SUMMARY
AND
CONCLUSION
Iodine is an essential element for all forms of life. It is required for thyroid function and it is a requisite for normal growth, development and functioning of the brain and body. It is necessary in micro quantities for the synthesis of thyroid hormones by the thyroid gland. Thyroid hormones have physiological effects, causing alteration in essentially all metabolic pathways and organs; they modulate oxygen consumption and the metabolism of protein, carbohydrate, lipid and vitamins.

Normal daily requirement of iodine is 100-150 microgram for adolescents and adults. Ninety percent of daily iodine requirement is met from food, while the rest is met from drinking water.

Deficiency of dietary iodine may result in decreased synthesis of iodine containing thyroid hormone, thyroxine (T₄) and triiodothyronine (T₃), whereas prolonged and severe iodine deficiency may result in hypothyroidism (myxedema in children or cretinism in infants or young children). Iodine deficiency is the single most common cause of preventable mental retardation and brain damage in the world. Iodine cannot be stored for a long time in the body, tiny amounts must be consumed regularly, but food grown in iodine-poor soil will not provide sufficient dietary iodine. The main cause of iodine deficiency is low iodine content in food and water.

Iodine deficiency disorder (IDD) is a major public health problem in India. A district wise survey done by the Director General of Health Services (DGHS, 2000) shows that the prevalence rate of goiter in Gujarat ranged from 5.9 to 44%.

The most well known effect of iodine deficiency is goiter – swelling of the thyroid gland in the neck. However, it was realized that iodine deficiency causes a wide spectrum of disorders such as increased fetal loss, infant and childhood mortality, congenital anomalies and psychomotor disorders etc.

Goitrogens are certain chemical substances, which interfere with the metabolism of iodine in the body. Even excess of iodine is known to act as a goitrogen. Certain varieties of common foodstuff such as sorghum, finger millet, mustard and groundnut also are known to contain a fair amount of goitrogens.
The thiocyanate ion has a molecular volume and charge similar to that of iodide ion and competes with iodide for uptake into the thyroid gland (Thilly et al, 1993).

Studies indicate that very little information is available on iodine deficiency disorder in the local areas of Anand and Vallabh Vidyanagar. Therefore the present investigation was planned in three parts. These broadly consisted of water and food analysis, animal experimentation and human studies.

Studies were carried out in the two local cities of Anand and Vallabh Vidyanagar.

In the beginning of the study, iodine content was estimated from the most common sources of drinking water, commonly consumed foodstuff and from common salt. Cooking and storage losses of iodine were also estimated from the salt samples.

A total of twenty-three water samples from different borewells of Anand Municipality (n=13) and Municipal Burrough, Vallabh Vidyanagar (n=10) were collected and analyzed in triplicate for iodine content. Samples of foodstuff and salt were also collected from the local market of Vallabh Vidyanagar and analyzed for iodine content. To observe the extent of loss of iodine content from different varieties of salt samples, these were stored at room temperature for twelve months and storage loss was studied, samples were weighed in triplicate on every occasion and used for estimation of iodine content. Salt samples were also tested for iodine loss during cooking. Three varieties of salt namely, Tata salt, Shudh salt, and Annapoorana salt (commonly consumed in the local area) were cooked at varying intervals (0, 2, 5, 7, 10, 12, 15, 20, and 25 minutes) at boiling temperature in a fixed amount of water using both the open pan and closed pan methods. Iodine content of samples were estimated by the titrimetric method.

Iodine content of drinking water from the most common sources (Anand and Vallabh Vidyanagar) ranged from 28.61 to 38.29 μg/l indicating sufficient iodine levels and supporting the low clinical prevalence. Iodine content of foodstuff ranged from 2.03 to 74.88 μg/100g. Cereals (30.44-74.47 μg/100g), and
pulses (25.19-51.62 μg/100g) showed higher iodine content compared to lower levels in fruits (7.56-16.37μg/100g) and vegetables (2.10-8.12μg/100g), the least iodine content being observed in vegetables. Milk powder showed the highest iodine content (154.93μg/100g) among all the food samples analyzed.

Various branded powdered salt samples (9 in number) and one unbranded locally available crystal salt were analysed for iodine content. Salt iodine content in 8 of the branded (iodine labeled) powdered salt ranged from 8.47 to 50.43 ppm. One of the locally available branded salt samples did not show iodine labeling on the package and contained no iodine. Similarly the single locally available crystalline salt also did not show any iodine content.

Iodine levels in salt decreased with storage and at the end of 12 months the average loss of iodine was 17.43-23.28 %. Cooking losses of iodine from salt were 14.86% in open pan cooking and 24.84% for closed pan cooking, which increased with time of cooking. This is expected, as iodine is a volatile compound.

Animal experiments were conducted to explore the effects of different dietary levels of iodine on the developmental, metabolic and teratogenic aspects of albino rats. Three animal experiments were planned and conducted.

The present study was planned to determine the effect of different dietary levels of iodine and goitrogen on the growth of weanling albino rats for six weeks. In this animal experiment, weanling albino rats (32 in number, 6 females and 2 males in each group) were fed low iodine, normal iodine, normal iodine plus goitrogen (thiocyanate) or high iodine supplemented to a corn-starch based diet containing 10% casein protein, for six weeks to study the growth pattern, in the first phase of the experiment.

In the second phase animals from the first phase were continued on the same diet for a period of 37 weeks during which they were bred thrice within their own group to observe the effect of different dietary iodine levels on reproductive performance. Animals were then sacrificed, organ weights recorded, tissue protein, enzyme, glucose, hormonal levels and lipid profile were estimated.
In the second animal experiment, the progeny obtained from experiment-1 were studied for assessing the reproductive performance of the first generation. Animals were grouped into four groups (5 females and 2 males in each group) and were continued on the same respective diets as their parental generation for a period of thirty-one weeks. During the experimental period, animals were bred twice to observe their reproductive performance, after which they were sacrificed and parameters as mentioned in the first experiment were repeated.

The third animal experiment was planned to assess the protective effect of antioxidants such as vitamin C, E, and A as well as β-carotene against the toxicity induced by an anti-thyroid drug (methimazole – MMI). Thirty adult male rats (150-185 g) were divided into six groups, control group was fed a normal diet based on 10% casein protein. Other groups were fed control diet along with MMI (1g/kg diet), MMI plus vitamin C (2.5g/kg diet), MMI plus vitamin E (2.5g/kg diet), MMI plus vitamin A (80 mg/kg diet) or MMI plus β-carotene (24 mg/kg diet) supplemented diets for a period of six weeks. At the end of six weeks of experimental feeding animals were sacrificed, organ weights recorded, tissue protein, enzyme, glucose, hormonal levels and lipid profile were estimated.

At the end of the experimental feeding (six weeks) animals were sacrificed. Thyroid weights, serum thyroxine (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH), cholesterol, triglyceride (TG), protein and liver MDA (indicator of lipid peroxidation) levels were determined using standard laboratory procedures.

Weanling albino rats fed varying levels of iodine, with or without goitrogen indicated that for a short term feeding period of six weeks, feeding high iodine led to higher diet intake and weight gain even higher than the group fed normal iodine although no significant differences were observed. Gain in weight was poor in both the low iodine group and the normal iodine plus thiocyanate group, between the two the thiocyanate group showed lower weight gain.
These animals were continued further with their experimental feeding in order to observe their reproductive performance, then sacrificed at the end of 37 weeks and tissue parameters analysed.

Final body weights and weight gain at the end of 37 weeks of feeding showed a different trend compared to the short term 6-week feeding period. Body weights (173.50g) and weight gain were highest in the normal iodine group and lower in the low iodine or high iodine group by 22.67 and 21.17g, respectively. Gain in weight was least in the normal iodine plus thiocyanate group, 64.5g lower than the normal iodine group.

Liver and kidney weights were significantly higher in the normal iodine group while they were lower in the low or high iodine or thiocyanate group. A reverse trend was seen for thyroid weights.

For all the plasma parameters studied at the end of 37 weeks of feeding, blood haemoglobin serum, glucose, protein, HDL, T3 and T4 levels were lowered due to low or excess iodine or thiocyanate feeding in the diet, all these parameters being significantly higher in the animals fed normal iodine. Similarly feeding low or high iodine or thiocyanate in the diet significantly increased serum total cholesterol, LDL, triglycerides, VLDL, ALP, LDH and TSH levels as well as liver MDA levels (a marker of liver lipid peroxidation activity) compared to the normal iodine group. The low iodine group showed a marked increase in LDH levels feeding thiocyanate particularly increased TSH, LDL, TG and LDH levels while it decreased serum protein, T3 and T4 levels.

Histopathological study indicated hypertrophic changes in the thyroid gland on low or high iodine or thiocyanate feeding.

Animals went through three breeding cycles in this phase of the experiment. In the first breeding cycle (at 12 weeks of the feeding experiment) females in all groups conceived indicating that at this stage it was too early for the dietary changes to show any effect on reproductive performance. In fact in the first breeding cycle, feeding high iodine produced higher litter size and litter weights even higher compared to the normal iodine group. The second and third
breeding cycles produced results only in the normal iodine group, the low or high iodine or thiocynate fed rats were not able to conceive.

The first generation animals of the parental generation fed low, normal, normal+goitrogen or high iodine diets were taken for the second animal experiment. A total of 28 animals, 7 animals in each group (5 females +2 males) were continued on the respective low, normal, normal+thiocyanate or high iodine diets as their respective parents. Feeding was continued for 31 weeks, during which animals went through two breeding cycles within their own dietary groups. After 31 weeks, animals were sacrificed and body weights and organ weights recorded and tissue parameters analysed.

The same trend as observed in the parental generation was observed in the first generation animals fed different levels of dietary iodine or goitrogen, indicating that feeding low iodine or goitrogen in the diet can successively affect generations, in their growth, development and reproductive performance.

The third experiment was conducted to investigate the protective effect of antioxidants against methimazole (MMI) induced hypothyroidism in adult albino male rats.

MMI treatment significantly (p<0.01) depressed body weight, weight gain, liver weight, liver moisture and kidney weight. It also significantly depressed serum T3, T4 levels and increased serum TSH and liver MDA levels. A significant increase was also observed in plasma lipid profile, except HDL levels. MMI treatment also significantly decreased serum and liver protein, vitamin C, E and A levels.

Feeding the four antioxidants namely vitamin C, E, A and β-carotene produced a protective lowering or rise as the case may be, against the toxicity induced by MMI. All the four antioxidants produced a protective increase in body weights and weight gain countering MMI toxicity. Liver and kidney weights showed a protective increase in weight on feeding dietary antioxidants. Rats which received vitamin C, vitamin E, vitamin A or β-Carotene along with MMI, showed reduced thyroid weights (25.61-39.70 %) compared to MMI group
(P<0.001), lesser suppression of T₃ and T₄ levels (20.00-39.04 and 94.33-122.64 % higher respectively compared to MMI) and lesser increase in TG, cholesterol and MDA levels (25.23-52.24 %, 26.19-35.57 % and 17.97-21.01 % lower than MMI, respectively), which were statistically significant. The data suggests that the positive effect of antioxidants on thyroid gland could be due to the direct involvement of antioxidants on the thyroid gland.

Unlike serum T₃ and T₄ levels, TSH levels increased against MMI toxicity and feeding the antioxidants decreased TSH levels.

Serum protein, vitamin C, E and A levels decreased against MMI toxicity whereas feeding the antioxidants showed a protective effect. Similar results were observed for all the liver parameters such as protein, vitamin C, E and A.

Among the four antioxidant studied, vitamin C showed a protective effect for a larger number of parameters compared to vitamin E, A or β-carotene. Vitamin C feeding produced a protective lowering of thyroid weights as well as serum total cholesterol and T₄ levels. A rise in serum protein as well as serum and liver vitamin C levels were observed specially for vitamin C against MMI toxicity.

Vitamin E feeding produced a protective lowering of serum triglyceride levels and a raise in serum T₃ and serum vitamin E levels. Feeding vitamin A protectively increased serum T₃ as well as serum and liver vitamin A levels against MMI induced toxicity. Feeding β-carotene led to a protective decrease in serum cholesterol levels and a rise in liver protein as well as serum and liver vitamin A levels.

Among the four protective agents vitamin C lowered thyroid weights while both vitamin E and A produced a rise in serum T₃ levels, in the context of thyroid toxicity.
Human studies on iodine deficiency disorder were conducted on 6-12 year old school children and normal and hypothyroidic adult subjects of the local population.

The present study was carried out among school children (6-12 years) in the semi-urban area of Vallabh Vidyanagar town. Out of nine schools in the vicinity, three schools were randomly selected for the study. From these, a total 1596 children of 6-12 years including 818 boys and 778 girls were covered for the prevalence of clinical signs (by palpation) of iodine deficiency disorder. Further from the 1596 subjects covered, every 4th child was selected and thus a total of 402 children were studied for the following parameters: Anthropometry, urinary iodine levels as well iodine content of salt samples from their household. From the 1596 subjects children with positive clinical signs of iodine deficiency disorder (as per the standard given by WHO, 1999) were enrolled. Estimation of urinary iodine levels was carried out from the clinically identified cases of goiter for confirmation of iodine deficiency in them. The mothers of these subjects were subjected to pre-tested questionnaire on awareness regarding iodized salt, type and quantity of salt purchased and consumption of goitrogenic foods. Further 46 salt shops in the vicinity were covered where the shopkeepers were questioned on the type of salt sold, type of packing, frequency of salt procurement, storage of salt etc.

The overall prevalence rate of goitre among 6-12 year school children in the present study was 6.0150% (96/1596). At the same time prevalence of grade-II goitre was negligible in Vallabh Vidyanagar, indicating that the degree of prevalence was not very high.

A cross section of the children (n=402) studied showed mean height and weight to be 130.03 cm and 25.01 kg, respectively, no significant differences were observed in the mean height and weight between boys and girls.

The mean urinary iodine excretion of the 402 children was correlated with the iodine levels of salt consumed in their household. The mean urinary iodine excretion level was found to be 157.48 μg/l in these subjects. A range of 100-200
μg/l indicates adequate intake of iodine and optimal iodine nutrition as given by Delange et al (2002).

Looking at details, about 310 school children showed normal UIE, of these a majority (n=240) consumed adequate iodine containing salt, only a few (n=70) consumed low iodine salt. Majority of the subjects showing clinical signs of goitre had low urinary iodine excretion levels, thus confirming that goitre was diagnosed correctly.

From 1596 subjects screened 96 were identified with clinical IDD. Out of these 96 subjects studied, 89 suffered from grade I goitre. Out of these, 39 subjects were found to be mildly deficient (50-99 μg/l UIE) while 50 subjects were found to be moderately deficient (20-49 μg/l UIE) on the basis of urinary iodine excretion. A total of 92 subjects (95.88%) showed urinary iodine excretion levels below 100 μg/l.

Demographic data indicated that the households consisted of mainly Hindu families from a middle income group. Most of the household (82.3%) were aware of iodized salt as the majority of the mothers in these households were literate (86.9%). Most of the families purchased packed salt (96.6%), monthly (50%) in 1 kg packs (81.3%).

Information collected from 46 shopkeepers selling salt in the local vicinity indicated that a majority of the shops (82.6%) sold iodized salt. About 95.7% of the shops stored salt inside the rooms a majority of them indicated that (78.30%) the community in general purchased iodized salt.

Thus regular monitoring of the iodine content of salt at the consumer level is essential for the prevention and elimination of IDD and there is a need to improve awareness of this at all levels.

In this part of the study investigations were carried out to explore the effect of iodine deficiency in adult human beings. A total of 79 adult subjects (30 normal and 49 suffering from hypothyroidism) aged 30-70 years were selected from Vallabh Vidyanagar and Anand, Gujarat.
Adopting purposive sampling method the subjects were selected. Fasting blood and urine samples were collected for biochemical analysis. In addition subjects were asked to fill pre-tested questionnaires regarding awareness of IDD, consumption of iodized salt and frequency of consumption of goitrogenic foods in the household.

Biochemical analysis indicated that thyroid hormones (T<sub>3</sub>, T<sub>4</sub>) levels were significantly (P < 0.01) lower while TSH levels were significantly (P < 0.01) higher in the subjects suffering from hypothyroidism compared to normal subjects. Urinary iodine excretion levels were significantly (P < 0.05) lower in these subjects compared to normal subjects. This confirms the state of hypothyroidism.

Among the subjects, a majority were aware of iodized salt (hypothyroidic-67.34% compared to normal 76.66%). Majority of the normal subjects (73.33%) used iodized salt while only 46.93% of hypothyroidic subjects used iodized salt. This aspect is interesting and needs to be further investigated.

Conclusion

The study indicates adequate iodine levels in drinking water, food and salt samples commonly consumed in the local area of Vallabh Vidyanagar. This in turn indicates adequate iodine content of soil, indirectly. Loss of iodine from salt during storage for 12 months or during cooking was less than 30% and is not a cause of worry.

Animal experiments indicate that both low or high iodine or goitrogens in the diet can affect growth and tissue hormonal levels and alter the lipid profile, thus increasing the risk of cardiovascular disease. These also affect the reproductive performance of the animals. Further successive generations are affected by low or high iodine or goitrogens in the diet.

Antioxidant consumption in the diet should be promoted as all the four antioxidants which were studied here namely vitamin C, E, A and β-carotene showed protective effects, vitamin C showed a greater effect compared to the other three.
The human study on 6-12 year old children indicated a relatively low prevalence (6.0%) in school children. Therefore the local community here at present is not at any severe risk of developing IDD. Studying the iodine content of household salt and correlating it with urinary iodine excretion in school children indicated that a majority of the children showed normal urinary iodine excretion levels i.e. above 100 μg/dl and also consumed salt with adequate iodine content whereas children showing mild or moderate iodine deficiency on the basis of urinary iodine excretion consumed low iodine salt (<15 ppm), indicating that urinary excretion of iodine was a good indicator of the salt iodine intake. Clinical identification was in keeping with urinary iodine excretion levels in the IDD deficient subjects and the former is a useful tool for the identification of IDD in the community. Adult human hypothyroidic subjects showed lower serum T₃, T₄ levels and higher TSH levels compared to the normal subjects studied in keeping with the general pattern observed all over the world, in this condition.