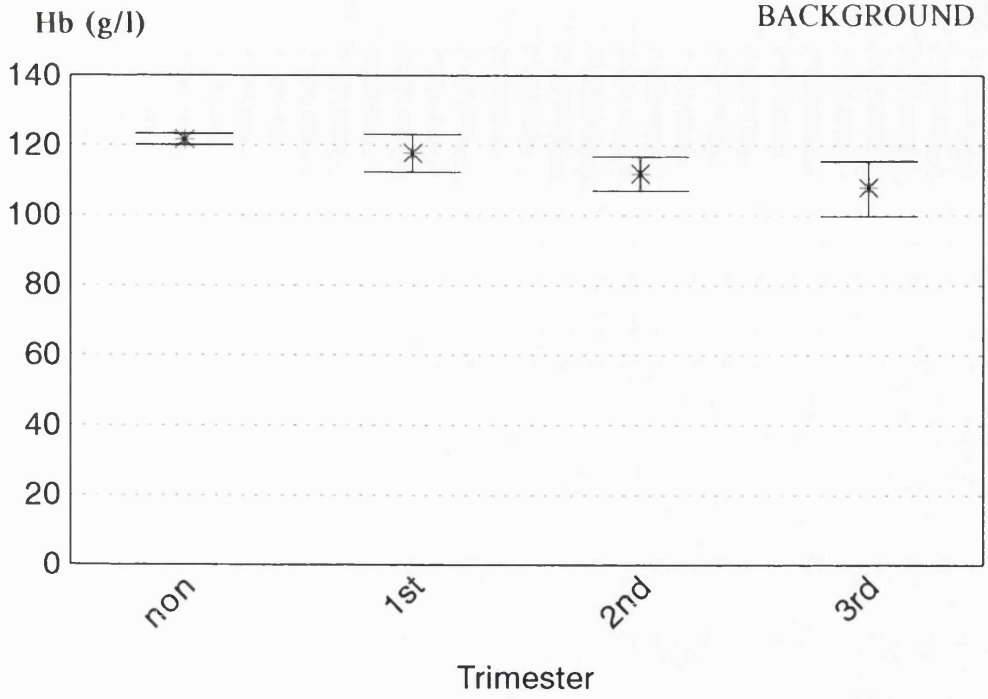


Figure 3.2.6.4.1. Mean Hb by trimester (n=520 for non-pregs, n=26 for 1st trim, n=37 for 2nd trim, n=39 for 3rd trim)

3.2.7. Ferritin

3.2.7.1. Ferritin Distribution

The distribution of serum ferritin in both non-pregnant and pregnant women was positively skewed with median serum ferritin concentrations of 12.6 and 7.1 μg/l for non-pregnant and pregnant women, respectively. A natural-log transformation approximately normalised the distributions which are shown in figures 3.2.7.1.1. and 3.2.7.1.2., overleaf. The (geometric) mean concentrations were:

13.8 μg/l (GSD 0.92, GSE O.10, 95% CI 11.3-16.8, n=88) for non-**pregnant**,

6.4 μg/l (GSD 1.33, GSE 0.22, 95% CI 4.1-10.0, n=36) for **pregnant** women.

The (geometric) mean ferritin concentration in non-pregnant women was significantly higher than in pregnant women ($p < 0.0005$, one way ANOVA).

3.2.7.2. Ferritin Category (Anaemia Status)

The proportions of women with ferritin levels below 10 μg/l, consistent with iron-deficiency anaemia, were 61.4% (±10.3%, n=88) and 75.0% (±14.7%, n=36) for non-pregnant and pregnant women, respectively, higher than indicated by the haemoglobin levels alone.

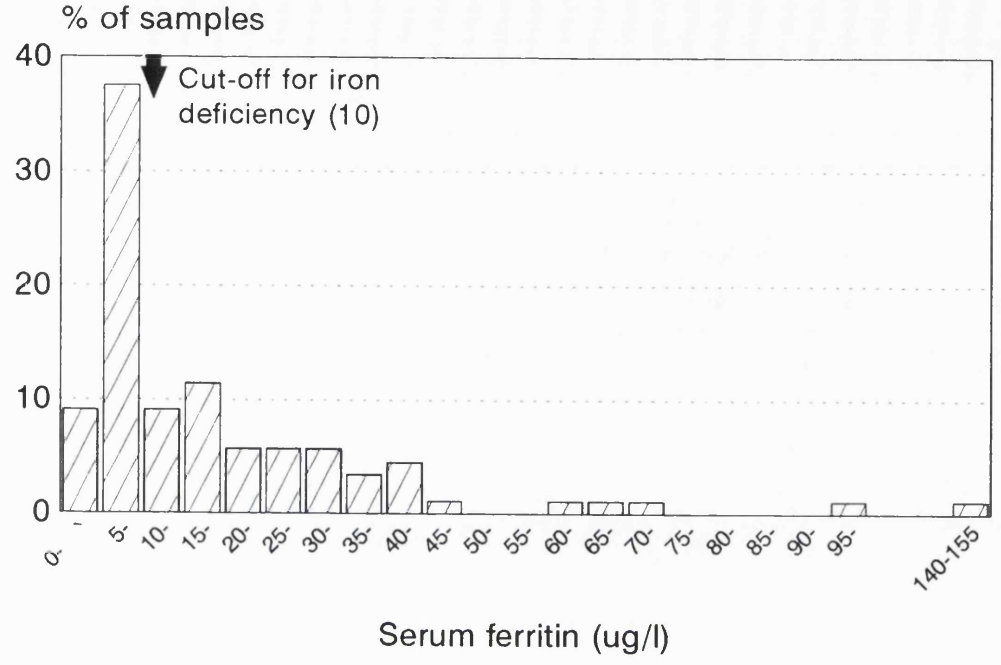


Figure 3.2.7.1.1. Ferritin distribution for non-pregnant women

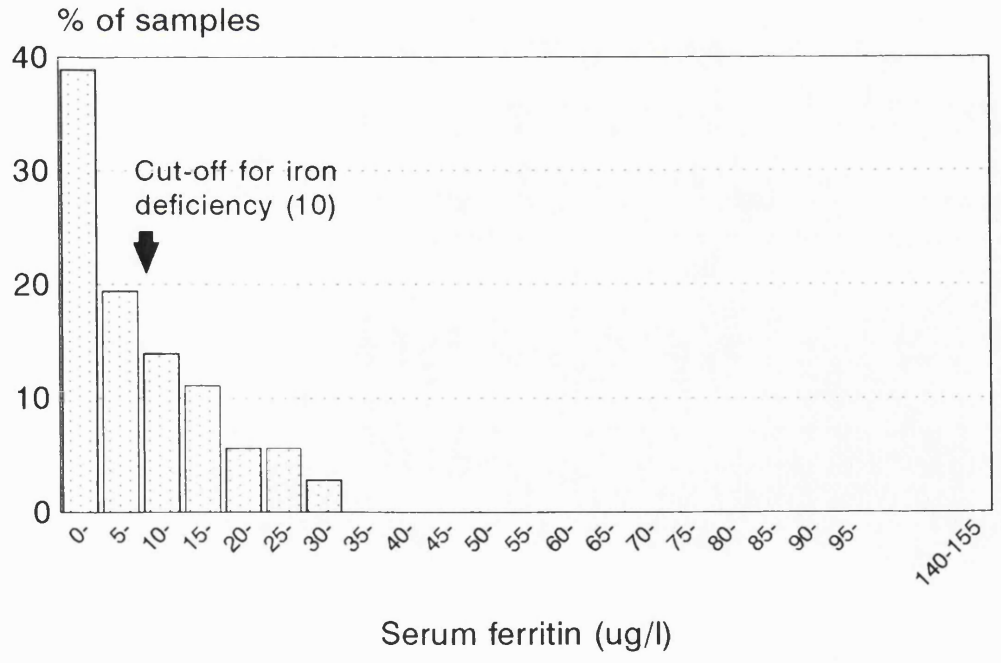


Figure 3.2.7.1.2. Ferritin distribution for pregnant women

3.2.8. Vitamin A

3.2.8.1. Vit A Distribution

Serum retinol concentration distributions were positively skewed, with median concentrations of 1.37 and 1.38 μmol/l, in non-pregnant and pregnant women, respectively. The distributions of vitamin A concentration for these women are shown in figures 3.2.8.1.1. and 3.2.8.1.2., overleaf. A natural log transformation approximately normalised the data to yield geometric mean concentrations of:

1.32 μmol/l (GSD 0.52, GSE 0.06, 95% CI 1.18-1.48, n=82) for **non-pregnant**,

1.34 μmol/l (GSD 0.52, GSE 0.09, 95% CI 1.12-1.59, n=36) for **pregnant** women.

3.2.8.2. Vit A Category (Deficiency Status)

Moderate deficiency (0.70 μmol/l < serum retinol < 1.05 μmol/l) was detected in:

14/82 (17.1% ±8.0) **non-pregnant** women,

9/36 (25.0% (±14.4%) **pregnant** women.

Severely deficiency (serum retinol < 0.70 μmol/l) was detected in:

8/82 (9.8% ±6.4%) **non-pregnant** women

3/36 (8.3% ±9.2%) **pregnant** women.

This suggests that Vitamin A deficiency may have been a considerable problem in this community. The iron and vitamin A results underline the generally poor micro-nutrient status of the women in this area.

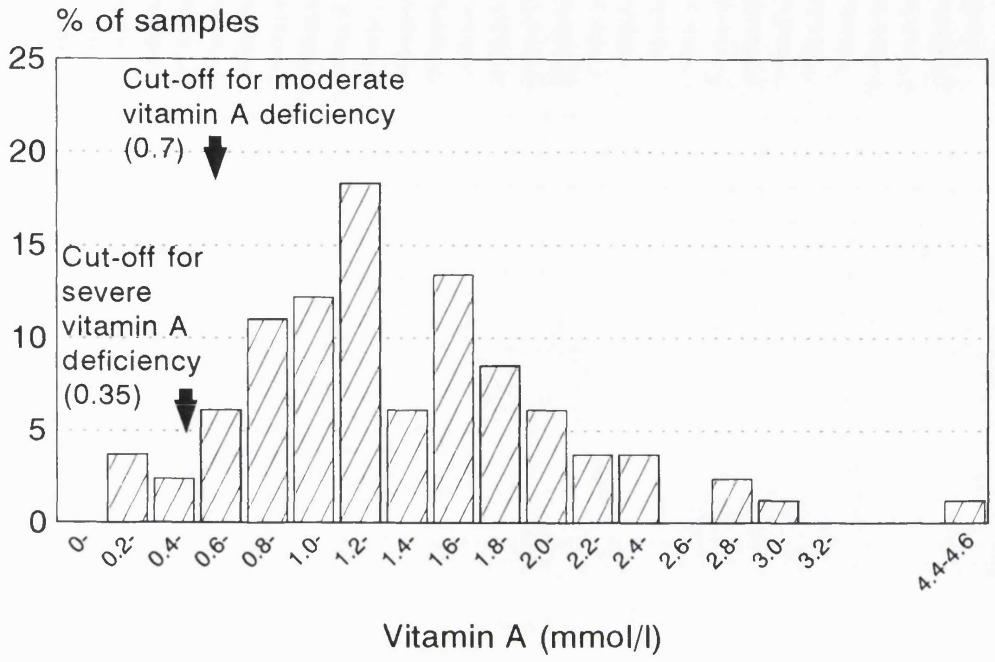


Figure 3.2.8.1.1. Vitamin A distribution for non-pregnant women

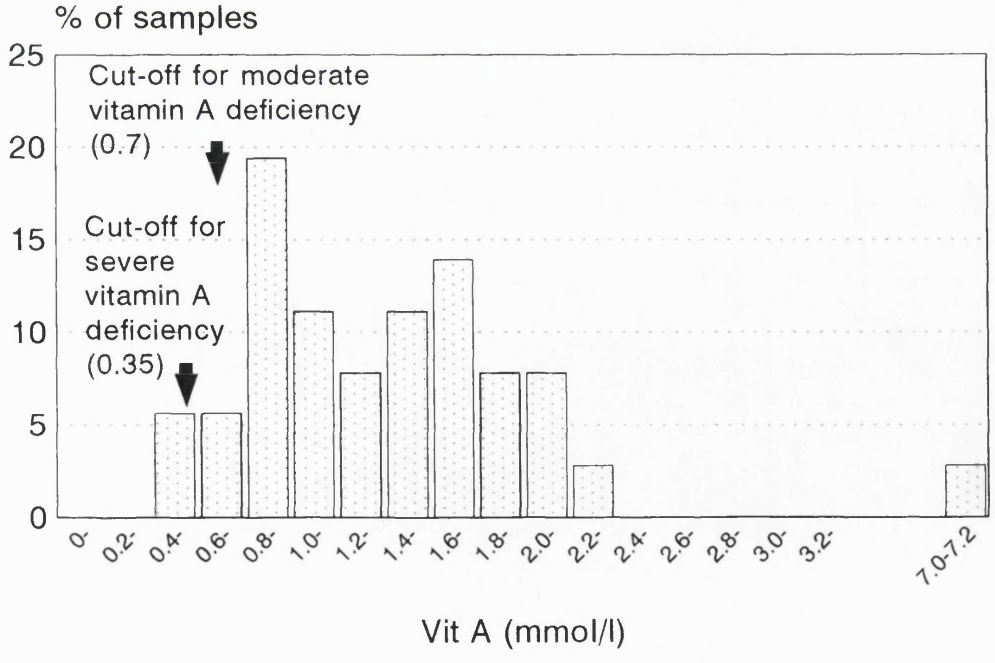


Figure 3.2.8.1.2. Vitamin A distribution for pregnant women

3.3 Paternal Characteristics

3.3.1. Paternal Age

Many women were unsure of their husband's age and gave an approximate age, leading to "nesting" of ages at 25, 30, 35 and 40. Over 40% of the women would not even guess their husband's age. The mean age of husbands was 33.9 ($\pm$1.9, n=61), the median age was 33 and the mode 40.

Women tended to be married to men older than themselves, with a mean age difference of 7.3 years ($\pm$1.4, n=61) a median difference of 7 years and a mode difference of 10 years. It should, however, be remembered that ages for both men and women were somewhat imprecise.

3.3.2. Paternal Education

The husbands tended to have had exposure to more education than the women and almost all the women interviewed knew how much education their husbands had received, in contrast to the number who would make a guess at their husband's age. This reflects the greater cultural importance of education level, compared with age, particularly in arranging marriage contracts. The level of education reached by the husbands is shown in figure 3.32.

3.3.3. Paternal Occupation

The level of education was reflected in the occupation of the husbands, with those receiving less education carrying out lower status jobs, such as a servant or labourer. The occupations are shown on figure 3.33. A large number of men in this area were in army "service", generally as low-paid clerks and "peons" though some were higher status regular soldiers (shown as "forces" on the figure). A few men were merchants or teachers and a small number were in overseas employment, generally in the Gulf.

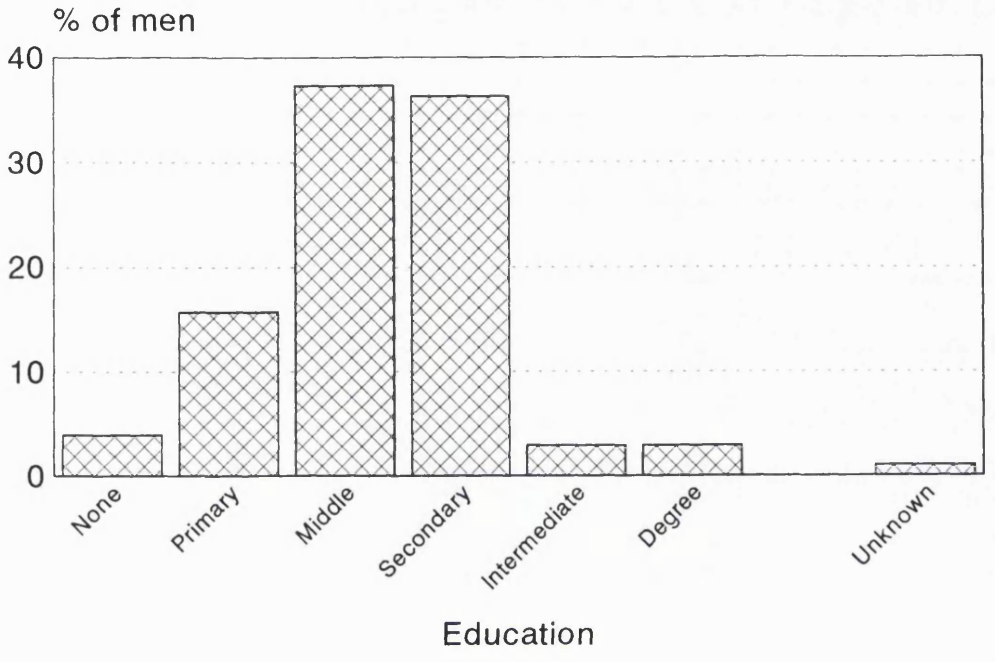


Figure 3.3.2. Paternal education (n=102)

Figure 3.3.3. Paternal occupation (n=102)

3.4. Baseline Thyroid Function in Non-pregnant Women

3.4.1. Summary

Table 3.4.1., overleaf, summarises the main biochemical findings.

3.4.2. Previous Exposure to Iodine

Women were shown the iodine capsules and asked if they had taken them previously. Only 15/732 (2.0%) of women reported having taken similar capsules previously and none had done so within the last 2 years. It was not thought necessary to exclude these women from the capsule distribution or from the analysis, as the protective effects of oral iodised oil are reported to last for less than 24 months (Benmiloud, Chaouki, Gutekunst *et al.*, 1994).

The small percentage of women who reported ever having received iodised oil capsules and the reported source of those capsules being Lehtrar Health centre, confirmed the wisdom of having moved the original study site from Lehtrar village to more remote centres, on the grounds of possible sample contamination by previous exposure to iodine supplementation.

Param. (units)	No	Mean	SD/ GSD	SE/ GSE	95% CI	% in category (±*)		
						hypo	eu	hyper
TSH (mIU/l)	509	1.91	0.65	0.04	1.76→2.08	14.3 (3.0)	82.2 (3.3)	3.3 (1.6)
TG (μ/l)	103	68.6	0.57	0.06	61.4→76.6	N/A	N/A	N/A
FT_4 (pmol/l)	137	8.15	0.60	0.07	7.37→9.01	62.0 (8.2)	32.8 (7.9)	5.1 (3.7)
TT_4 (nmol/l)	134	79.5	23.4	2.0	75.5→83.4	38.1 (8.3)	61.9 (8.3)	0
TBG (mg/l)	135	39.5	7.9	0.68	38.2→40.8	N/A	N/A	N/A
FT_3 (pmol/l)	135	4.89	0.32	0.03	4.63→5.15	2.2 (2.5)	86.7 (5.8)	11.1 (5.3)
TT_3 (nmol/l)	131	1.99	0.23	0.02	1.91→2.07	4.6 (3.6)	91.6 (4.8)	3.8 (3.3)
FT_3/FT_4 RATIO	133	0.596	0.63	0.05	0.535→0.664	N/A	N/A	N/A
TT_3/TT_4 RATIO	127	2.60	0.37	0.03	2.44→2.78	N/A	N/A	N/A

Table 3.4.1. Summary results for pre-supplementation thyroid parameters

N/A not applicable (allocation to thyroid category is inappropriate)

(*) (± x - to make 95% CI of the proportion: ≈2 x SEM)

arithmetic means for TT_4, TBG and TT_4/TBG ratio,

geometric means for TSH, TG, FT_4, FT_3, TT_3, FT_3/FT_4 ratio and TT_3/TT_4 ratio

TT_3/TT_4 ratio has been multiplied by 100 for convenience

3.4.3. Visible Goitre Rate (VGR)

For the reasons explained in section 2.7.2.6., baseline goitre assessment is presented only in terms of visible goitre - corresponding to grades 2 and 3 on the camp questionnaire. Table 3.4.3. below shows the proportion of women with recorded, visible goitre, according to pregnancy status.

Subjects - women	n	Number with visible goitre	Visible goitre rate (95%CI)
Non-pregnant	571	183	32.0% (±3.9)
Pregnant	100	41	41.0% (±9.8)
All	671	224	33.4% (±3.6)

Table 3.4.3. Visible goitre rate (VGR) at baseline.

In view of the unreliability of the goitre grading, further investigations into associations between goitre grade and other parameters are not reported.

3.4.4. Thyroid Stimulating Hormone (TSH)

3.4.4.1. Distribution of TSH

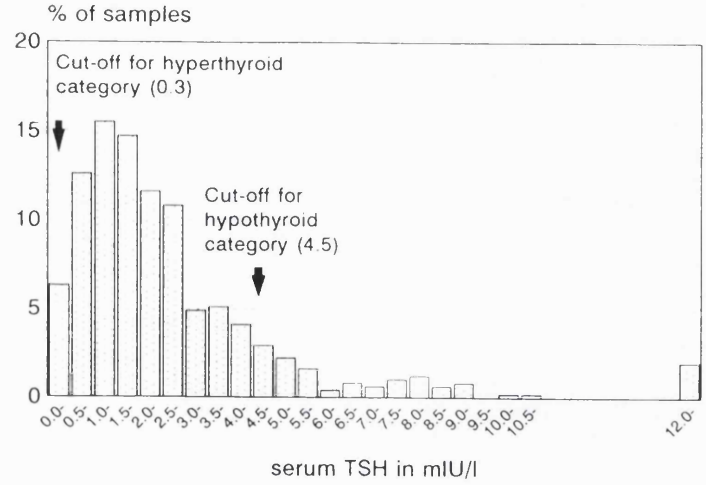


Figure 3.4.4.1. Distribution of TSH (n=509)

The TSH distribution was positively skewed with a median value of 2.04 mIU/l. A natural-log transformation produced a normal distribution of Ln(TSH).

3.4.4.2. Thyroid Status by TSH

Figure 3.4.4.2. shows the proportion of samples in each thyroid category, as defined by the cut-offs described in section 2.7.2.4.1.

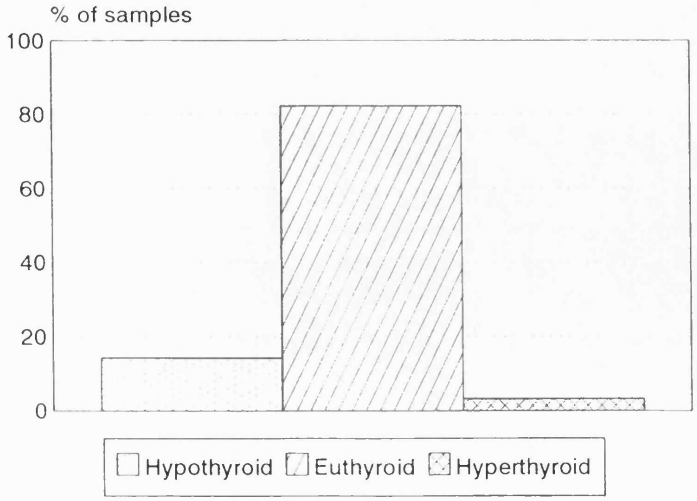


Figure 3.4.4.2. Thyroid status by TSH (n=509)

3.4.5. Thyroglobulin (TG)

3.4.5.1. Distribution of TG

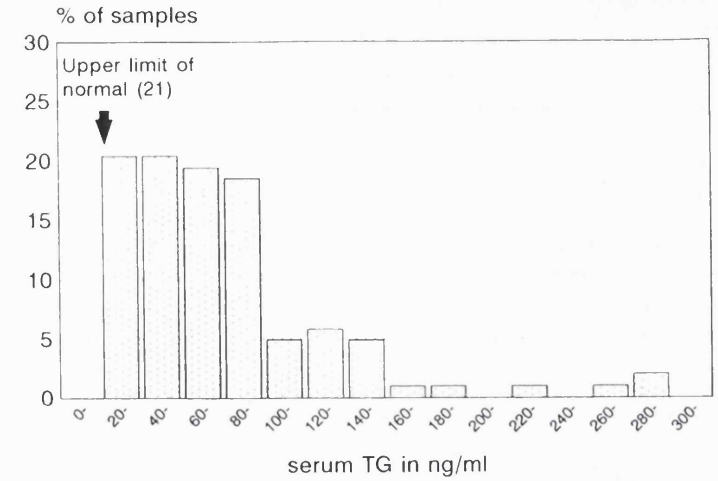


Figure 3.4.5.1. Distribution of TG (n=103)

The TG distribution was positively skewed, with a median value of 69.4 μg/l. A natural-log transformation produced a normal distribution for Ln(TG). All of the samples were "abnormal", according to the range defined in section 2.7.2.4.2.

3.4.5.2. TG and TSH

There was no significant difference in the (geometric) mean TG between the different thyroid status categories assigned by TSH concentration but there was a significant correlation between ln(TG) and ln(TSH), albeit a weak one ($r=0.20$, $p<0.05$).

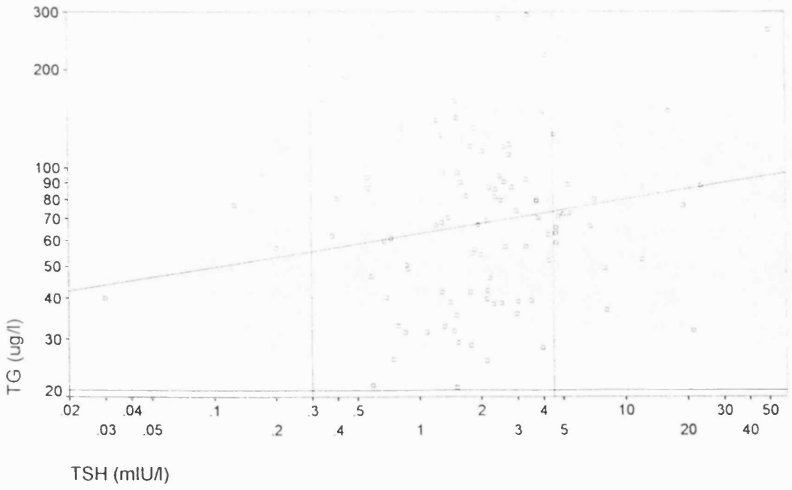


Figure 3.4.5.2. Scattergram of TG agaist TSH (n=102)

(horizontal and vertical lines denote the boundaries of the normal ranges)

3.4.6. Free Thyroxine (FT_4)

3.4.6.1. Distribution of FT_4

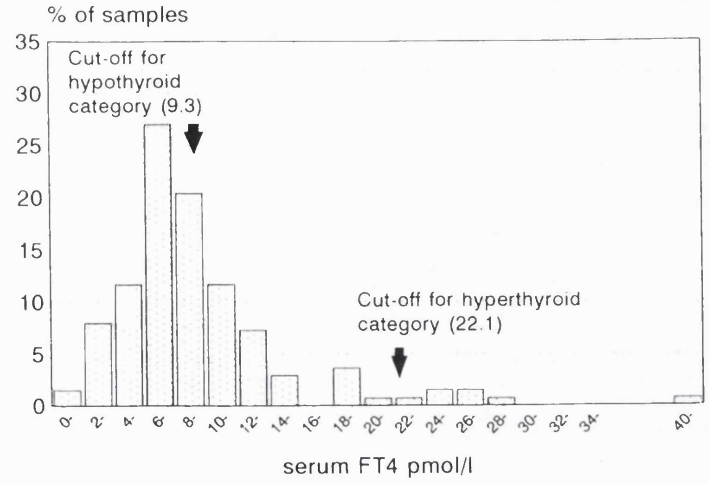


Figure 3.4.6.1. Distribution of FT_4 (n=137)

FT_4 distribution was positively skewed, with a median value of 8.17 pmol/l. A natural-log transformation produced a normal distribution for Ln(FT_4).

3.4.6.2. Thyroid Status by FT_4

Figure 3.4.6.2. shows the proportion of samples in each thyroid category, as defined by the cut-offs described in section 2.7.2.4.3.

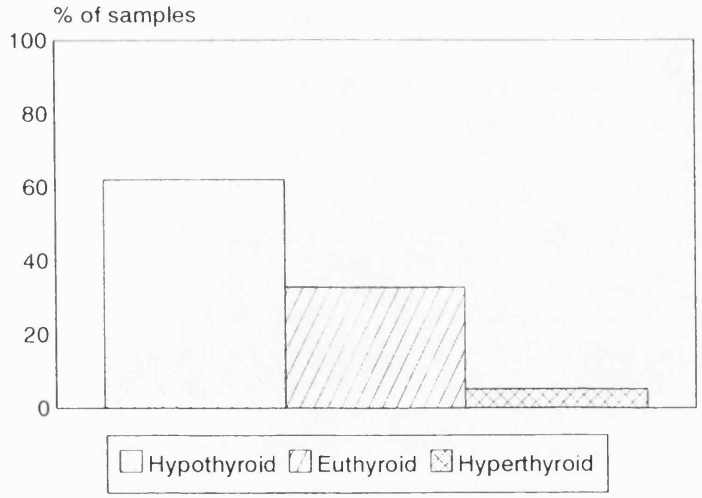


Figure 3.4.6.2. Thyroid status by FT_4 (n=137)

3.4.6.3. FT_4 and TSH

There was a weak but significant, negative correlation between ln(TSH) and ln(FT_4) ($r=-0.33$, $p<0.001$).

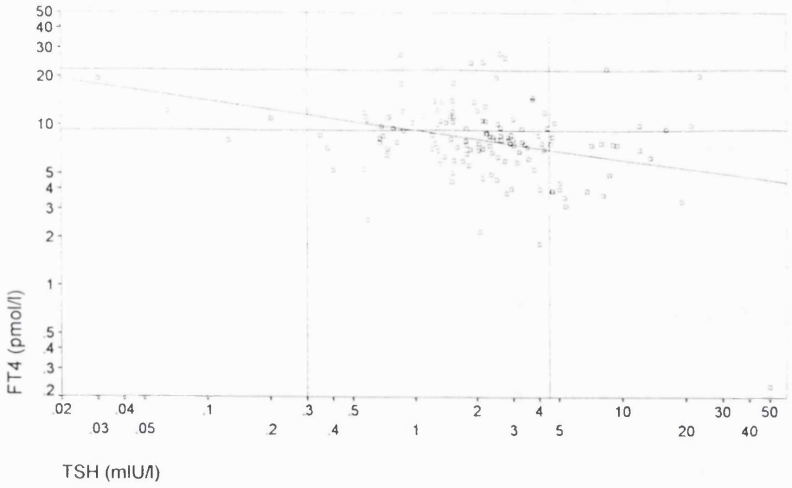


Figure 3.4.6.3. Scattergram of TSH against FT_4

(horizontal and vertical lines denote the boundaries of the normal ranges)

There was a significant difference in the (geometric) mean FT_4 between the different thyroid status groups assigned by TSH concentration such that the TSH-"hypothyroid" group had a lower mean FT_4 (5.7 pmol/l, GSD 0.85, GSE 0.17, 95% CI 4.0-8.1, n=25) than the TSH-"euthyroid" group (8.7 pmol/l, GSD 0.50, GSE 0.05, 95% CI 7.9-9.6, n=108) (p=0.0018, one way ANOVA). The TSH-"hyperthyroid" group was very small and had a mean FT_4 which was higher, but not significantly different from the other groups (12.0 pmol/l GSD 0.36, GSE 0.18, 95% CI 6.7-21.5, n=4).

There was no significant difference in the (geometric) mean TSH in the different thyroid status groups assigned by FT_4 concentration. Thus, raised TSH concentrations tended to be accompanied by low FT_4 concentrations but reduced FT_4 concentrations were not necessarily accompanied by high TSH concentrations.

Only 19+37=56/137 (40.9% ±8.3) samples were allocated to the same category, using TSH and FT_4 to assign thyroid status.

Thyroid Category	TSH-hypothyroid	TSH-euthyroid	TSH-hyperthyroid	Total
FT_4-hypothyroid	19	65	1	85
FT_4-euthyroid	5	37	3	45
FT_4-hyperthyroid	1	6	0	7
Total	25	108	4	137

Table 3.4.6.3. Thyroid category by TSH and FT_4

3.4.6.4. FT_4 and TG

There was a weak but significant correlation between ln(TG) and ln(FT_4) ($r=-0.37$, $p<0.001$).

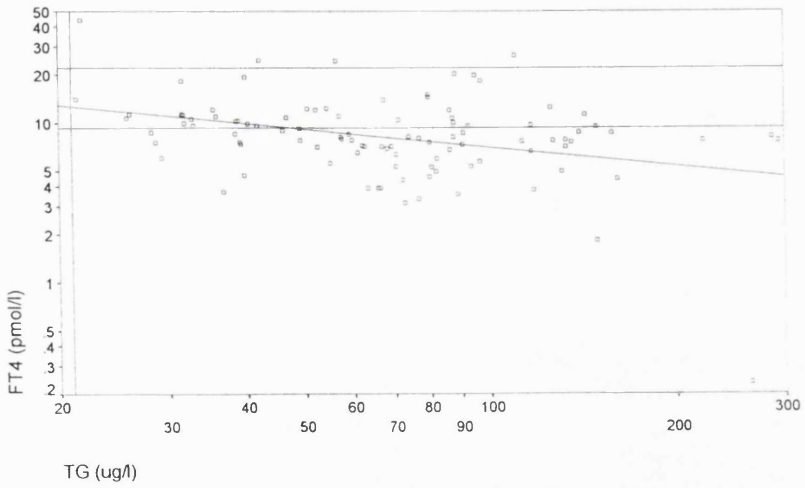


Figure 3.4.6.4. Scattergram of TG agaist FT_4 (n=101)
(horizontal and vertical lines denote the boundaries of the normal ranges)

There was a significant difference in the (geometric) mean TG between the thyroid status groups assigned by FT_4 concentration such that the FT_4-"hypothyroid" group had a higher mean TG (80.3 μg/l, GSD 0.52, GSE 0.07, 95% CI 67.6-91.8, n=60) than the FT_4-"euthyroid" group (54.3 μg/l, GSD 0.53, GSE 0.09, 95% CI 45.6-64.8, n=37) ($p<0.002$, one way ANOVA). The FT_4-"hyperthyroid" group had a lower mean, but not significantly so (48.9 μg/l, GSD 0.7, GSE 0.34, 95% CI 16.8-142.4, n=4).

3.4.7. Total Thyroxine (TT_4)

3.4.7.1. Distribution of TT_4

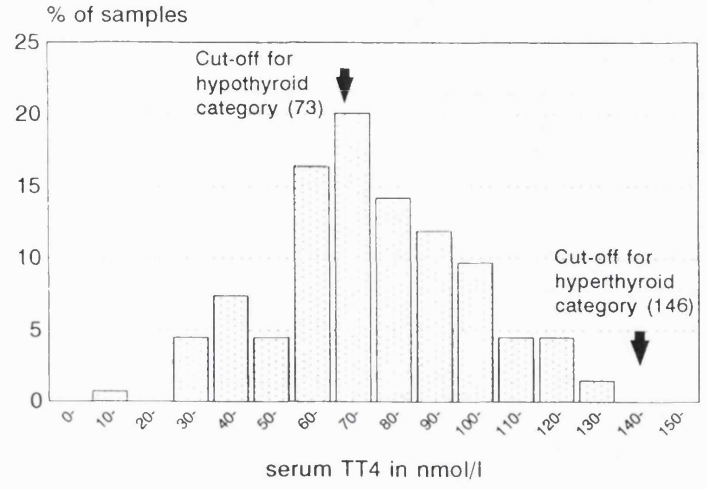


Figure 3.4.7.1. Distribution of TT_4 (n=134)

TT_4 concentrations were normally distributed.

3.4.7.2. Thyroid Status by TT_4

Figure 3.4.7.2. shows the proportion of samples in each thyroid category, as defined by the cut-offs described in section 2.7.2.4.4

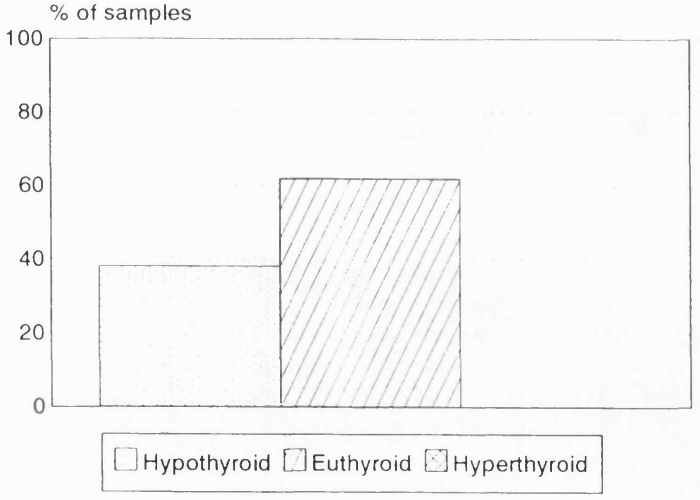


Figure 3.4.7.2. Thyroid Status by TT_4 (n=134)

3.4.7.3. TT_4 and FT_4

There was a significant correlation between TT_4 and $\ln(FT_4)$ ($r=0.63$, $p<0.001$).

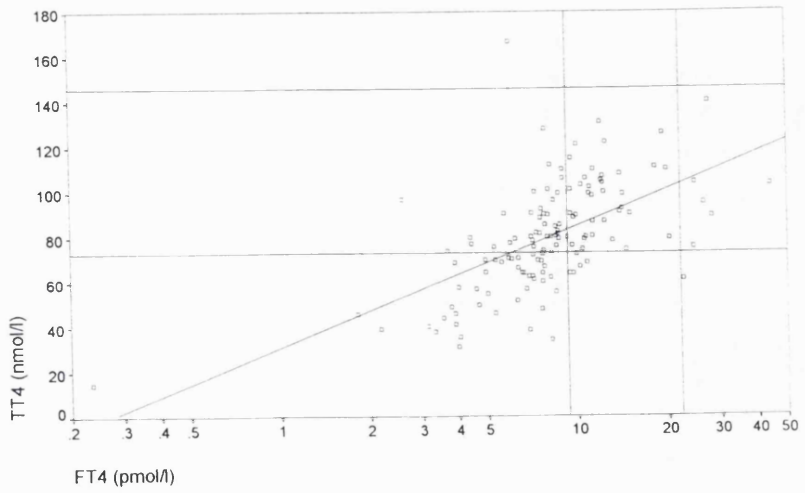


Figure 3.4.7.3. Scattergram of TT_4 against FT_4 ($n=131$)
(horizontal and vertical lines denote the boundaries of the normal ranges)

There was a significant association found when a X^2 test for a 2x3 table was performed on the cross-tabulation of thyroid category assigned by FT_4 concentration by that assigned by TT_4 concentration (Pearson X^2, $p<0.0001$) but less than two thirds ($44+37=81/131$ or 61.8% ± 8.4) of the samples were assigned to the same thyroid status category by TT_4 and FT_4. This is shown below in table 3.4.7.4.

Thyroid Category	TT_4-hypothyroid	TT_4-euthyroid	TT_4-hyperthyroid	Total
FT_4-hypothyroid	44	38	0	82
FT_4-euthyroid	5	37	0	42
FT_4-hyperthyroid	1	6	0	7
Total	50	81	0	131

Table 3.4.7.3. Thyroid category by TT_4 and FT_4

3.4.7.4. TT_4 and TSH

There was a fairly strong, significant correlation between TT_4 and ln(TSH) ($r=-0.48$, $p<0.001$).

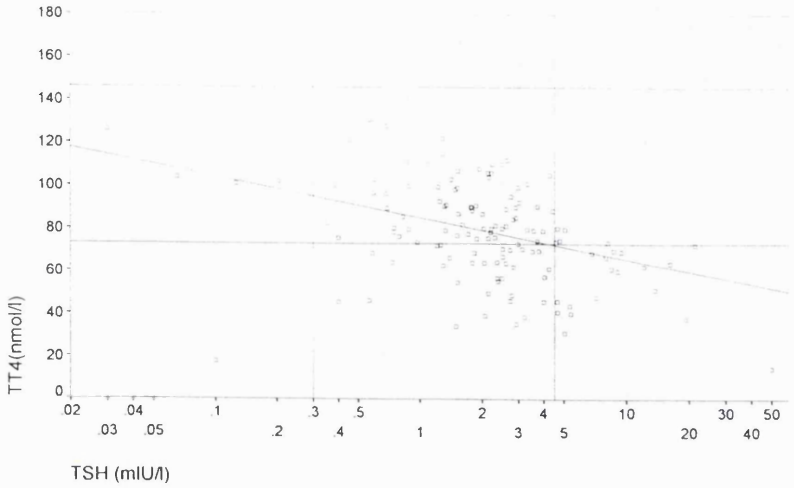


Figure 3.4.7.4. Scattergram of TT_4 against TSH (n=133)
(horizontal and vertical lines denote the boundaries of the normal ranges)

There was a significant difference in the (arithmetic) mean TT_4 concentration between the different thyroid status categories assigned by TSH concentration, such that the TSH-"euthyroid" group had a higher mean TT_4 (82.3 nmol/l, SD 21.3, SE 2.1, 95% CI 78.1-86.4, n=104) than the TSH-"hypothyroid" group (61.3 nmol/l, SD 21.2, SE 4.2, 95% CI 52.6-70.0, n=25). The few samples that were classified as TSH-"hyperthyroid" had a mean TT_4 of 108.3 nmol/l (SD 11.9, SE 5.9, 95% CI 89.3-127.2, n=4) which was significantly different from the other two groups ($p<0.0001$, one way ANOVA) but still lower than the median TT_4 in a group of "normal subjects".

There was also a significant difference in the (geometric) mean TSH concentration between the different thyroid status categories assigned by TT_4 concentration such that the TT_4-"hypothyroid" group had a higher mean TSH (3.58 mIU/l, GSD 0.91, GSE 0.13, 95% CI 2.76-4.63, n=51) than the TT_4-"euthyroid" group (1.56 mIU/l, GSD 0.45, GSE 0.11, 95% CI 1.25-1.95, n=82) ($p<0.0001$, one way ANOVA).

Only 18+71=89/133 (66.9% ±8.0) samples were allocated to the same category, using TSH and TT_4 to assign thyroid status.

Thyroid Category	TSH-hypothyroid	TSH-euthyroid	TSH-hyperthyroid	Total
TT_4-hypothyroid	18	33	0	51
TT_4-euthyroid	7	71	4	82
TT_4-hyperthyroid	0	0	0	0
Total	25	104	4	133

Table 3.4.7.4. Thyroid category by TSH and TT_4

3.4.7.5. TT_4 and TG

There was a weak but significant correlation between ln(TG) and TT_4 (r=-0.28, p=0.005).

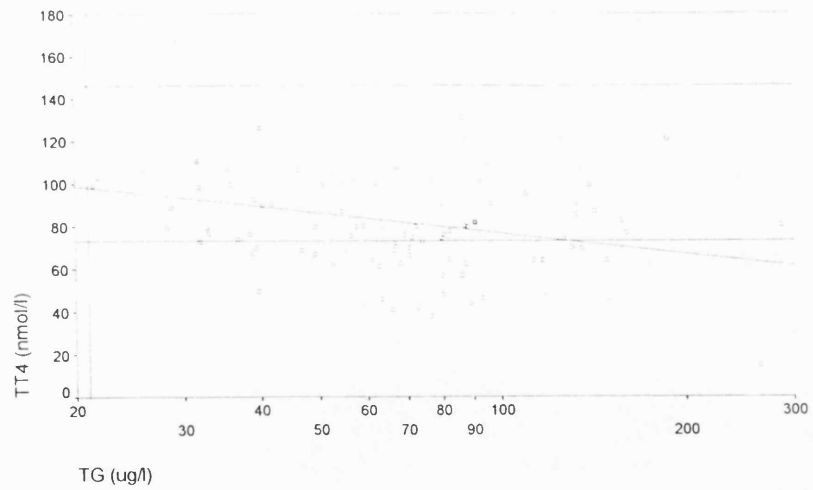


Figure 3.4.7.5. Scattergram of TT_4 against TG (n=101)
(horizontal and vertical lines denote the boundaries of the normal ranges)

There was no significant difference in the (geometric) mean TG concentration between the thyroid status categories assigned by TT_4 concentrations.

3.4.8. Thyroxine-Binding Globulin (TBG)

3.4.8.1. Distribution of TBG

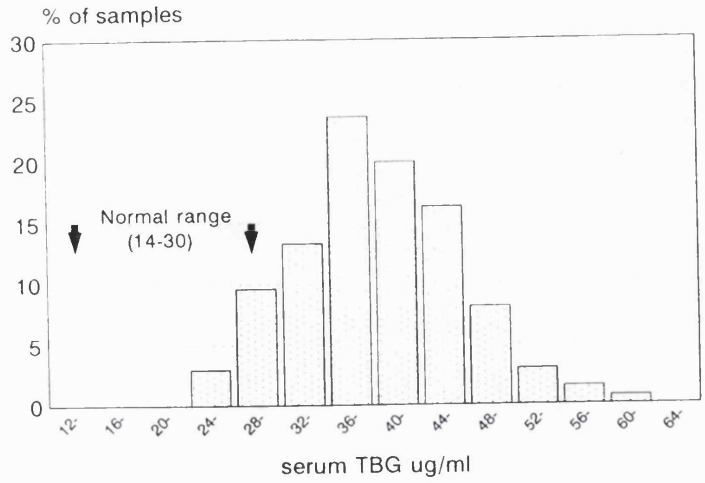


Figure 3.4.8.1. Distribution of TBG (n=135)

TBG concentrations were normally distributed.

Only 8/135 (5.9% ±4.0) samples had TBG concentrations below the upper limit of "normal", described in section 2.7.2.4.5.

3.4.8.2. TBG and TSH, TG, FT_4

There were no significant associations between TBG and TSH, TG, or FT_4 found by any of the methods described above.

3.4.8.3. TBG and TT_4

There was no significant correlation between TBG and TT_4.

There was a small but significant difference in the (arithmetic) mean TBG between the different thyroid status categories assigned by TT_4 concentration such that the TT_4-"hypothyroid" group had a lower mean TBG (36.5 mg/l, SD 8.9, SE 1.26, 95% CI 33.9-39.0, n=50) than the TT_4-"euthyroid" group (41.5 mg/l, SD 6.6, SE 0.73, 95% CI 40.0-42.9, n=81) (p=0.0004, one way ANOVA). Both these values, however, were well above the upper limit of "normal".

3.4.9. Free Triiodothyronine

3.4.9.1. FT_3 Distribution

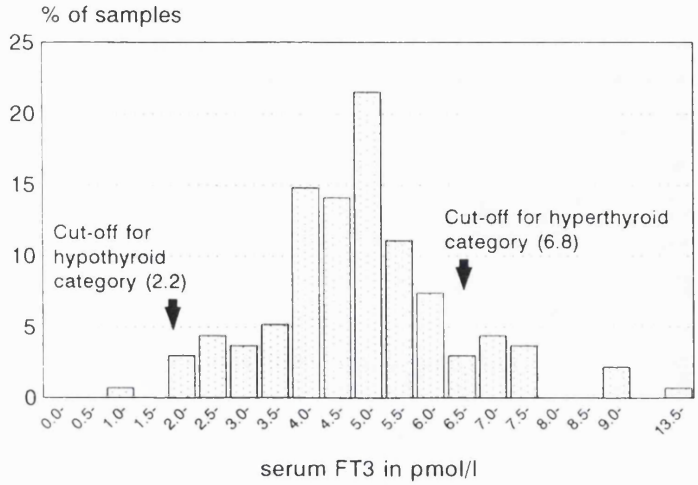


Figure 3.4.9.1. Distribution of FT_3 (n=135)

FT_3 distribution was positively skewed, with a median value of 5.1 pmol/l. A natural-log transformation produced a normal distribution for Ln(FT_3).

3.4.9.2. Thyroid Status by FT_3

Figure 3.4.9.2., below, shows the proportion of samples in each thyroid category, as defined by the cut-offs described in section 2.7.2.4.6.

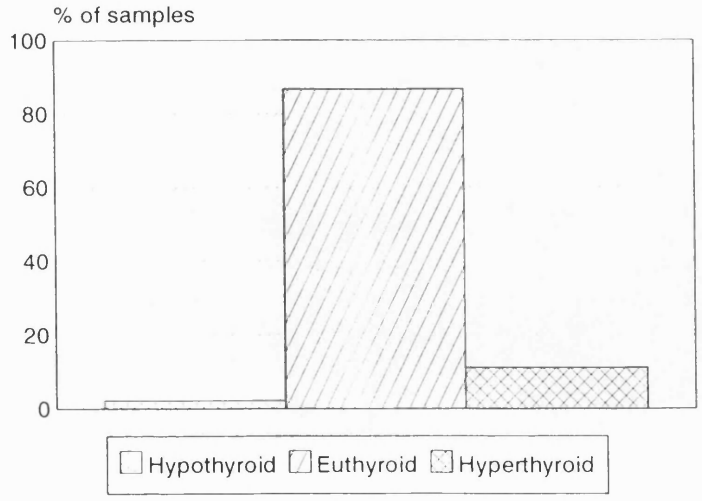


Figure 3.4.9.2. Thyroid status by FT_3 (n=135)

3.4.9.3. FT_3 and Other Parameters

There were weak but significant associations between $Ln(FT_3)$ and $Ln(FT_4)$ (corr. coeff. = -0.18, $p<0.05$) and between $Ln(FT_3)$ and TT_4 (corr. coeff. = 0.19, $p<0.05$) which are shown below in figures 4.2.9.3.1. and 3.4.9.3.2., respectively. There were, however, no other significant associations.

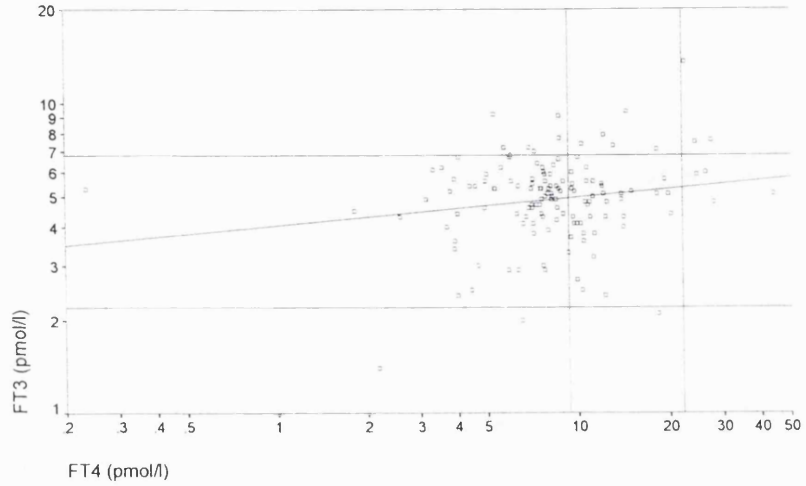


Figure 3.4.9.3.1. Scattergram of FT_3 against FT_4 (n=133)
(horizontal and vertical lines denote the boundaries of the normal ranges)

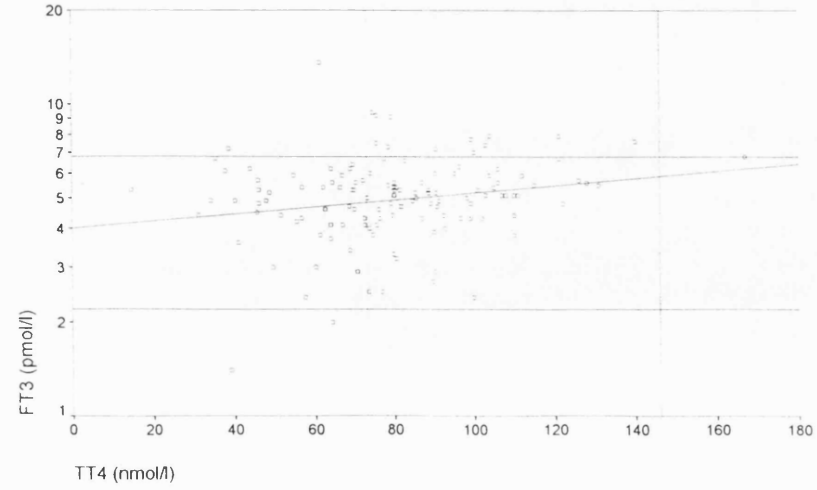


Figure 3.4.9.3.2. Scattergram of FT_3 against TT_4 (n=128)
(horizontal and vertical lines denote the boundaries of the normal ranges)

3.4.10. Total Triiodothyronine (TT_3)

3.4.10.1. Distribution of TT_3

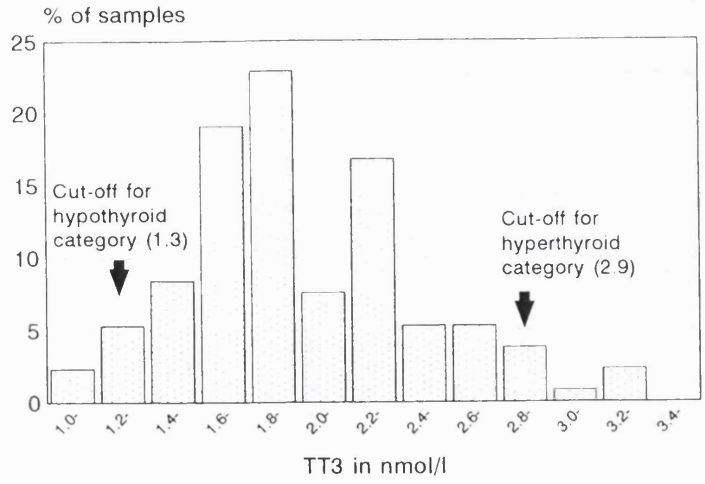


Figure 3.4.10.1. Distribution of TT_3 (n=131)

TT_3 distribution was positively skewed, with a median value of 2.0 nmol/l. A natural-log transformation produced a normal distribution for Ln(TT_3).

3.4.10.2. Thyroid Status by TT_3

Figure 3.4.10.2., below, shows the proportion of samples in each thyroid category, as defined by the cut-offs described in section 2.7.2.4.7.

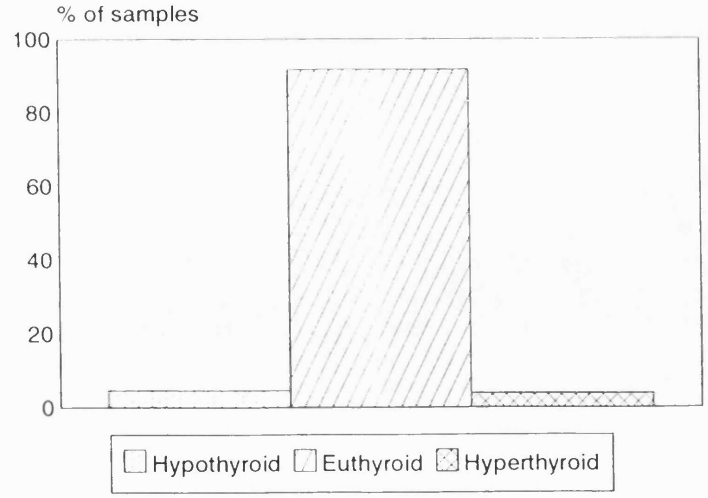


Figure 3.4.10.2. Thyroid status by TT_3 (n=131)

3.4.10.3. TT_3 and Other Parameters

There were weak but significant associations between $Ln(TT_3)$ and Ln(TSH) ($r=-0.23$, $p<0.01$), $Ln(TT_3)$ and TT_4 ($r=0.21$, $p<0.02$) and $Ln(TT_3)$ and TBG ($r=0.24$, $p<0.01$) which are shown below in figures 3.4.10.3.1., 3.4.10.3.2. and 3.4.10.3.3., respectively. There were, however, no other significant associations.

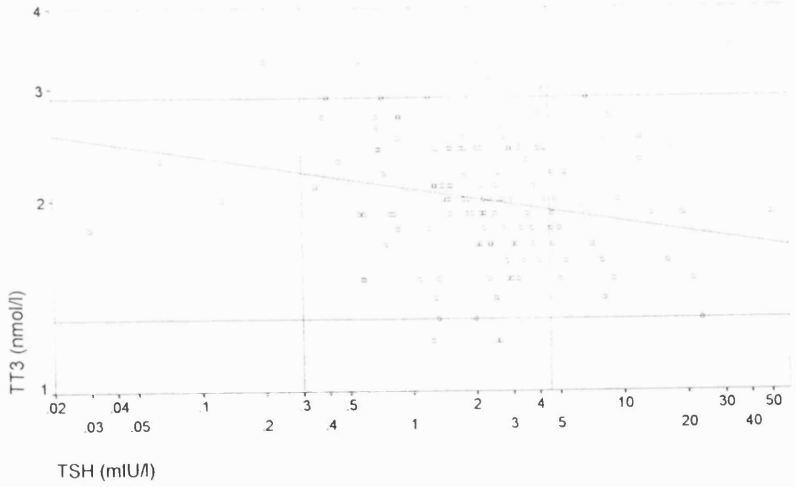


Figure 3.4.10.3.1. Scattergram of TT_3 against TSH (n=126)
(horizontal and vertical lines denote the boundaries of the normal ranges)

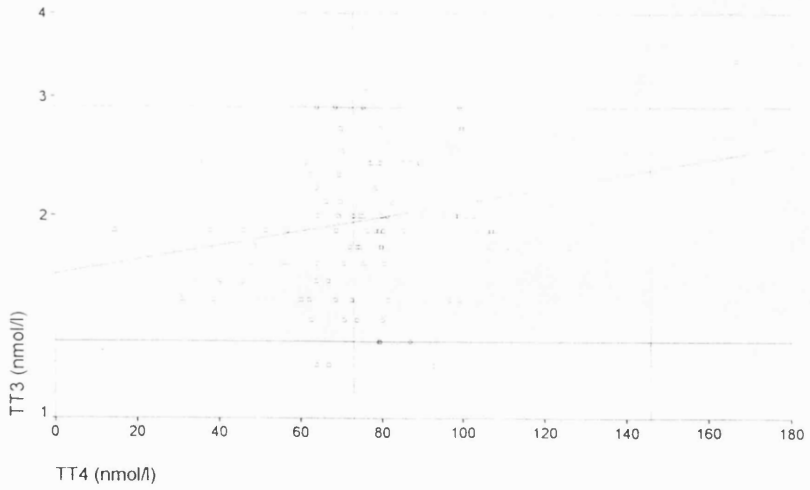


Figure 3.4.10.3.2. Scattergram of TT_3 against TT_4 (n=127)
(horizontal and vertical lines denote the boundaries of the normal ranges)

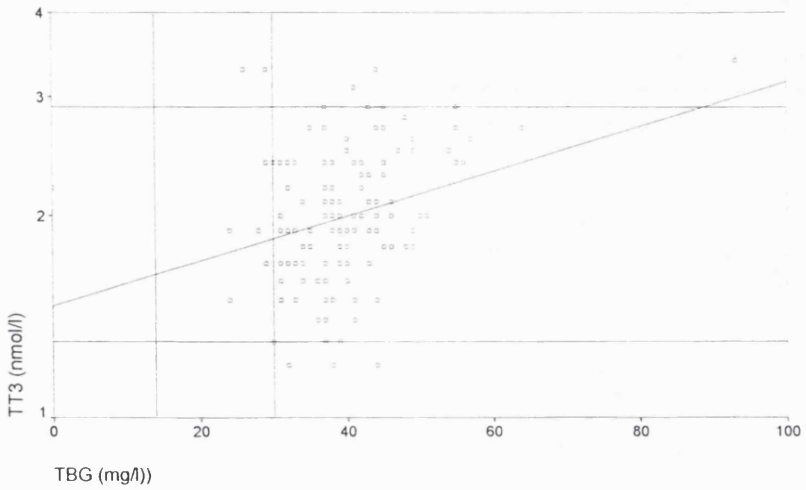


Figure 3.4.10.3.3. Scattergram of TT_3 against TBG (n=127)
(horizontal and vertical lines denote the boundaries of the normal ranges)

3.4.11. Triiodothyronine/Thyroxine (T_3/T_4) ratios

3.4.11.1. Free Hormones: the ratio is calculated as:

FT_3/FT_4 ratio = FT_3(pmol/l)/FT_4(pmol/l)

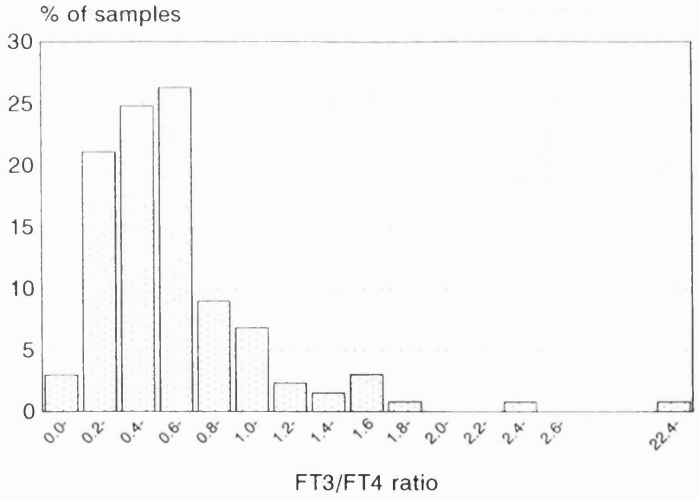


Figure 3.4.11.1. Distribution of FT_3/FT_4 (n=133)

The distribution of FT_3/FT_4 ratio was positively skewed with a median of 0.61. A natural-log transformation produced a normal distribution of Ln(FT_3/FT_4).

3.4.11.2. Total Hormones: the ratio is calculated as:

TT_3/TT_4 ratio = 100 X TT_3(nmol/l)/TT_4(nmol/l)

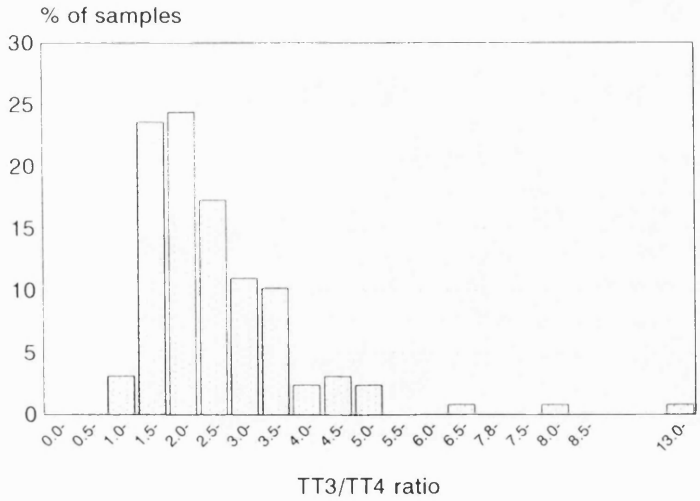


Figure 3.4.11.2. Distribution of TT_3/TT_4 (n=127)

The distribution of TT_3/TT_4 ratio was also positively skewed with a median of 2.5. A natural-log transformation produced a normal distribution of Ln(TT_3/TT_4).

3.5. Baseline Thyroid Function in Pregnant Women

3.5.1. Summary

Parameter (units)	Subjects (women)	mean	SD/ GSD	SE/ GSE	n
TBG (mg/l)	non-pregnant	39.5	7.9	0.68	135
	all pregnant	60.7	17.4	1.86	87
	first trimester	48.7	17.0	3.39	25
	second trimester	68.0	15.6	6.63	35
	third trimester	63.2	13.8	2.70	26
TT_4 (nmol/l)	non-pregnant	79.5	23.4	2.02	134
	all pregnant	105.3	31.2	3.37	86
	first trimester	95.5	30.6	6.13	25
	second trimester	112.3	33.2	5.62	35
	third trimester	105.4	27.8	5.57	25
TT_4/TBG ratio	non-pregnant	1.58	0.44	0.039	130
	all pregnant	1.40	0.37	0.040	86
	first trimester	1.58	0.43	0.085	25
	second trimester	1.30	0.34	0.057	35
	third trimester	1.12	0.28	0.057	25
FT_4 (pmol/l)	non-pregnant	8.15	0.60	0.051	137
	all pregnant	7.55	0.44	0.045	95
	first trimester	8.49	0.39	0.075	27
	second trimester	6.93	0.50	0.081	38
	third trimester	7.47	0.38	0.070	29
TT_3 (nmol/l)	non-pregnant	1.99	0.23	0.020	131
	all pregnant	2.42	0.26	0.028	81
	first trimester	2.17	0.29	0.058	25
	second trimester	2.68	0.23	0.040	33
	third trimester	2.42	0.19	0.040	22
FT_3 (pmol/l)	non-pregnant	4.89	0.32	0.028	135
	all pregnant	5.70	0.30	0.033	84
	first trimester	5.65	0.31	0.063	24
	second trimester	6.02	0.33	0.056	35
	third trimester	5.36	0.25	0.050	24
TT_4/TT_3 ratio	non-pregnant	2.60	0.37	0.033	127
	all pregnant	2.39	0.31	0.035	78
	first trimester	2.37	0.34	0.069	24
	second trimester	2.54	0.30	0.054	32
	third trimester	2.25	0.28	0.061	21

FT_4/FT_3 ratio	non-pregnant	0.596	0.63	0.054	133
	all pregnant	0.755	0.52	0.058	83
	first trimester	0.664	0.43	0.090	24
	second trimester	0.879	0.57	0.097	34
	third trimester	0.708	0.50	0.100	24
TSH (mIU/l)	non-pregnant	2.04	0.96	0.04	509
	all pregnant	2.02	1.10	0.11	98
	first trimester	1.87	1.30	0.25	27
	second trimester	2.07	0.99	0.16	39
	third trimester	2.34	0.80	0.15	29
TG (ng/ml)	non-pregnant	68.6	0.57	0.06	103
	all pregnant	83.9	0.52	0.07	61
	first trimester	74.2	0.64	0.14	20
	second trimester	82.6	0.40	0.08	25
	third trimester	99.2	0.52	0.14	15

Table 3.5.1. Summary results for thyroid parameters in pregnant women

3.5.2. Recruitment of Subjects

3.5.2.1. Number of Women Contacted

115 pregnant women attended the camps, about a third of the number who had been expected. 34/115 (29.6%) of these pregnant women came from the five key villages and 104/115 (90.4%) allowed blood samples to be taken.

3.5.2.2. Stage of Pregnancy

Women estimated their completed months of pregnancy and the date of their last menstrual period. These estimates of gestation time did not always agree, e.g. one woman said she was "about 3 months pregnant" but had not menstruated for 18 months, as she had been breast-feeding her previous child. Thus, 100 blood samples could be matched with the number of self-reported, completed months of pregnancy.

Due to the small number of pregnant women recruited and the uncertaintly over estimated month of pregnancy, results are presented only by trimester. The mean, reported number of completed months of pregnancy was 5.2 ($\pm$0.4, n=100), the median and mode were both 5 months.

Due to the poor response to follow-up (only 15 pregnant women, recruited at an iodine camp, attended a follow-up clinic, of whom only 4 allowed further blood sampling), it was not possible to confirm pregnancy stage by date of delivery.

Month	1	2	3	4	5	6	7	8	9	Total
No. of Women	4	11	13	8	20	14	13	9	8	100
Trimester	1			2			3			Total
No. of Women	28			42			30			100

Table 39.2.2. Reported stage of pregnancy

3.5.3. Visible Goitre Rate

As reported in section 3.4.3.2., 41/100 pregnant women had a recorded goitre of grade 2 or 3, giving rise to a VGR which was higher than that in non-pregnant women but not significantly so ($p > 0.07$). There were no significant differences in the VGR when analysed by trimester ($p > 0.4$ for differences in VGR between any two trimesters). Due to difficulties in goitre grading, discussed in section 2.7.2.6., no further analysis of VGR during pregnancy is presented.

3.5.4. TBG

3.5.4.1. TBG Distribution

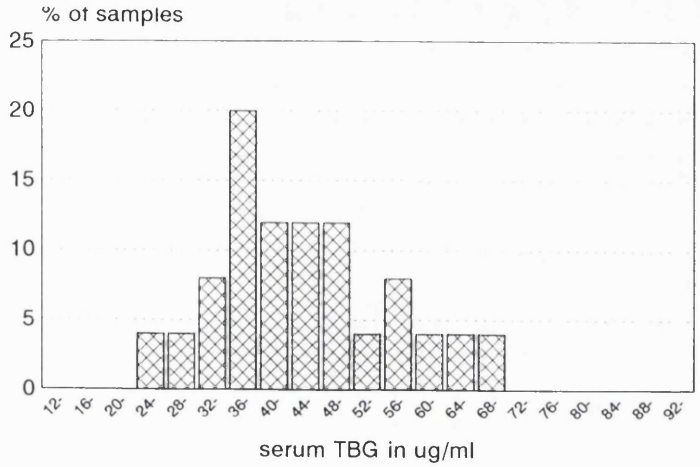


Figure 3.5.4.1.1. Distribution of TBG in first trimester (n=25)

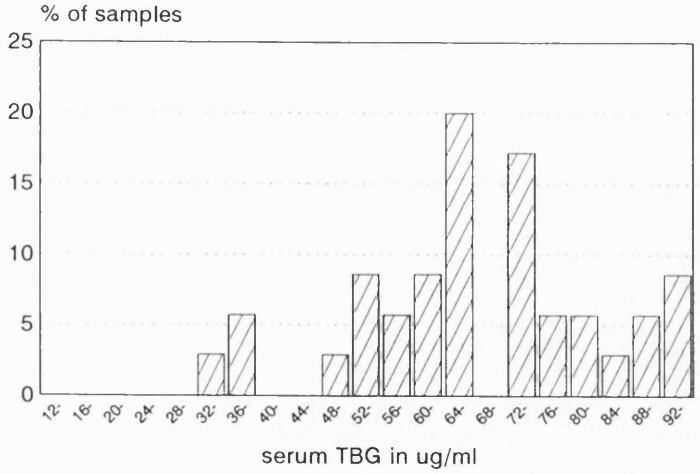


Figure 3.5.4.1.2. Distribution of TBG in second trimester (n=35)

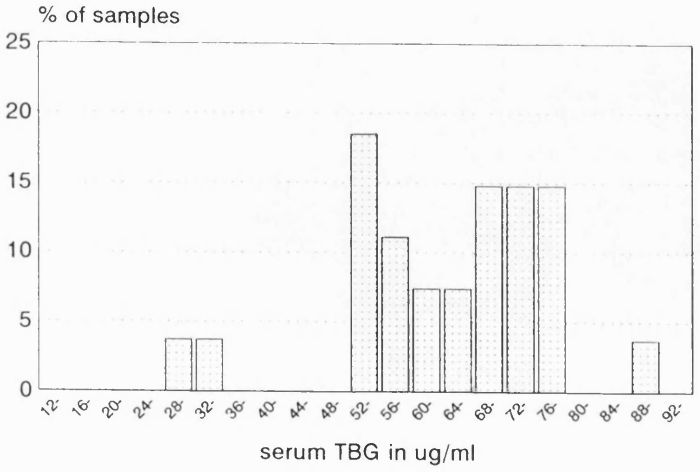


Figure 3.5.1.4.3. Distribution of TBG in third trimester (n=26)

3.5.4.2. Mean TBG

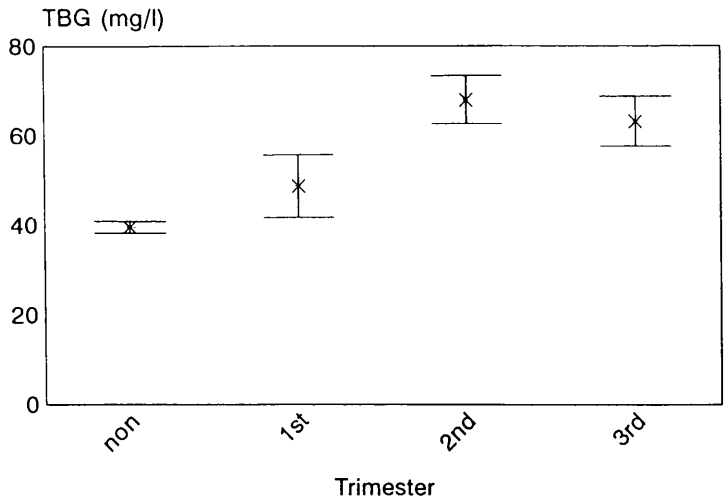


Figure 3.5.4.2. Arithmetic mean TBG concentration by trimester. (n=135 for non-pregs, n=25 for 1st trim, n=35 for 2nd trim, n=26 for 3rd trim)

There was a highly significant difference in mean TBG concentration between non-pregnant and pregnant women ($p < 0.0001$). There was also a significant increase in mean TBG between the first and second trimesters ($p < 0.0001$), with mean TBG in the third trimester similar to that in the second ($p > 0.2$) but significantly different to that in the first trimester ($p < 0.002$).

12/53 (22.6% $\pm$11.4) of women in the last 5 months of pregnancy had TBG concentrations above the upper limit of the absolute range (see section 2.7.2.4.5.) for this group.

3.5.5. TT_4

3.5.5.1. TT_4 Distribution

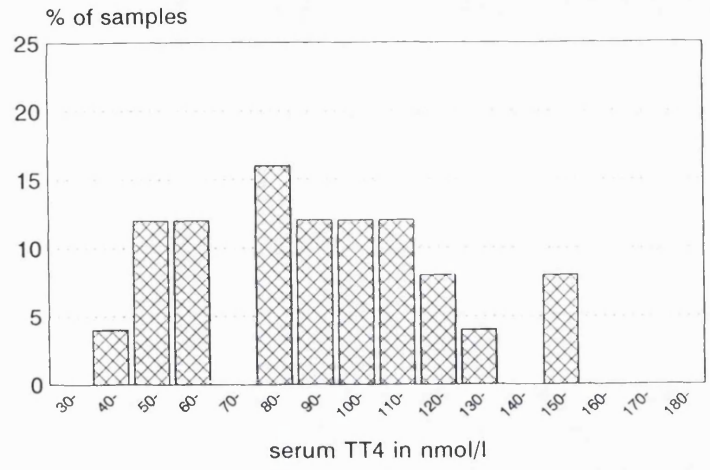


Figure 3.5.5.1.1. Distribution of TT_4 in first trimester (n=25)

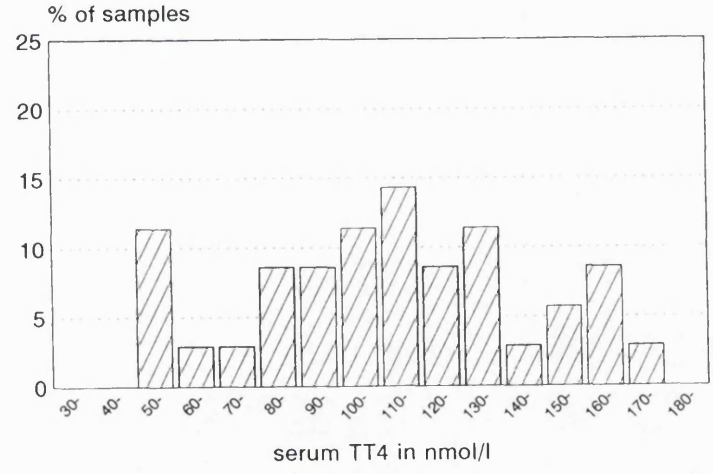


Figure 3.5.5.1.2. Distribution of TT_4 in second trimester (n=35)

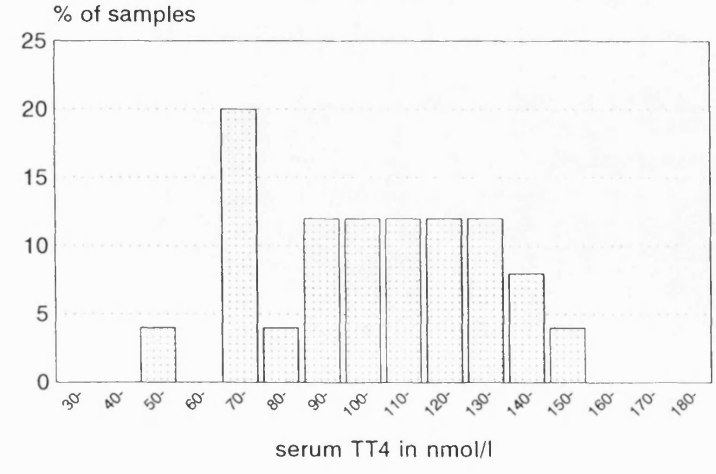


Figure 3.5.5.1.3. Distribution of TT_4 in third trimester (n=25)

3.5.5.2. Mean TT_4

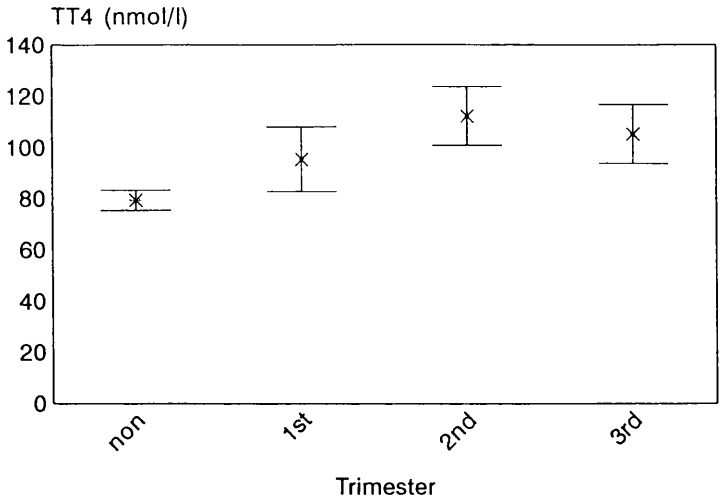


Figure 3.5.5.2. Arithmetic mean TT_4 concentration by trimester. (n=134 for non-pregs, n=25 for 1st trim, n=35 for 2nd trim, n=25 for 3rd trim)

As with TBG, there was a highly significant difference in mean TT_4 concentration between non-pregnant and pregnant women ($p<0.0001$) and an increase in mean TT_4 between the first and second trimester, although this was only marginally significant ($p=0.051$). The mean TT_4 concentration in the third trimester was intermediate between the values for first and second trimester and not significantly different from them ($p>0.2$, in both cases).

3.5.5.3. Thyroid Status by TT_4

37/54 (68.5% $\pm$12.5) women in the last 5 months of pregnancy had TT_4 concentrations below the absolute range of "normal values" for this group (see section 2.7.2.4.4.).

3.5.6. TT_4/TBG Ratio

3.5.6.1. TT_4/TBG Ratio Distribution

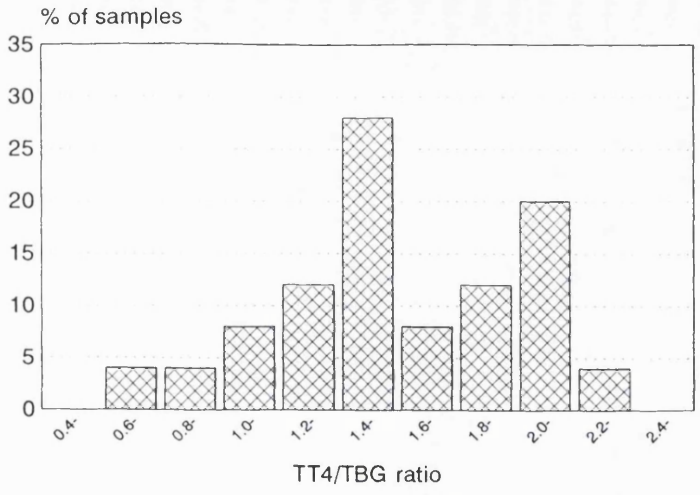


Figure 3.5.6.1.1. Distribution of TT_4/TBG in first trimester (n=25)

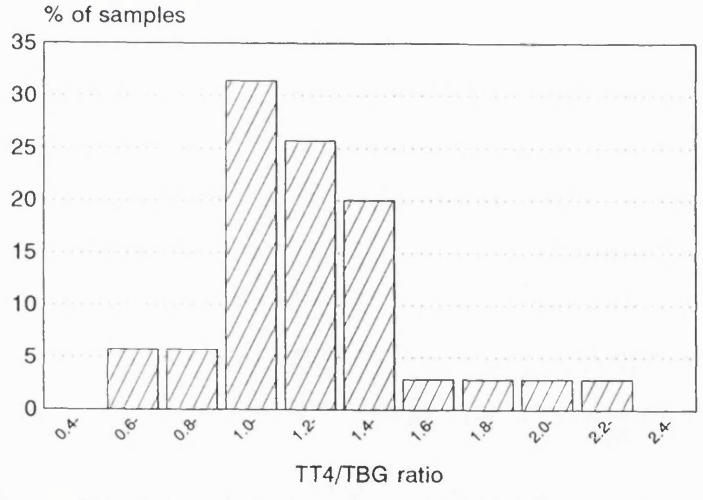


Figure 3.5.6.1.2. Distribution of TT_4/TBG in second trimester (n=35)

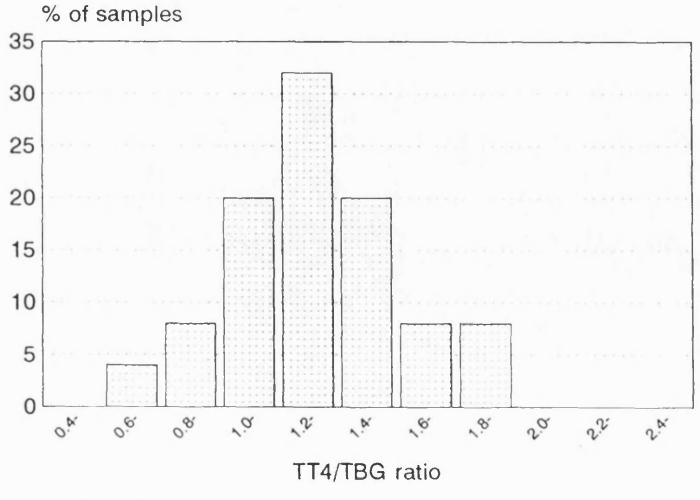


Figure 3.5.6.1.3. Distribution of TT_4/TBG in third trimester (n=25)

3.5.6.2. Mean TT_4/TBG

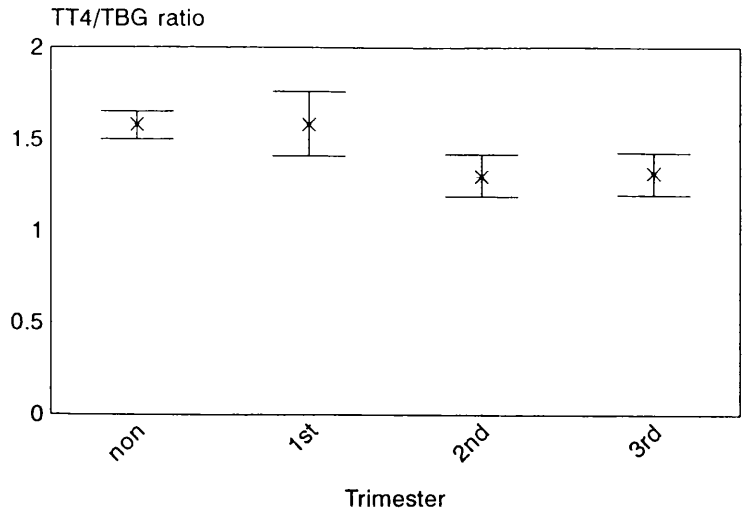


Figure 3.5.6.2. Arithmetic mean TT_4/TBG concentration by trimester (n=130 for non-pregs, n=25 for 1st trim, n=35 for 2nd trim, n=25 for 3rd trim)

There was a significant difference in mean TT_4/TBG ratio between non-pregnant and pregnant women ($p < 0.003$) with a significant decrease in mean TT_4/TBG ratio between the first and second trimester ($p < 0.006$). Mean TT_4/TBG ratio in the third trimester was similar to that in the second ($p > 0.8$) and significantly lower than that in the first trimester ($p < 0.02$).

3.5.7. FT_4

3.5.7.1. FT_4 Distribution

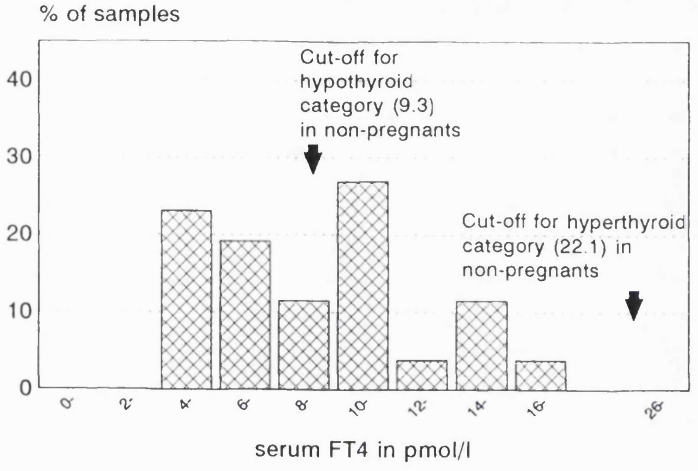


Figure 3.5.7.1.1. Distribution of FT_4 in first trimester (n=27)

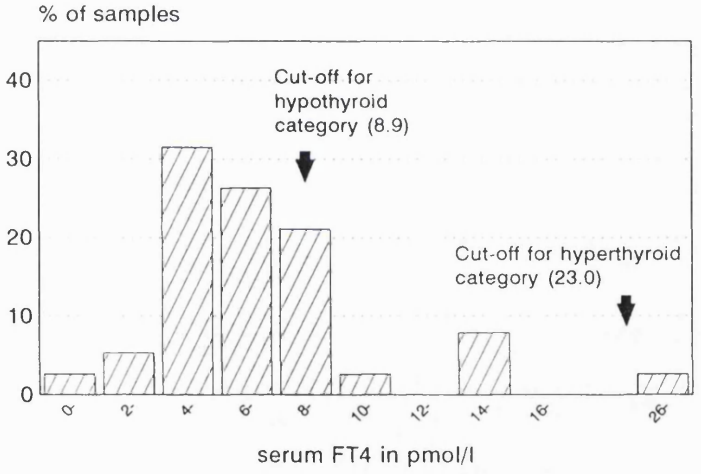


Figure 3.5.7.1.2. Distribution of FT_4 in second trimester (n=38)

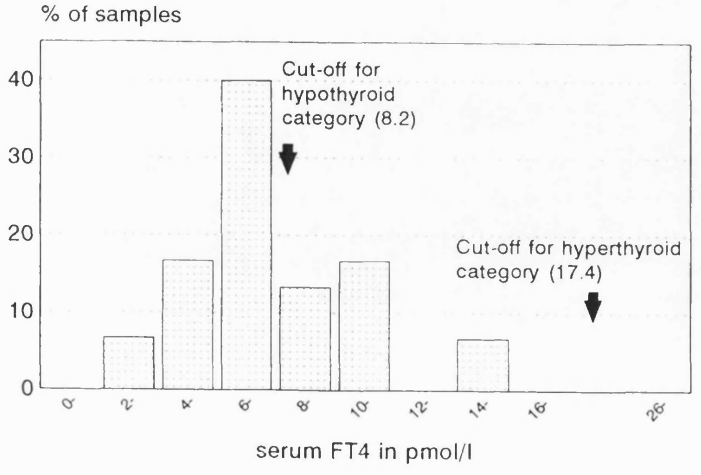


Figure 3.5.7.1.3. Distribution of FT_4 in third trimester (n=29)

3.5.7.2. Mean FT_4

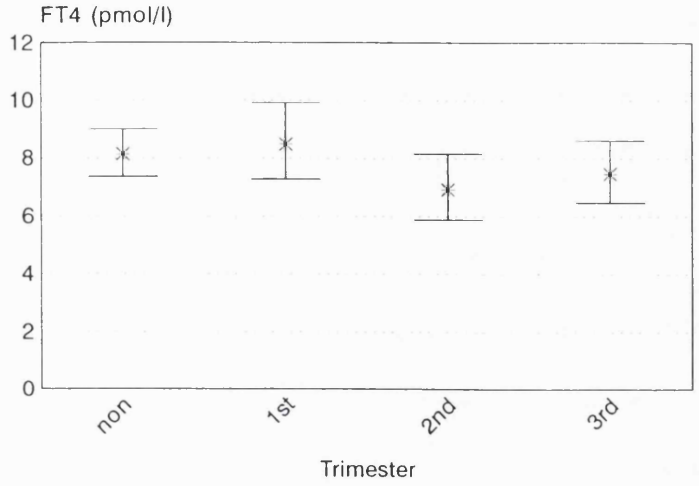


Figure 3.5.7.2. Geometric Mean FT_4 concentration by trimester. (n=137 for non-pregs, n=27 for 1st trim, n=38 for 2nd trim, n=29 for 3rd trim)

Mean FT_4 concentration did not differ significantly between pregnant and non-pregnant women ($p > 0.2$) or between the three trimesters ($p > 0.05$ between any two timesters, although there was a trend to a slightly elevated level in the first trimester, followed by a decrease in later pregnancy.

3.5.7.3. Thyroid Status by FT_4

The mean FT_4 concentrations for the second and third trimesters were lower than the bottom limits of the corresponding ranges for these trimesters (see section 2.7.2.4.3.) and the mean for the first trimester was low compared with the range for non-pregnant women as well as for the other trimesters. No women had FT_4 levels above the upper limit of the corresponding range. FT_4 concentrations **below** the corresponding "normal ranges" were found in:

14/27 (51.9% $\pm$19.2) first trimester women,

29/38 (76.3% $\pm$14.0) second trimester women,

19/29 (65.5% $\pm$18.0) third trimester women.

3.5.8. TT_3

3.5.8.1. TT_3 Distribution

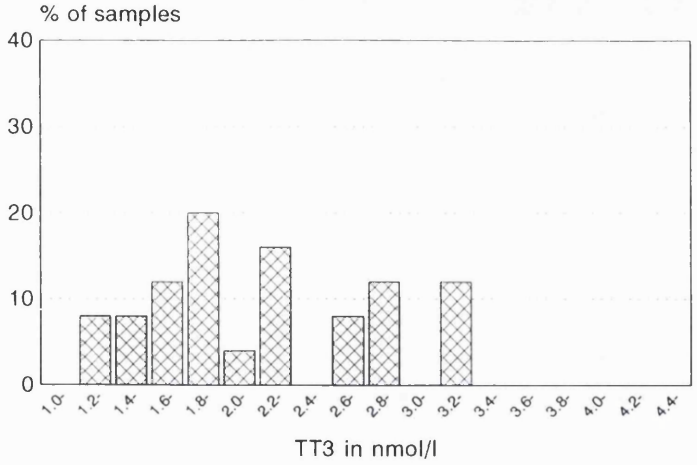


Figure 3.5.8.1.1. Distribution of TT_3 in first trimester (n=25)

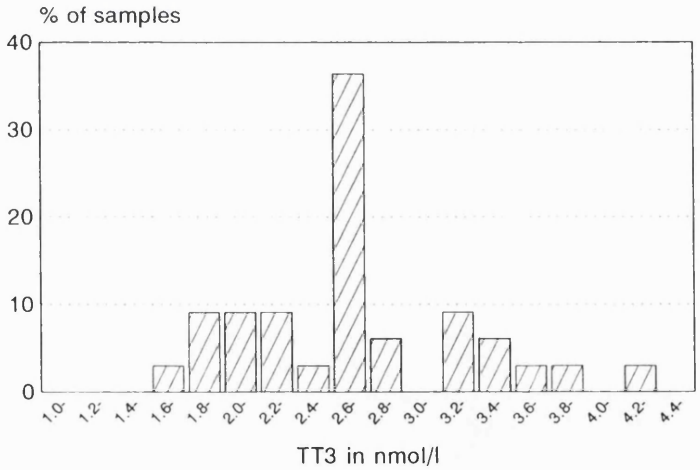


Figure 3.5.8.1.2. Distribution of TT_3 in second trimester (n=33)

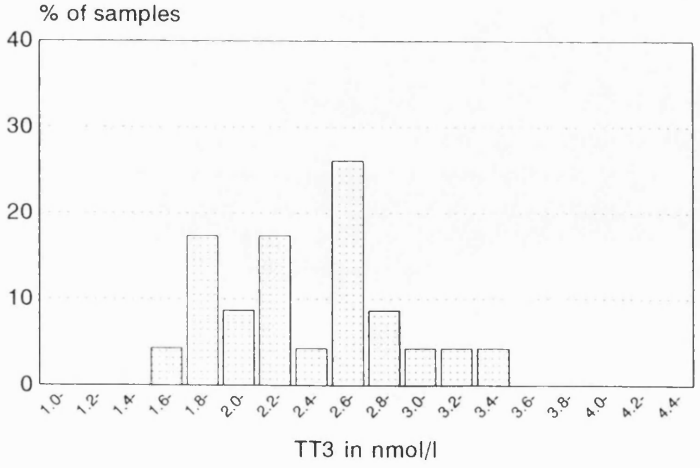


Figure 3.5.8.1.3. Distribution of TT_3 in third trimester (n=22)

3.5.8.2. Mean TT_3

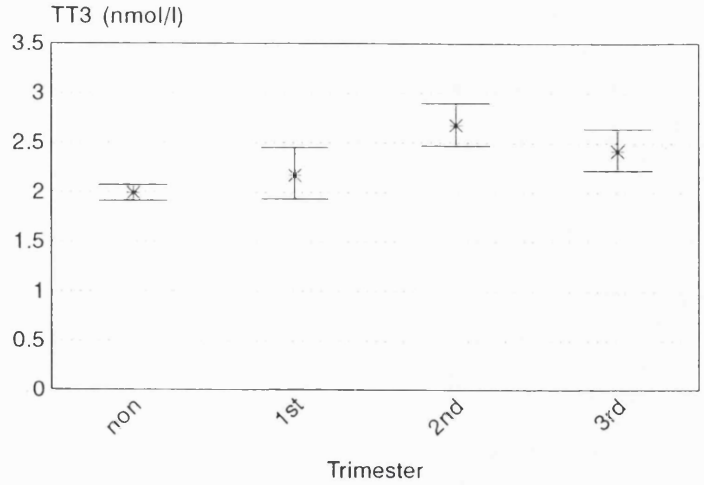


Figure 3.5.8.2. Geometric mean TT_3 concentration by trimester. (n=131 for non-pregs, n=25 for 1st trim, n=33 for 2nd trim, n=22 for 3rd trim)

As with TBG and TT_4, there was a highly significant difference in mean TT_3 between non-pregnant and pregnant women ($p<0.0001$) and a significant increase in mean TT_3 between the first and second trimesters ($p<0.005$). Mean TT_3 fell slightly in the third trimester and was not significantly different from first or second trimester mean concentrations ($p>0.1$ and $p>0.05$, respectively).

3.5.8.3. Thyroid Status by TT_3

The means for each trimester were within the normal limits for pregnancy (see section 2.7.2.4.7.) and very few samples had TT_3 values towards or beyond these limits. For the last 5 months of pregnancy:

2/49 (4.1% ±5.7) had TT_3 higher than 3.8,

6/49 (6.1% ±6.8) had TT_3 lower than 2.0

3.5.9. FT_3

3.5.9.1. FT_3 Distribution

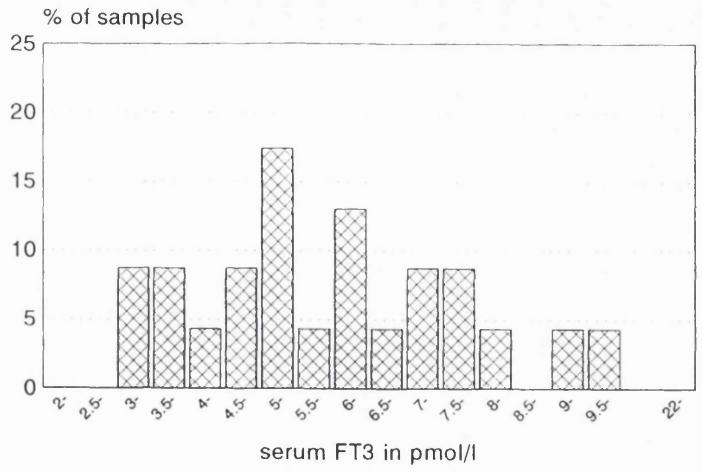


Figure 3.5.9.1.1. Distribution of FT_3 in first trimester (n=24)

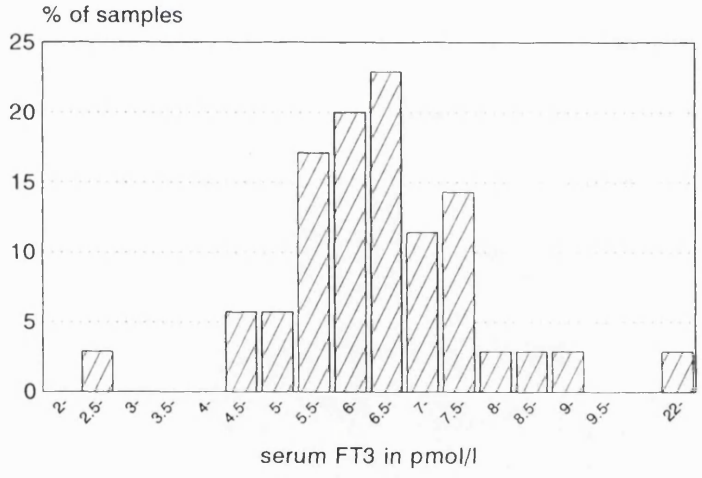


Figure 3.5.9.1.2. Distribution of FT_3 in second trimester (n=35)

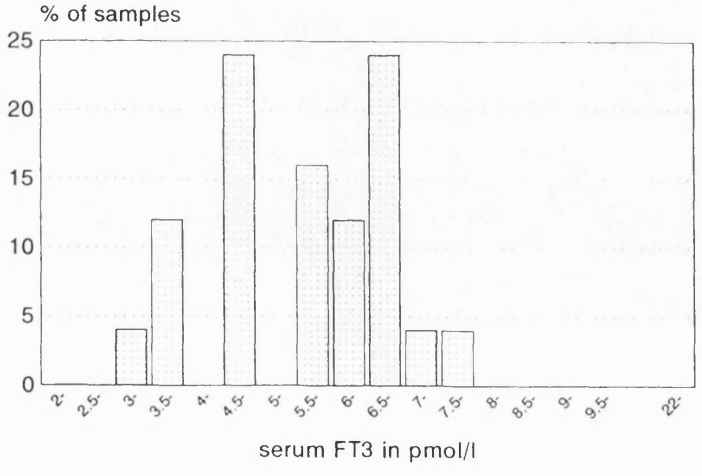


Figure 3.5.9.1.3. Distribution of FT_3 in third trimester (n=24)

3.5.9.2. Mean FT_3

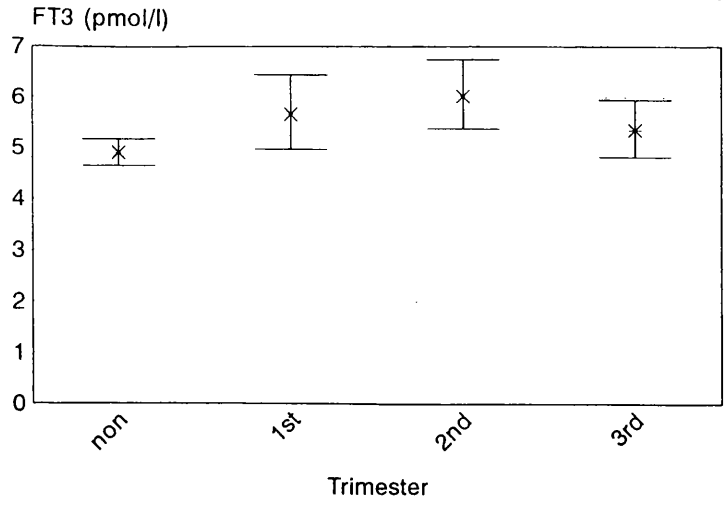


Figure 3.5.9.2. Geometric mean FT_3 concentration by trimester. (n=135 for non-pregs, n=24 for 1st trim, n=35 for 2nd trim, n=24 for 3rd trim)

There was a highly significant difference between mean FT_3 concentrations in non-pregnant and pregnant women ($p<0.0005$). There was a small increase in mean FT_3 between the first and second trimesters, followed by a small decrease in the third, although these differences were not significant ($p>0.4$, $p>0.5$ and $p>0.1$ for differences in mean between first and second, first and third and second and third, respectively).

3.5.9.3. Thyroid Status by FT_3

As with TT_3, the means for each trimester were within the normal limits for pregnancy (see section 2.7.2.4.6.) and very few samples had TT_3 values towards or beyond these limits. No samples had FT_3 values below the corresponding "normal ranges" and FT_3 levels above the "normal range" were found only in::

5/24 (20.8% ±17.1) first trimester women,

8/37 (21.6% ±13.7) second trimester women,

2/24 (8.3% ±11.6) third trimester women.

3.5.10. T_3/T_4 Ratios

3.5.10.1. Mean TT_3/TT_4 Ratio

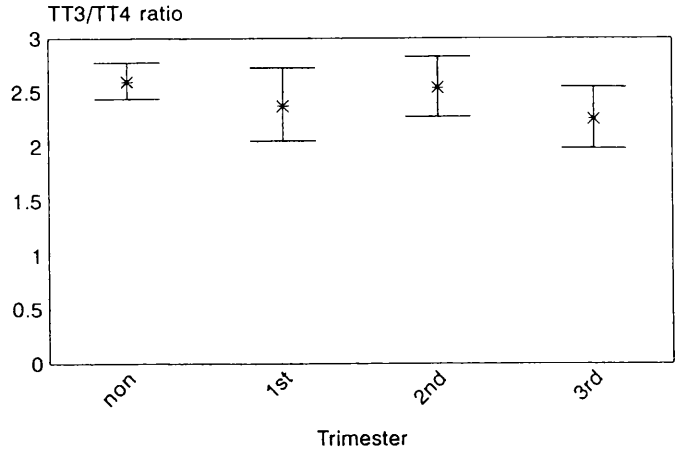


Figure 3.5.10.1. Geometric mean TT_3/TT_4 ratio by trimester (n=127 for non-pregs, n=24 for 1st trim, n=32 for 2nd trim, n=21 for 3rd trim)

3.5.10.2. Mean FT_3/FT_4 Ratio

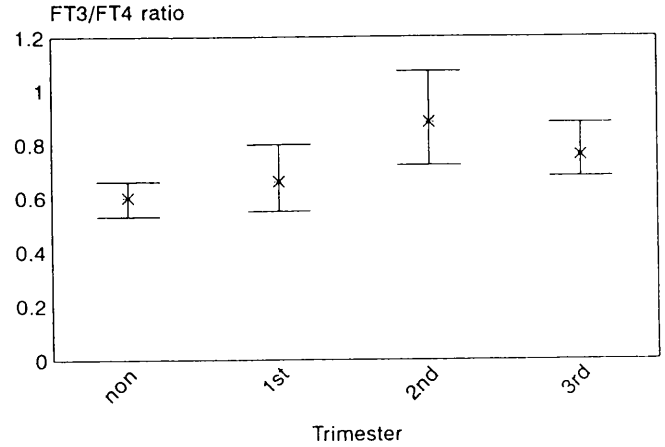


Figure 3.5.10.2. Geometric mean FT_3/FT_4 ratio by trimester (n=133 for non-pregs, n=24 for 1st trim, n=34 for 2nd trim, n=24 for 3rd trim)

There was no significant difference in mean TT_3/TT_4 ratios between non-pregnant and pregnant women ($p>0.05$) or between the three trimesters ($p>0.4$, $p>0.1$, $p>0.5$ between first and second, second and third, and first and third trimesters, respectively). There was a significant difference in FT_3/FT_4 ratio between non-pregnant and pregnant women ($p<0.005$). The mean ratio rose significantly between the first and second trimester ($p<0.05$) and fell slightly in the third, although the mean was not significantly different from either the first or second trimester ($p>0.5$ and $p>0.1$, respectively).

3.5.11. TSH

3.5.11.1. TSH Distribution

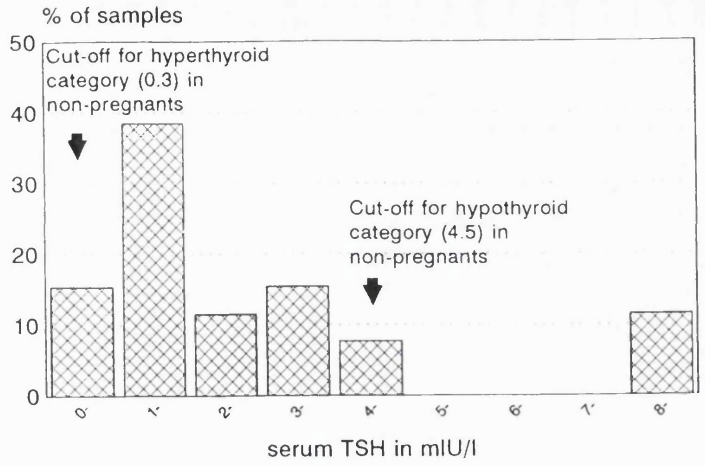


Figure 3.5.11.1.1. Distribution of TSH in first trimester (n=27)

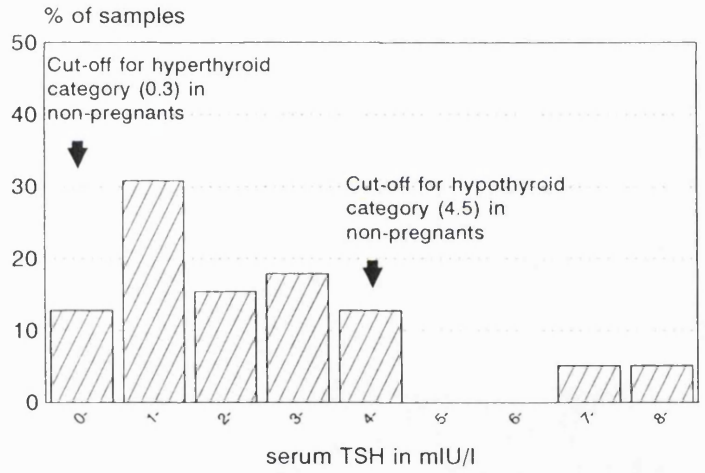


Figure 3.5.11.1.2. Distribution of TSH in second trimester (n=39)

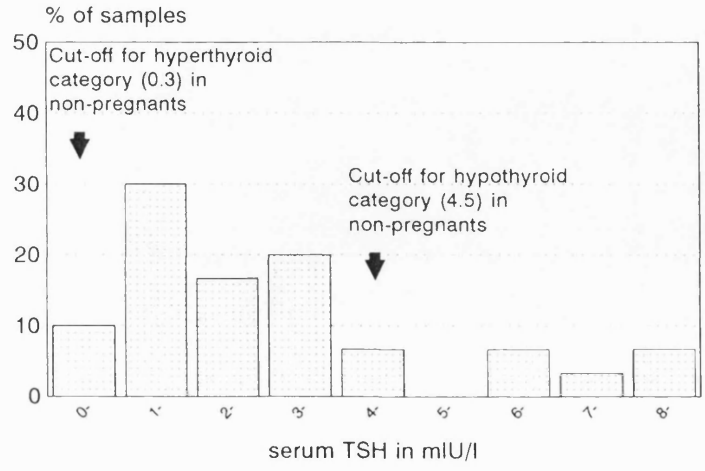


Figure 3.5.11.1.3. Distribution of TSH in third trimester (n=29)

3.5.11.2. Mean TSH

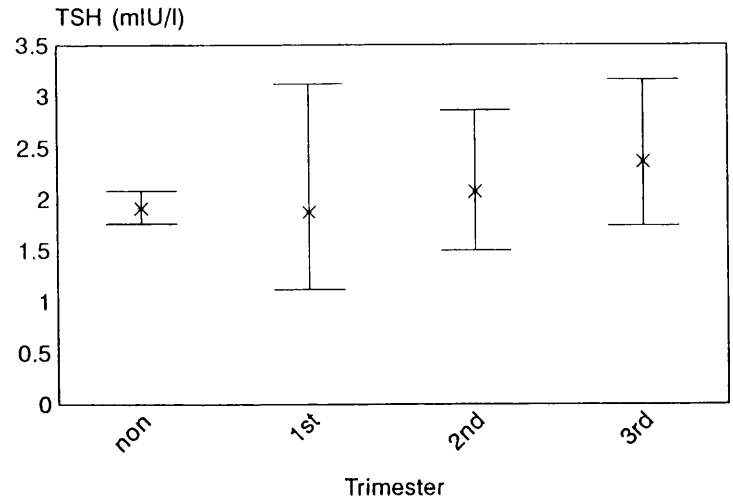


Figure 3.5.11.2. Geometric mean TSH concentration by trimester. (n= 509 for non=pregs, n=27 for 1st trim, n=4.3 for 2nd trim, n=29 for 3rd trim)

There was no significant difference between mean TSH concentration in non-pregnant and pregnant women ($p>0.6$). There was a slight upward trend in mean TSH, from first to third trimester, although this was not significant ($p>0.7$, $p>0.6$, $p>0.4$ between first and second, second and third, and first and third trimesters, respectively).

3.5.11.3. Thyroid Status by TSH

TSH levels below the "normal" range for non-pregnant women (see section 2.7.2.4.1.) were found in:

3/27 (11.1% ±12.4) first trimester women,

1/4.3 (2.6% ±5.1) second trimester women,

1/29 (3.4% ±6.9) third trimester women.

TSH levels above the "normal" range for non-pregnant women were found in:

5/27 (18.5% ±15.3) first trimester women,

7/39 (17.9% ±12.5) second trimester women,

6/29 (20.7% ±15.4) third trimester.

These proportions are similar to those found in non-pregnant women in the study area.

3.5.12. TG

3.5.12.1. TG Distribution

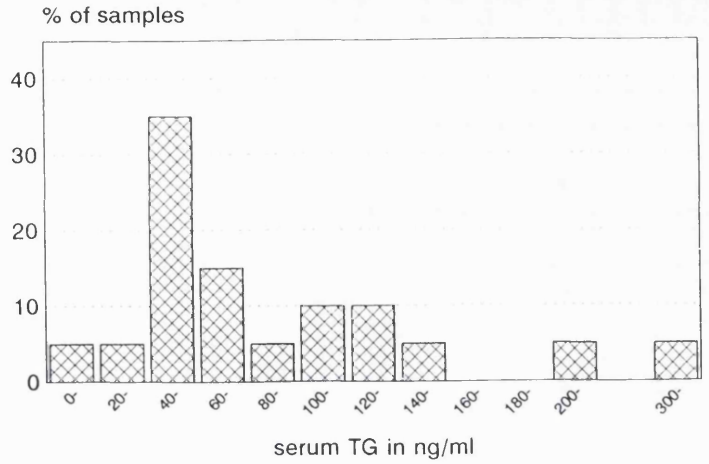


Figure 3.5.12.1.1. Distribution of TG in first trimester (n=20)

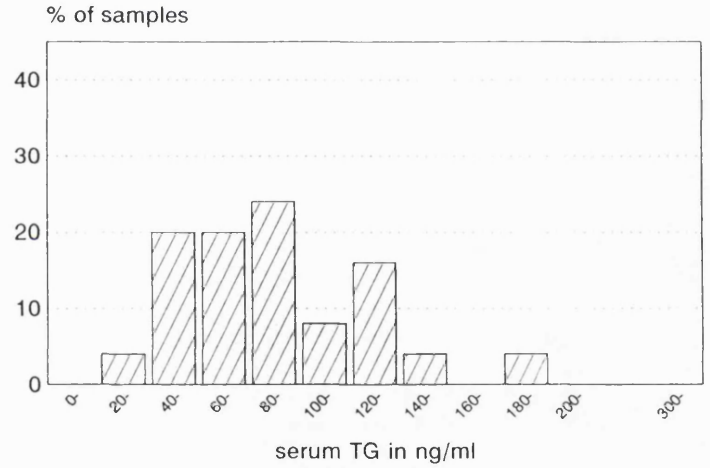


Figure 3.5.12.1.2. Distribution of TG in second trimester (n=25)

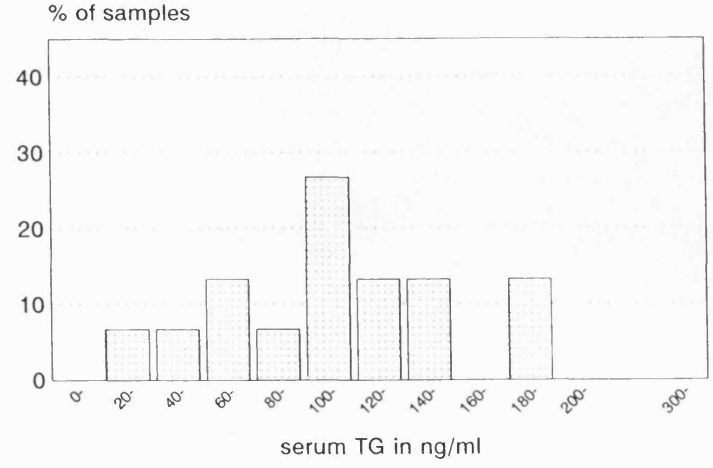


Figure 3.5.12.1.3. Distribution of TG in third trimester (n=15)

3.5.12.2. Mean TG

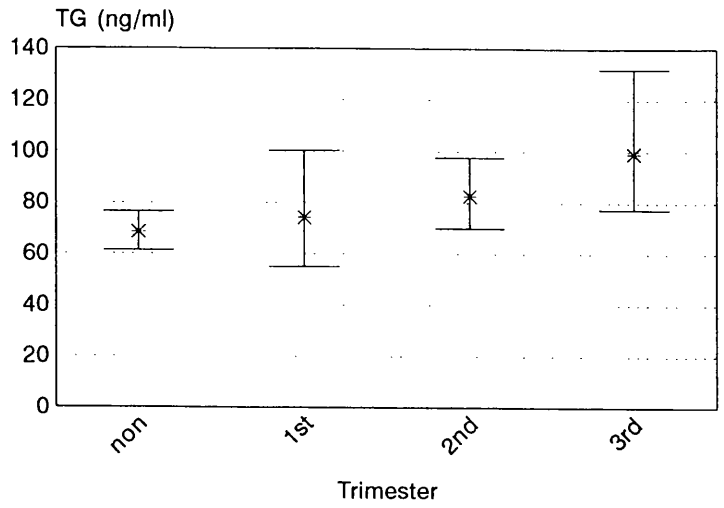


Figure 3.5.12.2. Geometric mean TG concentration by trimester. (n=103 for non-pregs, n=20 for 1st trim, n=25 for 2nd trim, n=15 for 3rd trim)

There was a significant difference in mean TG between non-pregnant and pregnant women ($p<0.03$) and an upward trend in mean TG concentration in the three trimesters, although there were no significant differences between the means in the three trimesters ($p>0.4$, $p>0.2$, $p>0.1$ between first and second, second and third and first and third trimesters, respectively).

3.6. Follow-up Thyroid Function in Non-pregnant Women

3.6.1. Summary

Summary results are presented in table 3.6.1., overleaf

Notes for table 3.6.1.

N/A not applicable (allocation to thyroid category based on this parameter is not appropriate)

(*) (± x - to make 95% CI of the proportion: ¸2 x SEM)

(**) p-value for (appropriate) mean paired difference:

arithmetic means for TT_4, TBG and TT_4/TBG ratio

geometric means for TSH, TG, FT_4, FT_3, TT_3, FT_3/FT_4 ratio and TT_3/TT_4 ratio

TT_3/TT_4 ratio has been multiplied by 100 for convenience

3.6.1.2. Response to Follow-up

As described in sections 2.3 and 2.4, the response to follow-up clinics varied from village to village. In 6 of the original 16 villages, including 2 of the key villages (Naniah and Golla), it was not possible to obtain any follow-up venous bloods, whilst in others, only a limited number of women were willing to allow a second sample to be taken. It is interesting to note that one of the five key villages (Behl Chakka) provided 40/116 (34.5%) of the follow-up samples (see table A.2.3.6., appendix 2.3.).

3.6.1.3. Follow-up Interval

The follow-up bloods were collected at the first round of follow-up clinics, held 10-14 weeks after the iodine camps, i.e. in November/December 1993. Data were pooled and **"post-supplementation"** results are presented for this 10-14 weeks period.

PARA-METER (UNIT)	NO. OF PRS	PRE-SUPPLEMENTATION				POST-SUPPLEMENTATION				p-VALUE (**)
		MEAN (95% CI)	%HYPO (±*)	%EU (±*)	%HYPER (±*)	MEAN (95% CI)	%HYPO (±*)	%EU (±*)	%HYPER (±*)	
TSH (mIU/l)	101	2.11 (1.72→2.60)	14.9 (7.0)	82.2 (9.4)	3.0 (3.4)	0.65 (0.50→0.85)	3.0 (3.4)	75.2 (8.5)	21.8 (8.2)	<0.0001
TG (ng/ml)	55	66.7 (57.7→77.2)	N/A	N/A	N/A	50.0 (42.7→58.6)	N/A	N/A	N/A	<0.02
FT_4 (pmol/l)	95	8.23 (7.19→9.43)	58.9 (10.0)	34.7 (9.7)	6.3 (5.0)	13.3 (11.7→15.1)	27.4 (9.1)	52.6 (10.2)	20.0 (8.2)	<0.0001
TT_4 (nmol/l)	80	80.9 (75.7→86.1)	32.5 (10.4)	67.5 (10.4)	0	108 (102→115)	12.5 (7.4)	81.3 (5.0)	6.3 (5.4)	<0.0001
TBG (mg/l)	79	39.4 (38.0→40.8)	N/A	N/A	N/A	38.7 (36.7→40.7)	N/A	N/A	N/A	NS
FT_3 (pmol/l)	79	4.77 (4.41→5.16)	3.8 (4.3)	87.3 (7.5)	8.9 (6.4)	5.28 (4.88→5.72)	2.5 (3.5)	75.9 (13.7)	21.5 (9.2)	NS
TT_3 (nmol/l)	78	1.98 (1.88→2.09)	7.7 (6.0)	88.5 (7.2)	3.8 (4.3)	1.86 (1.74→1.99)	5.1 (5.0)	87.2 (7.5)	7.7 (6.0)	NS
FT_3/FT_4 RATIO	78	0.53 (0.46→0.60)	N/A	N/A	N/A	0.40 (0.35→0.45)	N/A	N/A	N/A	<0.0001
TT_3/TT_4 RATIO	72	2.53 (2.31→2.77)	N/A	N/A	N/A	1.73 (1.63→1.83)	N/A	N/A	N/A	<0.0001

Table3.6.1.Summary of pre- and post-supplementation thyroid parameters

3.6.2. TSH

3.6.2.1 TSH Distribution

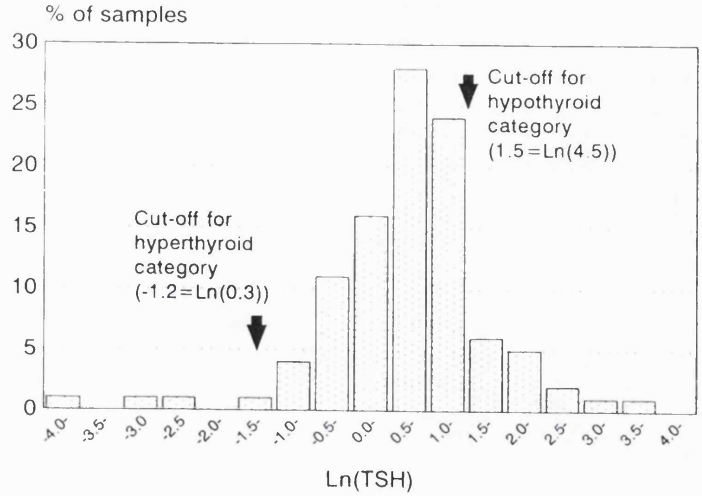


Figure 3.6.2.1.1. Pre-supplementation Ln(TSH) Distribution (n=101)

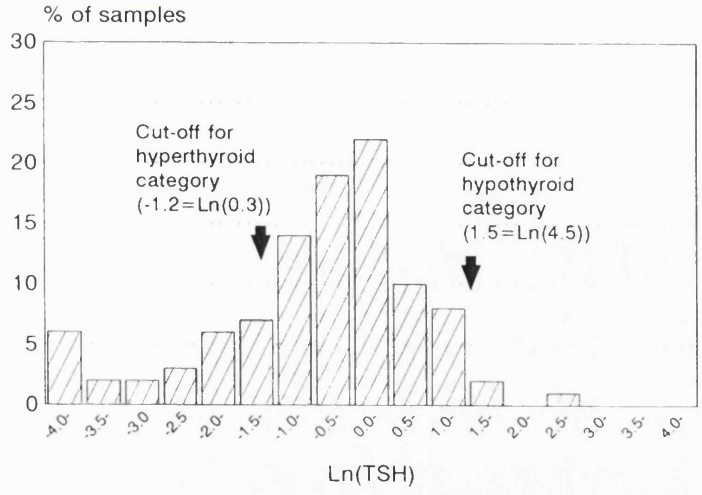


Figure 3.6.2.1.2. Post-supplementation Ln(TSH) Distribution (n=101)

The geometric mean paired difference between pre- and post-supplementation TSH concentrations was highly significant (mean ratio of pre-supplementation concentration to post-supplementation concentration = 3.27:1, 95% CI 2.59-4.12, $p<0.0001$, paired t-test) as was the non-parametric paired test of difference ($p<0.0001$, Wilcoxon matched-pairs signed-ranks test).

3.6.2.2. Thyroid Status by TSH

The proportion of samples in each category, defined in section 2.7.2.7.1., before and after supplementation are shown below.

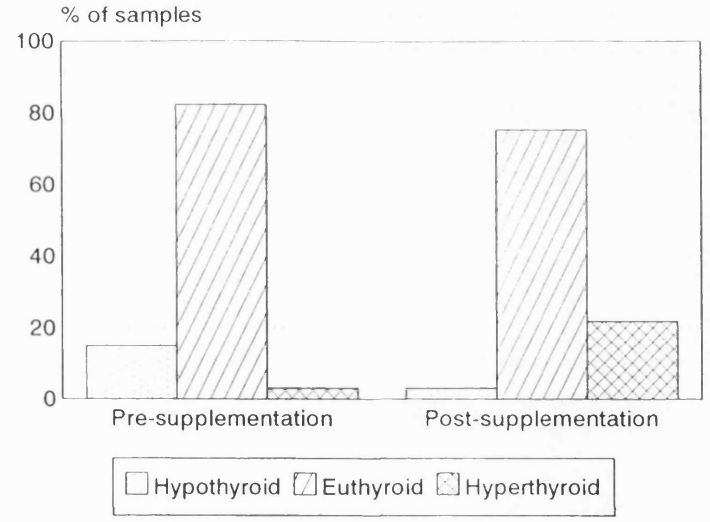


Figure 3.6.2.2. Pre- and post-supplementation "thyroid status", by TSH (n=101)

3.6.2.3. Individual Changes in TSH

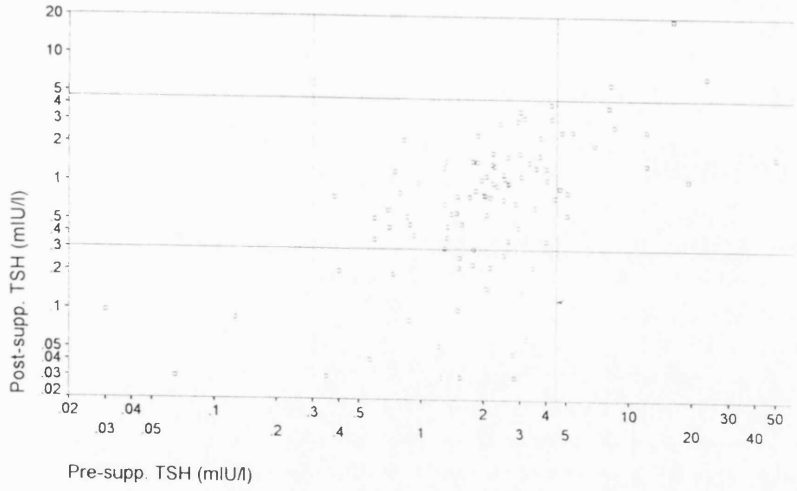


Figure 3.6.2.3. Pre- and post-supplementation TSH (n=101)
(horizontal and vertical lines indicate boundaries of normal ranges)

Subjects with **initially raised TSH**, classified as **TSH-"hypothyroid"**:

3/15 remained TSH-"hypothyroid",

12/15 became TSH-"euthyroid".

All 3 subjects who remained TSH"hypothyroid" had initially had one other thyroid hormone (FT_4 ,TT_4 or TT_3) in the "hypothyroid" range, with all others being "euthyroid". Following supplementation, one subject was "hypothyroid" by both free and total thyroxine but maintained normal levels of T_3, one subject had become FT_3 - "hyperthyroid", with all other parameters normal and one subject had remained with a low FT_4 but become "hyperthyroid" by TT_4, FT_3 and TT_3.

Of the 12 subjects who moved from TSH-"hypothyroid" to TSH-"euthyroid", 10 had sub-normal thyroxine levels (8 of these had low levels of both free and total hormone) before supplementation of whom only 4 had subnormal thyroxine levels afterwards (none of whom were low in both free and total hormone). One subject with initially elevated levels of FT_3 and FT_4 (but subnormal TT_4) remained so after supplementation and 3 subjects, all with initially low T_4 , had elevated levels of one or two thyroid hormone parameters after supplementation.

Subjects with **initially normal TSH**, classified as **TSH-"euthyroid"**:

64/83 remained TSH-"euthyroid"

19/83 became TSH-"hyperthyroid";

Of the 64 subjects who had normal levels of TSH both before and after supplementation, 48 had sub-normal thyroxine levels (19 of these had low levels of both free and total hormone) before supplementation of whom only 16 had subnormal thyroxine levels afterwards (only 3 of whom were low in both free and total hormone). 3 of these subjects also became FT_3- and/or TT_3 -"hypothyroid", possibly indicating an acute, inhibitory "Wolff- Chaikoff Effect" in response to the large dose of iodine given. 4 subjects who initially had raised levels of FT_4 (with normal levels of TT_4) maintained (or further increased) their FT_4 concentrations (although TT_4 remained normal). 7 other subjects, initially with normal or low thyroxine levels became FT_4-"hyperthyroid".

Of the 19 subjects, initially TSH-"euthyroid" who became hypothyrotropinemic, one

subject with an initially raised FT_4 level experienced a further increase in FT_4 and in addition became FT_3-"hyperthyroid" (though maintained normal levels of both total hormones). 9 subjects became "hyperthyroid" by thyroxine level (6 by free hormone, 2 by total hormone and 1 by both) and of these, 6 had associated increases in free and/or total T_3 to become T_3-"hyperthyroid" of whom only one had been T_3-"hyperthyroid" before supplementation.

Subjects with **initially suppressed TSH**, classified as **"TSH-hyperthyroid"**:

3/3 remained TSH-"hyperthyroid"

Of the 3 subjects with supressed TSH levels before and after supplementation, one was "euthyroid" in all other parameters, both before and after supplementation. One, with an initially low FT_4, became "hyperthyroid" by all 4 thyroid hormone measures (free and total T_3 and T_4, indicating true iodine-induced/enhanced hyperthyroidism and one, initially "euthyroid" by most parameters but with a raised FT_3, became "hypothyroid" in both free and total T_4.

3.6.3. TG

3.6.3.1. TG Distribution

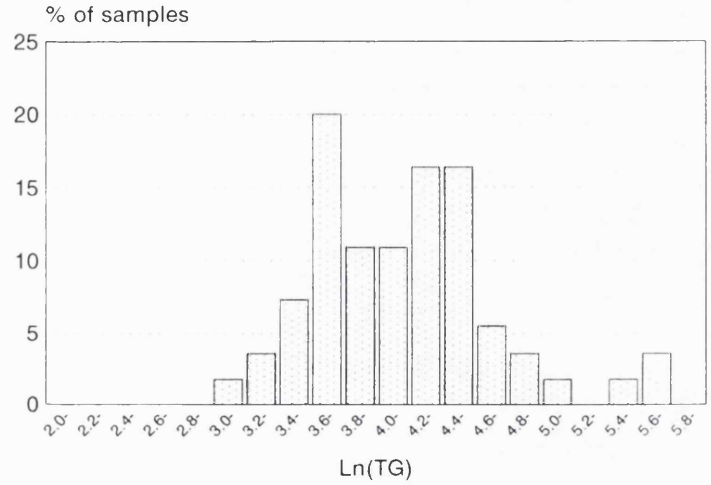


Figure 3.6.3.1.1. Pre-supplementation Ln(TG) distribution (n=55)

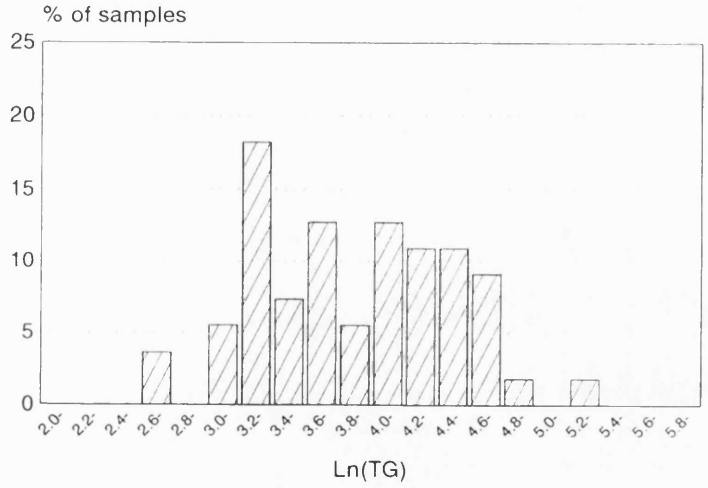


Figure 3.6.3.1.2. Post-supplementation Ln(TG) distribution (n=55)

The geometric mean paired difference between pre- and post-supplementation TG concentrations was statistically significant (mean ratio of pre-supplementation concentration to post-supplementation concentration = 1.33:1, 95% CI 1.06-1.68, $p < 0.02$, paired t-test) as was the non-parametric test of difference ($p < 0.03$, Wilcoxon matched-pairs signed-ranks test).

Before supplementation, all 55 women had serum TG concentrations above the upper limit of normal (21 ng/ml) and after supplementation, only 2/55 (3.6% ±5.1) had serum TG concentrations below this value.

3.6.3.2. Individual Changes in TG

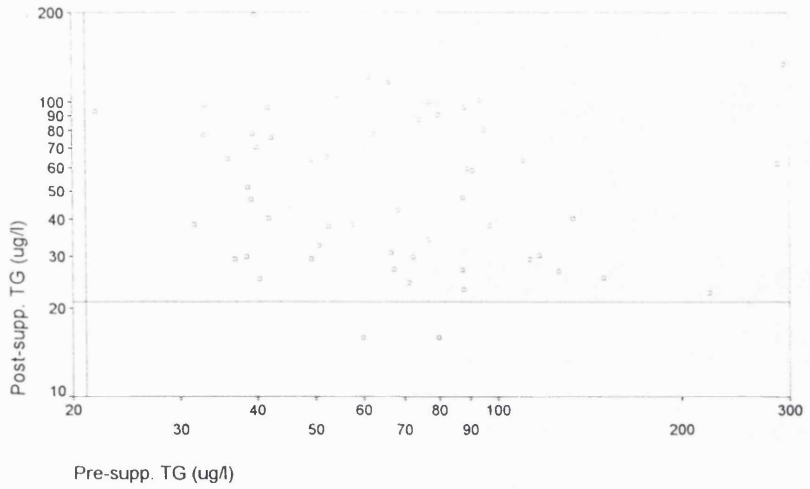


Figure 3.6.3.2. Pre- and post-supplementation TG (n=55)
(horizontal and vertical lines indicate boundaries of normal ranges)

3.6.4. FT_4

3.6.4.1. FT_4 Distribution

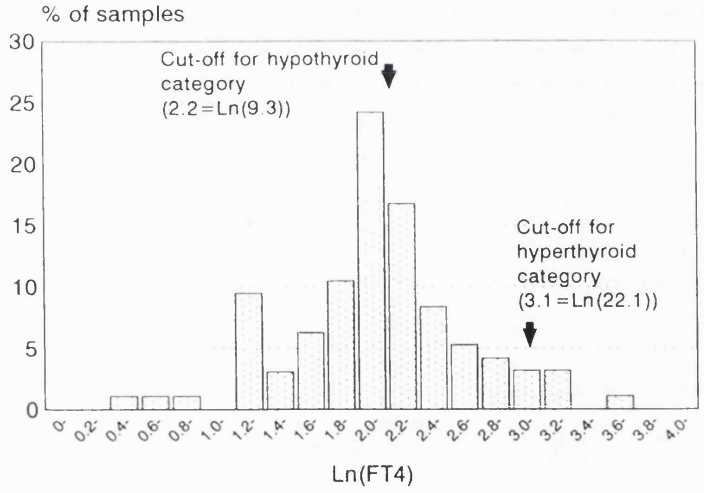


Figure 3.6.4.1.1. Pre-supplementation Ln(FT_4) distribution (n=95)

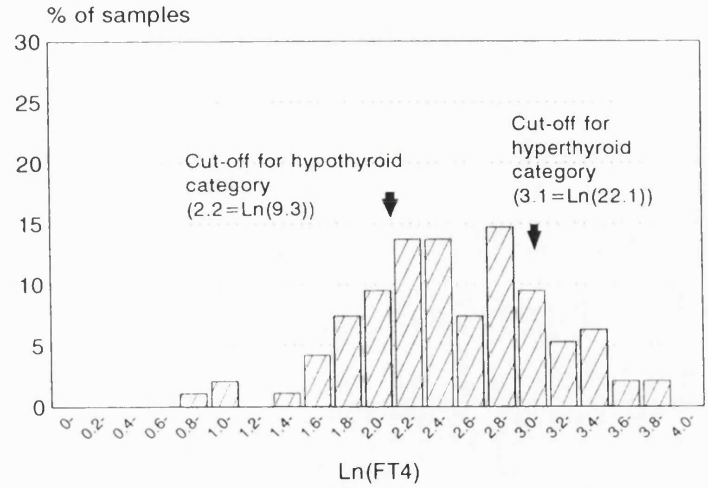


Figure 3.6.4.1.2. Post-supplementation Ln(FT_4) distribution (n=95)

The geometric mean paired difference between pre- and post-supplementation FT_4 concentrations was highly significant (mean ratio of pre-supplementation concentration to post-supplementation concentration = 0.62:1, 95% CI 0.54-0.71, $p < 0.0001$, paired t-test) as was the non-parametric paired test of difference ($p < 0.0001$, Wilcoxon matched-pairs signed-ranks test).

3.6.4.2. Thyroid Status by FT_4

The proportion of samples in each category, defined in section 2.7.2.4.3., before and after supplementation are shown below.

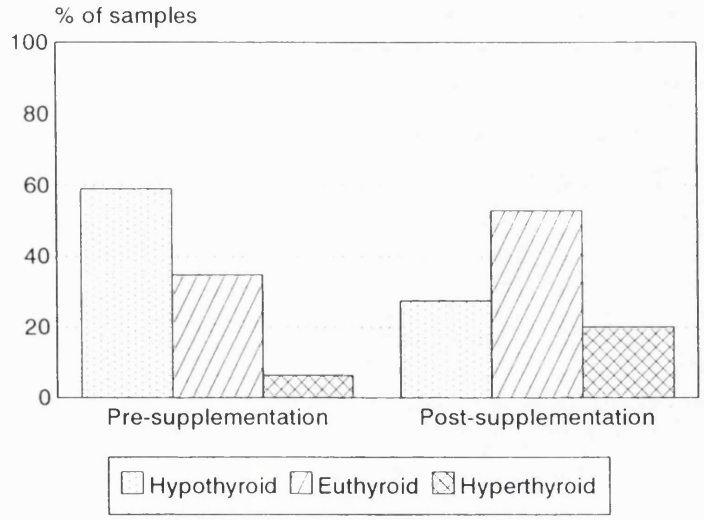


Figure 3.6.4.2. Pre- and post-supplementation "thyroid status", by FT_4 (n=95)

3.6.4.3. Individual Changes in FT_4

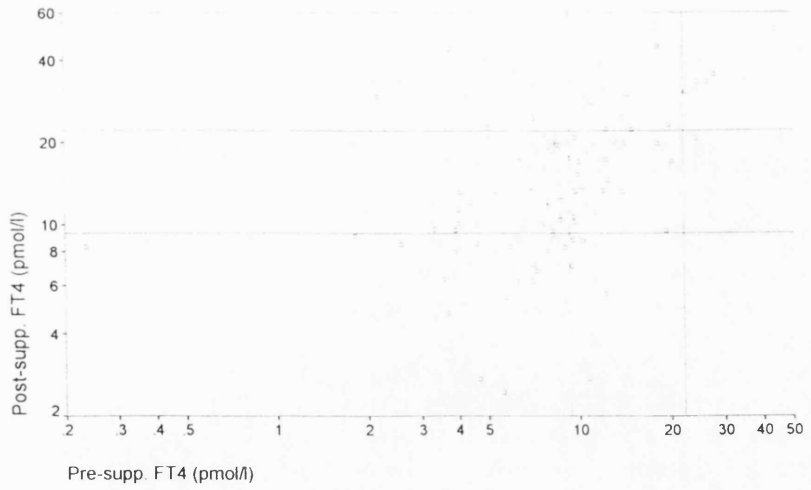


Figure 3.6.4.3. Pre- and post-supplementation Ln(FT_4) (n=95)
(horizontal and vertical lines indicate boundaries of normal ranges)

Subjects with **initially low FT_4**, classified as **FT_4-"hypothyroid"**:

21/56 remained FT_4-"hypothyroid"

29/56 became FT_4-"euthyroid"

6/56 became FT_4-"hyperthyroid"

Of the 21 subjects who remained hypothyroxinemic after supplementation, 4 initially also had raised TSH levels, 1 of whom still had raised TSH after supplementation. Low TT_4 levels were present in 9 of these subjects before supplementation (including 3 of the subjects with raised TSH) and 3 after supplementation (2 of whom did not have low TT_4 before supplementation). Although iodine supplementation did not move these subjects into the "euthyroid" category defined by FT_4 cut-offs, in most subjects FT_4 rose to levels higher than pre-supplementation.

Of the 29 subjects who were initially FT_4-"hypothyroid" and who became FT_4-"euthyroid", 14 were associated with initially low TT_4 levels (4 of whom also had raised TSH levels) which all became normal after supplementation. 2 of these 29 subjects became "hyperthyroid" in TT_4, FT_3, TT_3 and TSH with a further 5 becoming "hyperthyroid" by one or more of these parameters. With the few exceptions detailed above, these subjects are those in whom thyroid function has been normalised, following iodine supplementation.

Of the 6 subjects who were initially hypothyroxinemic but became hyperthyroxinemic, 4 were associated with a fall in TSH to "hyperthyroid" levels of whom 2 were also associated with increases in TT_4, FT_3 and TT_3 to "hyperthyroid" levels. These subjects appear to be those in whom correction of iodine deficiency, with a large dose of iodine, has led to true biochemical hypothyroidism, although it should be remembered that no clinical signs of hyperthyroidism were reported.

Subjects with **initially normal FT_4**, classified as **FT_4-"euthyroid"**:

20/33 remained FT_4-"euthyroid"

5/33 became FT_4-"hypothyroid"

6/33 became FT_4-"hyperthyroid"

Almost all of the 20 FT_4-"euthyroid" subjects who remained so after supplementation,

were associated with normal values of all other thyroid hormone parameters both before and after supplementation, although a few had one other parameter outside the normal range, either before or afterwards. These subjects appear to be those who have normal thyroid function before supplementation and who are able to adjust to the large dose of iodine, maintaining normal thyroid function, despite the increased availability of large amounts of iodine.

Of the 8 subjects, initially FT_4-"euthyroid" who became hyperthyroxinemic, 5 manifested no other changes in category, remaining euthyroid by other parameters. 3 developed raised FT_3 levels, of whom one also had a suppressed TSH level. These subjects appear to be those who respond to increased availability of iodine by manufacturing more thyroid hormone but in whom TSH is not (greatly) suppressed.

Of the 5 subjects, initially FT_4-"euthyroid" who became hypothyroxinemic, 2 were associated with euthyroid function of other parameters both before and after supplementation, one had suppressed TSH levels both before and afterwards and one had raised TSH levels both before and afterwards.

Subjects with **initially raised FT_4**, classified as **FT_4-"hyperthyroid"**:

5/6 remained FT_4-"hyperthyroid"

1/6 became FT_4-"euthyroid".

Of the 5 subjects who remained hyperthyroxinemic, only one was associated with other "hyperthyroid" parameters before supplementation, this subject having a very high FT_3 level, which further increased on supplementation, but low TT_4 and high TSH. After supplementation, one of these subjects had suppressed TSH and increased FT_3, indicating some enhancement of "hyperthyroid" status by free hormones. There may be some defect in total thyroxine production, although free hormones are maintained at high levels. The increased TSH is difficult to explain.

The one subject who moved from "hyperthyroid" to "euthyroid" status by FT_4 in fact, experienced only a small decrease in FT_4, from a value slightly above the normal range. It is therefore unlikely that this subject was truly "hyperthyroid" functionally.

3.6.5. TT_4

3.6.5.1. TT_4 Distribution

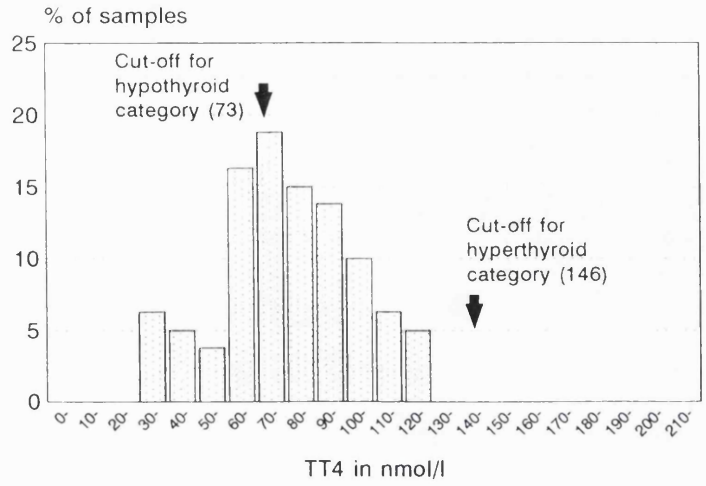


Figure 3.6.5.1.1. Pre-supplementation TT_4 distribution (n=80)

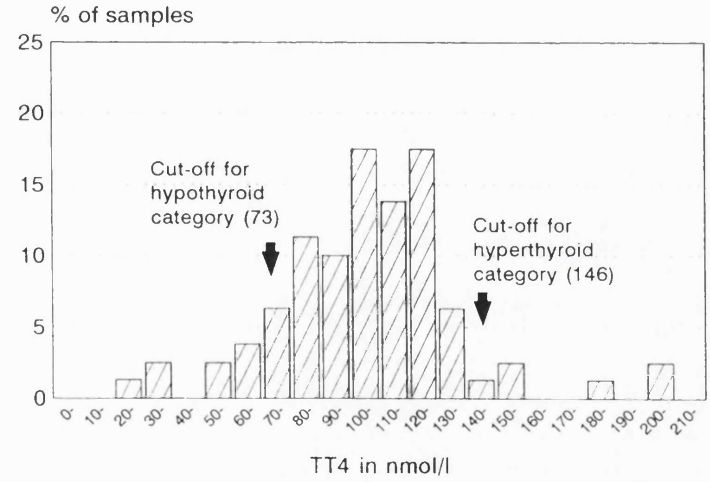


Figure 3.6.5.1.2. Post-supplementation TT_4 distribution (n=80)

The arithmetic mean paired difference between pre- and post-supplementation TT_4 concentrations was highly significant (mean increase in TT_4 concentration, following supplementation was 24.9 nmol/l, 95% CI 16.7-33.1, $p<0.0001$, paired t-test) as was the non-parametric paired test of difference ($p<0.0001$, Wilcoxon matched-pairs signed-ranks test).

3.6.5.2. Thyroid Status by TT_4

The proportion of samples in each category, defined in section 2.7.2.4.4., before and after supplementation are shown below.

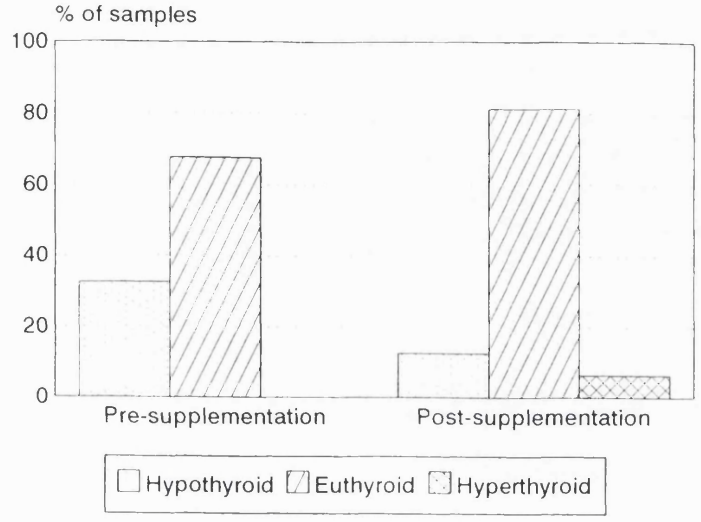


Figure 3.6.5.2. Pre- and post-supplementation "thyroid status", by TT_4 (n=80)

3.6.5.3. Individual Changes in TT_4

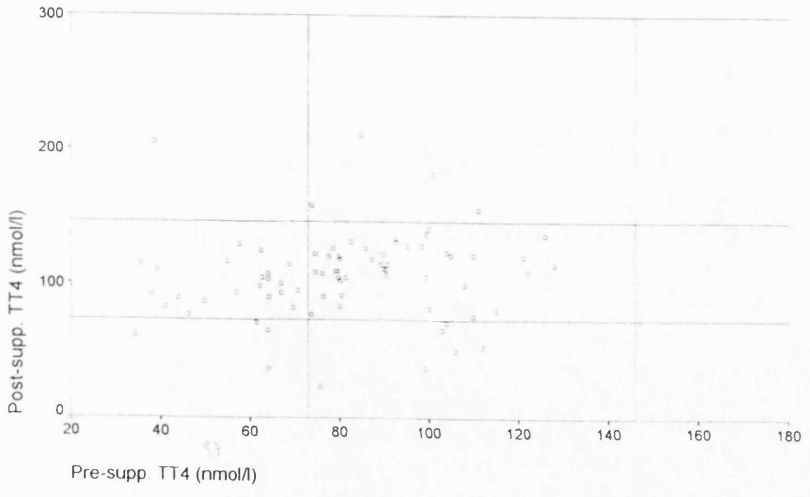


Figure 3.6.5.3. Pre- and post-supplementation TT_4 (n=80)
(horizontal and vertical lines indicate boundaries of normal ranges)

Subjects with **initially low TT_4**, classified as **TT_4-"hypothyroid"**:

4/25 remained TT_4-"hypothyroid"

21/25 became TT_4-"euthyroid"

1/25 became TT_4-"hyperthyroid"

Of the 4 subjects who remained hypothyroxinemic, 2 were associated with raised TSH levels before supplementation, one of which remained high afterwards and the other of which was associated with high FT_4 and FT_3 levels, both before and after supplementation, possibly indicating a defect in total thyroxine synthesis.

Of the 21 subjects initially TT_4-"hypothyroid" who became "euthyroid", 19 were associated with low levels of FT_4 before supplementation, of whom 5 also had raised TSH levels. After supplementation, only 3 subjects had persistently low FT_4, but 6 subjects had raised FT_4 levels and/or reduced TSH, indicating some degree of "hyperthyroidism".

The one subject, who moved from being TT_4-"hypothyroid" to TT_4-"hyperthyroid", initially had a low FT_4 with high FT_3 and normal TSH, indicating compensation for low thyroxine production by increased T_3 synthesis, but after supplementation the TSH level was dramatically suppressed and both FT_3 and TT_3 increased. The TT_4 concentration in this subject was grossly elevated (to 205 nmol/l) following supplementation. This suggests that, following supplementation and the increased availability of iodine, thyroid hormone synthesis was increased and continued at an elevated level, despite the accompanying fall in TSH stimulation, probably due to the continued increased iodide trapping.

Subjects with **initially normal TT_4**, classified as **TT_4-"euthyroid"**:

44/54 remained TT_4-"euthyroid"

6/54 became TT_4-"hypothyroid"

4/54 became TT_4-"hyperthyroid"

Of the 44 subjects who remained TT_4-"euthyroid" throughout the study, 16 had initially low levels of FT_4, 7 of which persisted after supplementation. In 7 of these 44 subjects, an increase in FT_4 was seen, to supranormal levels, compared with no elevated FT_4

levels before supplementation. In 8 subjects a suppression of TSH to subnormal levels was observed, of whom only 3 had previously had a low TSH. 7 subjects had supranormal FT_3 and/or TT_3 following supplementation, compared to only 3 before. This group of subjects thus represents those in whom thyroid function is generally normal in most parameters before supplementation, having adapted to the iodine deficiency. Some of these subjects remained "euthyroid" in all parameters and many experienced a mild degree of biochemical hyperthyroidism, as defined by one or more parameters, following supplementation.

Of the 6 subjects who moved from TT_4-"euthyroid" to TT_4-"hypothyroid", 2 were associated with decreases in FT_4, and T_3 (both free and total, where measured) and involved large decreases in TT_4 to very low levels, possibly indicating true iodine-induced hypothyroidism (Wolff-Chaikoff Effect) . 2 subjects experienced only small decreases in TT_4, and may therefore not represent a true change in status but rather a minor fluctuation.

Of the 4 subjects who moved from TT_4-"euthyroid" to TT_4-"hyperthyroid", in one subject this was an isolated change, all other parameters being normal before and after supplementation. In 2 subjects the increase in TT_4 was accompanied by increases in FT_4, FT_3 and TT_3 levels to subnormal values and decreases in TSH to supranormal values. These subjects may thus represent a response of true iodine-induced hyperthyroidism, as detailed above. In one subject, initial accompanying "hypothyroid" values for FT_4 and TSH were slightly improved, though not into "euthyroid" categories defined by them and T_3, initially normal became elevated.

There were no subjects with initially raised TT_4.

3.6.6. TBG

3.6.6.1. TBG Distribution

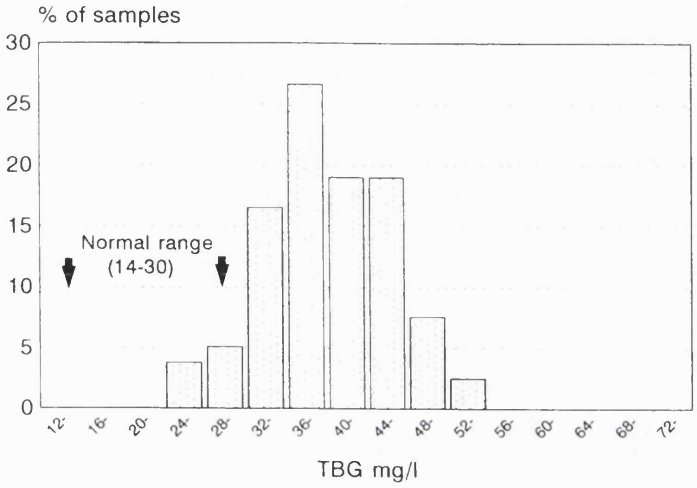


Figure 3.6.6.1.1. Pre-supplementation TBG distribution (n=79)

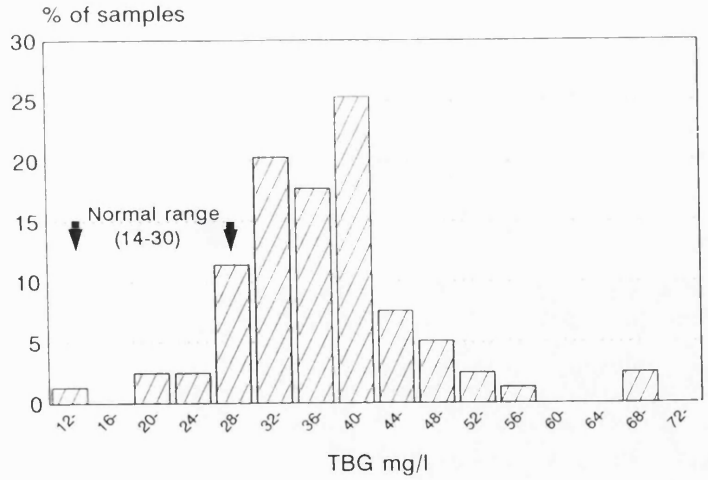


Figure 3.6.6.1.2. Post-supplementation TBG distribution (n=79)

The arithmetic mean paired difference between pre- and post-supplementation mean TBG concentrations was not significant ($p > 0.4$, paired t-test,) neither was the non-parametric paired test of differences ($p > 0.5$, Wilcoxon matched-pairs signed-ranks).

3.6.6.2. Individual Changes in TBG

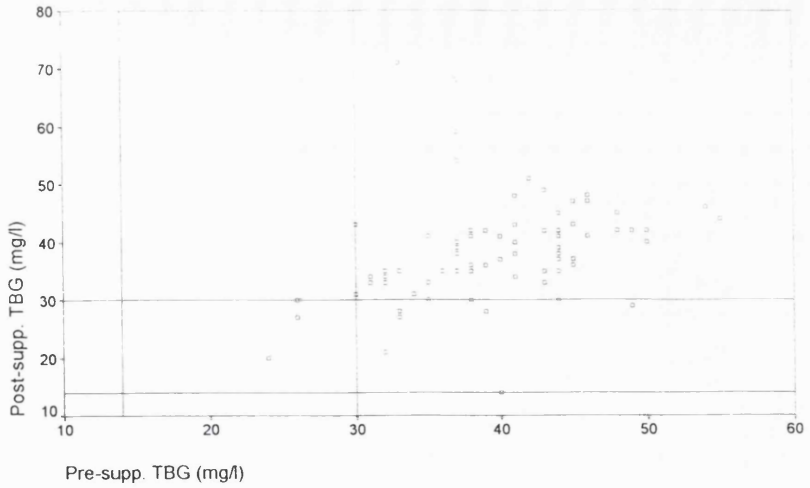


Figure 3.6.6.2. Pre- and post-supplementation TBG (n=79)
(horizontal and vertical lines indicate boundaries of normal ranges)

3.6.7. FT_3

3.6.7.1. FT_3 Distribution

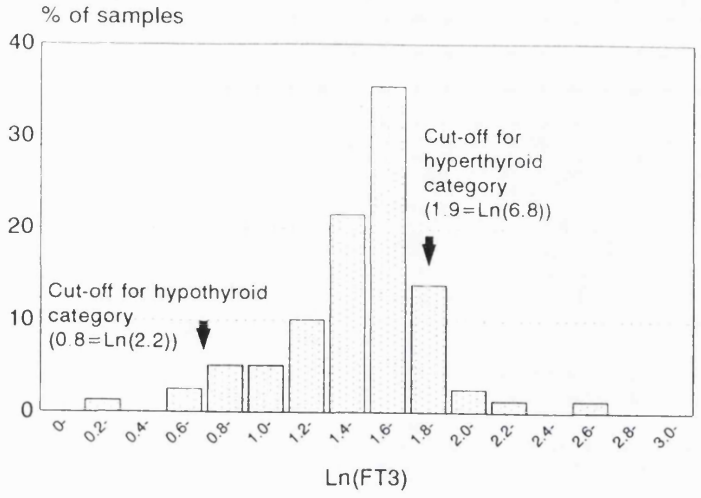


Figure 3.6.7.1.1. Pre-supplementation Ln(FT_3) distribution (n=79)

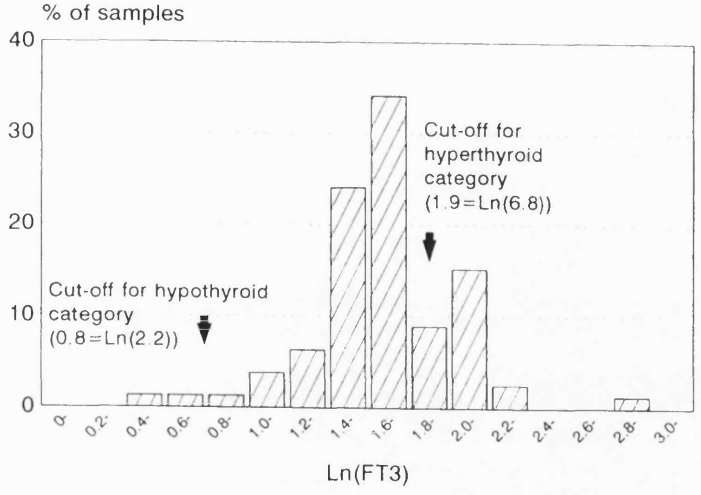


Figure 3.6.7.1.2. Post-supplementation Ln(FT_3) distribution (n=79)

The geometric mean paired difference between pre- and post-supplementation FT_3 concentrations was not significant ($p > 0.05$, paired t-test,) nor was the non-parametric paired test of difference ($p > 0.05$, Wilcoxon matched-pairs signed-ranks).

3.6.7.2. Thyroid Status by FT_3

The proportion of samples in each category, defined in section 2.7.2.4.5., before and after supplementation are shown below. Changes in these categories were not significant ($p > 0.1$).

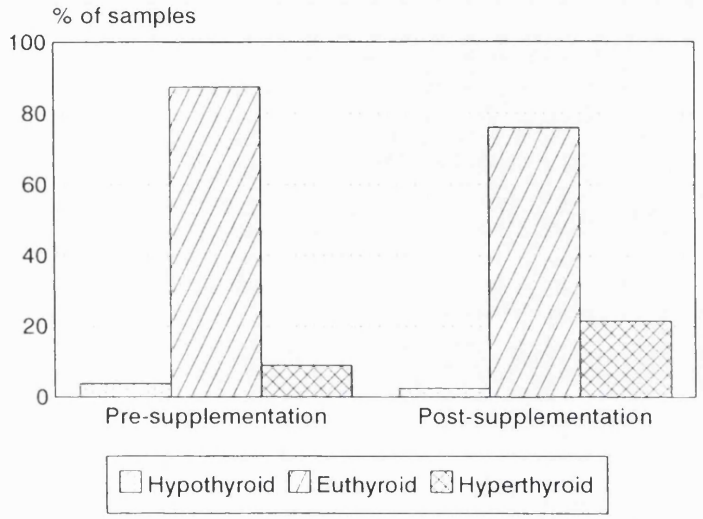


Figure 3.6.7.2. Pre- and post-supplementation "thyroid status", by FT_3 (n=79)

There were no significant differences in the proportion of subjects in each category before and after supplementation.

3.6.7.3. Individual Changes in FT_3

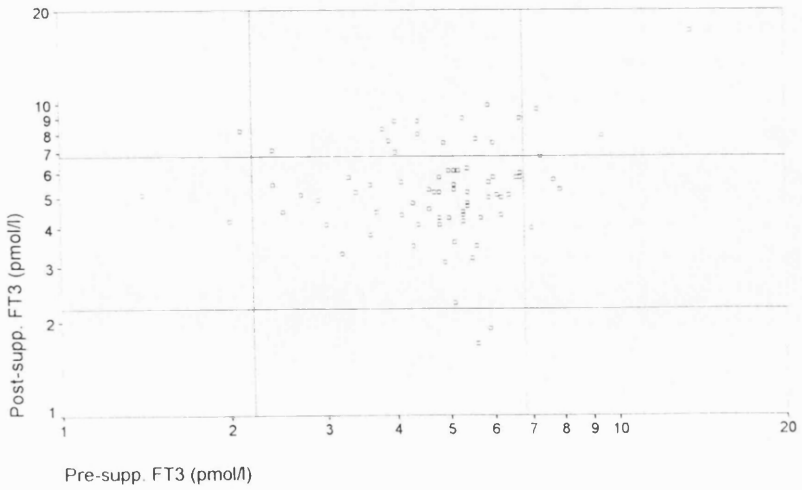


Figure 3.6.7.3. Pre- and post-supplementation Ln(FT_3)
(horizontal and vertical lines indicate boundaries of normal ranges)

Subjects with **initially low FT_3**, classified as **FT_3-"hypothyroid"**:

none remained FT_3-"hypothyroid"

2/3 became FT_3-"euthyroid"

1/3 became FT_3-"hyperthyroid"

The 2 subjects initially classified as FT_3-"hypothyroid", who became FT_3-"euthyroid", were both associated with subnormal levels of T_4 (both free and total) before supplementation and with suppressed TSH afterwards. In one of these subjects, FT_4 was slightly supranormal after supplementation. These 2 subjects appear to have responded to supplementation with iodine by increasing both T_4 and T_3 production which has the effect of reducing TSH levels by feedback inhibition.

The one subject in whom FT_3 increased dramatically from subnormal to supranormal had initially been euthyroid by all other parameters but became "hyperthyroid" by both TSH and FT_4, following supplementation. This subject is thus in an iodine-induced biochemically hyperthyroid state.

Subjects with **initially normal FT_3**, classified as **FT_3-"euthyroid"**:

54/69 remained FT_3-"euthyroid"

2/69 became FT_3-"hypothyroid"

13/69 became FT_3-"hyperthyroid"

In the 54 subjects who remained FT_3-"euthyroid" throughout, there are many differences in the details of individual thyroid profiles but the general pattern is one of an increase in T_4 and T_3, so that many "hypothyroid" categorisations are corrected to "euthyroid" following supplementation. There are, however, also many cases where individual parameters which were classified as "euthyroid" (or even "hyperthyroid") before supplementation become "hyperthyroid" afterwards. This is also true of the 4 subjects who moved from FT_3-"hyperthyroid" to FT_3-"euthyroid" categories.

The 2 subjects in whom FT_3, though initially normal, decreased to subnormal levels initially had slightly subnormal FT_4 levels which reduced further, following supplementation. These subjects, who also exhibited a reduction in TT_4 to subnormal levels, appear to be manifesting some of the biochemical indicators of iodine-induced

hypothyroidism (Wolff-Chaikoff effect).

Of the 13 subjects initially FT_3-"euthyroid", who became FT_3-"hyperthyroid", 11 were associated with one or more "hypothyroid" other parameters before supplementation but only 3 were so associated afterwards. Conversely, only 2 were associated with a "hyperthyroid" category of any another parameter before supplementation, whereas 9 were so associated afterwards. In these subjects, it would appear that "hypothyroid" status has largely been corrected, although some overcorrection has occurred and some "euthyroid" states have now become "hyperthyroid".

Subjects with **initially high FT_3**, classified as **FT_3-"hyperthyroid"**

3/7 remained FT_3-"hyperthyroid"

4/3 became FT_3-"euthyroid"

In the 3 subjects who remained FT_3-"hyperthyroid", there were concomitant increases in both FT_4 and TT_4, although the level of FT_3 rose dramatically only in the subject with a very high initial FT_3. This suggests that the extra iodine available following supplementation is mainly used for thyroxine synthesis in these subjects.

A similar process to that occurring in the 2 subjects who moved from FT_3-"euthyroid" to FT_3-"hypothyroid" may be occurring in the 4 subjects who moved from FT_3-"hyperthyroid" to FT_3-"euthyroid". These subjects initially had slightly low FT_4 levels. After supplementation these were further depressed and the subjects also became TT_4-"hypothyroid".

3.6.8. TT_3

3.6.8.1. TT_3 Distribution

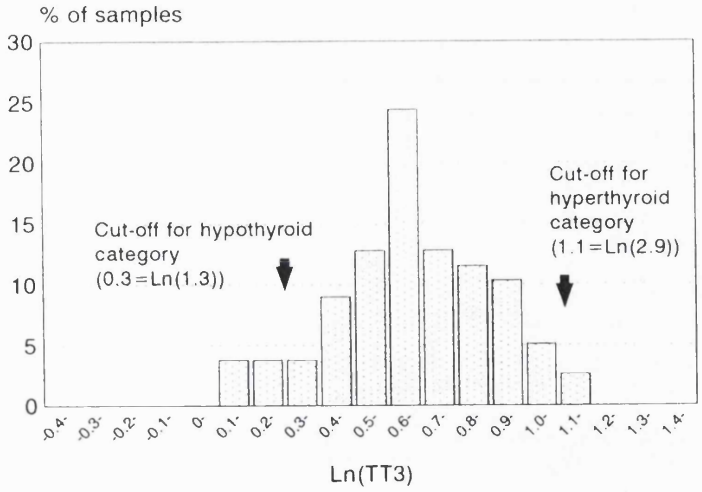


Figure 3.6.7.1.1. Pre-supplementation Ln(TT_3) distribution (n=78)

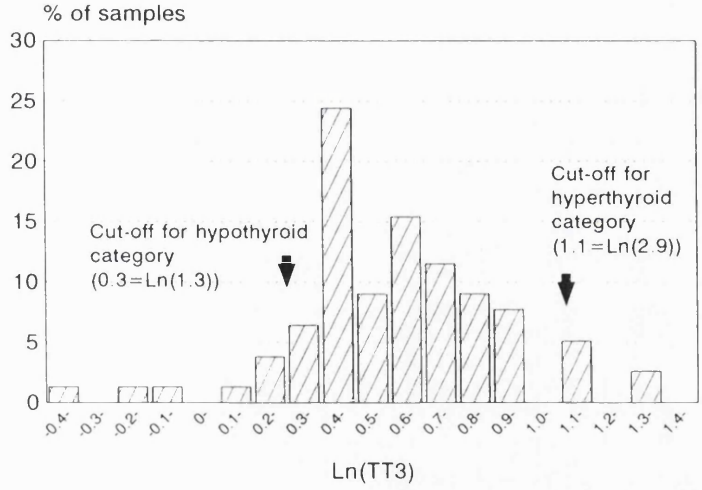


Figure 3.6.7.1.2. Post-supplementation Ln(TT_3) distribution (n=78)

The geometric mean paired difference between pre- and post-supplementation TT_3 concentrations was not significant ($p > 0.2$, paired t-test,) neither was the non-parametric paired test of difference ($p > 0.2$, Wilcoxon matched-pairs signed-ranks).

3.6.8.2. Thyroid Status by TT_3

The proportion of samples in each category, defined in section 2.7.2.4.6., before and after supplementation are shown below.

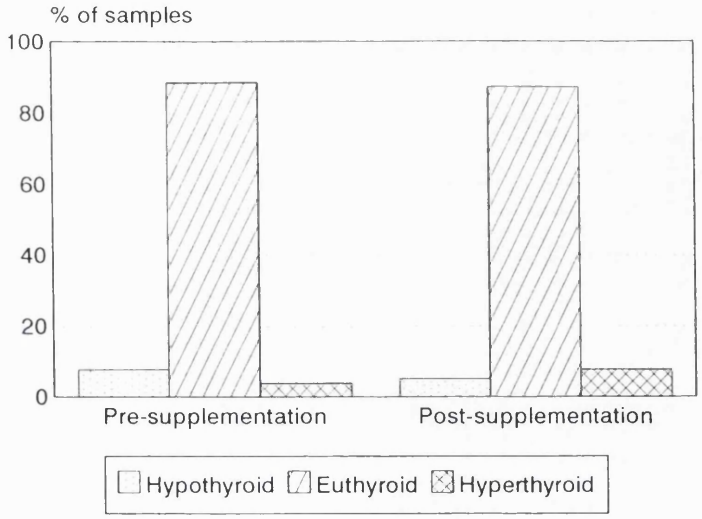


Figure 3.6.8.2. Pre- and post-supplementation "thyroid status", by TT_3 (n=78)

As with FT_3 there were no significant differences in the proportion of subjects in each category before and after supplementation,

3.6.8.3. Individual Changes in TT_3

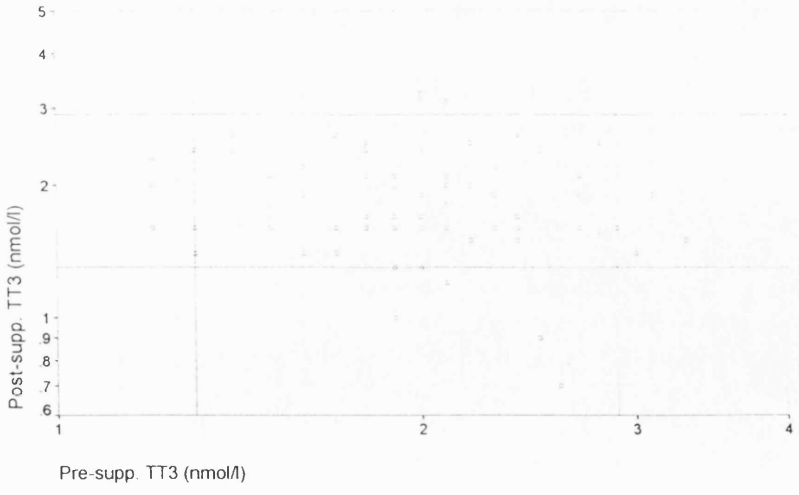


Figure 3.6.8.3. Pre- and post-supplementation Ln(TT_3) (n=78)
(horizontal and vertical lines indicate boundaries of normal ranges)

Subjects with **initially low TT_3**, classified as **TT_3-"hypothyroid"**:

all 6 became TT_3-"euthyroid";

Of the 6 subjects with initially subnormal TT_3 values, 2 were also associated with low TT_4 values and one with elevated TSH. All other parameters were normal. After supplementation, FT_4, TT_4, FT_3 and TT_3 all rose and TSH fell in all 6 subjects, such that all TT_3 values were in the normal range. The subject with elevated TSH remained with a supranormal value for this parameter (and was now associated with a raised FT_3). In 2 other subjects, one or two parameters were pushed into the hyperthyroid range. This group of subjects represents those in whom thyroid function was not severely compromised before supplementation and in whom correction of the iodine deficiency induced a small degree of biochemical hyperthyroidism, albeit in a few isolated parameters.

Subjects with **initially normal TT_3**, classified as **TT_3-"euthyroid"**:

56/69 remained TT_3-"euthyroid"

7/69 became TT_3-"hypothyroid"

6/69 became TT_3-"hyperthyroid"

As with FT_3, the 56 subjects who remained TT_3-"euthyroid" were associated with a wide range of thyroid profiles, although the general pattern was one of a few "hypothyroid" other parameters before supplementation and a few "hyperthyroid" other parameters afterwards.

In all 7 subjects who experienced a decline in TT_3 value, such that they moved from TT_3-"euthyroid" to TT_3-"hypothyroid" categories, FT_3 and FT_4 increased. In 6 of these subjects TT_4 decreased and in one TT_4 increased. Following supplementation, there was a change in the ratio of free to bound hormone.

All 6 subjects in whom TT_3 was initially normal and became elevated, the increase in TT_3 was accompanied by increases in FT_3, TT_4 TT_4 and decreases in TSH. 5 of these subjects also became "hyperthyroid" by 2 or 3 other parameters. These subjects were those in whom true iodine-induced hyperthyroidism was seen.

Subjects with **initially high TT_3**, classified as **TT_3-"hyperthyroid"**:

all 3 subjects became TT_3-"euthyroid".

Complete thyroid profiles were available for only 2 of the 3 subjects who moved from TT_3-"hyperthyroid" to TT_3-"euthyroid" and revealed a concomitant decrease in TT_4 and increases in FT_3 and FT_4, again illustrating a change in bound and unbound fractions.

3.6.9. T_3/T_4 Ratio

The ratios were calculated as described in section 3.4.11.

3.6.9.1. FT_3/FT_4 Ratio

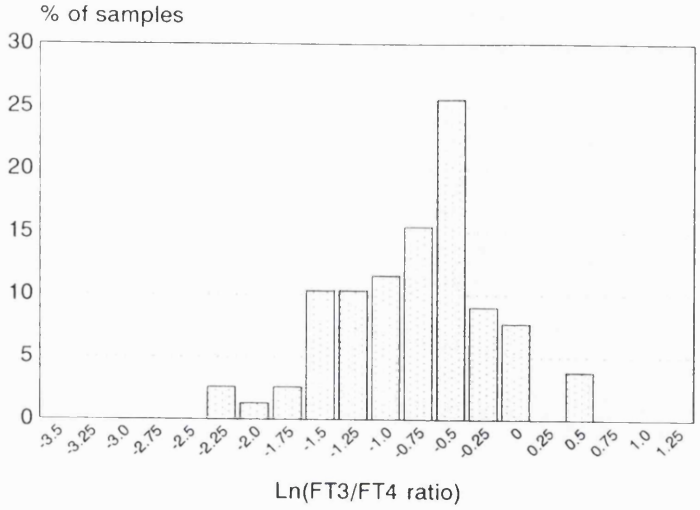


Figure 3.6.9.1.1. Pre-supplementation Ln(FT_3/FT_4) distribution (n=78)

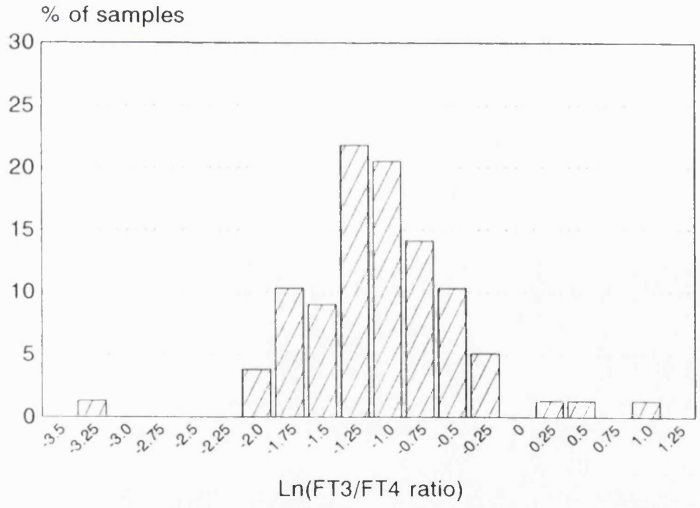


Figure 3.6.9.1.2. Post-supplementation Ln(FT_3/FT_4) distribution (n=78)

The geometric mean paired difference between pre- and post-supplementation FT_3/FT_4 ratios was highly significant (mean ratio of pre-supplementation FT_3/FT_4 ratio to post-supplementation FT_3/FT_4 ratio = 1.33:1, 95% CI 1.17-1.51, $p<0.0001$, paired t-test) as was the non-parametric paired test of difference ($p<0.0001$, Wilcoxon matched-pairs signed-ranks test).

3.6.9.2. TT_3/TT_4 Ratio

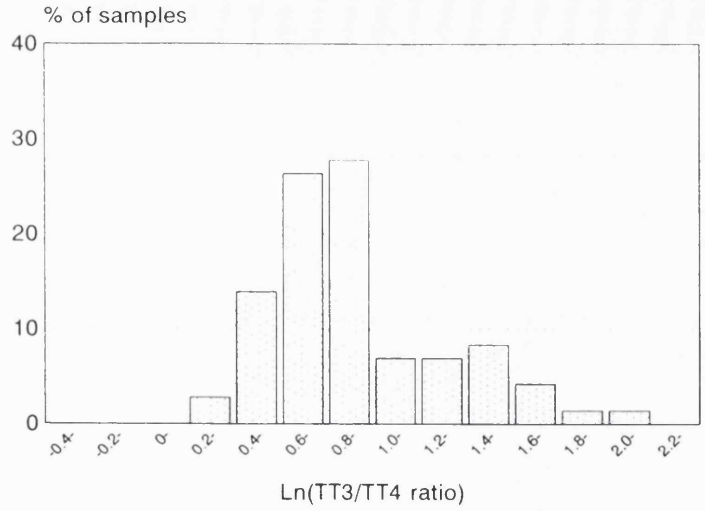


Figure 3.6.9.2.1. Pre-supplementation Ln(TT_3/TT_4) distribution (n=72)

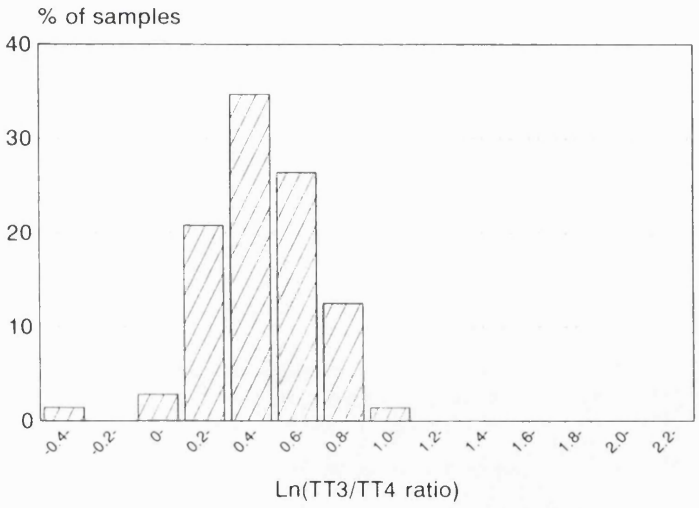


Figure 3.6.9.2.2. Post-supplementation Ln(TT_3/TT_4) distribution (n=72)

The geometric mean paired difference between pre- and post-supplementation TT_3/TT_4 ratios was highly significant (mean ratio of pre-supplementation TT_3/TT_4 ratio to post-supplementation TT_3/TT_4 ratio = 1.46:1, 95% CI 1.33-1.61, $p < 0.0001$, paired t-test) as was the non-parametric paired test of difference ($p < 0.0001$, Wilcoxon matched-pairs signed-ranks test).

CHAPTER 4 - DISCUSSION

4.1. Limitations of Field Work

This study highlighted many of the difficulties of fieldwork in a developing country, particularly in gaining access to women in a conservative, Muslim culture in a remote, rural area. With the benefit of hindsight, there are several recommendations which could be made about conducting similar work in future.

4.1.1. Location

As originally conceived, the study involved daily clinics in one Rural Health Centre, which was already functioning and was situated in a nuclear village, Lehtrar. Staff in the study were expected to stay at the RHC during the week. By the time the study was actually launched, iodine supplementation camps had already been announced, without full consultation and consideration, in 16 sites, many of which were not located in well-defined, cluster communities. The scattered nature of the dwellings in this area and the remoteness of certain clinic sites, would not have been too much of a problem for clients, if the politics and culture had not hindered women from attending the clinics. This meant that the area was not really suitable for a study requiring intense follow-up and frequent contact between staff and clients. In addition, it was no longer possible to stay in the clinic sites, overnight.

4.1.2. Timing

A longer recruitment and training period would have greatly benefitted the study, with more time for team-building, as well as technical training in form filling, goitre grading, infant and adult measurement, blood-taking and immunisation. It would also have been more sensible to launch the iodine camps **after** the Monsoon rains, so that the first round of follow-up clinics was not disrupted by the bad weather and landslides, leading to community disappointment and loss of goodwill. The elections could not have been predicted when the study was planned. They had profound negative consequences, dividing communities, and information about clinics was used as a political tool - withheld or released as convenient. Some of the effects of community hostility could, however, have been mitigated by better choice of health committees.

4.1.3. Staffing

Given the numbers of women we expected to see in the iodine camps, and the number of activities planned both for the camps and for the follow-up clinics, it would have been helpful to have four, rather than two community nurses, although recruitment of even this small number had proved to be extremely difficult. It is difficult to see how recruitment could have been more successful, given the cultural constraints on women travelling out to the villages to work and the lack of trained women in the villages.

For continuity in community relations and reliability in goitre grading, as well as personal commitment to the project, two or more (preferably female) doctors would have been better than the constantly changing team of medical students and junior housemen who generally made up the medical staff at the clinics. Again, cultural constraints in recruiting women doctors greatly impeded the study, as village women were reluctant to discuss their medical concerns with men.

There were problems in relationships between the senior investigator and the medical team; issues of authority and communication caused difficulties. In retrospect, a male (preferably medically qualified) senior investigator would have had more status and authority in organising the field work, particularly among the male doctors. He would not, however, have had access to the village women, and in this culture, a husband and wife team would probably have been the most acceptable unit to lead the study. These issues emphasise the considerable difficulties of working in such a community in collaboration with a medical team. Some of the difficulties were anticipated but not resolved, others were not anticipated.

4.1.4. Community Relations

These were good at the start of the study but deteriorated for some of the reasons outlined in sections 2.2.2. and 2.5.3., particularly during the election period and it would be important, in a future study, to ensure that the health committee included people from several political groups, and that the primary aim was to promote better health in the community, rather than furthering individuals' political careers.

It would also be desirable to have women on the committees, although this goes against the cultural norms of the community. Probably the most important way of maintaining good community relations is to be reliable and respectful, qualities the team did not always exhibit, given some of the constraints above.

Having seen the enthusiasm of one of the local female teachers, in bringing her pupils to an iodine camp, it would be prudent to use the teachers in mobilising community support for the study, particularly in encouraging women to attend the clinics, and explaining the benefits of iodine and the importance of the supplementation programme. The local teachers are very supportive of the parallel study of school children and their school achievement and cognitive function and could be wonderful advocates for the project.

4.2. Baseline Thyroid Function in Non-pregnant Women

The pattern of thyroid hormones was typical of those seen in other areas of moderate iodine deficiency, with elevated TSH (and TG), subnormal T_4 and normal or slightly raised T_3 levels, giving rise to elevated T_3/T_4 ratios. Tables, with details of results from other studies may be found in appendix 4.2.

4.2.1. Goitre

The results reflect a lower prevalence of visible goitre than casual observation, during the planning stages of the research, had suggested (Baksh, personal communication). In addition, the rate was much lower than that found in the same area, a few years previously, where goitre was reportedly almost universal (98% $\pm$2.7) in children aged 5-12 years (Zia, 1990). These comparisons suggest that VGR may have been under-estimated in the present study, probably due to the reasons described in section 2.7.2.6.

The prevalence of visible goitre in non-pregnant and pregnant women combined was, however, higher than found during the earlier GOP survey in this area (GOP, 1969), when a VGR of 21.5% ($\pm$5.6) was reported for women > 15 years, pregnant and lactating women, combined. The selection criteria for the earlier survey are not known and further comparison, for example, by pregnancy status, was not possible because lactating women in the earlier survey were grouped with pregnant women, whereas in the current study, they were grouped with non-pregnant women, as it was not possible, in the camp situation, to ascertain whether or not they were currently breast feeding.

The VGR in pregnant women was higher than in non-pregnant women, supporting the hypothesis that pregnancy represents a further stress on the iodine-deficient thyroid (Abdoul-Khair, Crooks, Turnbull *et al.*, 1964), although the difference was not statistically significant (Pearson X^2, $p=0.10$). These groups are not, strictly, comparable because the pregnant population was represented by a smaller age range than the non-pregnant population (see section 3.2.1.).

Other studies in iodine deficient areas, have reported widely varying VGR and TGR, e.g a recent study in the Sudan reported a TGR of 90% and VGR of 28% among women aged 15-44 (Elnagar, Eltom, Karlsson *et al.*, 1995), whereas another study in Guinea reported a lower TGR of 78% with a higher VGR of 48% among women (Konde, Ingenbleek, Daffe *et al.*, 1994). As described in section 2.7.2.6., goitre grade is notoriously difficult to estimate and inter-observer variation is large. Comparison between different studies, therefore, may not be very meaningful.

4.2.2. TSH

TSH has been used since the mid 1980s as a first-line test for thyroid dysfunction (Wilkinson, Rae, Thomson *et al.*, 1993) and was used to screen all women for suspected hypothyroidism in the five villages where the second phase of follow-up was concentrated, as well as being measured in pregnant women, those who attended with infants and those who came for follow-up testing. There are, accordingly, more results available for baseline TSH levels than for any other thyroid function parameter, as shown in table 3.4.1., and more studies with comparable measurements of TSH (see table A.4.2.2.

The median TSH concentration (2.04 mIU/l) was higher than that given for the reference range of the kit (1.68 mIU/l) and the proportion of women classified as TSH-"hypothyroid" (more correctly, **"hyperthyrotropinemic"**) was much higher than the 2.5% expected in a population of normal subjects. This indicates that there is some degree of thyroid hyperstimulation occurring in this population. This increased TSH secretion is probably in response to low levels of circulating thyroid hormones, discussed below, and stimulates the iodine-deficient thyroid to increase secretion of the hormones.

4.2.2.1. TSH in Other Studies

Other studies in iodine-deficient areas have shown similar elevated levels of TSH, although there is wide variation in findings, at least partly due to a difference in methods for measuring TSH and in cut-offs chosen for "abnormality" (Chopra, Hershman, and Hornabrook, 1975; Maberly, Corcoran and Eastman, 1982; Lazarus, Parkes, John *et al.*, 1992; Tonglet, Bourdoux, Minga *et al.*, 1992; Konde, Ingenbleek, Daffe *et al.*, 1994; Elnagar, Eltom, Karlsson *et al.*, 1995). Median TSH concentrations range from 1.3 mIU/l (Lazarus, Parkes, John *et al.*, 1992) to 18 mIU/l (Chopra, Hershman, and Hornabrook, 1975) and the proportion of subjects with "raised" TSH range from 9.5% (>4.13 mIU/l) (Lazarus, Parkes, John *et al.*, 1992) to 43.7% (>4.0 mIU/l) (Elnagar, Eltom, Karlsson *et al.*,1995).

4.2.3. TG

It has been suggested that thyroglobulin is a more sensitive indicator of iodine deficiency than thyrotropin, reflecting thyroidal compensation for iodine deficiency by increased production of the matrix in which the thyroid hormones are formed, to increase iodide trapping (Mißler, Gutekunst and Wood, 1994). TG concentrations may thus be elevated before the concentrations of the hormones themselves are low enough to stimulate pituitary production of TSH via loss of feedback inhibition.

All samples had TG levels above the "normal range", suggesting excessive stimulation of the thyroid gland, in an attempt to increase the amount of thyroid hormones released.

4.2.3.1. TG in Other Studies

Few studies have reported measurement of TG, and interest in TG as a proxy indicator of thyroid stimulation is fairly recent. However, these results are similar to those found in a study to develop and evaluate dry blood spot assays for TSH and TG measurement in children in an iodine deficient area of Zimbabwe (Mißler, Gutekunst and Wood, 1994). In that study, 9.2% ($\pm$3.3) of children had raised TSH (defined as a TSH concentration $>$ 4.5 mIU/l) and 59.9% ($\pm$6.0) had raised TG (defined as TG concentration $>$ 20 μg/l). Our study seems to indicate an even higher degree of thyroid hyperstimulation, possibly due to more severe iodine deficiency, although the populations are not, strictly, comparable.

4.2.3.2. TG and TSH

TSH does not appear to be the major determinant of TG production in this population, as the correlation between these two was weak. Small changes in TG production caused by TSH stimulation may be "hidden" by the larger effect of the iodine deficiency itself, or other components of thyroid status are more important in influencing TG production.

Wachter *et al.* report that TSH and TG were significantly correlated only in schoolchildren, no relationship being apparent in adult subjects (Wachter, Pickardt, Gutekunst *et al.*, 1987).

4.2.4. FT_4

Elevated TSH and/or TG levels indicate some increase in activity in the thyroid gland, as it is stimulated in an attempt to increase production of thyroid hormones, but evaluation of the extent of hormone deficiency can only occur by direct measurement of the hormones concerned. Since the hormones are metabolically active in their free, rather than bound, forms and T_4 is present in much larger quantities than T_3, FT_4 was the next test of choice for evaluating thyroid function.

The median FT_4 concentration (8.17 pmol/l) was much lower than that given with the kit (13.1 pmol/l) and the proportion of women classified as FT_4-"hypothyroid" (more correctly, **"hypothyroxinemic"**) was high (62.0% ±8.2), supporting the earlier statement that TSH levels were raised in response to low T_4 levels.

4.2.4.1. FT_4 in Other Studies

The routine measurement of free thyroxine is a fairly recent development and earlier studies have generally included measurement of total thyroxine or some estimate of free thyroxine, based on measurements of total thyroxine, thyroxine-binding globulin, T_3 uptake etc. (see section 1.10.2.). There are thus, fewer studies available for comparison of FT_4 than for TSH but those which are available show the same general pattern of lowered FT_4 or FTI, compared with a non-iodine deficient population. Even more so than for TSH, the wide variety of methods and reference ranges used make precise comparisons difficult but mean FT_4 concentrations were approximately 20-50% lower than the corresponding reference range means (Chopra, Hershman, and Hornabrook, 1975; Lazarus, Parkes, John *et al.*, 1992; Konde, Ingenbleek, Daffe *et al.*, 1994).

4.2.4.2. FT_4 and TSH

The negative correlation between TSH and FT_4 (natural log-transformed data) is similar to that found in other studies, although the relationship is weaker. This may be because correlation between these parameters is generally poor in the "normal range", where the majority of TSH concentrations lie (J.Butler, personal communication), but it may be due to methodological problems with measurement of FT_4, discussed in section 4.2.5.4.

In Senegal, the correlation between ln(TSH) and FT_4 had a coefficient of r=-0.477 and p-value p=0.041 and of the 48 subjects with raised TSH, 43 had low FT_4, suggesting that FT_4 was mainly responsible for feedback regulation of pituitary response in these subjects (Lazarus, Parkes, John *et al.*, 1992). In Guinea and PNG, TSH and FT_4 were also reportedly negatively correlated (Konde, Ingenbleek, Daffe *et al.*, 1994; Chopra, Hershman, and Hornabrook, 1975).

The proportion of subjects in the various thyroid categories differs considerably between these two parameters, questioning the usefulness of using only one parameter to describe or monitor iodine deficiency, although this may be partially explained by the methodological problems discussed below.

The observation that raised TSH concentrations tended to be accompanied by low FT_4 concentrations but reduced FT_4 concentrations were not necessarily accompanied by high TSH concentrations suggests that there were two groups of subjects:

The first group consisted of subjects in a steady state of uncompensated thyroid hormone production, in whom decreased circulating FT_4 did not cause increased pituitary TSH secretion, through the normal negative feedback mechanism. The second group consisted of subjects who had not quite achieved a compensated state in whom decreased circulating FT_4 led to increased TSH secretion which was, nevertheless, insufficient to normalise FT_4 levels.

4.2.4.3. FT_4 and TG

The results show that there is an inverse relationship between thyroglobulin production and serum FT_4 concentration, overlaid on a generally high TG production for the whole population. This suggests that where circulating FT_4 levels were particularly low, thyroglobulin production was further stimulated.

4.2.5. TT_4

Measurement of total thyroxine allows further examination of the thyroxine-producing activity of the thyroid gland in iodine deficiency, as differences in thyroxine production may not be accurately reflected in FT_4 concentrations if there are also abnormalities in binding proteins in iodine deficiency. In addition, many older studies have measured TT_4 and not FT_4 so measurement of TT_4 allowed comparisons with previous work.

4.2.5.1. TT_4 in Other Studies

Comparison of the findings with other studies, again reveals wide variation in reference ranges used and in specific findings, although the trend is towards low mean TT_4 values and large proportions of subjects with hypothyroxinemia (Chopra, Hershman, and Hornabrook, 1975; Maberly, Corcoran and Eastman, 1982; Tonglet, Bourdoux, Minga *et al.*, 1992; Konde, Ingenbleek, Daffe *et al.*, 1994; Elnagar, Eltom, Karlsson *et al.*, 1995). The proportion of women with "low TT_4" (<50 nmol/l) ranged from 16% in Zaire (Tonglet, Bourdoux, Minga *et al.*, 1992) to 68% in Sudan (Elnagar, Eltom, Karlsson *et al.*, 1995).

4.2.5.2. TT_4 and FT_4

A much higher proportion of women were classified as "hypothyroid" by FT_4 concentration compared with those so-classified by TT_4 concentration (62.0% $\pm$8.2 and 38.1% $\pm$8.3, respectively). In addition, the correlation between these two parameters, when $Ln(FT_4)$ was plotted against TT_4, was not as high as expected for what should have been two estimates of essentially similar variables. In the absence of binding protein abnormalities, a correlation coefficient of > 0.8 would be expected for determination of TT_4 and FT_4 in a "normal population" but in this case the correlation coefficient in only 0.63 ($p<0.001$).

These differences suggest either a difference in specificity of detection between the two estimates, or that a higher proportion of thyroxine than might be expected is in bound, rather than free, form. An unexpectedly high proportion of bound thyroxine should be reflected in elevated binding protein concentrations, examined below.

The negative correlation between TT_4 and Ln(TSH) ($r=-0.48$, $p<0.001$) is similar to that between $\ln(FT_4)$ and ln(TSH) and supports the conclusions drawn in section 4.2.4.2. It is also similar to the finding in PNG (Chopra, Hershman, and Hornabrook, 1975), of an inverse relationship between Ln(TSH) and TT_4 where $r=-0.52$ and $p<0.001$). In the PNG study, TSH was markedly higher and TT_4, markedly lower than in this study in Pakistan, hence the closer correlation between parameters, since fewer samples fell within the "normal range" where correlation between these parameters is generally poor (see section 4.2.4.2.).

The weak association found between TT_4 and TG is similar to that found between FT_4 and TG and support the suggestion that low circulating thyroxine is associated with increased TG production, although this effect is small when compared to the overall increase in TG concentration in this population.

4.2.5.3. TT_4 and TSH

There was closer agreement in assigning thyroid categories than when TSH and FT_4 were used but there was still considerable discrepancy between thyroid status assigned by different parameters, again indicating that one measurement of thyroid status does not adequately describe the situation.

4.2.5.4. Problems With the Measurement of FT_4

The discrepancy in thyroid category classification, according to these two parameters, was larger than expected and may be explained by then-unknown problems with the method of estimating FT_4. The "Serozyme" FT_4 kit uses a FT_4 analogue, which competes with FT_4 in the sample serum, for a limited (and quantified) amount of FT_4 binding sites. Quantification of the amount of bound analogue then allows calculation of the amount of bound FT_4 and hence the concentration in of FT_4 in the serum. This is a classic "competitive binding assay" which relies on the assumption that there is no interaction between the analogue and the T_4 binding proteins.

Since the use of this assay in the study, this assumption has been shown to be false () and there appears to be considerable interference with the assay when albumin levels are particularly low. This may be the case where there is chronic undernutrition (which did not appear to be the case with these subjects, but which cannot be ruled out), or where there is underlying chronic illness (both AGP and CRP were measured and found to be normal in these subject, but again, this situation cannot be eliminated).

In order to further investigate the possibility that FT_4 was under-estimated in this population, there are plans to measure serum albumin and to re-estimate FT_4 using a new "second generation" RIA FT_4 assay. This possible under-estimation of FT_4 may also explain the difference in thyroid status classifications by total and free T_4.

4.2.6. TBG

Most circulating T_4 (and T_3) is bound to TBG, with smaller amounts bound to albumin and thyroxine-binding pre-albumin (TBPA) (see section 1.10.) so measurement of TBG was chosen to give the clearest indication of the binding protein status in this population. In view of the discrepancies outlined above, between findings based on measurement of FT_4 or of TT_4, measurement of TBG was undertaken to elucidate these discrepancies. Unfortunately, few other studies have measured TBG in iodine-deficient populations, although TBG is often raised in hypothyroidism (Teitz, 1995).

Although it is not appropriate to assign samples to thyroid categories, based on TBG alone (see section 2.7.2.4.5.) it may be noted that TBG concentrations, in this study in Pakistan, were much higher than those found in the reference "normal population"

4.2.6.1. TBG and TT_4

There was no significant correlation between TBG and TT_4, probably due to the massive amount of TBG present in most samples. This has the effect of masking the normal TBG-TT_4 relationship seen in non-iodine deficient individuals.

4.2.7. FT_3

Many women with low serum T_4 concentrations did not have significantly raised TSH, suggesting that a steady-state had been reached in which the low levels of circulating T_4 did not lead to increased TSH production and further thyroid stimulation to increase T_4 production. The other thyroid hormone, triiodothyronine, was therefore also measured.

Many women in this population had elevated FT_3 levels. The low proportion of subjects classified as T_3-"hypothyroid" (2.2% $\pm$2.5) and the high proportion classified as T_3-"hyperthyroid" (11.1% $\pm$5.3), given the assignments made by other thyroid parameters, indicate the inappropriateness of these terms, in iodine deficiency, based on one parameter. More correctly, we should describe those subjects with low FT_3 as **"hypotriiodothyroninemic"** and those with high FT_3 as **"hypertriiodothyroninemic"**.

4.2.7.1. FT_3 in Other Studies

These results are supported by earlier studies, in which mean FT_3 levels were supranormal and in which many subjects were found to have FT_3 levels towards or beyond the upper limit of "normal" (Chopra, Hershman, and Hornabrook, 1975; Lazarus, Parkes, John *et al.*, 1992; Konde, Ingenbleek, Daffe *et al.*, 1994). The phenomenon of low normal or subnormal T_4, coupled with high normal or supranormal T_3 is referred to as **"T_3-euthyroidism"** and is often seen in areas of iodine deficiency (Larsen, 1992).

This state represents an attempt by the thyroid to maintain euthyroidism while unable to manufacture sufficient thyroxine for metabolic requirements. This state of T_3-euthyroidism is found even in some subjects with no palpable goitre, indicating that a compensatory mechanism, in response thyroxine insufficiency, is in operation even before any clinical signs of disease are present (Konde, Ingenbleek, Daffe *et al.*, 1994).

The weak nature of the few associations found, indicates that the FT_3 concentration was maintained at fairly high, "normal" levels whatever the thyroid status defined by other parameters. This is similar to the findings in the studies above.

The absence of an inverse relationship between FT_3 and TSH, indicates that FT_3 does not play a major role in the feedback regulation of pituitary response. This is in contrast to the strong negative correlation between FT_4 and TSH discussed above. However, it is likely that the high levels of TSH are directly responsible for stimulating the hypersecretion of T_3 in order to maintain euthyroidism (Larsen, 1992).

In the PNG study (Chopra, Hershman, and Hornabrook, 1975), serum FT_3 (but not TT_3) correlated positively with TSH ($r=0.41$, $p<0.01$), indicating that TSH may, indeed, be responsible for the high levels of FT_3 present, although the scatter was very high.

4.2.8. TT_3

For completeness, samples were analysed for TT_3, thus allowing comparison with older studies, conducted before the routine use of FT_3, and the calculation of TT_3/TT_4 ratios for comparison purposes.

The median value for TT_3, 2.0 nmol/l was the same as that given with the assay kit, for a "normal healthy" population, indicating that TT_3 levels are not elevated to the same degree as FT_3 levels. This is confirmed by the small proportion of subjects with either subnormal or supranormal levels of TT_3 (4.6% $\pm$3.6 and 3.3% $\pm$3.3, respectively).

4.2.8.1. TT_3 in Other Studies

In other studies where TT_3 has been measured, this has not been the case and TT_3 levels have generally been elevated, compared with some control population (Chopra, Hershman, and Hornabrook, 1975; Maberly, Corcoran and Eastman, 1982; Tonglet, Bourdoux, Minga *et al.*, 1992; Konde, Ingenbleek, Daffe *et al.*, 1994; Elnagar, Eltom, Karlsson *et al.*, 1995). In particular, the study in Sudan (Ingenbleek, Daffe *et al.*, 1994) found that 54.7% of subjects had an elevated TT_3 concentration. As with other parameters, it is important to note that reference ranges differ greatly between studies.

That TT_3 was not strongly associated with other parameters confirms that T_3 levels are maintained at normal or high normal levels, despite falling T_4 or rising TSH levels.

4.2.9. T_3/T_4 Ratios

Very few samples contained sub-normal concentrations of T_3, whether the free or bound hormone was measured, demonstrating the ability of the thyroid gland to switch to T_3 production, rather than T_4, under conditions of limited iodine supply to the gland. The enhanced production of T_3 compensating for the reduction in T_4 may be seen in the higher than normal T_3/T_4 ratios.

Preferential secretion of T_3, rather than T_4, under conditions of iodine deficiency represents an increase in efficiency of thyroid hormone production on two counts:

1. Firstly, T_3 contains only 3 atoms of iodine, rather than 4, so is "less expensive" in terms of scarce iodine resources.

2. Secondly, T_3 is 4-5 times more metabolically active than T_4, which also acts as a pro-hormone and undergoes de-iodination in the peripheral tissues to form about 80% of the circulating T_3, among other metabolites (Larsen, 1992). Production of T_3, rather than T_4, reduces the need for this peripheral conversion, thus reducing loss of iodine during recycling processes.

T_4 is, however, the metabolically-active thyroid hormone in the brain, so there is some concern that even if "normal" T_3 concentrations are maintained in iodine deficiency, low T_4 concentrations may compromise brain function, particularly during developmental stages *in utero* and during childhood (see section 1.5.).

4.2.9.1. T_3/T_4 Ratio in Other Studies

Several other studies, in which both T_3 and T_4 were measured have demonstrated this increase in T_3/T_4 ratio, as the thyroid, under conditions of iodine deficiency, increases its efficiency at maintaining peripheral levels of T_3 (Chopra, Hershman, and Hornabrook, 1975; Maberly, Corcoran and Eastman, 1982; Lazarus, Parkes, John *et al.*, 1992; Tonglet, Bourdoux, Minga *et al.*, 1992; Elnagar, Eltom, Karlsson *et al.*, 1995).

4.3. Baseline Thyroid Function in Pregnant Women

The pattern of thyroid hormones seen in pregnant women in this iodine-deficient population is one of an exaggerated rise in TBG, with T_4 increases lower than expected in a normal pregnancy, resulting in a decreasing TT_4/TBG ratio and FT_4 levels, throughout pregnancy. In contrast, T_3 rose as in a normal pregnancy, as the iodine-deficient thyroid switched production from T_4 to T_3. TSH levels remained fairly high and constant, throughout pregnancy and TG levels were even higher than the elevated levels found in non-pregnant women and continued to rise throughout pregnancy. There is particular concern that low T_4 during pregnancy may be detrimental to foetal growth and development and this situation is therefore worrying. Tables, with details of results from other studies may be found in appendix 4.3.

4.3.1. TBG

During a normal pregnancy, the most striking alteration in thyroid function is the increase in TBG concentration, mediated by high concentrations of oestrogen, particularly in early pregnancy. The non-pregnant women in this study had highly elevated TBG concentrations and the increase in TBG during pregnancy was particularly marked in this population, with TBG values far exceeding those usually found in non-iodine deficient women.

4.3.1.1. TBG in Other studies

Few studies of iodine-deficient women in pregnancy have reported TBG concentrations but those which do, note that TBG increases to a greater extent than TT_4 so that TBG saturation is decreased (see section 4.3.3.) (Glinoer, de Nayer, Bourdoux *et al.*, 1990; Berghout, Endert, Ross *et al.*, 1994). These studies both took place in areas of only marginal deficiency (Belgium and Holland, respectively) so that TBG levels, though raised, were lower than in the Pakistani study.

4.3.2. TT_4

The proportional rise in TT_4, from first to second trimester, was not as great as the increase in TBG, suggesting that in this population, thyroxine synthesis is limited and unable to keep pace with increased TBG secretion, although the difference was not statistically significant (17.6% ±15.5 and 39.6% ±20.0, respectively). This was also reflected in the low mean concentrations of TT_4, compared with "normal pregnancy".

4.3.2.1. TT_4 in Other Studies

This situation of relatively low total thyroxine (i.e. inadequate increases in thyroxine concentration, despite rising levels of TBG) has been observed elsewhere (Pretell and Stanbury, 1974; Medeiros-Neto, Walfish, Almeida *et al.*, 1978; Thilly, Delange, Lagasse *et al.*, 1978; Silva and Silva, 1981; Glinoer, de Nayer, Bourdoux *et al.*, 1990)

Pretell and Stanbury (1974) report a high proportion of pregnant women with low TT_4 in a goitrous area of Peru. Although pregnant women had TT_4 levels higher than non-pregnant women in the same area, 27/45 pregnant women had TT_4 below the "normal range", defined by pregnant women in a non-iodine deficient area in the USA, and 44/45 women had a TT_4 concentration below the mean value of the "normal range"

Pharoah *et al.* (Pharoah, Ellis, Ekins *et al.*, 1976), report that 25/147 (17.0% ±6.1) pregnant women, in the remote highlands of PNG, had "very low" TT_4, defined as < 32 nmol/l, where the "normal range" was 58-148 nmol/l. They comment that there is a high mortality among the offspring of the "very low" TT_4 mothers, compared with offspring of women who had TT_4 over 32 nmol/l, although the numbers in the study were too small to yield a statistically significant result.

These studies all support the suggestion that both the initial values and the rises in TT_4, during pregnancy, are lower in iodine-deficient populations than in those in whom there is no iodine deficiency. This is important because low levels of thyroxine have been shown to be associated with increased levels of miscarriage, stillbirth and congenital deformity (McMichael, Potter and Hetzel, 1980).

4.3.3. TT_4/TBG

Given that TBG concentrations were altered in pregnancy and that TBG concentrations greatly influence the concentration of TT_4 in non-iodine-deficient pregnant women (see sections 1.16.3 and 1.10.), the relationship between TT_4 concentration and stage of pregnancy was investigated in our iodine-deficient population.

The TT_4/TBG ratio is a measure of TBG saturation, corresponding to the fractional occupancy of TBG binding sites by T_4. It is expressed as a mass ratio, calculated as:

$$TT_4/TBG \text{ ratio} = TT_4(\mu g/dl)/TBG \text{ (mg/dl)}$$

TT_4 concentration was converted from nmol/l to μg/dl by multiplying by 777/10,000
TBG concentration was converted from μg/ml to mg/dl by dividing by 10

(It can be converted to the mole ratio by multiplying by 0.073 and expressed as a percentage by multiplying by 100, where molecular weight of TT_4 is taken as 777 and TBG as 57,000)

In the study population, the TT_4/TBG ratio fell during pregnancy, as the iodine-deficient thyroid failed to secrete sufficient thyroxine to keep pace with the increase in TBG. Glinoer *et al.* (Glinoer, de Nayer, Bourdoux *et al.*, 1990), observed a similar decrease in TBG saturation, with TT_4/TBG ratios decreasing throughout pregnancy from 5.4 in the first trimester to 3.8 in the third, where the "normal range", quoted for non-pregnant women was 3.8-8.2.

4.3.4. FT_4

FT_4 concentrations are generally slightly elevated in early pregnancy and gradually fall to low-normal values in the latter half of a normal pregnancy, as described in section 1.17.4. The disturbances in TBG and TT_4 concentrations, described above, should also be reflected in FT_4 concentrations in these iodine-deficient pregnant women. Decreasing TBG saturation is expected to reduce FT_4.

As described above, TT_4 concentrations rose rapidly, during early pregnancy, in this iodine-deficient population, but failed to keep pace with increases in TBG, resulting in decreases in the TT_4/TBG ratio and in FT_4 concentrations. These studies also indicate that the fall in FT_4 concentration, during pregnancy, is enhanced by iodine deficiency, in addition to beginning at a lower level that in iodine-sufficient subjects.

4.3.4.1. FT_4 in Other Studies

Relatively low FT_4 concentrations have been reported in a number of other studies of iodine-deficient pregnant women. In most of these studies, FT_4 was found to be similar to or slightly higher than in non-pregnant subjects in the first trimester but lower in the second and third trimesters (Pretell and Stanbury, 1974; Silva and Silva, 1981; Glinoer, de Nayer, Bourdoux *et al.*, 1990; Pedersen, Laurberg, Iversen *et al.*, 1993; Chaouki and Benmiloud, 1994; Berghout, Endert, Ross *et al.*, 1994).

Pharoah *et al.* (Pharoah, Ellis, Ekins *et al.*, 1976), report that 27/97 (27.8% $\pm$9.0) pregnant women in PNG had "very low" FT_4, defined as $<$ 1.3 pmol/l, where the "normal range" was 2.6-14.8 pmol/l. As with TT_4, they found a higher mortality among the offspring of the "very low" FT_4 mothers, compared with offspring of women who had FT_4 over 1.3 pmol/l (9/27 children died before the age of 4 who had "very low TT_4" mothers, compared with 8/70 who did not) , and in the case of the free hormone, the difference was significant.

4.3.5. TT_3

In a normal, healthy pregnancy, the huge increase in circulating TBG is accompanied by increases in both TT_4 and TT_3, with TT_4/TBG TT_3/TBG ratios maintained. In non-pregnant individuals in this community, TT_4 concentrations were low, but TT_3 concentrations were maintained at normal levels, in order to make more efficient use of the iodine available, as discussed in section 4.2.9.

Examination of the TT_3 and FT_3 levels in pregnant women revealed that a similar pattern of enhanced secretion of T_3 occurred in pregnancy, indicating that TT_3 levels were maintained within appropriate ranges throughout pregnancy, in contrast to the fall in levels of TT_4, towards the second half of pregnancy.

4.3.5.1. TT_3 in Other Studies

Other workers have observed similar patterns of TT_3 concentrations in iodine-deficient populations, with the majority reporting an increase in TT_3 during pregnancy (Medeiros-Neto, Walfish, Almeida *et al.*, 1978; Silva and Silva, 1981; Glinoer, de Nayer, Bourdoux *et al.*, 1990; Berghout, Endert, Ross *et al.*, 1994). Only in extreme iodine deficiency (in Zaire) was TT_3 seen to fall (Thilly, Delange, Lagasse *et al.*, 1978). Pharoah *et al.* (Pharoah, Ellis, Ekins *et al.*, 1976) found very few (10/113) (8.8% $\pm$5.3) pregnant women with TT_3 concentrations below the normal range of 85-176 ng/dl (1.31-2.70 nmol/l) and these women appeared to experience high pregnancy wastage (although numbers were too small for statistical analysis).

4.3.6. FT_3

The pattern of mean FT_3 during pregnancy was the reverse of the pattern seen in FT_4, where mean concentrations were lower in the second trimester, compared with the first and then rose slightly in the third. In the case of FT_3, mean concentrations rose in the second trimester and fell a little in the third, further supporting the suggestion that FT_3 levels were maintained in iodine deficiency, despite relative decreases in FT_4 in later pregnancy.

4.3.6.1. FT_3 in Other Studies

These results agree with those of other workers, where FT_3 was raised in pregnancy, compared to non-pregnant individuals, (Silva and Silva, 1981) although work in areas of milder iodine deficiency has shown a small decrease in FT_3 throughout pregnancy (Glinoer, de Nayer, Bourdoux *et al.*, 1990; Berghout, Endert, Ross *et al.*, 1994). As with TT_3, Pharoah *et al.* (Pharoah, Ellis, Ekins *et al.*, 1976) found very few (6/98, 6.1% $\pm$4.8) women with FT_3 concentrations below the normal range of 6-12 pmol/l

4.3.7. T_3/T_4 ratios

In non-pregnant women, both free and total T_3/T_4 ratios were elevated.

4.3.7.1. T_3/T_4 Ratios in Other Studies.

Other studies, have found little variation in the ratios during pregnancy, with the ratios being elevated, compared with non-pregnant controls (Medeiros-Neto, Walfish, Almeida *et al.*, 1978; Silva and Silva, 1981; Glinoer, de Nayer, Bourdoux *et al.*, 1990; Pedersen, Laurberg, Iversen *et al.*, 1993; Berghout, Endert, Ross *et al.*, 1994. Supplementation with iodine, however, reduced the ratios to levels similar to that in non-pregnant controls, as T_4 production increased with the increase in available iodine.

The exception was a study in Zaire, where iodine deficiency was very severe and TT_3 particularly low, so that the TT_3/TT_4 ratio was similar to that in subjects in an iodine-sufficient population (Thilly, Delange, Lagasse *et al.*, 1978).

4.3.8. TSH

In a normal, healthy pregnancy, TSH concentration is normal or low in the first trimester and rises (within the normal range for non-pregnant subjects) later in pregnancy (Glinoer, de Nayer, Bourdoux *et al.*, 1990). In non-pregnant women, in the study population, TSH levels were raised.

As with non-pregnant women, mean TSH was higher than in non-iodine deficient populations but there was little fluctuation in TSH levels throughout pregnancy. It is possible that the already raised levels of TSH in this population, even before pregnancy, mean that changes in hCG concentrations, which tend to suppress TSH during the first trimester, are not so influential as in non-iodine deficient populations.

4.3.8.1. TSH in Other Studies

This situation has also been reported by other workers (Pretell and Stanbury, 1974; Medeiros-Neto, Walfish, Almeida *et al.*, 1978; Thilly, Delange, Lagasse *et al.*, 1978; Silva and Silva, 1981; Glinoer, de Nayer, Bourdoux *et al.*, 1990; Pedersen, Laurberg, Iversen *et al.*, 1993; Chaouki and Benmiloud, 1994; Berghout, Endert, Ross *et al.*, 1994).

Pharoah *et al.* (ref 8), report that 25/122 (20.5% $\pm$ 7.2) women had "elevated" TSH, defined as $>$ 10 mIU/l, where the "normal range" was $<$ 5 mIU/l. They noted that there was a significantly higher mortality among the offspring of the "elevated" TSH mothers, compared with offspring of women who had TSH under 5 mIU/l.

Pedersen *et al.* (ref 13), report a gradual increase in TSH concentration, over pregnancy, to 121% of the initial value, in unsupplemented women, which was not seen in supplemented women. This implies that the stimulatory effect of increasing levels of TSH is only required where there is insufficient iodine to meet the increased demand for thyroid hormones in pregnancy.

4.3.9. TG

It has already been noted that TG concentrations were greatly elevated in non-pregnant individuals in this area and that the visible goitre rate was slightly (though non-significantly) higher in pregnant women, compared with non-pregnant women, and in the third trimester compared with the first. Thyroglobulin concentrations give an indication of synthetic activity in the thyroid gland and are expected to increase under the conditions of oestrogen-stimulation of thyroxine production, seen during pregnancy.

This was, indeed the case, with higher mean TG levels seen in the pregnant women, compared with the non-pregnant, and the extra stress of pregnancy, on the already over-worked thyroid, causing a rise in TG throughout pregnancy (although this was not significant).

4.3.9.1. TG In Other Studies

Fewer studies have investigated TG during pregnancy, in iodine-deficient populations but those which have, similarly found that TG levels were higher than in non-pregnant women and rose during pregnancy, particularly towards the end (Glinoer, de Nayer, Bourdoux *et al.*, 1990; Pedersen, Laurberg, Iversen *et al.*, 1993).

In the Belgian study (Glinoer, de Nayer, Bourdoux *et al.*, 1990), two thirds of the women exhibited a significant increase in TG levels over the course of pregnancy and there was a wide variation in TG values between individuals. In Denmark, (Pedersen, Laurberg, Iversen *et al.*, 1993) there was a dramatic reduction in TG levels, to 41% of the initial value, one year after iodine supplementation during pregnancy. This is in contrast to the significant increase in TG, over pregnancy, in non-supplemented women.

These results support the findings in non-pregnant women of raised TG levels in iodine deficiency. The further increases in TG during pregnancy, appear to be a consequence of the iodine deficiency, rather than pregnancy *per se*, because no such increase was seen in the iodine sufficient subjects in Holland and TG levels were actually stabilised or reduced following supplementation with iodine.

4.4. Follow-up Thyroid Function in Non-pregnant Women

The overall pattern is one of increased thyroxine production, as more iodine becomes available to the thyroid gland, which, in turn, acts through negative feedback inhibition to reduce levels of TSH. T_3 levels, which were generally maintained at normal levels, before supplementation, remained in the high normal range. Many subjects moved from being in a "hypothyroid" category, defined by one or more thyroid parameters, to being "euthyroid" and it would appear that supplementation with 400 mg of iodine, in IPSO, has increased the thyroxine production of these previously iodine-deficient women sufficiently to begin to normalise thyroid function.

Some subjects, however, became biochemically "hyperthyroid", with raised T_4 or T_3 and/or suppressed TSH levels. There does not appear to have been any clinical signs of thyrotoxicosis, although examination in the field clinic setting was limited. Any biochemical hyperthyroidism may well have been transient and a longer follow-up period would have allowed investigation of the possible "normalisation" of these levels over a period of several months to a year. It is hoped that longer term follow up of these subjects might be possible, particularly as iodised salt is introduced.

Tables, with details of results from other supplementation studies may be found in appendix 4.4.

4.4.1. TSH

The dramatic reduction in (geometric) mean TSH and the decrease in the proportion of TSH-"hypothyroid" subjects, following supplementation, suggests that hyperstimulation of the thyroid by elevated levels of TSH is no longer necessary to maintain thyroid function and that normalisation of TSH function has occurred in a number of subjects.

4.4.1.2. TSH in Other Studies

These results are supported by several other studies, in which median (or mean) TSH and/or proportion of subjects with "high" TSH were reduced, following supplementation with a variety of doses, at time intervals between 3 months and 1 year. In Zaire, TSH levels remained low (but normal) for up to 6 months with an oral dose of 47 mg I and up to 12 months with an oral dose of 112 mg I, (Tonglet, Bourdoux, Minga *et al.*, 1992) while observers in Malaysia noted that 2 years after supplementation with im 480 mg I, 10% of subjects had TSH elevated above the normal limit, compared to 24% prior to injection and 0% one year afterwards (Maberly, Corcoran and Eastman, 1982). In Senegal, there was no significant increase in TSH at one year, compared with the 6-month value (Lazarus, Parkes, John *et al.*, 1992) and in Sudan, TSH levels remained low for up to 12 months at the 3 doses used (Elnagar, Eltom, Karlsson *et al.*, 1995).

In this study in Pakistan, however, the proportion of subjects classified as TSH-"hyperthyroid" (more correctly, "hypothyrotropinemic") increased, following supplementation, indicating that there was a group of subjects in whom TSH production was inhibited to sub-normal levels. This may have been due to a sudden increase in the production of thyroxine, following the increased availability of iodine, which was a transitory effect, but it had not returned to normal 2-3 months after supplementation. It may also be that iodine deficiency was masking latent thyrotoxicosis in these subjects, which became frank, once the deficiency was corrected. Thyroid overactivity was then seen in these subjects.

It had, initially, been hoped that weekly follow-up in a few centres would allow detailed examination of any acute effects of iodine supplementation, but this was not possible.

Some studies have reported biochemical "hyperthyroidism" a few days after supplementation, notably with greatly increased thyroxine and concomitant reduction in TSH concentration (Mantovinovic, 1980; Bourdoux, Goset, Sindabarira *et al.*, 1993). Such "iodine-induced hyperthyroidism" (called "**Jodbasedow syndrome**") is generally reported as "transient", although there is a paucity of data to support this and further investigation of the acute effects of iodine supplementation are needed (Ermans, 1994).

Elnagar *et al.* report an increase in the proportion of subjects with suppressed TSH for up to 12 months, with the increase peaking at 3 months, to proportions similar to our findings. The reported TSH-"hyperthyroidism" is sometimes prolonged, particularly in the case of older subjects or those with nodular goitre (Elnagar, Eltom, Karlsson *et al.*, 1995) but it was not possible to obtain longer-term follow-up data on our sample.

4.4.2. TG

The small decrease in mean TG concentration did not indicate a normalising of TG secretion, following iodine supplementation, as the post-supplementation changes appeared to quite random (figure 3.6.3.2.) and mean value was still well above the "upper limit of normal" quoted with the kit. Hyperfunction of the thyroid, as measured by TG concentration was, thus, not much diminished following supplementation, and most women remained "abnormal" with respect to TG concentration.

This smaller lowering of TG secretion, compared to TSH secretion, may be because TG secretion takes longer to "reset", following iodine supplementation. TG is generally elevated in the presence of goitre (Teitz) and the continuing presence of goitre in many of the women (although not recorded) may also be a factor in the small reduction seen in TG concentrations.

There are no other studies where TG has been measured before and after supplementation, in non-pregnant women, although one study reported a decrease in TG, following iodine supplementation of mildly-deficient pregnant women in Denmark (Pedersen, Laurberg, Iversen *et al.*, 1993).

4.4.3. FT_4

The significant and substantial increase in mean FT_4 concentration is due to increased synthesis and subsequent secretion of thyroxine, in the thyroid, following an increase in the availability of iodine for incorporation into iodinated thyroglobulins. The mean FT_4 moves from being below the "normal range" to being well within it, indicating normalisation of thyroxine production by a large proportion of the population.

Following supplementation, the proportion of women classified as FT_4-"hypothyroid" was greatly reduced, representing normalisation of FT_4 function in many subjects, however, the proportion or women classified as FT_4-"hyperthyroid" increased, representing a group of subjects where FT_4 production occurred at supra-normal levels, due to a sudden increase in the availability of iodine. Such an increase in thyroxine production would be expected to be accompanied by a fall in TSH (as discussed below, this was, indeed the case in some subjects) but it may be that it takes the thyroid some time to "reset" and reach a steady state where thyroxine and thyrotropin values are both within the normal range.

4.4.3.1. FT_4 in Other Studies

In Sudan, There was a steady increase in FTI up to 3 months after supplementation and a small reduction at 12 months, the levels reached being similar for all 3 doses used. With the highest dose (800 mg I) an increase in FTI was only observed after 6 weeks and the authors speculate that this may be due to acute inhibition of thyroxine production via a Wolff-Chaikoff effect. In addition, 4 subjects were observed with transient or persistent biochemical thyrotoxicosis, one of whom had raised thyroxine at one year. In common with our subjects, none complained of symptoms of thyrotoxicosis at follow-up, nor were clinical signs observed (Elnagar, Eltom, Karlsson *et al.*, 1995). In Senegal, FT_4 concentrations were sustained at an increased level at one year and reported no thyrotoxicosis, possibly because, as in our study, no follow-up samples were taken soon after supplementation (Lazarus, Parkes, John *et al.*, 1992).

The increased availability of iodine for incorporation into thyroglobulins, coupled with

the increased iodide trapping of the iodine-deficient gland, caused an increase in thyroxine production, which in turn, reduced pituitary TSH secretion by negative feedback inhibition. In these subjects, it would appear that the thyroid machinery was producing excessive amounts of thyroxine and that a steady state, with normal levels of thyroxine and thyrotropin had not yet been reached, at three months. This is not an unusual state, immediately following supplementation (Mantovinovic, 1980; Bourdoux, Goset, Sindabarira *et al.*, 1993).

4.4.4. TT_4

The increase in mean TT_4 supports the increase in FT_4, described above. Fewer subjects were initially classified as having low TT_4 than FT_4 and this may explain the proportionately lower increase in mean TT_4 (although the difficulties in estimating FT_4., outlined in section 4.2.5.4. should also be remembered). The proportion of women classified as TT_4-"hypothyroid" was reduced, representing normalisation of TT_4 function in some subjects, but the proportion classified as TT_4-"hyperthyroid" increased, representing a group of subjects in whom thyroxine synthesis was hyperstimulated, as described in section 4.4.3.

4.4.4.1. TT_4 in Other Studies

In Malaysia, there was a sustained increase in TT_4 at 2 years, following a similar dosing regime to that used in Pakistan. No comment is made on possible iodine overload or transient thyrotoxicosis and in both cases first follow-up was at 6 months (Maberly, Corcoran and Eastman, 1982). In contrast to these, In Zaire, there was no change in TT_4 5 days after supplementation, a marked increase at 3 months and a small reduction from the highest attained level at one year. There was no evidence of inhibition of thyroid function by a Wolff-Chaikoff effect but one subject had transient biochemical hyperthyroidism (Tonglet, Bourdoux, Minga *et al.*, 1992).

4.4.5. TBG

The pre- and post-supplementation concentrations of TBG were not significantly different, indicating that TBG synthesis was not much influenced by the administration of iodine. The concentration of TBG was already so high that it may be that "resetting" the TBG-synthesising process requires a much longer period. As described earlier, TBG is so dependent on many other factors that its use as a tool for measuring thyroid function is severely limited there was wide variation in TBG both before and after supplementation and there are few samples which lay within the "normal range".

4.4.6. FT_3

As noted in section 4.2.7., FT_3 was high-normal or high in most subjects, before supplementation. It is therefore not surprising that FT_3 concentration was little affected by the iodine supplementation. The lack of influence of iodine supplementation on T_3 synthesis is supported by the reduction in the (already small) proportion of subjects classified as FT_3-"hypothyroid" being non-significant. The increase in the FT_3-"hyperthyroid" proportion, in contrast to FT_4, was also non-significant.

42.7.3. FT_3 in Other Studies

Few studies have reported FT_3 concentrations before and after supplementation in non-pregnant adults. The small (and non-significant) increase in FT_3 contrasts with the significant decrease in FT_3, from 6.4 to 4.7 pmol/l, reported in Senegal, which was sustained at one year (Lazarus, Parkes, John *et al.*, 1992).

4.2.7. TT_3

As with FT_3, the non-significant decrease in mean TT_3 indicates that T_3 synthesis was little affected by iodine supplementation, in contrast with T_4 synthesis.

42.8.3. TT_3 in Other Studies

In Sudan, TT_3 was basically unchanged throughout their one year follow-up period (Elnagar, Eltom, Karlsson *et al.*, 1995), and a similar situation is reported in Zaire (Tonglet, Bourdoux, Minga *et al.*, 1992). These results contrast with those observed in Malaysia, where a significant increase in TT_3 at one year and a return to baseline levels at 2 years were reported. There was also one thyrotoxic subject with TT_3 highest at 2 years (Maberly, Corcoran and Eastman, 1982).

4.4.8. T_3/T_4 Ratios

The significant decrease in the T_3/T_4 ratios, following supplementation, was mainly due to increases in T_4, as T_3 did not change significantly during the study.

The decrease in the T_3/T_4 ratio is an indication that more thyroxine is available for use in the body. As discussed in section 1.5., this is particularly important for brain function and foetal development, and it is expected that those women who became pregnant subsequent to the supplementation were better able to provide sufficient thyroxine for early foetal growth and development. It is interesting (and a little discouraging) to note that the "normalisation" of thyroid function was not completed in all women by three months after supplementation and that a longer time may be needed between supplementation and conception to ensure foetal protection against the adverse effects of iodine deficiency.

4.4.8.1.T_3/T_4 Ratios in Other Studies

A similar decrease in the ratio is reported by in Senegal, which was sustained at one year. The authors suggest that FT_3/FT_4 ratio may be a more optimal index than other hormone measurements for assessing thyroid function, although they do not give a **"normal range"**, with which to compare the ratio in iodine deficiency (Lazarus, Parkes, John *et al.*, 1992).

Tonglet *et al.*, in Zaire, do not themselves report the value of the decrease in TT_3/TT_4 ratio, observed in their study, so it is not possible to comment on its statistical significance, although it is of the same order of change as in our study. Likewise, Maberly *et al.*, in Malaysia, do not calculate TT_3/TT_4 ratio themselves but examination of the data reveals a (non-significant) small increase in the ratio at one year, following a non-significant fall in TT_4 and a significant rise in TT_3. At 2 years, this is reversed and a decrease in the ratio is seen, due to a now significant rise in TT_4 and a return of TT_3 to pre-supplementation levels.

CHAPTER 5 - CONCLUSIONS AND RECOMMENDATIONS

5.1. Disadvantaged Status of Women

In this community, women's access to education and health care was generally poor and reflected the disadvantaged status of women in this strictly conservative society.

Few women had ever attended school (37%) and only one quarter had completed primary education. Very few women had gone on to middle or secondary school and only two women, in our sample of 104, had completed secondary school. This exclusion from formal education was reflected in the women's occupations, with almost all women describing themselves as "housewives", and just two teachers in the sample (the same two women who had completed their secondary education.)

Access to health care was poor, in terms of both physical absence of, or distance from, facilities and lack of permission to visit such facilities where they did exist. In the study area, Basic Health Units (BHU), designed to cover the whole population in small areas, were not fully functional. Some were uncompleted or unstocked, others were under-equipped or under-staffed. Those staff who were posted to the BHUs were doing their best, often under difficult circumstances, and were supportive of the study but many women lived far from their "local" BHU and found walking there difficult.

For many women, the main access problem was in obtaining permission and a chaperon to attend a functioning BHU, where one was available and physically accessible to the women. The seclusion of women in this area made it very difficult for them to consult male health personnel but also meant that female health personnel were rare. These issues of access were also experienced in the mobile clinics run during the study, such that women found it difficult to attend the clinics.

The disadvantaged status of women, particularly in obtaining health care, was also reflected in the high rate of pregnancy losses (9.4%) and infant mortality rates (IMR) (91.1/1000), compared with an IMR of 6.4/1000 in UK, and the fact that none of the women who attended our camps had received Tetanus Toxoid during pregnancy, a recommendation approved by the government and practised in urban hospitals.

5.2. Best Supplementation Route

Given the poor attendance at the iodine camps, mass supplementation with IPSO does not appear to be the best means of reaching this community with iodine.

Although there was a preference for injections where illness was perceived, there was a general dislike of needles used on a large scale, in the camp situation. This meant that there was a poor uptake of tetanus toxoid immunisations, offered to pregnant women, although there was more enthusiasm for infant immunisations. The logistics of a mass injection programme and the associated safe disposal of needles in this remote area, as well as the community antipathy toward needles for preventive health care, make mass supplementation by injection of IPSO unattractive in this area.

Supplementation by oral IPSO also appears to be of limited value, in view of the small proportion of the community reached by the iodine camps. The remote and scattered nature of the dwellings in this area mean that it is unlikely that complete coverage of any particular target group could be achieved by having a centralised supplementation programme, even using several sites.

Long-term delivery of iodine may, therefore, be best achieved through iodised salt, which needs to be actively promoted and marketed in the area, as there is currently little interest in the product. In this area, it is the community leaders, local politicians and businessmen who are most able to influence the uptake of iodised salt and their co-operation needs to be actively encouraged, in addition to the introduction and enforcement of relevant legislation.

The price differential, between iodised and non-iodised salt, and the strikingly different appearance of the two salts, as currently sold, means that community acceptance of iodised salt can probably only be achieved once universal salt iodisation has been enforced. Techniques to iodise the more favoured large crystals of rock-salt may need to be introduced, to ensure customer acceptability of iodised salt.

5.3. Target Groups

Given the poor representation of women in community leadership structures (all the health committees in our study area were exclusively male) and the concentration of decision-making powers in the hands of male household heads, there is a need to educate the respected, older men about iodine deficiency and encourage them to support iodised salt purchase and consumption within the community.

Health education, including iodine deficiency awareness, is currently part of the primary school curriculum but, given the low school enrolment in the area, there is a need to widen the availability of the information and targeting community leaders and household heads, as well as local merchants who may be persuaded to stock iodised salt and promote its use, may be the best way to disseminate knowledge concerning ID and its prevention in this community.

5.4. Screening for / Monitoring ID

5.4.1. Goitre Size

Goitre surveys have traditionally been used as a technique for rapid screening and monitoring of ID in populations but there are difficulties associated with them, some of which were highlighted in this study. Acceptability was low in this culture, when inspection of women was undertaken by male health workers, and accuracy of grading suffered as a result.

In addition, there did not appear to be any strong associations between presence of a goitre and biochemical indices for individual women, although the high overall goitre rate was accompanied by biochemical disturbances in the community as a whole. Even careful goitre grading, therefore, may not yield very useful information concerning an individual's current iodine or thyroid status.

5.4.2. Urinary Iodine Determination

Urine collection for iodine determination appeared to be fairly acceptable in this community, although storage problems meant that samples were not actually analysed in this study. Urinary iodine determination is a non-invasive way to monitor iodine intake and availability, although it does not indicate the functional thyroid status.

5.4.3. Blood Biochemistry

Biochemical determinations, although more costly to the project and less acceptable to the women, as blood samples needed to be taken, provided more accurate information on the biochemical status of individual women and allowed investigation of the complex changes in thyroid function over the period of the follow-up.

Determination of the complete thyroid profile, before and after supplementation, required serum from venous samples and was a useful research tool but expensive and not appropriate to screening in a large programme. For community screening, both initially and in monitoring supplementation programmes, fewer determinations of thyroid status need to be made and community acceptability can be increased by the use of finger-prick blood-spot determinations.

The results of biochemical determinations on non-pregnant women (both before and after supplementation) indicate that, in a community, different estimates of the extent of thyroid dysfunction will be made, according to the parameter measured. e.g. 14.3% of the women had high TSH (> 4.5 mIU/l), indicating some degree of excessive thyroid stimulation, and could be termed TSH-"hypothyroid", but 62.0% of the women had low FT_4 (< 9.3 pmol/l), indicating subnormal production of thyroxine, and could be termed FT_4-"hypothyroid". When TT_4 measurement was performed, 32.5% of women were found to have low TT_4 (< 73 nmol/l).

As has been discussed, this discrepancy in the determinations of the proportion of "hypothyroid" subjects occurs because the thyroid compensates for lack of iodine by producing relatively more T_3 than T_4, compared to the situation when iodine is not a constraint. This means that T_4 levels may be low but T_3 concentrations are maintained at normal levels, so that TSH levels may not be abnormally high. Thus, determination of TSH alone, could lead to an under-estimate of the extent of thyroid disturbances in the community, whereas a determination of FT_4 alone, could lead to an overestimate of uncompensated thyroid dysfunction.

In populations where thyroxine production itself is not critical, provided that T_3 levels are maintained, e.g. non-pregnant women, adult men, it may be sufficient to screen for iodine deficiency and monitor changes, following supplementation, using TSH alone. For populations where thyroxine itself is thought to be important, e.g. mental development and school achievement in schoolchildren, foetal growth and development in pregnant women, FT_4 may be the more appropriate determination for monitoring iodine deficiency. Where FT_4 cannot be measured, TT_4 determination is recommended.

Where financially viable, a combination of **both** TSH **and** thyroxine determinations will probably give the best means of monitoring the iodine deficiency. As these determinations can both be done on blood-spots, collection of a few drops of blood from a finger-prick, onto a strip of filter-paper, will allow both determinations to be carried out with minimal distress to the subject and work for the field staff.

5.5. Risk of Iodine-Induced Thyrotoxicosis

It is important to note that no symptoms of thyrotoxicosis were reported, nor were any clinical signs observed, during the follow-up period. Measurement of thyroid hormones indicated a rise in the proportion of women with supressed TSH (from 3% to 22%) or with elevated T_4 (from 6% to 20% for the free hormone and from 0% to 6% for the total hormone), indicating an increase in biochemical "hyperthyroidism" but no clinical changes were observed. In contrast to the rumours circulating among some health professionals in Pakistan, we found no short-term adverse effects of iodine supplementation.

5.6. Timing of IPSO Supplementation in Pregnancy

It was not possible to obtain follow-up samples from women during pregnancy but some inferences may be drawn from the follow-up on non-pregnant women, 10-14 weeks after supplementation. If we assume that similar changes, due to the supplementation, occur in pregnant women, superimposed on the normal fluctuation of thyroid hormones, due to the stage of pregnancy, it is clear the "normalisation" of thyroid function will not occur in all women by 10-14 weeks after supplementation.

Not all subjects experienced "normalisation" of thyroid function in all of the parameters measured by 10-14 weeks. Some subjects experienced "normalisation" in most or all parameters but many did not; some becoming "normal" in a limited number of parameters and others seemingly being "over-corrected" in some parameters. It is possible that where subjects became biochemically "hyperthyroid" in one or more parameters, this was a transient phenomenon and may have been resolved within a few months, but the lack of longer-term follow-up means that this is speculation.

It is clear, however, that normalisation of thyroid function cannot be guaranteed within 3 months, so that even if supplementation is carried out at the beginning of pregnancy, normal maternal thyroid function may not be present during the critical period of early foetal development, before the foetal thyroid begins functioning, when maternal thyroid is the only source of thyroid hormones.

Thus, targeting iodine supplementation at pregnant women may not be beneficial to the foetus they are carrying, at the time of supplementation, but may be useful in building up iodine stores before the next pregnancy. In the study population, the next child was often conceived within a year of the birth of the previous one, so that supplementation in pregnancy could be beneficial for the next child, although explanation of this, to the mother, could be confusing.

In attempting to improve the iodine status of women, ready for pregnancy, it may be better to offer iodine supplementation at the time of formal engagement or marriage, although cultural sensitivities about this would need to be carefully investigated. Alternatively, iodine supplementation could be offered at the time of delivery, by the TBAs, with an explanation that this would help during the next pregnancy.

Ultimately, the best way to improve iodine status in pregnant women and the whole community is to ensure the long term consumption of iodised salt and this must remain a priority for national and international agencies, hoping to improve iodine status.

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ABBREVIATIONS

AJK	Azad Jammu and Kashmir
BMR	basal Metabolic Rate
CI	confidence interval
CQ	camp questionnaire
CNS	central nervous system
CSF	cerebro-spinal fluid
DIT	diiodotyrosine
Ferr	ferritin
FHQ	family history questionnaire
FSH	follicle-stimulating hormone
FT_3	free triiodothyronine
FT_4	free thyroxine
GOP	Government of Pakistan
GSD	geometric standard deviation
GSE	geometric standard error
Hb	haemoglobin
hCG	human chorionic gonadotropin
I^*	radioactively-labelled iodine
IA	immunoassay
ICCIDD	International Consultative Committee on Iodine Deficiency Disorders
IDD	iodine deficiency disorders
IM	intra-muscular
IMA	immunometric assay
IPSO	iodised poppy seed oil
KI	potassium iodide
LH	luteinizing hormone
MIT	monoiodotyrosine
NA	Northern Areas (Pakistan)
NIH	National Institute of Health
NWFP	North Western Frontier Province
or	oral

PBI	protein-bound iodine
PDD	Planning and Development Division
PEM	protein-energy malnutrition
PNG	Papua new Guinea
ppm	parts per million
REY	reproductive exposure years
RIA	radioimmunoassay
rT_3	reverse triiodothyronine
SD	standard deviation
SE	standard error
TBG	thyroxine-binding globulin
T_3	triiodothyronine
T_4	thyroxine
TBA	traditional birth attendant
TBC	thyroxine-binding capacity
TG	thyroglobulin
TGR	total goitre rate
THBI	thyroid hormone binding index
THBR	thyroid hormone binding ratio
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
TT_3	total triiodothyronine
TT_4	total thyroxine
UIE	urinary iodine excretion
UNICEF	United Nations Children's Fund
Vit A	vitamin A
VGR	visible goitre rate
WHO	World Health Organisation

APPENDIX 1 - INTRODUCTION

A.1.21. IDD in Pakistan - Early Surveys

VILLAGE	POPULATION	NO. WITH GOITRE	GOITRE RATE (%)
Basin	93	11	11.8
Umphris	385	77	20.0
Damyal	181	34	18.8
Majinpharri	718	143	20.0
Kyok	229	62	26.6
Sonyar	458	112	24.5
Kashrote	128	58	45.3
TOTAL	**2192**	**497**	**22.7**

Table A.1.21.1. Goitre prevalence in villages in the Chitral and Gilgit valleys.
Source: adapted from McCarrison, 1910.

TOWN	NO. EXAM.		TOTAL % GOITRE			% GOITRE	
	BOY	GIRL	BOY	GIRL	BOTH	VIS.+	NOD.#
Gujrat	380	91	11.6	10.0	11.3	0.2	0.1
Jhelum	358	418	29.9	28.9	29.4	0.8	0.1
Rawalpindi	440	252	30.7	52.4	38.6	3.3	0.3
PUNJAB TOTAL	1178	761	24.3	34.4	28.3	1.5	0.2
Abbottabad	295	75	40.0	36.0	39.2	1.1	0.3
Peshawar	208	164	14.9	31.7	22.3	1.1	0.3
Mardan *	220	156	0.0	0.6	0.3	0.0	0.0
Saidu Sharif	344	76	13.4	18.4	14.3	0.0	0.0
NWFP TOTAL *	847	315	23.0	29.5	24.8	0.7	0.2
Chitral	637	0	71.0	---	71.0	5.5	14.8
Balakot	543	97	77.2	72.2	76.4	11.6	8.0
NA TOTAL	1180	97	74.8	72.2	73.9	8.6	11.4

Table A.1.21.2. Goitre rates in selected townsin northern Pakistan.

Source: adapted from WHO, 1960.

+ visible # nodular

* results from Mardan are not included in the total, as the goitre rates are very different from all other towns and there may have been some difficulties in measurement technique here.

Some of the goitre rates or numbers examined have been recalculated due to discrepancies in the original table.

APPENDIX 2 - METHODOLOGY

A.2.1. Original Study Design

A.2.1.1. Aim

To compare the effects on foetal and infant growth and development of supplementation of iodine-deficient pregnant women with oral Iodised Poppy Seed Oil (IPSO) preconceptually, early and later in pregnancy.

This project was carried out in conjunction with research into the effect of iodine supplementation on cognitive function of schoolchildren and a projected timetable of events for these two projects is given in table 2.1.12.

A.2.1.2. Hypotheses to be Tested

1. Supplementation of iodine-deficient women with oral IPSO improves the thyroid status of the woman as measured by:

- urinary iodine
- TSH
- thyroxine.
- goitre grade

2. A single dose of oral IPSO provides enough iodine to protect against iodine deficiency for up to 24 months.

3. Oral supplementation of the preconceptual and pregnant women affects the thyroid status of the foetus. This was to be tested by comparison of maternal and neonatal thyroid status at or near delivery, using the above indicators.

4. Oral supplementation preconceptually and in early, rather than late, pregnancy is more advantageous to foetal growth and development. The effect on physical growth was to be measured by:

- birth weight
- length at birth

whilst the effect on brain growth was to be evaluated by :

- head circumference
- neurological development at, or near, birth.

5. Maternal supplementation, with oral IPSO, just prior to or during pregnancy continues to affect infant growth and development during the first year of life. The differential effects of preconceptual, early and late supplementation was to be compared by measuring:

- linear growth (weight and height increments)
- brain development (head circumference, neurological and cognitive functions)

A.2.1.3. General Design

Preconceptual and pregnant women were to be followed up from supplementation with oral IPSO, through to birth of their infants and for 12 months afterwards. They were to be retrospectively assigned to "pre", "early" or "late" groups, according to when they were given IPSO, relative to the birth of their infant. These three groups were to be compared in the parameters listed above and described more fully below.

A.2.1.4. Recruitment

"Iodine camps" were to be held in 4 villages, in Lehrar Union Council, in the first month of the study, to which all women between the ages of 15 and 40 were invited and at which attenders were to be given a standard dose of oral IPSO (2 capsules each, containing a total of 400mg iodine). This Union Council had a population of approximately 22,500, of whom 16% were estimated to be married women of childbearing age (Lehtrar, 1991). The potential target population was thus 3,600 women and community leaders estimated that almost all of these women would attend the iodine camps.

The number of women expected to attend, at each centre was about 1000. All women who gave birth within the following 12 months, and their infants, were to be recruited into the study, following culturally-appropriate, informed consent (see A.2.3.1.).

The mother and infant pairs were to be assigned to "pre" group if birth occurred more than 40 weeks after supplementation, to "early" group if birth occurred 32-40 weeks after supplementation (i.e. supplementation was during the first 8 weeks of pregnancy) and to "late" group if birth occurred 0-24 weeks after supplementation (i.e. supplementation was after 16 weeks of pregnancy.)

A.2.1.5. Exclusion Criteria

Women with gross or nodular goitre would not be offered IPSO because of the possibility of iodine-induced hyperthyroidism in such individuals. They were to be referred to a surgeon with expertise in thyroid surgery. It was not thought likely that many women would be in this group.

A.2.1.6. Sample Size

Sample size was calculated using the expected differences in outcome indicators to be measured, so that a difference could be detected between the two groups at a significance level of 5%, with a power of 80%. Calculations were carried out for the sample size needed to detect a difference in means e.g. birthweight and the difference in proportions of samples in a particular category e.g. proportion of low birth weight ($<$ 2500g) babies. These calculations, an example of which is shown below, indicated that 75 subject pairs were required in each group. To allow for an expected drop-out of 30-40%, 120 mothers were to be recruited into each group.

A.2.1.6.1. Example of Sample Size Calculation

Studies in the Gambia suggested that we might expect a difference of approximately 200 g in mean birthweights, from approximately 2800 g to 3000 g and a decrease of 15% in low birthweight babies from approximately 25% to 5%, between early and late supplemention groups, (Prentice, Cole, Foord *et al.*, 1987; Greenwood, Armstrong, Byass *et al.*, 1992).

The following formula was used to calculate sample size for comparison of two means:

$$n > \frac{(u+v)^2(\sigma_1^2+\sigma_2^2)}{(\mu_1-\mu_2)^2}$$

where:

u=one-sided percentage point of the normal distribution corresponding to 100%-power since power = 80%, 100%-power=20%, u=0.84

v=percentage of the normal distribution corresponding to a (two-sided) significance level of 5%, i.e. v=1.96

σ_1=estimated standard deviation of birthweights in late-supplemented group, σ_1=400g

σ_2=estimated standard deviation of birthweights in early-supplemented group, σ_2=400g

μ_1=estimated mean birthweight in late-supplemented group, μ_1=2800

μ_2=estimated mean birthweight in early-supplemented group, μ_2=3000

i.e. $n > \frac{(0.84+1.96)^2(400^2+400^2)}{(2800-3000)^2}$, $n > \frac{(7.84)(320000)}{(40000)}$

$\underline{n > 63}$

The following formula was used to calculate sample size for comparison of two proportions:

$$n > \frac{\{u\sqrt{[\pi_1(1-\pi_1)+\pi_2(1-\pi_2)]}+v\sqrt{[2\pi(1-\pi)]}\}^2}{(\pi_2-\pi_1)^2}$$

where:

u=0.84, as above

v=1.96, as above

π_1=proportion of low birthweight babies in late-supplemented group, π_1=0.2

π_2=proportion of low birthweight babies in early-supplemented group, π_2=0.05

$\pi=\frac{(\pi_1+\pi_2)}{2}$ =0.125

i.e. $$n > \frac{\{0.84\sqrt{[(0.2x0.8)+(0.05x0.95)]}+1.96\sqrt{[2x0.125x0.875]}\}^2}{(0.05-0.2)^2}$$

$$n > \frac{\{0.84\sqrt{[0.16+0.048]}+1.96\sqrt{[0.219]}\}^2}{(-0.15)^2}, \quad n > \frac{\{0.84x0.456+1.96x0.468\}^2}{0.023}$$

$$n > \frac{\{0.383+0.916\}^2}{0.023}, \quad n > \frac{\{1.299\}^2}{0.023}, \quad n > \frac{1.688}{0.023}$$

$$\underline{n > 75}$$

Similar calculations were performed for other outcome parameters, with smaller sample sizes found, given the estimated difference in means or proportions expected.

A.2.1.6.2. Attainment of Sample Size

The fertility rate in the study area was not accurately known but discussion with community leaders indicated that women experienced an average of 8 live births, i.e. an average of 1 birth every 3 years, between the ages of 15 and 40. This would give a rate of about 27 conceptions per month per 1000 women. Recruitment into the "early" group would thus be approximately 55 per 1000 women. In order to recruit 120 women into this group, 2200 women would need to be supplemented. This number represented approximately 60% of the target age-group female population but community leaders advised that the camps would be popular and well-attended.

This 2200 was expected to contain about 390 women, eligible for inclusion in the "late" group, so a random selection of approximately one third of these women could be made, to form the "late group". In addition, about 180 women would be eligible for inclusion in the "pre" group so two thirds of these could be randomly selected to form the "pre" group. Randomisation was to be through the use of random number tables.

Alternatively, if measurement and analysis time permitted, all of those eligible for inclusion in the "pre" and "late" groups were to be included and differences between sub-groups (e.g. 16-26 vs 27-40 weeks), could then be studied.

A.2.1.7. Follow-up

Following the iodine camps, weekly clinics were to be held, to which supplemented women who gave birth within 12 months of supplementation, were be invited. At these clinics, infant growth monitoring and immunisations were to be offered.

Each infant was to be seen within one week of birth, by a traditional birth attendant (TBA) who would encourage attendance at the clinic for BCG vaccination. The adjusted birthweight would be calculated from a graph of infant weights obtained on different days during the first week post-delivery.

Thereafter, the infant was to be seen at 3, 4, and 5 months for first, second and third triple vaccines, respectively, at 9 months for measles and at one year of age, when final

neurological and cognitive testing would be performed.

At each visit, weight, length head and chest circumference was to be measured. At the first and last visits, a venous blood sample was to be taken from the mother, a heel-prick blood from the infant, and a urine sample was to be obtained from the mother, for determination of urinary iodine.

The expected number of births in the study was 27 per month per 1000 women, giving a maximum number of infants requiring an immunisation of 5x27 (=135) per month per 1000 women. Thus, if 2200 women were to be supplemented, up to 300 infants could be expected at clinic each month, approximately 70 a week. Even if 3000 women attended the iodine camps, the maximum expected number of clinic attenders seen on one day of a 3-days-in-the-field clinic would be 30 and this was thought to be a manageable number, given the field staff described below.

A.2.1.8. Field Workers

The clinics were to be run by a community nurse, with assistance from a medical doctor and nutritionist. The iodine camps were expected to employ all three and possibly be assisted by other doctors and nurses from the department of Community Mental Health in Rawalpindi General Hospital. Neurological and cognitive development was to be assessed by two project clinical psychologists, assisted by a psychiatrist.

In addition, local "motivators" were to be employed to remind the women to when attend clinics with their infants, visiting them in their homes, a few days before the clinic. In this way, it was hoped that drop-out would be minimised.

A.2.1.9. Parameters to be Measured and Documented

A.2.1.9.1. Weight

At the time of immunisations and at one year of age, infant weight was to be measured at the clinics. Portable electronic scales (Soehnle Multina 8300.00, CMS Weighing, London), positioned on a firm, level surface and checked with standard weights, were be used to measure nude weight to the nearest 10g.

A.2.1.9.2. Length

This was be measured at the clinics, using a Harpenden Infantometer length board (CMS Weighing, London), to the nearest 1mm.

A.2.1.9.3. Head Circumference

The greatest occipito-frontal circumference was to be measured, to the nearest 1mm, using a narrow fibre-glass tape, at the clinics.

A.2.1.9.4. Urinary Iodine

Urine samples were to be obtained from the women before supplementation, soon after delivery (within one month, at the BCG clinic) and at the time of their infant's developmental assessment. Specimens were to be stored and analysed, using the perchloric acid method, at NIH.

A.2.1.9.5. Thyroid Hormones

Venous samples were to be taken from the women on the same occasions that urine specimens were collected, i.e. before supplementation, at delivery and one year later. All bloods were to be immediately analysed for haemoglobin, using a portable, battery-operated "Hemocue" analyser.

Women with severe anaemia (Hb$<$ 8g/l) were to be offered a short course of iron tablets, those with mild to moderate anaemia (8$\leq$ Hb $<$11 g/l) were to be given nutrition advice and all women would be offered a multi-vitamin preparation, as it was considered culturally inappropriate to give tablets to some women but not to others.

Blood samples were to be separated, using a battery-operated centrifuge, within 4 hours, and the serum stored in an ice box for transfer to a NIH for storage at -20°C. Samples were to be analysed for TSH and FT_4, using the Serono "Serozyme" kits.

A.2.1.9.6. Neurological Assessment

This was to be done soon after birth, by those running the clinic, and at one year of age, by the project clinical psychologists. The Dubovitz scoring method was to be used.

A.2.1.9.7. Cognitive Function

This was to be assessed at one year, using a locally-developed scale, by the clinical psychologists.

A.2.1.9.8. Goitre Grade

This was to be assessed according to the WHO classifications discussed in section 7.1., using both observation and palpation, before supplementation, at delivery and one year after delivery.

A.2.1.10. Data Collection

Each woman supplemented at the camps, was to have a short form, detailing name, address and government ID number (if any). Every woman who gave birth within 12 months of supplementation was then to complete a more detailed form, designed to allow systematic entry of all the above parameters, both for herself and for her infant, and using encoding to facilitate entry of data into a computer. Each mother-infant pair was to be given a project number at the time of entry into the study.

A.2.1.11. Data Analysis

Data was to be analysed using the EPI INFO programme (EPI-INFO, 1991) to determine whether the following were likely to be true at a 95% level of significance:

1.(a) The percentage of women classified as "severely iodine deficient" ($< 2\mu g$ I/dl Urine) is reduced, following supplementation with a single dose of oral IPSO.

(b) The mean urinary iodine is reduced, following supplementation with a single dose of oral IPSO.

2.(a) The percentage of women classified as "hypothyroid" (TSH > 4.5 IU/dl, TT_4 < 73 nmol/l or FT_4 < 9.3 pmol/l) is reduced, following supplementation with a single dose of oral IPSO.

(b) The mean TSH is reduced and the mean TT_4 and FT_4 are increased, following supplementation with a single dose of oral IPSO.

3. The goitre grade of a woman is reduced following supplementation with a single dose of oral IPSO.

4. Mean birth weight, head circumference and neurological development at birth are all greater in infants whose mothers were supplemented preconceptually and in "early" rather than "late" pregnancy.

5. Mean increments in weight, length and head circumference are all greater in infants whose mothers were supplemented preconceptually and in "early" rather than "late" pregnancy.

6. Mean neurological and cognitive developmental scores are both greater at one year in infants whose mothers were supplemented preconceptually and in "early" rather than "late" pregnancy.

A.2.1.12. Timetable for Study

DATE	DOCTOR	NUTRIT.	NURSE	PSYCH.	LAB
MAY 93	----------Training----------				
JUNE 93	Iodine Camps			Training	
JULY 93	Clinics	Weekly clinics	Weekly clinics		
AUG 93	Schools			Baseline testing of school children	
SEPT 93	Weekly clinics	NIH			
OCT 93					UrI/TF
NOV 93		Weekly clinics		Review	
DEC 93	Schools			Post - iodine testing - school children	
JAN 94					
FEB 94	Clinics	NIH			UrI/TF
MAR 94	Schools	Weekly clinics		End of year IQ testing - school children	
APR 94					
MAY 94	Weekly clinics	NIH			UrI/TF
JUNE 94		Clinics		Infant develop-ment testing at weekly clinics, of babies on or near first birthday	
JULY 94		UK			
AUG 94		NIH			UrI/TF
SEPT 94		Weekly clinics			
OCT 94					
NOV 94		NIH			UrI/TF
DEC 94		Weekly clinics			
JAN 95					
FEB 95		NIH			UrI/TF
MAR 95	Schools	Weekly Clinics			
APR 95					
MAY 95		NIH			UrI/TF
JUNE 95	Write-up, London				

Table A.2.1.12. Timetable of project activities

A.2.3. Iodine Camps

A.2.3.1. Consent Form - in Translation

I, ...

of .....

understand that my child, ...

will be examined regularly to measure his/her weight, length, head circumference, arm circumference and development. In addition, I understand that one blood sample will be taken using a heel prick.

I agree to the procedures which have been explained to me by

.....

I understand that if I wish to withdraw my child from the study at any time I am free to do so without affecting any chance of treatment of my child.

Signed .....

Date .....

Thumbprint

A.2.3.2. Camp Questionnaire

CAMP QUESTIONNAIRE ID NO _____

CAMP LOCATION _____ CODE _____ DATE _____/_____/_____

FULL NAME _____

DATE OF BIRTH ____/____/____

FULL ADDRESS _____ ADDRESS CODE_____

HUSBAND / FATHER'S NAME _____

MARITAL STATUS:

SINGLE=1 ENGAGED=2 MARRIED=3 WIDOWED=4

DIVORCED=5 NOT KNOWN=9

DATE OF LAST PERIOD ___/___/___

DO YOU THINK YOU ARE PREGNANT ? NO=0 YES=1 NOT KNOWN=9

HEALTH CHECK - COMMENTS _____

- TREATMENT _____

- REFERRAL _____

- TABS _____

GOITRE GRADE: 0=0 1A=1 1B=5 2=2 3=3 NOT KNOWN=9

- NODULAR NO=0 YES=1 NOT KNOWN=9

- REFERRAL NO=0 YES=1 NOT KNOWN=9

CLINICAL SIGNS - HYPOTHYROIDISM NO=0 YES=1 NOT KNOWN=9

- HYPERTHYROIDISM NO=0 YES=1 NOT KNOWN=9

IODINE CAPSULES TAKEN PREVIOUSLY? NO=0 YES=1 NOT KNOWN=9

IF YES, WHEN _____/_____/_____

TYPE OF SALT USED REGULARLY _____

URINE SAMPLE TAKEN NO=0 YES=1 NOT KNOWN=9

BLOOD SAMPLE? NONE=0 CAPILLARY=1 VENOUS=2 UNKNOWN=9

HAEMOGLOBIN RESULT _____g/l

FEEDBACK IRON TABS=1 DIETRY ADVICE=2 OK=3

NOT KNOWN=9

IODINE CAPS TAKEN TODAY NO=0 YES=1 NOT KNOWN=9

A.2.3.6. Summary of Camp and Clinic Data

Location	Camp code	Date	No. at camp	No. of pregs.	F-up time (wks)	No. at f-up clinic
Biaga	1 *K*	15/08/93	160	13	10	4
Kamkot Haider	12	16/08/93	84	10	10	3
Kahuti	16	19/08/93	95	15	N/A	0
Bagga	6	22/08/93	132	7	13	18
Ariari	9	23/08/93	112	9	11	14
Mouri	7	24/08/93	115	10	14	6
Surba	8	25/08/93	106	3	10	12
Behl Chakka	2 *K*	28/08/93	93	3	12	40
Durnuyian **	3 *K*	29/08/93	94(32)	11(4)	14	7
Burhad	11	01/09/93	91	11	12	2
Deergran	13	04/09/93	43	2	N/A	0
Golla	5 *K*	05/09/93	22	2	N/A	0
Naniah	4 *K*	07/09/93	115	5	N/A	0
Chaman	10	08/09/93	55	1	10	10
Karl	14	19/09/93	58	6	N/A	0
Phutha Parian	15	20/09/93	32	3	N/A	0
Total			**1432**	**115**		**116**

K Key village

** includes 2 camps held in same BHU - numbers in brackets are for 30/09/9

No. at camp=number of women supplemented at the camp

No. of pregs.=number of pregnant women identified at the camp

F-up time=interval between camp and first clinic at which samples were taken, in weeks

No. at f-up clinic=number of non-pregnant women who attended for follow-up clinics

Table A.2.3.6. Summary of recruitment camp and follow-up clinic contacts.

A.2.4. Follow-up Clinics I

A.2.4.1. Mother - General Information Questionnaire

MOTHER - GENERAL INFORMATION ID NO _____

CLINIC LOCATION _____ CODE _____ DATE _____/_____/_____

FULL NAME _____

DATE OF BIRTH _____/_____/_____

EDUCATION: NONE=0 PRIMARY=1 MIDDLE=2

SECONDARY=3 INTERMEDIATE=4 DEGREE=5 UNKNOWN=9

OCCUPATION _____ CODE _____

HUSBAND / FATHER'S NAME _____

HUSBAND / FATHER'S EDUCATION _____ CODE _____

HUSBAND / FATHER'S OCCUPATION _____ CODE_____

IS YOUR HUSBAND RELATED BY FAMILY TO YOU?

UNRELATED=1 1st COUSIN=2 2nd COUSIN=3

DISTANT RELATION=4 NOT KNOWN=9

DATE OF DELIVERY _____/_____/_____

PLACE OF DELIVERY: HOME=1 CLINIC=2 HOSPITAL=3

OTHER=4 (4=_____) NOT KNOWN=9

NUMBER OF CHILDREN EXCLUDING LATEST BABY:

(a) - STILL LIVING _____

(b) - BORN LIVE BUT DIED (i) UNDER 24 HOURS _____

(ii) UNDER 7 DAYS _____

(iii) UNDER 1 MONTH _____

(iv) UNDER 1 YEAR _____

(v) UNDER 5 YEARS _____

(vi) OVER 5 YEARS _____

(c) - STILLBORN _____

(d) - MISCARRIAGES _____

(e) - INDUCED ABORTIONS _____

BABY'S NAME_____ ID N0_____

SEX 1=MALE 2-=FEMALE BABY'S DOB ____/____/____

A.2.4.2. Mother - Clinic Sheet Questionnaire

MOTHER - CLINIC SHEET MONTH _____ ID NO _____

CLINIC LOCATION _____ CODE _____ DATE _____/_____/_____

FULL NAME _____

DATE OF BIRTH _____/_____/_____

WEIGHT (NOT MONTH 1) _____ kg

HEIGHT (NOT MONTH 1) _____mm

HEALTH CHECK - COMMENTS _____

- TREATMENT _____

- REFERRAL _____

- TABS _____

GOITRE GRADE:

0=0 1A=1 1B=5 2=2 3=3 NOT KNOWN=9

- NODULAR NO=0 YES=1 NOT KNOWN=9

- REFERRAL NO=0 YES=1 NOT KNOWN=9

CLINICAL SIGNS OF - HYPOTHYROIDISM NO=0 YES=1 NOT KNOWN=9

- HYPERTHYROIDISM NO=0 YES=1 NOT KNOWN=9

URINE SAMPLE TAKEN NO=0 YES=1 NOT KNOWN=9

BLOOD SAMPLE TAKEN

NONE=0 CAPILLARY=1 VENOUS=2 NOT KNOWN=9

HAEMOGLOBIN RESULT _____g/l

FEEDBACK IRON TABS=1 DIETRY ADVICE=2 OK=3

IODINE CAPSULES TAKEN PREVIOUSLY? NO=0 YES=1 NOT KNOWN=9

IF YES, WHEN _____/_____/_____

IODINE CAPS TAKEN TODAY NO=0 YES=1 NOT KNOWN=9

A.2.4.3. Baby - Clinic Sheet Questionnaire

BABY - CLINIC SHEET ID NUMBER _____

CLINIC LOCATION _____ CODE _____ DATE _____/_____/_____

FULL NAME _____SEX 1=MALE 2=FEMALE

DATE OF BIRTH _____/_____/_____

BABY'S FOOD: EXCLUSIVELY BREASTFED=1

MAINLY BREASTFED=2 MAINLY WEANED=3

COMPLETELY WEANED=4 NOT KNOWN=9

HEALTH CHECK - COMMENTS _____

- TREATMENT _____

- REFERRAL _____

- TABS _____

GOITRE GRADE:

0=0 1A=1 1B=5 2=2 3=3 NOT KNOWN=9

- NODULAR NO=0 YES=1 NOT KNOWN=9

- REFERRAL NO=0 YES=1 NOT KNOWN=9

CLINICAL SIGNS OF - HYPOTHYROIDISM NO=0 YES=1 NOT KNOWN=9

- HYPERTHYROIDISM NO=0 YES=1 NOT KNOWN=9

WEIGHT _____ kg

LENGTH _____ mm

HEAD CIRCUMFERENCE _____ mm

URINE SAMPLE TAKEN NO=0 YES=1 NOT KNOWN=9

BLOOD SAMPLE TAKEN

NONE=0 CAPILLARY=1 VENOUS=2 NOT KNOWN=9

IMMUNISATION GIVEN: NONE=0 BCG=5 1ST TRIPLE=1

2ND TRIPLE=2 3RD TRIPLE=3 MEASLES=4 OTHER=6

NOT KNOWN=9

MOTHER'S NAME_____ ID NUMBER_____

A.2.5. Follow-up Clinics II

A.2.5.1. Family Information Questionnaire

FAMILY INFORMATION

CLINIC LOCATION _____ DATE _____/_____/_____

MOTHER:

GIVEN NAME_____ ID NUMBER_____

AGE _____YEARS

EDUCATION LEVEL:

NONE PRIMARY MIDDLE SECONDARY INTERMEDIATE DEGREE

NOT KNOWN

OCCUPATION _____

HUSBAND:

HUSBAND'S NAME _____

AGE _____YEARS

EDUCATION LEVEL:

NONE PRIMARY MIDDLE SECONDARY INTERMEDIATE DEGREE

NOT KNOWN

OCCUPATION _____

IS YOUR HUSBAND RELATED BY FAMILY TO YOU ?:

UNRELATED 1st COUSIN 2nd COUSIN DISTANT RELATION

NOT KNOWN

BABY:

BABY'S NAME_____ ID NUMBER_____

DATE OF BIRTH OF BABY: _____/_____/_____

SEX: MALE FEMALE

PLACE OF DELIVERY:

HOME CLINIC HOSPITAL OTHER

(IF OTHER, SPECIFY_____)

WHO WAS IN ATTENDANCE AT BIRTH:

DOCTOR NURSE TBA OTHER NONE

(IF OTHER, SPECIFY_____)

A.2.5.2. Brief Pregnancy History Questionnaire

BRIEF PREGNANCY HISTORY

MOTHER'S NAME _____ID NUMBER_____

ASK THE MOTHER ABOUT ALL HER CHILDREN, BOYS AND GIRLS, WHETHER STILL LIVING OR NOT, AND ALL THE PREGNANCIES SHE HAS HAD WHICH WHICH HAVE NOT BEEN COMPLETED. TELL HER THAT THE ANSWERS ARE COMPLETELY CONFIDENTIAL AND WILL HELP US TO GIVE BETTER CARE TO HER AND OTHER WOMEN IN HER COMMUNITY.

NUMBER OF CHILDREN NOT INCLUDING THE LATEST BABY:

(a) - STILL LIVING _____(BOYS)_____(GIRLS)

(b) - BORN LIVE BUT DIED

(i) UNDER 24 HOURS	_____(B)_____(G)
(ii) UNDER 7 DAYS	_____(B)_____(G)
(iii) UNDER 1 MONTH	_____(B)_____(G)
(iv) UNDER 1 YEAR	_____(B)_____(G)
(v) UNDER 5 YEARS	_____(B)_____(G)
(vi) OVER 5 YEARS	_____(B)_____(G)

(c) - STILLBORN _____(B)_____(G)

(d) - MISCARRIAGES _____

(e) - INDUCED ABORTIONS _____ (STRESS AGAIN THAT THIS IS IN STRICT CONFIDENCE)

IF THE MOTHER REPORTS NO LOSSES, THANK HER FOR HELPING US BY ANSWERING OUR QUESTIONS.

IF THE MOTHER REPORTS ANY CHILDREN WHO HAVE DIED OR ANY PREGNANCY LOSSES THROUGH (c), (d) OR (e), ASK FURTHER QUESTIONS TO FIND OUT WHAT HAPPENED BY USING THE LONGER PREGNANCY HISTORY QUESTIONNAIRE.

APPENDIX 3 - RESULTS

A.3.1. Background Results

A.3.1.3. Knowledge, Attitudes and Practice in Childbearing

A.3.1.3.1. Context of Discussion

Guided focus group discussions were held in the 5 key villages, to investigate beliefs and practices surrounding pregnancy, birth and child-rearing. The groups contained a cross-section of married women from the village, all of whom had children, as it was not considered appropriate to discuss child-bearing with unmarried girls present. The senior women in the village, often including one or more traditional birth attendants (TBAs), were the most vocal in discussion and provided much of the information, although younger women, who might have been exposed to more education and possibly a wider variety of views at school, also contributed to the discussion.

Occasionally, male household heads were present in the home where the discussion took place, and they were invited to participate in a limited part of the discussion only, as their presence restricted the topics women could discuss and tended to dominate the discussion, if allowed to stay. They were interviewed separately by the investigator on more general matters of health and in this way contributed to the background information without inhibiting discussion of more targeted topics.

There were minor variations in the responses to questions posed by the investigator in the different villages but, in general, similar beliefs and practices prevailed in all the villages. Some of the topics covered are presented below.

A.3.1.3.2. Behavioral Changes in Pregnancy

There appeared to be little obvious change in behaviour during pregnancy and women reported that their work patterns were unchanged until close to the time of delivery. They were, however, concerned about walking over the steep hill sides and many reported miscarriages, caused by falling over, when collecting water, carrying heavy loads or working in the fields. They were reluctant to move far from their homes during pregnancy, because of this fear of falling.

Most women reported that their diets were unchanged in pregnancy, although many said that they knew pregnant women should be given "better" foods to "help the baby grow". In this culture men were served with food first and the choice meat, if eaten at all, was given to them. Women eat separately, after the men and are often not served breakfast, as they do not "go out to work". No particular taboos, concerning foods during pregnancy, were reported.

A.3.1.3.3. Delivery

There were no trained midwives in the study area and women were generally assisted at delivery by female relatives, such as mothers-in-law or older sisters, who have themselves had children, and TBAs from the village. A number of women reported giving birth without assistance because there were no other married women in the household at the time of birth, or because the TBA did not arrive in time. Birth takes place in the husband's house (or father-in-law's, if the husband does not have his own dwelling) but no special arrangements were reported to prepare the house for a birth.

After birth, the cord is tied with cotton and cut with a razor blade. Sometimes an ointment, made from grasses and herbs, is applied to the cord but often it is left untreated. The woman is generally left to recover alone and the baby removed and fed honey-water or a special tea, by the mother-in-law. It is the mother-in-law's responsibility to care for the baby for the first 2-3 days, during which time the baby sleeps separately, in his own bed.

A.3.1.3.4. Feeding Practices

For the first few days, the baby is fed honey-water and milk, as the colostrum is perceived as unsuitable for the baby. The preferred milk was reported as buffalo but if this was not available, goat's or cow's milk was given. On the third or fourth day, the mother starts to feed the baby herself, and breast milk was widely reported as the best food for the baby. Some women, however, reported that they were too weak or did not produce enough milk to satisfy their baby and so continued to give supplementary milk.

Tea and honey-water continue to be given, along with other milks, which are gradually introduced into the diet. At around 6 months of age, bread, crumbled into milk or water, is introduced into the diet although some women reported that they did not introduce solids until the child was a year old. Lentils, rice and vegetables are given to older infants and breast feeding is generally continued until the child is two years old, or until the start of the next pregnancy.

3.1.4. Reproductive Data

3.1.4.1. Recruitment of Subjects

Reproductive histories were obtained from 104 women, presenting at a follow-up clinic during pregnancy, or with an infant. They were asked about the number of previous pregnancies, pregnancy losses and young child deaths they had experienced.

3.1.4.2. Reproductive Rates

6.2% (±2.6) of reported pregnancies resulted in miscarriages and 3.2% (±1.6) in stillbirths, giving a combined foetal loss rate of 9.4% (±2.7) of pregnancies (94 (±27) /1000 pregnancies). Neonatal and infant mortality rates were 53.2 (±22.1) and 91.1 (±28.4)/1000 live births, respectively. These and other losses are shown in table A.3.1.4.2.

3.1.4.3. Reproductive Profile

An examination of the rate/1000 REY for the various parameters suggests that an average woman will be pregnant 3.8 times every 10 years - i.e. will experience a pregnancy slightly more often than once every three years, during her reproductive life. For a fertile period of 25 years (between the ages of 16 and 40), this means that an average woman would experience 9.5 pregnancies (25x381/1000), 0.6 miscarriages (25x23.6/1000), 0.3 stillbirths (25x12.2/1000), 0.8 infant deaths (25x31.4/1000) and have 7.8 children still alive (25x310/1000) at the end of her reproductive period.

An alternative way of expressing this data is to say that in this community 6 out of 10 women can expect to experience a miscarriage, 3 out of 10 a stillbirth and 8 out of 10 an infant death. These figures accord well with the estimates reported by the senior

women and men in the focus group discussions, although the reported family size (6-7 children) is smaller than that calculated using REY. Table A.3.1.4.3. also shows loss rates/REY.

notes for table A.3.1.4.3. overleaf:

(*) late miscarriages tend to be reported as stillbirths

(+) perinatal deaths include stillbirths and deaths in the first 7 days

Parameter	no.	Rate /1000 pregnancies	Rate /1000 total births	Rate /1000 live births	Rate /1000 REY
Reproductive exposure years (REY)	1145	---	---	---	----
Pregnancies	436	---	---	---	381 (±14.4)
Miscarriages (*)	27	61.9 (±22.6)	---	---	23.6 (±4.5)
Total births	409	938 (±22.6)	---	---	357 (±14.2)
Stillbirths	14	32.1 (±16.2)	34.2 (±17.6)	---	12.2 (±3.2)
Livebirths	395	906 (±27.4)	966 (±24.1)	---	345 (±14.0)
Deaths under 24 hr	9	20.6 (±13.3)	22.0 (±14.2)	22.8 (±14.7)	7.9 (±2.6)
Deaths on days 2-7	6	13.8 (±10.8)	14.7 (±11.7)	15.2 (±12.1)	5.2 (±2.1)
Perinatal deaths (+)	29	66.5 (±23.4)	70.9 (±24.9)	---	25.3 (±4.6)
Deaths on days 8-28	6	13.8 (±10.8)	14.7 (±11.7)	15.2 (±12.1)	5.2 (±2.1)
Neonatal deaths	21	48.2 (±20.1)	51.3 (±21.4)	53.2 (±22.1)	18.3 (±4.0)
Deaths in months 2-12	15	34.4 (±16.8)	36.7 (±18.2)	38.0 (±18.8)	13.1 (±3.4)
Infant deaths	36	82.6 (±25.8)	88.0 (±27.5)	91.1 (±28.4)	31.4 (±5.2)
Deaths in years 2-5	2	4.6 (±6.3)	4.9 (±6.8)	5.1 (±7.0)	1.7 (±1.2)
Deaths under 5 years	38	87.2 (±26.5)	92.9 (±28.1)	96.2 (±29.1)	33.2 (±5.3)
Deaths over 5 years	2	4.6 (±6.3)	4.9 (±6.8)	5.1 (±7.0)	1.7 (±1.2)
Children still alive	355	814 (±36.5)	868 (±32.8)	898 (±29.8)	310 (±13.7)

Table A.3.1.4.3. Reproductive losses

A.3.1.4.4. Comparison with Other Areas

Table A.3.1.4.4. compares reproductive loss rates with those in Lahore, an iodine-sufficient area of the Punjab, Pakistan, with national data, with data from Bangladesh and with UK statistics. The stillbirth, neonatal and infant mortality rates in the study area, in Lahore and in Bangladesh are all similar and much higher than in UK, although all these studies were fairly small, so detailed comparisons are not possible. The infant mortality rate is close to that reported for the country as a whole (95/1000), by UNICEF (Unicef, 1993), indicating that, though the sample is small, it may be representative of the population as a whole. Caution is, however, required in interpreting results based on such a small sample, selected under conditions of considerable bias.

Rate	Pakistan Study Area	Villages near Lahore (iodine-sufficient)	Pakistan national data	Bangladesh	England and Wales
Stillbirth	34	26 (1)	N/A	N/A	4.3 (4)
Perinatal mortality	71	60 (1)	56 (2)	N/A	7.5 (4)
Infant mortality	91	119 (1)	95 (3)	94 (3)	6.4 (4)

Table A.3.1.4.4. Pregnancy loss comparisons

Sources

(1) Hagekull, Nazir, Jalil *et al.*, 1993

(2) GOP, 1987b

(3) UNICEF, 1995

(4) OPCS, 1992

Definitions

stillbirth rate = number of stillbirths per 1,000 total births

perinatal mortality rate = number of stillbirths and deaths within the first week of life per 1,000 total births

infant mortality rate = number of deaths in the first year of life per 1,000 live births

A.4. Discussion

The following studies are referred to in these tables, with the reference numbers in brackets:

(ref 1) Chopra, Hershman, and Hornabrook, 1975

(ref 2) Maberly, Corcoran and Eastman, 1982

(ref 3) Lazarus, Parkes, John *et al.*, 1992

(ref 4) Tonglet, Bourdoux, Minga *et al.*, 1992

(ref 5) Konde, Ingenbleek, Daffe *et al.*, 1994

(ref 6) Elnagar, Eltom, Karlsson *et al.*, 1995

(ref 7) Pretell and Stanbury, 1974

(ref 8) Pharoah, Ellis, Ekins *et al.*, 1976

(ref 9) Medeiros-Neto, Walfish, Almeida *et al.*, 1978

(ref 10) Thilly, Delange, Lagasse *et al.*, 1978

(ref 11) Silva and Silva, 1981

(ref 12) Glinoer, de Nayer, Bourdoux *et al.*, 1990

(ref 13) Pedersen, Laurberg, Iversen *et al.*, 1993

(ref 14) Chaouki and Benmiloud, 1994

(ref 15) Berghout, Endert, Ross *et al.*, 1994

A.4.2. Baseline Thyroid Function in Non-pregnant Women

Area Date (ref)	Selection criteria	TSH (mIU/l) Median (95%CI)	Normal range	% "high" TSH (95%CI)
PNG 1974 (1)	M&F: goitrous non-goitrous	 15 (sd 25) 18 (sd 48)	1-7 "high" = >10	 34 31
Malaysia 1982 (2)	M&F	N/A	up to 5.0	24
Senegal 1992 (3)	M&F	1.3	up to 4.13	9.5 (±1.3)
Zaire 1992 (4)	M&F visible goitre	2.9 (2.0-3.8)	0.4-4.0	28.0 (±18.5)
Guinea 1994 (5)	F goitre: stage 0 stage 1 stage 2 stage 3	 1.5 (0.3-3.2) 2.6 (0.6-8.5) 3.7 (1.0-30) 18 (1.2-142)	N/A	N/A
Sudan 1995 (6)	M&F	3.5 (2.8-4.9)	0.4-4.0	43.7

Table A.4.2.2. TSH in other studies

Area Date (ref)	Selection criteria	FT_4 (pmol/l) mean (sd)	Normal range	% "low" FT_4 (95%CI)
PNG 1974 (1)	M&F: goitrous non-goitrous	 27.1 (12.9) 25.8 (10.3)	36.1 (6.5) mean (sd)	N/A
Senegal 1992 (3)	M&F	11.9 (3.5)	over 8	44
Guinea 1994 (5)	F goitre: stage 0 stage 1 stage 2 stage 3	 12.4 (3.0) 11.4 (2.7) 10.3 (2.5) 7.6 (1.4)	19.1 (3.4) mean (sd)	N/A
Sudan 1995 (6)	M&F	7.7 (0.9) (FTI)	10-27	73.8

Table A.4.2.4. FT_4 in other studies

Area Date (ref)	Selection criteria	TT_4 (nmol/l) Mean (sd)	Normal range	% low TT_4 (95%CI)
PNG 1974 (1)	M&F: goitrous non-goitrous	 76 (33) 89 (37)	107 (27) mean (sd) "low" = <64	 36 20
Malaysia 1982 (2)	M&F	92 (22)	N/A	N/A
Zaire 1992 (4)	M&F, visible goitre	62 (26)	50-150	16
Guinea 1994 (5)	F goitre: stage 0 stage 1 stage 2 stage 3	 91 (12) 83 (11) 75 (10) 55 (8)	119 (14) mean (sd)	N/A
Sudan 1995 (6)	M&F	41 (3)	50-150	67.4

Table A.4.2.5. TT_4 in other studies

Area Date (ref)	Selection criteria	FT_3 (pmol/l) Mean (sd)	Normal range
PNG 1974 (1)	M&F: goitrous non-goitrous	 10.4 (2.7) 10.2 (2.1)	5.8 (1.6) mean (sd)
Senegal 1992 (3)	M&F	6.3 (1.3)	N/A
Guinea 1994 (5)	F goitre: stage 0 stage 1 stage 2 stage 3	 4.3 (0.8) 4.4 (0.7) 4.8 (0.8) 4.3 (0.8)	4.2 (0.7) mean (sd)

Table A.4.2.7. FT_3 in other studies

Area Date (ref)	Selection criteria	TT_3 (nmol/l) Mean (sd)	Normal range	% "high" TT_3 (95%CI)
PNG 1974 (1)	M&F: goitrous non-goitrous	 2.6 (0.8) 2.4 (0.8)	1.9 (0.5) mean (sd) "high" = >3.2	 12 16
Malaysia 1982 (2)	M&F	1.9 (0.3)	N/A	N/A
Zaire 1992 (4)	M&F, visible goitre	3.2 (0.6)	1.4-3.2	N/A
Guinea 1994 (5)	F goitre: stage 0 stage 1 stage 2 stage 3	 2.0 (0.4) 2.2 (0.5) 2.4 (0.5) 2.2 (0.5)	1.9 (0.3) mean (sd)	N/A
Sudan 1995 (6)	M&F	3.4 (0.1)	1.4-3.2	54.7

Table A.4.2.8. TT_3 in other studies

Area Date (ref)	selection criteria	T_3/T_4 ratio (Free or Total)	Controls Mean	% "high" TT_3 (95%CI)
PNG 1974 (1)	M&F: goitrous non-goitrous	(total) (free) 3.5 0.38 2.9 0.40	1.5 (total) 0.16 (free)	N/A
Malaysia 1982 (2)	M&F	2.2 (total)	N/A	N/A
Senegal 1992 (3)	M&F	0.55 (free)	N/A	N/A
Zaire 1992 (4)	M&F, vis. goitre	5.2 (total)	N/A	N/A
Guinea 1994	F goitre: stage 0 stage 1 stage 2 stage 3	(total) (free) 2.2 0.35 2.7 0.38 3.3 0.46 3.9 0.57	0.16 (total) 2.2 (free)	N/A
Sudan 1995 (6)	M&F	8.3 (total)	N/A	N/A

Table A.4.2.9. T_3/T_4 Ratios in other studies

A.4.3. Baseline Thyroid Function in Pregnant Women

Area (ref)	Subjects	TBG (mg/l) mean (±SD)
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	21.2 (±0.3) 28.5 (±0.4) 31.5 (±0.3) (11-21)
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	20.1 (±3.0) 42.5 (±3.7) 48.8 (±6.2) 48.3 (±4.3)

Table A.4.3.1. TBG in other studies

Area (ref)	Subjects	TT_4 (nmol/l) mean (±SD/SE)
Peru (7)	Unsupplemented pregnant women (20-39 weeks) Supplemented pregnant women (20-39 weeks) Non-pregnant women in the same area Pregnant controls (N.American)	67 (±17) SD 101 (±21) 53 (±22) 93 (N/A)
Brazil (9)	Women at delivery	166 (±5) SE
Zaire (10)	Unsupplemented women at delivery Supplemented women at delivery Belgian controls at delivery	148 (±9) SE 203 (±9) 197 (±9)
Chile (11)	Unsupplemented pregnant women (11-30 weeks) (21-40 weeks) Supplemented pregnant women (9-32 weeks) (15-40 weeks) Non-pregnant adults, same area	121 (±28) SD 120 (±34) 112 (±31) 161 (±23) 99 (±23)
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	138 (±3) SE 148 (±3) 148 (±3) (50-150)
Denmark (13)	Unsupplemented pregnant women (17-18 weeks)	178 (161-207) 95% CI
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	105 (±11) SD 165 (±16) 153 (±19) 158 (±26)

Table A.4.3.2. TT_4 in other studies

Area (ref)	Subjects	FT_4 (pmol/l) mean (±SD/SE)
Peru (7)	Unsupplemented women at delivery Supplemented women at delivery	16.1 (±0.52) SD 21.5 (±0.26)
Chile (11)	Unsupplemented pregnant women Supplemented pregnant women Non-pregnant adults, same area	19 (±5) SD 29 (±5) 29 (±5)
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	17.9 (±0.3) SE 14.5 (±0.1) 13.4 (±0.1) (10-26)
Denmark (13)	Unsupplemented pregnant women (17-18 weeks)	10.5 (9.1-11.1) 95% CI
Algeria (14)	Unsupplemented women in first trimester Unsupplemented women at delivery Supplemented women at delivery (Non-pregnant reference range)	11.6 (±0.06) SE 11.2 (±0.00) 15 (9.0-24.5)
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	13.7 (±2.0) SD 13.5 (±4.1) 11.2 (±2.8) 10.2 (±1.6)

A.4.3.4. FT_4 in other studies

Area (ref)	Subjects	TT_3 (nmol/l) mean (±SD/SE)
Brazil (9)	Women at delivery Control women at delivery	3.4 (±0.2) SE 2.3 (±0.1)
Zaire (10)	Unsupplemented women at delivery Supplemented women at delivery Belgian controls at delivery	2.6 (±0.2) SE 2.4 (±0.1) 3.4 (±0.1)
Chile (11)	Unsupplemented pregnant women (10-40 weeks) Supplemented pregnant women (10-40 weeks) Non-pregnant adults, same area	2.8 (±0.4) SD 2.7 (±0.6) 1.9 (±0.4)
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	3.15 (±0.03) SE 3.55 (±0.05) 3.58 (±0.03) (1.40-3.20)
Denmark (13)	Unsupplemented pregnant women (17-18 weeks)	2.05 (1.86-2.29) 95% CI
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	1.94 (±0.30) 2.88 (±0.49) 2.83 (±0.47) 2.88 (±0.50)

Table A.4.3.5. TT_3 in other studies

Area (ref)	Subjects	FT_3 (pmol/l) mean (±SD/SE)
Chile (11)	Unsupplemented pregnant women (10-40 weeks) Supplemented pregnant women (10-40 weeks) Non-pregnant adults, same area	5.1 (±0.9) SD 5.2 (±0.9) 4.6 (±2.2)
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	5.0 (±0.1) SE 4.2 (±0.1) 3.8 (±0.1) (3-11)
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	4.55 (±0.63) SD 4.64 (±0.88) 3.72 (±0.67) 4.01 (±0.75)

Table A.4.3.6. FT_3 in other studies

Area (ref)	Subjects	TT_3/TT_4 ratio	FT_3/FT_4 ratio
Brazil (9)	Women at delivery	2.0	N/A
Zaire (10)	Unsupplemented women at delivery Supplemented women at delivery Belgian controls at delivery	1.8 1.2 1.7	N/A
Chile (11)	Unsupplemented pregnant women Supplemented pregnant women Non-pregnant adults, same area	2.7 1.7 1.9	0.27 0.18 0.16
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	2.3 2.4 2.5 (1.0-2.3)	0.27 0.29 0.28 (N/A)
Denmark (13)	Unsupplemented pregnant women (17-18 weeks)	1.3	N/A
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	1.9 1.8 1.9 1.8	0.33 0.33 0.35 0.30

Table A.4.3.7. T_3/T_4 ratios in other studies.

Area (ref)	Subjects	TSH (mIU/l) mean (±SD/SE)
Peru (7)	Unsupplemented women at delivery Supplemented women at delivery American pregnant controls	4.49 (±1.68) SD 3.72 (±0.41)
Brazil (9)	Women at delivery Control women (iodine sufficient)	9.5 (±0.58) SE 3.1 (±0.3)
Zaire (10)	Unsupplemented women at delivery Supplemented women at delivery Belgian controls at delivery	8.7 (±1.1) SE 5.4 (±0.5) 3.1 (±0.3)
Chile (11)	Unsupplemented pregnant women (11-30 weeks) (21-40 weeks) Supplemented pregnant women (9-32 weeks) (15-40 weeks) Non-pregnant normal range	3.0 (±2.1) SD 2.6 (±1.6) 2.8 (±1.4) 1.5 (±0.8) < 5
Belgium (12)	First trimester women Second trimester women Third trimester women Immediate post-partum (Non-pregnant reference range)	0.75 (±0.04) SE 1.05 (±0.04) 1.29 (±0.04) 1.89 (median) (0.2-4.0)
Denmark (13)	Unsupplemented pregnant women (17-18 weeks)	1.63 (1.28-1.91) 95% CI
Algeria (14)	Unsupplemented pregnant women first trimester Unsupplemented women at deliviery Supplemented women (Non-pregnant reference range)	3.9 (±0.01) SE 4.1 (±0.01) 2.1 (0.1-4.0)
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	1.5 (±0.7) SD 0.9 (±0.6) 1.1 (±0.6) 1.4 (±0.6)

Table A.4.3.8. TSH in other studies

Area (ref)	Subjects	TG (μg/l) mean ($\pm$SD/SE)
Belgium (12)	First trimester women Second trimester women Third trimester women (Non-pregnant reference range)	31 (2) SE 31 (2) 38 (2) < 5
Denmark (13)	Unsupplemented pregnant women (17-18 weeks)	11.5 (6.0-17.0) 95% CI
Holland (15)	Women before pregnancy First trimester women Second trimester women Third trimester women	55.1 (32.9) SD 63.1 (39.9) 57.3 (29.3) 58.4 (39.2)

Table A.4.3.9. TG in other studies

A.4.4. Follow-up Thyroid Function in Non-pregnant Women

Area Date (ref)	Normal range (mIU/l)	Pre-supplementation		Post-supplementation		Route (mg/I) Dose	F-up time (mths)
		median	% high	median	% high		
Malaysia 1982 (2)	< 5	N/A	24	N/A	0	im 480	12
Senegal 1992 (3)	< 4.13	1.5	N/A	0.58	N/A	or 480 960	6
Zaire 1992 (4)	0.4-4.0	2.4 2.7	N/A	1.2 1.2	N/A	or 47 118	3
Sudan 1995 (6)	0.4-4.0	4.8 1.8 3.8	51.2 36.1 43.6	1.1 1.0 0.6	6.5 0 0	or 200 400 800	3

Table A.4.4.1. TSH in other supplementation studies

Area Date (ref)	Normal range (pmol/l)	Pre-supplementation	Post-supplementation	Route Dose (mg I)	F-up time (mths)
		mean FT_4 (pmol/l)	mean FT_4 (pmol/l)		
Senegal 1992 (3)	> 8.0	10.5	12.8	or 480 960	6
Sudan 1995 (6)	10-27 (FTI)	6.4 7.7 9.0	17 17.5 20	or 200 400 800	3

Table A.4.4.3. FT_4 in other supplementation studies

Area Date (ref)	Normal range (nmol/l)	Pre-supplementation	Post-supplementation	Route Dose (mg I)	F-up time (mths)
		TT_4 (pmol/l) mean (sd)	TT_4 (pmol/l) mean (sd)		
Malaysia 1982 (2)	N/A	92 (22)	103 (40)	im 480	12
Zaire 1992 (4)	50-150	62 (27) 57 (26)	90 87	or 47 118	3

Table A.4.4.4. TT_4 in other supplementation studies

Area Date (ref)	Normal range (nmol/l)	Pre-supplementation	Post-supplementation	Route Dose (mg I)	F-up time (mths)
		TT_3 (nmol/l) mean (sd)	TT_3 (nmol/l) mean (sd)		
Malaysia 1982 (2)	N/A	1.9 (0.3)	2.5 (0.9)	im 480	12
Zaire 1992 (4)	1.4-3.2	3.2 (0.6) 3.2 (0.5)	"no sig. change"	or 47 118	3
Sudan 1995 (6)	1.4-3.2	3.6 3.3 3.3	"remained basically unchanged"	or 200 400 800	3

Table A.4.4.6. TT_3 in other supplementation studies