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# *Chapter*

# 3

## **SUBJECTS, DIET AND GENERAL METHODS**

### **3.1. PLACES OF STUDY**

India (Figure 3.1) is a subcontinent and represents one-sixth of the world's population. It has two main divisions: north and south India and has 29 States. In addition there are seven union territories. The diet, culture, climate, language spoken in particular area and clothing change with the State. The main cereal consumed by north Indians is wheat and by south Indians is rice. Cheese, chocolates and cornflakes are not consumed routinely as Indian diet.

More than a third of the population of India is too poor to be able to afford an adequate diet hence generalised malnutrition and other deficiency disorders are common.

There are many environmental problem like deforestation; soil erosion; air pollution from industrial effluent and vehicle emissions; water pollution from raw sewage and runoff of agricultural pesticides; tap water is not potable throughout the country; huge and growing population is overstraining natural resources.

July 2001 estimate showed that population of India was 1,029,991,145. Thirty three percent of this population is children below 14 years of age and 62% are adults in the age range of 15 to 64 while remaining 5% are old people in 65 years and over range.

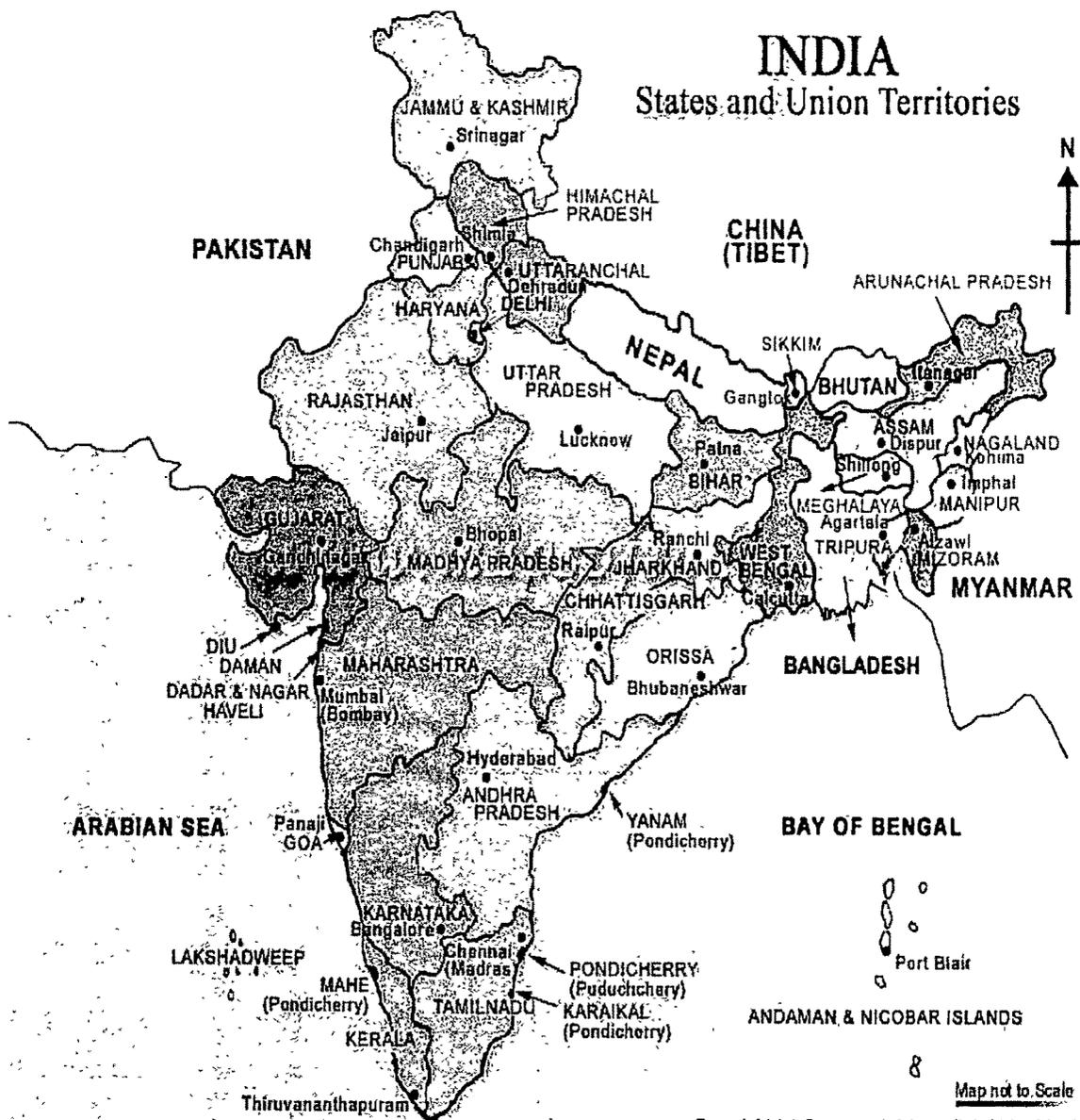
Endemic goitre and cretinism are widely distributed over a broad Himalayan and sub-Himalayan belt. One conservative estimate suggested that about 150 million people were at risk of IDD, 54 million had goitre, 2.2 million suffered from cretinism and an estimated 6.6 million were affected by milder neurological deficits attributable to environmental iodine deficiency (Pandav, 1994). In addition, about one-fifth of pregnant women are at considerable risk of giving birth to children who will not reach their optimum physical and mental potential because of maternal iodine deficiency (Vir, 1995). Next survey estimated that 200 million people in India were exposed to the risk of iodine deficiency, and 70 million suffered from goitre and other IDD (IDD & Nutrition Cell, 1998).

There are more and more isolated pockets of endemic goitre being reported (by palpation) from different parts of rest of India. Thus, goitre has been reported to be prevalent in endemic proportions along the hilly tracts of central India, particularly along the Chhota-Nagpur Plateau, the Aravalli range in Rajasthan, Auragabad in Bombay City, and also along the higher reaches of the Western Ghats, in the tea estates of Kerala and Karnataka. A survey of goitre in eight villages of the Deccan plateau by Krishnamachari showed an average prevalence rate of 50%. Goitre prevalence, in endemic proportion has been reported from the Narmada valley by Edibam et al, the average prevalence rate observed being 37.7%. A recent study of goitre among school children of Delhi showed prevalence rates that range from 15 to 30% in different schools.

There were two reasons to study IDD in India: 1. Severe goitre is restricted to it and in the search to find all the consequences of iodine deficiency on

health, the data from this area would be extremely helpful because they would present the most severe anomalies. This will thus be a starting point to define more subtle complications. 2. In the areas of severe goitre, where iodine salt prophylaxis is not feasible, injection of iodized oil, a safe alternative could be used.

**FIGURE 3.1. MAP OF INDIA SHOWING STATES AND UNION TERRITORIES**



Although solutions for IDD control have existed for many decades in India, the severity of the problem of IDD has increased. The reasons for this apparent increase are firstly, more detailed surveys being carried out in remote areas; and secondly, the difficulty of applying in developing countries the existing solutions that are more suitable for developed countries. In this regard, it is interesting to note the overlap between the remoteness of an area and severity of iodine deficiency. This is probably one of the reasons why few of the iodine prophylaxis programs based on the distribution of iodinated salt have met with success in developing countries, even though this solution has been recommended by the WHO for more than 30 years (WHO 1994). There is therefore an urgent need to develop other strategies and methods to control iodine deficiency that is a major public health problem and is the leading preventable cause of mental impairment worldwide. The cumulative consequences in iodine-deficient populations spell diminished performance for the entire economy of affected nations. The impact of IDD on intellectual development and resulting brake on socioeconomic development has played a significant role in mobilising scientists, public health administrators and political leaders the world over to deal effectively with IDD.

## **GUJARAT**

### **BACKGROUND**

Gujarat (Figure 3.2.) was formed as a separate State in the Union of India in 1960. It has 6.19% of the total geographical area of India. As per provisional figures published after Census 2001, the population of Gujarat stood at 5,05,97,000 constituting 4.93% of that of India.

Coastline of Gujarat with two gulfs is the longest in the country that produces 65 % of the salt in India hence offers tremendous scope for promoting salt-based industries.

Mango, Onion, Psyllium, Papaya, Potato, Cotton, Banana, Garlic, Groundnut, Guava, and Carrot are exported.

The Gujarat State has nineteen districts (Figure 3.2.) and a population consisting of both urban and rural segments in most of the districts. As iodine content of drinking water is different according to the geographic location of the place, the study included two different places i.e. Baroda is on plain land and Dang is on mountain. Marked differences existed in the intake of dietary iodine in the population as diet differed completely in both districts due to habits and availability.

The status of iodine nutrition was expected to be different because there was a ban on sale of non-iodized salt in Dang district but non-iodized salt was freely available in Baroda District.

**FIGURE 3.2. MAP OF GUJARAT WITH ITS DISTRICTS**



## **BARODA**

Baroda is one of the 19 districts of Gujarat State (Figure 3.2.) and has a rich historical background. It is one of the most important centres for textile, chemical and oil industries today. It occupies a unique position on the educational, cultural and industrial map of India.

It is one of the most cosmopolitan cities and has welcomed a wide variety of people from all over India and also from all over the world due to education and work opportunities. Maharaja Sayajirao University is the only institute where medium for teaching is English. The dominant industrial groups are chemicals (GSFC, GACL and IPCL) and pharmaceutical, cotton textiles and machine tools, Gujarat Refinery and many Multinational firms. There is proliferation of academic activities and it has a strategically important geographical location. The outstanding trait about Baroda's cultural life is that it is remarkably cosmopolitan; dynamic, ever changing and alive.

The city is on the major rail and road arteries joining Mumbai with Delhi and Mumbai with Ahmedabad because National Highway passes through the city. Baroda is known as a 'Gateway to the Golden Corridor'. The Population in 1998 was 1,400,000 (approx.) and 71.11% of total population (males 76.21%; females 65.41%) are educated.

Baroda district had never been investigated for IDD.

## **DANG**

This district is a remote mountainous area (Figure 3.2.) where soil and drinking water may be poor in iodine. Dang district has a predominantly tribal population.

This place was investigated in the past for goitre prevalence that was 44% by palpation (Desai VK, 1994) but the severity of IDD is not confirmed by urinary iodine measurement and thyroid ultrasound so far.

The sale of non-iodized salt has been banned in the district since this survey in 1994.

## **HIMACHAL PRADESH**

This hilly and mountainous State is located on the north-west (Figure 3.3.). State is wrapped in snow most of the time as it is nestled in the Himalayas, the world's mightiest mountain ranges, and constitutes 10.54 % of the Himalayan landmass. There is wide variation in altitudes from 350 meters to 6975 meters above mean sea level thus ranging from low hills to high mountains.

Population in 1991-Census was 5170877 persons with the majority of population being rural (91.3 %). Thus there is an urban population of 0.45 million persons and a rural population of 4.72 million persons. There are 976 Females per 1000 Males in 1991 census. Agriculture is the main occupation of the people and the State is known as Fruit Bowl (Apple stone and citrus fruits) of India. Crops like wheat, maize and pulses are grown here. The difficult hilly terrain and limited resources has not deterred the State in attempting to solve the problem of accessibility by establishing and locating health institutions as close to the people as possible. A dedicated team of health providers has tried its best to ameliorate the sufferings of the human being. Himachal Pradesh has made a definite niche in the health framework.

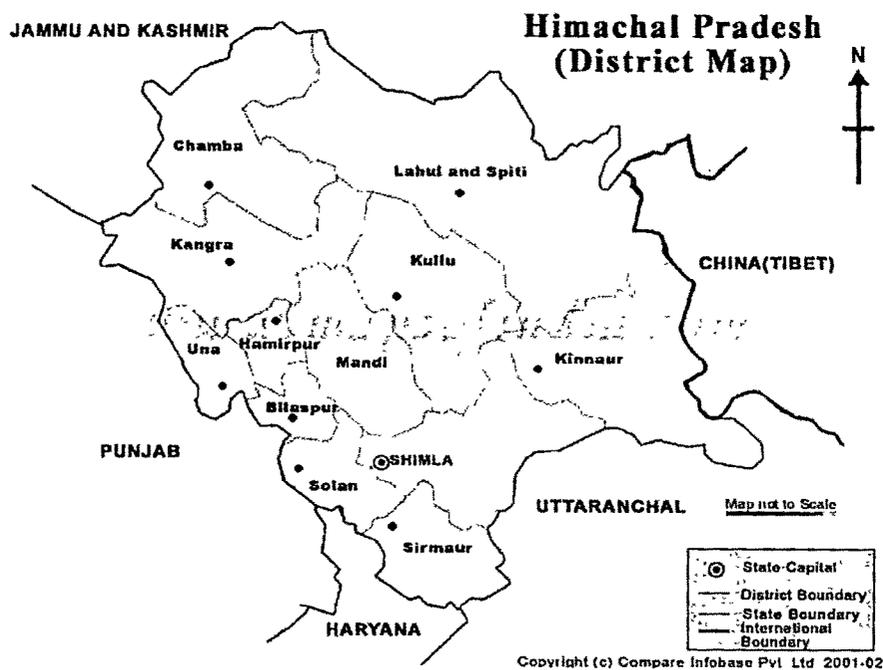
Being a Sub-Himalayan belt, goitre is still a nutritional deficiency disorder of some concern in the State. Prevalence of goitre, which was quite high previously in Shimla (41.6 % in 1974) came down to 2-13 % in 1996 for

various age groups as per Nutritional Survey carried out by Department of Women and Social Welfare in HP. During 1999-2000, 1,17,699 samples of salt were analyzed out of which only 78 samples were found to be without iodine.

As per nutritional survey conducted by Ministry of Women and Social Welfare Govt. of India, which covered 10 districts of Himachal Pradesh, 0.5 percent infants were found to be suffering from Marasmus. Eighty one percent children suffered from mild to moderate degree of malnutrition and only 4% suffered from severe degree. Eight percent of adults suffered from Chronic Energy Deficiency (CED). Goitre was the major nutritional deficiency disorder (2-13 %) in various age groups.

There are 12 Districts and Shimla is the State Capital. Shimla was discovered in 1819 on the altitude of 2159 meters and was declared as "summer capital" of India."

**FIGURE 3.3. HIMACHAL PRADESH WITH ITS DISTRICTS**



## **TAMIL NADU**

Tamil Nadu, southern state, is referred to as the cradle of ancient Dravidian culture. Tamil Nadu is a bastion of Hinduism at its most vigorous, whose past endures into the present. The Aryans never brought their meat-eating influence to the extreme south of India, so Tamil Nadu is more or less totally vegetarian. Dosas and idlis have become enormously popular all over the country. Mounds of rice accompany every meal, sweets are a favourite, and coffee is more popular than tea.

## **CHENNAI (FORMERLY MADRAS)**

Chennai is a major metropolis city and the capital of Tamil Nadu. Chennai is India's fourth largest city and Formerly known as Madras, the city's history dates back to more than 2000 years. It is the most convenient point of entry to South India, and a fast-growing software and retail center. Chennai has an international airport, a sea port and a rail and road network that links it to all major towns and cities. Being a port town it has always attracted traders and merchants and these included the French, the Portuguese, and the British. When the British established their supremacy in the 19th century the city became the Madras Presidency, one of the four divisions of British India. Today, Chennai is one of India's booming cosmopolitan city. It is the center of the Tamil film industry – the slickest in the country. Chennai is also known for its good educational institutions and some of India's hottest retail outlets.



We studied the subjects irrespective of castes, religions, and residence. As 34% of the population was children, 62% adults and 4% over 60 years, we studied all three categories. The children below 10 years were born after the implementation of Universal Salt Iodization in India in 1974 whereas adults might be exposed to iodine deficiency in their early life. The children were easily accessible from boarding schools for rich and poor and surveys for adults were performed from households in various villages or from schools and institutes.

Urban subjects were from Baroda, Chennai and Shimla cities of Gujarat, Tamil Nadu and Himachal Pradesh respectively. Rural subjects were from rural part of Baroda and tribal subjects were from Dang district in Gujarat.

The studied villages of Baroda rural were in the range of 50 kilometres from Main City. These were Muval and Tentlav in North and South Baroda. The studied villages of Dang district that is 350 kilometres from Baroda were wide spread throughout the district. These were Saputara, Rutambhara, Rambhas, Baripada, Dediapada and Vaghai.

### 3.3. DIET

In Gujarat there are two to three main meals per day: breakfast, lunch and dinner. Breakfast in some communities is as big as lunch and dinner; consisting of pancakes and vegetables. Traditional Lunch has rice, wheat flour pancakes, vegetables, salads, pickles, curd and pulses. Traditional dinner has pancakes made from pearl millet or jowar or wheat with vegetables and milk or buttermilk. In between meals snacks (samosa, pettis, pakoda and dalvada) are consumed that also contain vegetables, pulses and various flours like chickpea and wheat. There is no fix limit for these small snacks.

There was one survey conducted on the type of diet in Gujarat in 1998 that showed substantial differentials in food consumption patterns by background characteristics. Diet (fruits, eggs, and chicken, meat, or fish) varied according to residential areas (urban or rural), education (literate or illiterate), caste/tribe (scheduled, upper castes like Brahmins, Vaishnav, Bania, Harijan) and religions (Muslim or Hindu). Consumption of milk or curd, green leafy vegetables, and especially fruits increases substantially with education and the standard of living. Seventy-one percent of women in Gujarat never ate chicken, meat, or fish, and eggs. These women did not even cook meat that made meals for their children and family devoid of all sorts of meat. On the daily bases these vegetarian families consumed milk, curd, pulses or beans and vegetables. The families consuming meat used it once or twice in a week. Only 1% of these consumed meat daily in one main meal. The cost of meat is high hence it is mixed with other vegetables or pulses.

Every family consumes at least 3-5 kilograms of vegetables, one to two kilograms of various flours, 300-500 grams of oil, 100 gms of fresh herbs, 100-200 gms pulses and 2-3 litres of milk per day (only rich people). Thus one person consumes at least one kilogram of vegetables, cereals, pulses,

fruits and at least 250 ml of milk. The most common beverage is tea, from a minimum of 2 to 8 cups. Chocolates, desserts, wine and cheese do not form a part of the regular meal. The non-vegetarian families also consume at least one kilogram per person per day of vegetables, cereals and pulses. Fruits are seasonal and their use depends according to availability, affordability and price.

### **VEGETABLES**

There are a wide variety of vegetables in Gujarat and some of them are exclusive to this State. Giloda, Parvel, Egg plant, Ladies finger, Valod Papdi, Surti Papdi, Cauliflower, Tuver, Lilva, Guar, Snake beans, Tomatoes, Onions, Coriander, Fenugreek leaves, Spinach, Tandaljo, Dil leaves, Ginger, Potato, Sweet potato, Amorphophalus, Chillies, Garlic fresh green, Turmeric fresh, Ambo fresh, Yam ratarhu, Kankoda, Dudhi, Galka, Tooree, Lotus shoots, Bitter gourd Karela, Radish, Cucumber, Carrot, Mogree and many other vegetables are eaten by people. Most of these vegetables have no assigned English names.

### **FRUITS**

Banana is available throughout the year in Gujarat. There are many seasonal cheap fruits consumed in bulk like guava, bor, grapes, watermelon, honey dew melon, apple, mango, pear and chikoo. Costly fruits are strawberries, mulberries and cherries. Amongst the dry fruits; cashewnuts, almonds, walnuts are costly but peanut and coconut are the cheap ones hence consumed in bulk.

### ***SPICES***

Red chili powder, coriander powder, turmeric powder, and garam masala (mixture of spices: cloves, cinnamon, cardamom, nutmeg, black pepper ground together).

### ***SALT***

Iodine is an important micronutrient and is the single most important and preventable cause of mental retardation worldwide. Iodine deficiency can be avoided by using salt that has been fortified with iodine. In 1983–84, the Government of India adopted a policy to achieve universal iodization of edible salt by 1992. In 1988, the Prevention of Food Adulteration Act was amended to fix the minimum iodine content of salt at 30 parts per million (ppm) at the manufacturing level and 15 ppm at the consumer level (MOHFW, 1994). The Government of India had advised all states and union territories to issue notifications banning the sale of edible salt that is not iodized. However, the ban on non-iodized salt was lifted in September 2000.

The iodised salt National Health survey was conducted in Gujarat in 1998–99. The results of this survey based on consumption of different types of iodised salt are shown in Table 3.1. as percent distribution of households by degree of iodization of salt according to selected background characteristics.

**TABLE 3.1. NATIONAL HEALTH SURVEY GUJARAT, 1998–99  
ON CONSUMPTION OF IODISED SALT**

<b>Background characteristic</b>	<b>Not iodized</b>	<b>7 ppm</b>	<b>15 ppm</b>	<b>30 ppm</b>	<b>Number of households</b>
<i>Type of place of residence</i>					
Large city	7.8	7.8	7.8	76.4	736
Small city	26.9	14.7	11.9	46.5	325
Town	28.2	12.7	17.1	41.8	633
Rural area	37.4	16.7	17.1	28.7	2,238
<i>Religion of house- hold head</i>					
Hindu	29.7	14.6	15.2	40.4	3,529
Muslim	30.4	14.1	13.5	41.7	317
Other	19.9	1.1	8.2	70.8	85
<i>Caste/tribe of household head</i>					
Scheduled caste	30.0	17.4	16.5	36.1	576
Scheduled tribe	54.4	9.7	11.4	24.3	774
Other backward class	26.1	17.2	19.6	36.8	929
Other	19.6	13.5	13.3	53.4	1,651
<i>Standard of living index</i>					
Low	44.3	16.1	16.6	22.7	979
Medium	29.8	16.3	16.6	37.2	1,838
High	16.0	9.2	10.6	64.2	1,111
<b>Total</b>	<b>29.5</b>	<b>14.2</b>	<b>14.9</b>	<b>41.2</b>	<b>3,932</b>

Note: Total includes 1 and 4 households with missing information on caste/tribe and the standard of living index, respectively, which are not shown separately.

## **GOITROGENS:**

Apart from iodine deficiency in food and water, a series of other factors in the diet and processes contribute to goitrogenesis both deteriorating or mitigating the cascade of iodine deficiency-induced growth and proliferation of the thyroid gland.

Among the compounds contributing to goitrogenesis are plant derived substances such as C- or N- glycosides, which produce cyanate, isocyanate, goitrin, flavonoids or other goitrogens known to interfere with thyroid hormone synthesis.

## **FLAVONOIDS IN DIET:**

These are ubiquitously distributed in more than 4000 varieties of plants as their secondary metabolites. These integral colored pigments are polycyclic phenolic compounds either in free, conjugated or methylated form. Western diet may contain only up to 2 gms flavonoids but the vegetarian diet in developing countries especially in Gujarat (India) may contain > 10 gms. These are eliminated rapidly after absorption by liver in the conjugated form either as sulfate or glucuronate. Sometimes these flavonoids are metabolized by GIT microbial flora prior to absorption. In vitro animal studies have shown that flavonoid interfere with hormone system of the host by cellular signal transduction, alterations in gene expression, interference with metabolic reactions and structural remodeling. Isoflavonoids act as phytoestrogens and genistein acts by interfering with signal transduction.

Flavonoids exert multiple actions in biological systems depending upon their chemical structure. Flavonoids having at least one free aromatic phenolic group act as an antioxidant. Monocyclic and polycyclic flavonoid interfere with the thyroid axis and contribute to goitrogenesis by their interference with

pituitary function and TSH secretion, effects on thyroid gland directly or indirectly through the effects on binding and distributing protein. *Mellissa off*, *Lycopus virg.* or *Lycopus europ* and *Thymus serpyllum* are the flavonoid compounds studied in rats for their effect on pituitary gland and they inhibit TSH secretion and LH and testosterone. These effects are confirmed with synthetic flavonoid F 21388. Anti TSH action and TSH decrease results in decreased thyroid hormone secretion.

#### ***Flavonoid content in food***

One kilogram of Onion contains 1.5 grams of quercetin, 0.4 grams of luteolin and 0.9 grams of Kaemferol. Tea, Guava and Chillies contain flavonoid luteolin, which is 1.5, 1.2 and 1.7 grams per kilogram of them respectively. Onion leaves, Carrot and Celery also contain flavonoid luteolin but the content is low ie 0.4, 0.04 and 0.08 gms/kg respectively. Flavonoid apigenin content of Chinese cabbage, Garlic, French peas, Snake gourd, Celery and Guava varies between 0.2 to 0.6 (grams) per kilogram. Catechin is another flavonoid of Tealeaves and apple.

### **3.4. GENERAL METHODS**

#### **3.4.1. ASSESSMENT OF IDD STATUS BY APPROPRIATE INDICATORS**

The two major categories are whether the assessments are clinical (thyroid size and cretinism) or biochemical (urinary iodine and thyroid-related hormones). Criteria for selection include acceptability, technical feasibility, cost, performance, and availability of reference data. Measuring thyroid size by palpation and ultrasound did the clinical assessment of IDD in the present

study whereas biochemical assessment included urinary iodine and blood spots TSH measurements (first four surveys).

### **3.4.1.1. CLINICAL INDICATORS (THYROID SIZE)**

Iodine is an essential component of the thyroid hormones, and the iodine deficient thyroid cannot make sufficient hormones for the body's needs. The pituitary gland responds to low levels of circulating thyroid hormone by increasing secretion of its hormone, thyroid stimulating hormone (TSH), which drives the thyroid to enlarge, work harder, and produce more hormone. The consequent thyroid enlargement is called a goitre. Goitre is a compensatory adaptation to iodine deficiency. Other conditions may also cause goitre, but these rarely reach a prevalence over 5% in an iodine-sufficient population. Thyroid size, when precisely assessed, is one of the most sensitive indicators of community iodine nutrition because it changes inversely in response to alterations in iodine intake with a lag of 6 to 12 months in children and young adults of less than 30 years (WHO, 1994).

#### **3.4.1.1.1. PALPATION**

Palpation requires no instrument beyond the examiner's fingers and the technique is inexact for only slightly enlarged thyroids. The examiner faced the subject and looked for visible thyroid enlargement. The subject was then asked to look up and extend the neck that made thyroid enlargement more visible. The examiner palpated the thyroid by sliding his thumbs along each side of the trachea between the thyroid cartilage ("Adam's apple") and the top of the sternum. The size and consistency of the thyroid was carefully noted. The subject was asked to swallow so that thyroid moved upward and its size could be defined. Definition of what constitutes a goitre by palpation is inexact. The statement that "a thyroid gland whose lateral lobes have a volume greater than the terminal phalanges of the thumbs of the person

examined will be considered goitrous" was used in these epidemiological studies of endemic growth, despite its empirical nature.

The preferred group for survey by palpation is school-age children, usually 6-12 years of age but we palpated all age population. The following simplified classification was used (WHO/UNICEF/ICCIDD, 1994).

1. Grade 0 - no palpable or visible goitre
2. Grade 1 - a mass in the neck consistent with an enlarged thyroid, that was palpable but not visible when the neck was in the normal position. The mass movement upward in the neck was noted when the subject swallowed.
3. Grade 2 - A swelling in the neck that was visible when the neck was in the normal position, and was consistent with an enlarged thyroid when the neck was palpated.

Usually a total goitre rate (including both grades 1 and 2) of 5% or more in schoolchildren 6-12 years of age indicates a public health problem according to ICCIDD. The cut off point of 5% allows some margin of error for the imprecision of goitre assessment and for goitres that may occur from problems other than iodine deficiency.

#### **3.4.1.1.2. ULTRASONOGRAPHY**

Ultrasonography is a safe non-invasive technique and the most commonly employed imaging technique for thyroid gland anatomy. High frequency sound waves in the megahertz range (ultrasound) are used to produce a photographic image. The subject remains comfortable during the test, which takes only a few minutes. The procedure was done with the subject reclining with the neck hyper-extended. Gel was applied over the skin of the neck (since air does not transmit ultrasound, skin over the thyroid was coupled to a

liquid medium) and then a probe with a frequency of 5.0 (in the first two surveys) to 7.5 megahertz (in all other surveys) was applied. Probe contained a piezoelectric crystal called a transducer that was held against each lobe of the subject's neck. Measurements of length, width and depth of each lobe were made and noted on the proformas. The portable ultrasound equipment was operated by electricity and it rapidly alternated as the generator of the ultrasound and the receiver of the signal that had been reflected by thyroid. The signal was organized electronically into numerous shades of Gray and was processed electronically to produce an image instantaneously (real-time). The air-filled trachea did not transmit the ultrasound. The thyroid gland was slightly echo-denser than the adjacent structures because of its iodine content. The volume of the each lobe (V) was calculated in millilitres (the specific gravity of thyroid tissue is 1 and this helps in changing the mm to ml) by the simple formulae

**WHO formula:**

$$TV (ml) = 0.000479 \times d (mm) \times w (mm) \times l (mm)$$

**Rotation ellipsoid model formula:**

$$TV (ml) = 0.00052 \times d (mm) \times w (mm) \times l (mm)$$

The thyroid volume was the sum of the volumes of both lobes. The volume of the isthmus was not included.

Thyroid volume was calculated using both formulae in personal computer after data entry and was expressed as a function of either for age or for body surface area. The median and 97th percentile for thyroid volume was calculated for age and BSA by gender. The results need to be compared with normative values for thyroid volume in iodine sufficiency, and these values must be established for each population group. Data of normative value for thyroid volume in iodine-replete schoolchildren as a function of age and body surface area have been presented for Europe (adopted by WHO as an international reference) and several other areas (USA, Switzerland and Malaysia). The upper limit of normal is taken as the volume at the 97th

percentile of the corresponding iodine sufficient group as adopted by WHO (Table 3.2. A and 3.2. B).

The body surface area is calculated using the formula of Dubois and Dubois (1916):

$$\text{BSA (m}^2\text{)} = W^{0.425} \times H^{0.725} \times 71.84 \times 10^{-4}$$

**TABLE 3.2. A: WHO RECOMMENDED UPPER LIMIT OF NORMAL THYROID VOLUME FOR AGE IN EUROPEAN IODINE-REplete CHILDREN AGED 6-15 YEARS**

Age (years)	Thyroid volume (ml)	
	Boys	Girls
6	5.4	5.0
7	5.7	5.9
8	6.1	6.9
9	6.8	8.0
10	7.8	9.2
11	9.0	10.4
12	10.4	11.7
13	12.0	13.1
14	13.9	14.6
15	16.0	16.1

**TABLE 3.2. B: WHO RECOMMENDED UPPER LIMIT OF  
NORMAL THYROID VOLUME FOR BODY SURFACE AREA  
(BSA) IN EUROPEAN IODINE-REPLETE CHILDREN AGED 6-  
15 YEARS**

<i>BSA</i>	<i>Thyroid volume (ml):</i>	
	<i>Boys</i>	<i>Girls</i>
0.8	4.7	4.8
0.9	5.3	5.9
1.0	6.0	7.1
1.1	7.0	8.3
1.2	8.0	9.5
1.3	9.3	10.7
1.4	10.7	11.9
1.5	12.2	13.1
1.6	14.0	14.3
1.7	15.8	15.6

In areas with a high prevalence of protein-energy malnutrition, the BSA reference is recommended.

The examination took few minutes per subject. The data were quantitative, and reproducible.

Epidemiological criteria for assessing the severity of IDD based on the prevalence of goitre in school-aged children are shown in the summary table 3.3.

**TABLE 3.3. SUMMARY OF IDD PREVALENCE INDICATORS  
AND CRITERIA FOR A SIGNIFICANT PUBLIC HEALTH  
PROBLEM**

Indicator	Target population	Severity of public health problem			
		None	Mild	Moderate	Severe
%Goitre grade > 0	SAC	<5	5.0-19.9	20.0-29.9	≥ 30.0
%Goitre Ultrasound (TV >97 <sup>th</sup> centiles)	SAC	<5	5.0-19.9	20.0-29.9	≥ 30.0
Median UI level (µg/l)	SAC	>100	50-99	20-49	< 20
TSH (>5mU/L whole blood)	Neonate	<3	3.0-19.9	20.0-39.9	≥ 40.0

SAC = School-age children

Numbers represent percentage of population

The cut-off data for adult population were not available hence 20 ml was considered as the upper limit of normal for adults of this study.

### **3.4.1.2. BIOCHEMICAL MARKERS**

#### **3.4.1.2.1. Urinary iodine**

Most iodine absorbed in the body eventually appears in the urine that makes urinary iodine excretion measurement a good marker of very recent dietary intake. In individuals, urinary iodine excretion can vary somewhat from day to day and even within a given day, but this variation tends to damp out in populations. The iodine concentration in casual urine specimens (child or adult) provides an adequate assessment of a population's iodine nutrition.

Since casual specimens were used, it was desirable to sample enough subjects from a given population to allow for difficult degrees of subject hydration and other biological variations. In general, 30 urine determinations from a defined sampling group are considered sufficient by WHO/ICCIDD/UNICEF but due to dietary diversification of our study population, different caste groupings according to religious bindings and economic diversity we determined urinary iodine for all subjects.

Urinary iodine determination were carried out in the "URINARY IODINE LABORATORY" of the Institute of Clinical Pathology and Medical Research (ICPMR), Western Sydney Area Health Services, Westmead. Collected urine samples of the first Gujarat survey (GSI) that was conducted in 1998 were brought to Australia personally. Autoanalyzer (Technicon II) determined the urinary iodine of these samples. As this method included the interfering substances, a method was developed to remove them and measure the true urinary iodine. Then the Autoanalyzer (Technicon II) was replaced to adapt method L (Hitachi) for urinary iodine measurements. For the next three surveys in Gujarat (GSII, GSIII, GSIV) and one survey in Himachal Pradesh (HPI), the urinary iodine from samples was measured by the kits available for urinary iodine assay from Bioclone Australia Pty Limited based on method L.

#### ***AUTOANALYZER (TECHNICON II) METHOD***

The urine samples (n=2322) of first two IDD surveys in Gujarat (GSI and GSII) in 1998-1999 were analysed by Technicon Autoanalyzer II (Garry et al, 1973) (1363 schoolchildren and 959 adults reported in chapter 4 and 5 respectively from GSI plus 530 schoolchildren and 472 adults reported in chapter 6 and 7 respectively from GSII). This method was simplified to avoid the digestion with strong acid by dialyzing urine as the Autoanalyzer had a dialysis unit and the process was automated to analyse iodine in the dialysate based on the reaction between cerium IV and arsenic III (Sandell-Kolthoff Reaction).

**Materials and Method:****Apparatus**

*Instrument:* Basic Autoanalyzer system produced by the Technicon Instruments Corporation, NY. It has various components like Manifold diagram, Dialyser to perform dialysis at 30°C with a type "C" membrane, Sampling that use sample rate at 60/hour and a Colorimeter that uses a 15 mm tubular flow cuvet and 410 nm filter.

**Reagents**

*Arsenious acid:* Dissolve 19.6 gms of arsenic trioxide powder and 14.0 gms of sodium hydroxide in about 500 ml water in a 2-liter volumetric flask. Carefully add 64 ml of concentrated sulfuric acid and then water to the 2-liter mark. Add 50 gms of sodium chloride and dissolve.

*Ceric ammonium sulfate:* Dissolve 10.5 gms of ceric ammonium sulfate and 52 ml of concentrated Sulfuric acid in 200 ml of water in a one liter volumetric flask, and allow to cool; then add water to a final volume of 1 liter.

*Stock iodine standard (100 µg/ml):* Dissolve 130.8 mg of potassium iodide in water and dilute to 1 liter.

*Intermediate stock iodine standard (1 µg/ml):* Dilute 10 ml of stock iodine standard (100 µg/ml) with water in 1 liter.

*Working iodine standard:* Dilute intermediate stock iodine standard (1 µg/ml) with 0.1 molar hydrochloric acid (9 ml of concentrated hydrochloric acid diluted to one liter with water) as follows:

Iodide standard	0.1 molar hydrochloric acid ml	Iodine concentration µg/l
5	95	50
10	90	100
20	80	200
30	70	300
40	60	400

Fresh standards were prepared every week.

***Procedure:***

The urine samples were acidified immediately after collection and frozen for later analysis. All reagents were pumped through reagent lines as shown in the manifold diagram (Garry et al, 1973) and the reagent baseline on the recorder was adjusted to 20 and 30% transmittance. Iodine working standards were analysed in an ascending order to establish the standard curve. This was followed by controls and urine samples.

The contamination of the system with iodine from highly concentrated urine samples was avoided by rapid initial rapid screening at a rate of 120 per hour. The urine samples above 200 µg/l were diluted and process was repeated once again.

The method had several potential sources of error and did not separate out the interfering substances (May SL et al, 1990). The method gave false results especially for the subjects from an iodine deficient belt having low urinary iodine. Hence a method was developed to remove interfering substances and the true urine iodine values were established. The results were expressed as micrograms of iodine per litre of urine (µg/l). Present

method showed a good correlation with UI measurement by the Ion Coupled Plasma Mass Spectrometer (ICPMS) and method A.

***Removal of Interfering substances (IS):***

The urine samples were diluted according to the requirement from the peak obtained in Autoanalyzer recording paper. The arsenic acid was added to a 200 $\mu$ l of diluted sample or neat sample (one having low total UI) and kept for one hour and then ceric ammonium sulfate solution was added and the mixture was kept in dark for 24 hours. This was read in the Autoanalyzer again and the calculation were done to arrive at true UI and the amount of IS. The standard curve was plotted and the standards of different concentrations of iodine were also run throughout.

For the other Gujarat (GSIII, GSIV), Himachal Pradesh (HPI) and Tamil Nadu surveys, the iodine determination in urine samples was carried out by the kits available from Bioclone Australia Pty Limited based on method L.

***URINARY IODINE ASSAY KIT:***

***PRINCIPLES:***

The oxidation of the sample is completed with ammonium persulphate for 1.5 hours at 90°C on a polypropylene microtitre plate and this removes thiocyanate and nitrates (potential interfering substances). The use of special sealing cassette during this digestion prevents the loss of vapour or cross contamination. After digestion the sample is transferred to a polystyrene microtitre plate where arsenic solution and cerium solution are added. Photometric absorbance readings are then taken at 405nm using a microtitre plate reader. The colour in the sample is inversely proportional to the amount of iodine present, which can be quantitated with a standard curve.

**Procedure**

**Digestive step**

**Plate A (polypropylene)**

1. Pre-heat the sealing cassette to 90°C (oven) for 60 minutes.
2. Add 10ml of Iodine-Free Water to the ammonium persulphate bottle and allow to dissolve
3. Add 50µl of standard or urine sample to plate A
4. Add 100µl of ammonium persulphate solution to each well of plate A
5. Place plate A in to the pre-heated the sealing cassette and tighten
6. Incubate at 90°C (oven) for 90 minutes
7. Remove plate A from oven using gloves and place in to a tray containing approximately 1.5 cm of ice or water
8. Allow to cool for approximately 15 minutes
9. Open the sealing cassette and remove plate A

↓ (Sample + Oxidiser)

↓ 50 µl + 100 µl

↓ 96 Samples

Heating in Sealing Cassette (90°C, 1.5 hr)

**Assay**

**Plate B (polystyrene)**

- (1) Transfer 50µl from each well of plate A to the equivalent well of Plate B
- (2) Add 100µl of arsenic solution to each well
- (3) Add 50µl of cerium solution to each well without interruption in 1-2 minutes
- (4) Incubate stationary at room temperature for 30 minutes
- (5) Read plate using a Microtitre plate reader at 405nm

Transfer 50 µl of the Reaction Mixture Plate B

Sandell-Kolthoff Reaction for 20-30 minutes

(As<sup>3+</sup> reagent 100 µl + Ce<sup>4+</sup> reagent 50 µl)

Microplate reader (405 nm)

***Instruments and solutions***

4. Bottle A (Ammonium persulphate)
5. Bottle B (Arsenic solution)
6. Bottle C (Cerium solution)
7. Standard set of iodine solution
8. Iodine free distilled water
9. PS plate (polystyrene)
10. PP plate (polypropylene)
11. Pipette
12. Tray
13. Paper towel

***Calculation of results***

Create a standard curve: plot standard value against the log of absorbance at 405 nm to produce a linear standard curve

Determine values of unknowns by reading the log of sample OD off the standard curve and obtaining a concentration

A summary table shows cut-off points proposed for classifying iodine nutrition into different degrees of public health significance in the table 3.2. Frequency distribution curves were necessary for full interpretation, because urinary iodine values from populations were not normally distributed; therefore, the median values were used rather than the mean. An indicator for iodine deficiency elimination is a median iodine concentration of 100 micrograms per liter, i.e., 50% of the samples should be above 100 micrograms iodine per liter urine, and not more than 20% of samples should be below 50 micrograms per liter (table 3.4).

**TABLE 3.4. CRITERIA FOR MONITORING PROGRESS  
TOWARDS ELIMINATING IDD AS A SIGNIFICANT HEALTH  
PROBLEM:**

<i>Indicator</i>	<i>Goal</i>
1. <b><i>Salt iodization</i></b> Proportion of households consuming Effectively iodized salt	> 90 %
2. <b><i>Urinary iodine</i></b> Proportion below 100 µg/l Proportion below 50 µg/l	< 50 % < 20 %
3. <b><i>Thyroid size</i></b> In school children 6 – 12 years of age: Proportion with enlarged thyroid , by palpation or ultrasound	< 5 %
4. <b><i>Neonatal TSH</i></b> Proportion with levels > 5 mU/l whole blood	< 3 %

The urinary iodine concentration is currently the most practical biochemical marker for iodine nutrition. It assesses iodine nutrition only at the time of measurement, whereas thyroid size reflects iodine nutrition over months or years. Therefore, populations may have attained iodine sufficiency by median urinary iodine concentration, yet goitre may persist, even in children.

Accordingly, the new category of > 300 micrograms iodine/L was proposed for addition to the classification of iodine nutrition by urinary iodine concentration as "more than adequate" by WHO in 1999.

### **3.4.1.2.2. BLOOD ASSAYS**

Two blood constituents, thyrotropin (TSH) and thyroglobulin (Tg), can serve as surveillance indicators. In a population survey, blood spots on filter paper or serum samples can be used to measure TSH. Determining serum concentrations of the thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3), is usually not recommended for monitoring iodine nutrition, because these tests are more cumbersome, more expensive, and less sensitive as indicators.

#### **TSH**

Few drops of whole blood were collected on filter paper by a finger prick from all subjects. Blood spots were dried and stored in a plastic bag. They were transported personally from India to Sydney and were stable.

#### **BIOCLONE NEONATAL TSH ELISA METHOD**

TSH levels of the blood spot were measured using commercially available Bioclone neonatal TSH Elisa kits for the quantitative determination of TSH.

#### **PRINCIPLES:**

It is an enzyme-linked immunoassay incorporating a biotinylated anti-TSH polyclonal antibody (Antibody reagent) and an anti-TSH monoclonal antibody bound to the microwells. It is an amplified method, utilising the biotin-streptavidin linkage to increase the signal generated. The polyclonal-monoclonal pair gives rapid incubation times and enhanced specificity. The TSH is first eluted from the blood spot and at the same time the eluted TSH binds to the anti-TSH antibody on the microwell. During the next incubation, a

'sandwich' is formed between added biotinylated antibody, the antibody on the microwell and the eluted sample TSH antigen. The plate is washed to remove unbound material. Streptavidin-peroxidase (Amplification Reagent) is then added and binds to the biotinylated antibody at many sites. The plate is washed again to remove unbound streptavidin-peroxidase, then 3,3', 5,5'-tetramethylbenzidine (TMB) substrate solution is added. The substrate solution reacts with the enzyme to produce color in direct proportion to the amount of antigen in the sample. From photometric absorbance readings a standard curve is constructed and the TSH in patient samples can be quantified.

**REAGENTS:**

***NEONATAL TSH Coated microwell***

There are 5 x 96 well plate holder containing microwells coated with mouse anti TSH monoclonal antibody. Ready to use.

***NEONATAL TSH Antibody Reagent***

A bottle containing 50 ml biotinylated sheep anti-TSH polyclonal antibody in a buffered solution containing bovine serum albumin, non-immune animal sera and a blue dye. Contained sodium azide, 0.2% w/v and thiomersal, 0.01% w/v. Ready to use.

***NEONATAL TSH Amplification Reagent***

A bottle containing 50 ml streptavidin-peroxidase (streptavidin from *S. avidinii*) in a buffered solution containing bovine serum albumin and a violet dye. Contained Bronidox L 0.2% v/v and thiomersal, 0.02% w/v. Ready to use.

***Elution Buffer***

A bottle containing 50 ml of a buffer with bovine serum albumin. Contained sodium azide, 0.1% w/v.

***Wash Concentrate***

250 ml of a 15 x concentrated wash solution. Contained thiomersal, 0.15% w/v.

***Substrate Solution***

A bottle containing 50 ml of 3, 3', 5, 5' tetramethylbenzidine (TMB) and hydrogen peroxide in a stabilising solution.

***Stopping Solution***

A bottle containing 30 ml of 1 M sulphuric acid.

***Human TSH Blood Spots- Standards and Controls***

One set of Standards and Controls supplied on a sheet of filter paper. Contained dried blood spots with six different TSH standard levels, labelled A through to F (nominal values: 0, 5, 10, 25, 100, 250 m IU/L whole blood), and two controls, labelled 1 and 2 (nominal values: 15 and 60 m IU/L)

***Equipment***

Disc punch

Forceps

Repeating pipette

One litre measuring cylinder

Absorbent tissue

Timer

Plate Shaker

Microplate reader with 650 nm and 405 filter

**Procedure**

1. Place a single 3-mm spot (standard, control and samples) in a well. Record the position of each sample on the plate.
2. Pipette 100  $\mu$ l of Elution Buffer in to all wells.
3. Cover plate with lid and incubate for one hour on a plate shaker at room temperature.
4. Pipette 100  $\mu$ l of NEONATAL TSH Antibody Reagent (blue) in to wells.
5. Cover plate with lid and incubate for one hour on a plate shaker at room temperature.
6. After incubation, remove discs.
7. Wash the plate. Aspirate the liquid and rinse each well four times with 250  $\mu$ l of wash solution. After the final wash, invert the plate and tap firmly on absorbent tissue to remove any remaining wash buffer. Ensure that no air bubbles remain in the wells before proceeding to the next step.
8. Pipette 100  $\mu$ l NEONATAL TSH Amplification Reagent (violet) in to each well.
9. Cover the plate and incubate for 10 minutes on a plate shaker at room temperature.
10. After incubation, wash the plate as before. Invert and tap the plate firmly on absorbent tissue.

**Kinetic Read**

1. Pipette 100  $\mu$ l Substrate Solution in to all wells. This step should be carried out smoothly and without interruption. Timing of incubation step is measured from the addition of Substrate Solution to the first well.
2. Cover the plate with lid and incubate for 10 minutes stationary at room temperature.

3. Pipette 50  $\mu$ l Stopping Solution in to all wells in the same timed sequence as for Substrate Solution addition
4. Place the plate in to the microplate reader and carry out reading at 450 nm within 5 minutes of the last step.

### ***Calculations***

The software plotted a standard curve upon manual entry of standard and unknown samples values from the reader printout and calculation were done automatically.

The cut-off TSH values for describing the severity of IDD as a public health problem are not available for the target population of school-aged children and adults hence those available for neonates (Table 3.3.) were applied to studied population.

### ***Interpretation, presentation of results and availability of reference data:***

Biochemical parameters (UI and blood TSH) are measured on a continuous scale but their results do not have normal Gaussian distribution. The use of means and standard deviations alone may be inappropriate (unless logarithmic transformation of means and standard deviations to get normal distribution is performed) hence presentation of median or other centiles is ideal.

Interpretation of IDD status depends on availability of reference data as these help in establishing cut-off values and prevalence levels for use in identifying public health problems but the data for biochemical indicators in adults are not available. It is recommended (WHO) that the full distribution of results is presented using cut-off points to delineate the upper and lower tail of distribution.

### **3.4.2. DIETARY GOITROGENS ANALYSIS**

Cereals and pulses were studied for the amount of goitrogens present in them. The main cereals examined were wheat, rice, pearl millet, jowar, maize and nagli. The pulses were tuver dal (used on daily basis), udad dal, moong dal. The goitrogens studied were vitexin, apigenin, glycosyl-vitexin, luteolin, Aplin and luteolin-7-glucoside.

### **METHOD**

Cereal grains were powdered and subjected to soxhlet extraction for 3 hours using methanol as the extracting solvent. The extracts were then analyzed by liquid chromatography-mass spectrophotometry without any attempt to pre-concentrate. The mass spectrometer (Hewlett Packard-1100) was operated in selected ion monitoring mode to monitor ions such as 433 (vitexin), 449 (luteolin-7-glucoside), 565 (Aplin), 287 (luteolin) and 271 (apigenin).

### **3.4.3. ASSESSMENT OF PEM STATUS:**

The body standing height and weight was measured for each subject and for comparisons with WHO normative data, it was necessary to derive three indices up to the age of 18 years. These indices were weight-for-height, height-for-age and weight-for-age that gave rise to three main indicators of growth like wasting, stunting and undernutrition respectively based on Z-score system.

#### **3.4.3.1. WHO INDICATORS FOR CHILD GROWTH**

In children the three most commonly used anthropometric indices to assess their growth status are weight-for-height, height-for-age and weight-for-age (WHO, 1995). These anthropometric indices were interpreted as follows:

**Low weight-for-height:** Wasting indicated a recent and severe process of weight loss, which could be associated with acute starvation and severe disease. However, wasting might be the result of a chronic unfavorable condition. Usually when there is no severe food shortage, the prevalence of wasting is below 5%, even in poor countries but higher prevalence is reported for the Indian subcontinent. On the severity index, prevalence between 10-14% is regarded as serious, and above or equal 15% as critical.

**High weight-for-height:** "Overweight" is the preferred term for describing high weight-for-height. Even though there is a strong correlation between high weight-for-height and obesity as measured by adiposity, greater lean body mass can also contribute to high weight-for-height.

**Low height-for-age:** Stunted growth reflects a process of failure to reach linear growth potential as a result of sub-optimal health and/or nutritional conditions. On a population basis, high levels of stunting are associated with poor socioeconomic conditions and increased risk of frequent and early exposure to adverse conditions such as illness and/or inappropriate feeding practices. The worldwide variation of the prevalence of low height-for-age is considerable, ranging from 5% to 65% among the less developed countries. Therefore, for older children, it reflects a state of "having failed to grow" or "being stunted".

**Low weight-for-age:** Weight-for-age reflects body mass relative to chronological age. Both influence it: the height of the child (height-for-age) and weight (weight-for-height). In general terms, the worldwide variation of low weight-for-age and its age distribution are similar to those of low height-for-age.



### ***International reference population***

The designation of a child as having impaired growth implies some means of comparison with a "reference" child of the same age and sex. Thus, in practical terms, anthropometric values need to be compared across individuals or populations in relation to an acceptable set of reference values.

The database uses as a basis for comparison across countries the National Center for Health Statistics (NCHS) growth reference, the so-called NCHS/WHO international reference population. The international reference growth curves were formulated in the 1970s and these were originally planned to serve as a reference for the USA. The height curves for this reference are based on standing height measurements from 2 to 18 years of age. The samples in the reference consisted of healthy well-nourished US children.

The WHO adopted the reference curves of the NCHS for international use in the late 1970s based on the then growing evidence that the growth patterns of well-fed, healthy preschool children from diverse ethnic backgrounds are very similar. Differences of genetic origin are evident for some comparisons; however, these variations are relatively minor compared with the large worldwide variation in growth related to health and nutrition.

The adoption by WHO of the NCHS-based growth curves resulted in their wide international dissemination. Throughout the 1980s, several microcomputer-based software versions of the NCHS/WHO international growth reference were developed and supported by CDC and WHO.

WHO has advised that in a given population, a high prevalence of anthropometric deficit always indicate significant health and nutritional problems. At the same time, it is not only those individuals below the cut-off

point who are at risk; the entire population is at risk, and the cut-off point should be used only to facilitate the application of the indicator.

The information on Child Growth and Malnutrition complies with the following standardized format:

- systematic use of the National Center for Health Statistics (NCHS)/WHO international reference population;
- display of growth retardation prevalence for under-5-year-olds, as measured by the proportion of weight-for-age, height-for-age and weight-for-height below -2 and -3 standard deviations (Standard Deviations) (Z- scores);
- display of the prevalence of overweight, as measured by the proportion of children with weight-for-height above +2 Z-scores;
- display of Z-score means and Standard Deviations (SD) for the three indices;
- Stratification of the results according to age, sex, region, and rural/urban strata.

### ***WHO Z-score or standard deviation classification system***

There are three different WHO systems by which a group of children can be compared to the reference population: Z-scores (standard deviation scores), percentiles, and percent of median. For population-based assessment - including surveys and nutritional surveillance - the Z score is widely recognized as the best system for analysis and presentation of anthropometric data because of its advantages compared to the other methods.

We interpreted our data by using the Z-score classification system for three indices: weight-for-height, height-for-age and weight-for-age. The Z-score system expressed the anthropometric value as a number of standard

deviations or Z-scores below or above the reference mean or median value. For our population-based studies, this system was a major advantage that means a group of Z-scores were subjected to summary statistics such as the mean and standard deviation. The formula for calculating the Z score was:

$$\text{Z-score} = \frac{\text{observed value} - \text{median value of the reference populations}}{\text{standard deviation value of reference population}}$$

***Interpreting the results in terms of Z-scores had several advantages:***

The Z-score scale was linear and sex-independent.

***Cut-off points and summary statistics***

Our population-based assessment used cut-off-based prevalence and the summary statistics of the Z-scores: mean, standard deviation and frequency distribution where required.

***Prevalence-based reporting:***

Prevalence-based data was reported using a cut-off value, -2 and +2 Z-scores. The rationale for this was the statistical definition of the central 95% of a distribution as the "normal" range.

The WHO Global Database on Child Growth and Malnutrition uses a Z-score cut-off point of -2 SD to classify low weight-for-age, low height-for-age and low weight-for-height as moderate and severe under nutrition, and -3 SD to define severe under nutrition. The cut-off point of +2 SD classifies high weight-for-height as overweight in children.

The use of -2 Z-scores as a cut-off implied that 2.3% of the reference population would be classified as malnourished even if they were truly "healthy" individuals with no growth impairment. Hence, 2.3% could be

regarded as the baseline or expected prevalence. We did not subtract this baseline value in order to calculate the prevalence above normal from the observed value. This was not a problem because prevalence of underweight, wasting and stunting in our deprived populations was much higher than 2.3%.

The prevalence ranges used by WHO to classify levels of stunting, underweight, and wasting are shown in Table 3.5.

**TABLE 3.5. WHO CLASSIFICATION FOR SEVERITY OF MALNUTRITION ASSESSMENT BY PREVALENCE RANGES AMONG CHILDREN UNDER 5 YEARS OF AGE**

Indicator	Severity of malnutrition by prevalence ranges (%)			
	Low	Medium	High	Very high
Stunting	<20%	20-29	30-39	• 40
Underweight	<10	10-19	20-29	• 30
Wasting	<5	5-9	10-14	• 15

#### **3.4.3.2. Additional Anthropometric Indices:**

##### **Mid- Upper-Arm- Circumference (MUAC)**

It was accurately measured to the nearest cm with a non-extensible, calibrated and flexible steel tape at the mid-point between acromion and olecranon process with the right arm relaxed and hanging by the side. The tape was positioned at 90° angle to the long axis of the arm and did not compress the tissues. MUAC is a function of muscle mass, skin, subcutaneous fat, bone, and the neurovascular bundle of arm, and of these parameters, the amount of muscle, skin, and fat are determined by nutritional status.

**Thigh circumference (TC)**

It was measured at a level 15 cm proximal to the adductor tubercle of the right femur with the subject lying supine in bed with thigh and leg fully extended. The tape just made contact with the skin so that there was no compression to underlying soft tissue and was placed at 90° angle to the long axis of the limb segment.

**Triceps Skinfold Thickness (TSF)**

It was measured to the nearest mm with a Vernier/ Lange skinfold caliper having a pressure of 10g/ mm<sup>2</sup> of contact surface area. The measurement was taken over the triceps muscle halfway between the elbow and the acromion process of the scapula, with the skinfold parallel to the longitudinal axis of the upper arm. TSF is the direct measurement of the subcutaneous adipose tissue thickness.

**Derived Indices:**

**Arm Fat Area (AFA) (cm<sup>2</sup>):**

It was calculated by the formula

$$AFA = \frac{MUAC \cdot TSF}{2} - \frac{\pi \cdot (TSF)^2}{4}$$

**Arm muscle area (AMA) (cm<sup>2</sup>):**

It was calculated by the formula

$$AMA = \frac{[MUAC - \pi \cdot TSF]^2}{4\pi}$$

Where MUAC = mid- upper-arm-circumference (cm), TSF =triceps skinfold thickness (cm).

**Body mass index (BMI) :**

$$\text{BMI (kg/ m}^2\text{)} = \frac{\text{Weight (kg)}}{\text{Height (m}^2\text{)}}$$

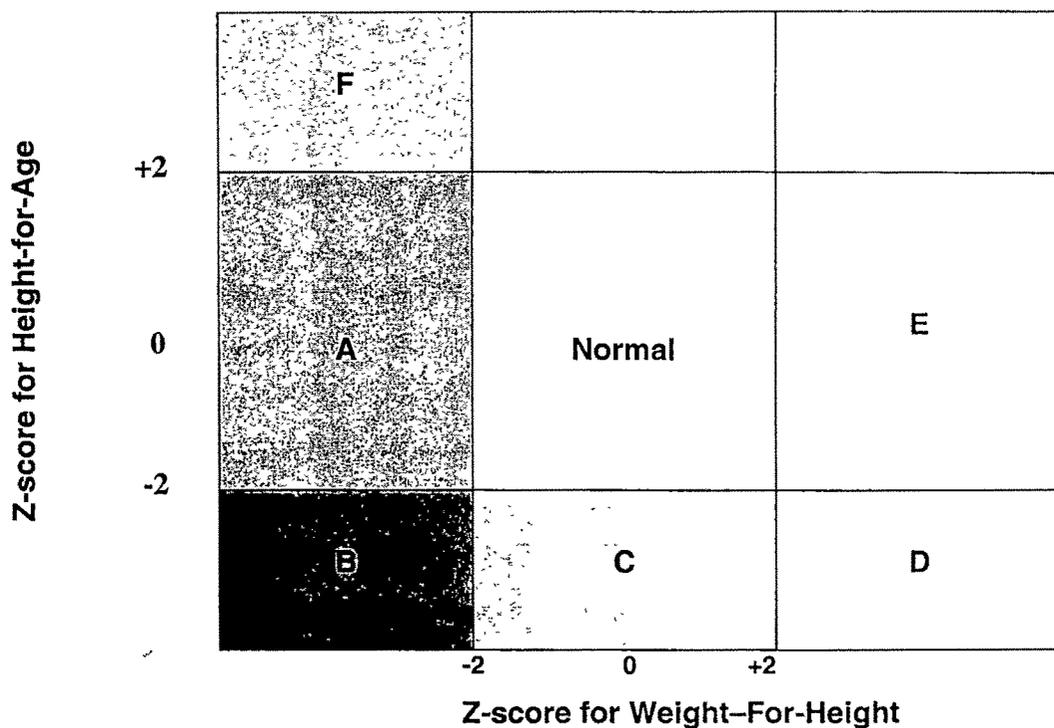
***General Principles used in the application of anthropometric indices***

Weight provided the assessment of overall energy balance. Height (standing) was used as an indicator to estimate past and chronic malnutrition. The Arm Muscle Area (AMA) reflected the reserves of muscle protein and was the measurement of lean body mass whereas Arm Fat Area (AFA) reflected the subcutaneous adipose tissue. Body Mass Index (BMI) was used with age-independent cut-off values to define thinness. The preferred indicator for thinness was weight-for-height in children (below 10 years) while BMI-for-age was used in adolescence.

**3.4.3.3. INDICATORS OF PEM THRESHOLD**

***Waterlow Classification Scheme: Combination height-for-age & weight-for-height***

This scheme uses plots to combine variables where index weight-for-height is the abscissa and height-for-age index is plotted on the ordinate as shown below.



**TABLE 3.6. WATERLOW CLASSIFICATION PRESENTS AN OVERVIEW OF THE THRESHOLDS OF MALNUTRITION IN A POPULATION IN TO ONE OF THE SIX GROUPS FROM A TO F**

Code	Combination of indices		Interpretation of Nutritional status
	Height-for-age	Weight-for-Height	
A	Normal	Low	Wasted
B	Low	Low	Stunted + wasted
C	Low	Normal	Stunted
D	Low	High	Stunted + obese
E	Normal	High	Obese
F	High	Low	wasted

### **3.5. SURVEILLANCE METHODS**

#### **3.5.1. ASSESSMENT OF IDD PREVALENCE**

India being a large country needed a prevalence survey by States that was further refined by districts. The cluster sampling units were made up of groups of study units in districts, villages and schools. Two stage cluster survey was preferred over "probability proportionate to size (PPS)". PPS is systematic selection from cumulative population list whereas in two-stage cluster sampling, there is random selection of schools. The sample size was calculated for the prevalence-based survey on relative and absolute precision. The sample size for Baroda district and for Dang district was based on the goitre prevalence of less than 5% in Baroda and 44% in Dang as reported by Dr VK Desai in 1994. Thirty clusters ensure a valid prevalence estimate (WHO, 1994). Usually recommended sample size for the collection of urine or blood spots is 300 (30x10 children per cluster) but we collected these from all the studied subjects due to variability of food types and consumption of iodized salt based on caste, religion, income and many other factors. The palpation is usually advised for all subjects and ultrasound in few for confirmation but most of the subjects had small goitres of 0 and 1 grade hence we did thyroid ultrasound of all subjects. Thus each subject of the GSI was investigated for IDD by four prevalence indicators (UI, TSH, palpation and ultrasound) but in the next surveys TSH measurements were dropped as per suggestions from ICCIDD (1999).

The main target groups for surveillance were preschool children from nursery and kindergarten schools, school-age children from primary and higher secondary section of the schools and women of childbearing age and other adults in households. The school-age children were useful because of easy access and because they reflected recent rather than remote iodine nutrition. Household screening of women 15-44 years targeted a particularly important group because they were potential mothers and the most damaging effects of

iodine deficiency are on the developing fetus. The usual sampling unit was either communities or schools. Each unit was one cluster in a defined geographical area. More than thirty clusters were studied altogether to ensure a valid prevalence estimate.

**School surveys** – At the time of initial surveys we approached areas (Baroda) with no existing prevalence data hence our first survey was based on a suspicion or prediction of IDD in that location. Schools for GSI were the most feasible type in the tribal belt of Dang district in Gujarat where goitre prevalence of 44% was known. These schools were Rutambhara girls school, Saputara boys school and Vaghai Boys school. All these schools were with lodging and boarding (run by Government funds). The rural children were from Muval and Tentlav villages of Baroda district but they were recruited from household visits along with adults. The adults and children in GSII were from tribal and rural belt schools and households. Schools were selected for GSIII survey on the basis of affluence from each corner (Basil from Tandalja, Bright from Karelibaug, Bhavans from Makarpura and Tejas from Ellora Park) and Centre (Rosary from Fatehganj) of the Baroda city. For GSIV two schools were selected from Shimla, one with boarding (Cotton Bishop School) had mainly boys and another had coeducation and boarding for some but the girls were from local area attending day school. We studied few boys from boarding mainly from Thailand and local area girls. Within each cluster, a specified number (usually more than 30) of children was selected by systematic random sampling. Each child provided a urine specimen. At least 30 samples are usually needed to assess differences between clusters and to recognize localities where iodine deficiency may still be a problem. During the survey, thyroid gland was palpated for general picture of overall IDD status. Ultrasonography was also performed for all so as to get a clear picture of thyroid assessment as goitre upon palpation were mainly 0 grade. Children and adults were assessed for vitamin A deficiency by xerophthalmia and night blindness.

**Analyzing results** - Data from surveys were analyzed by computer. Microsoft Excel spreadsheets were made for each survey and sorted out for age, BSA, gender etc. The median was used as the measure of central tendency. This meant that half the results in the distribution were above the median and half below. Another approach for values that were not distributed normally was to describe a distribution in certain percentiles, e.g., the 25th and 75th percentiles. With urinary iodine values, numbers and proportions below certain values, typically 100, 50, and 20  $\mu\text{g/L}$  were indicated for full distribution.

In carrying out a survey, only a sample of individuals from the entire population was assessed, so some degree of sampling error inevitably occurred. The Ninety-five percent confidence intervals were calculated and used to reflect the range in which the true population value was likely to lie.

### **3.5.2. IDENTIFICATION OF HIGH PREVALENCE AREAS**

The first goal was to identify high prevalence goitre areas in geographic foci based on the large number of children that had goitre. Survey reports invite focus intervention efforts of Government. The main interventions for controlling IDD are fortification of salt with potassium iodate and supplementation with iodised oil as shown in chapter 2.

### **3.5.3. CRITERIA FOR MONITORING PROGRESS TOWARDS ELIMINATING IDD AS A SIGNIFICANT PUBLIC HEALTH PROBLEM**

Table 3.4. Show the recommended criteria for use as core indicators in monitoring progress towards the goal of eliminating IDD as a significant public health problem. Criteria include both IDD status indicators and a control programme process indicator. Thyroid enlargement in more than 5% and

urinary iodine proportion below 100 µg/l in more than 50% of the population (all segments) studied signals a public health concern.

#### **3.5.4. STATISTICAL METHODS:**

Proportion, mean, standard deviation, median, interquartile range have been used to describe the data as appropriate. Statistical analysis was performed using SPSS version 6.1.2. Thyroid volume was logarithmically transformed and the Kolmogorov-Smirnov test was used to verify normality of the transformed data. Differences in thyroid volume and other nutritional parameters between districts were tested using the Mann-Whitney test. T-tests were used to test for gender differences in anthropometric and clinical parameters. Linear regression analysis was used to test for association between thyroid volume and other parameters of IDD status, dietary factors and PEM variables. The Bonferroni method was used to correct the significance levels for multiple comparisons.