Chronic Intake of Iodized Retail Table Salt(S) As Reflected On Thyroid Function and Certain Metabolic Aspects in Experimental Rats

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Abstract: Effective salt iodization is a prerequisite measure to combat disorders of iodine deficiency. However, it appears that the problem still exists. Iodized table salts (fine and coarse retailed forms) which are available in the Egyptian market were selected to test their effect, according to their iodization level. Male albino rats were used, divided into four groups and received oral salt solutions in different concentration levels for 90 days as follows; group I: served as control and received no salt solution, group II: referred to low iodine dose, group III: as moderate iodine dose, group IV: expressed as high iodine dose. Body weights were recorded and blood samples were collected, at the start of the study and at 1 month interval for 3 successive months' and processed for determination of thyroid hormones, mainly plasma free T3, fT4, TSH, fructosamine, insulin, and qualitative C-reactive protein (CRP), being followed by histological examination of thyroid tissues. Salt intake of different iodine concentrations induced significant increase in TSH, fT4, and CRP. However, fT3, fructosamine, and insulin demonstrated significant decrease.

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Key words Retail iodized table salt, fT3, fT4, TSH, Fructosamine, Insulin, CRP

1. Introduction

Thyroid disorders are common endocrine problems encountered in the African continent [1]. Sever iodine deficiency may lead to certain metabolic disorders which include endemic goiter, hypothyroidism, cretinism, decreased fertility rate, increased infant mortality, and mental retardation [2]. Despite substantial progress against iodine deficiency diseases (IDD), it is estimated that 125 million people in Africa (20% of the population) remain iodine deficient [3]. Nutritional iodine status therefore represents an important factor dealing with thyroid dysfunction and thyroid autoimmunity [4]. WHO indicated that more than two-thirds of the five billion people living in countries affected by IDD have now access to iodized salt [5] However, excessive intake of dietary iodine may be associated with increased risk for hypothyroidism and/or hyperthyroidism [6-8], as reflected by increased thyroid volume, specifically in children, due to hyperthyroidism state [9].

Governmental regulations specify salt iodination for at least 50 mg of iodine per kg of salt (50 ppm), in a consideration for the probability of iodine loss during storage and distribution, keeping therefore a level of not less than 15 mg/kg of iodine [10].

Population-based studies of adults have shown that the prevalence and incidence of thyroid diseases differ from one region to other, varying with regional iodine intake [11, 12]. In developing countries with moderate or greater IDD, hypothyroidism due to iodine substrate insufficiency is the more common of the two [13, 14], and more common in individuals from a lower socio-economic status [14]. In support of these findings, it has been observed that in areas of moderate deficiency, as regional iodine intake increases, the incidence of hyperthyroidism gradually declines while hypothyroidism increases [15-18]. For example, following the initiation of iodine supplementation in deficient regions, there is a marked rise in certain disturbances, represented by the incidence formation of thyroglobulin and thyroperoxidase autoantibodies [16, 17].

Moreover, individuals having hyperthyroid state may suffer from impaired glucose tolerance (IGT), as observed during oral glucose loading. This may be attributed to abnormal glucose metabolism in muscle, fat, and liver, or by the sluggish secretion of insulin and accelerated degradation. It may be also due to increased glucose absorption by a hyperkinetic gastrointestinal tract [19].

Previously, a decade ago, monitoring of the iodine concentration of household salt indicated that 62.4% of households used adequately iodized salt having at least 15 ppm iodine [10], which may refer to under-iodized process. Due to the frequent occurrence of thyroid disorders, proper monitoring and control of iodine intake to the population is a cost-effective alternative for diagnosis and to fix therapeutic measures for individuals suffering from thyroid disease.

Hence, the present study aimed mainly to monitor the effect of low or high iodized salt intake, using salts

with different iodization levels already available in the Egyptian markets, on thyroid hormones profile and glucose homeostasis. For such reason, first, we evaluated iodine content of retail fine and coarse table salts of different brands sold in Egypt. Second, evaluation of thyroid function where plasma fT3, fT4, and TSH were performed, additionally, plasma fructosamine and insulin, and finally CRP in groups of experimental rats, which have received iodized salt for 3 successive months'.

Table salt iodine content

Samples of retail table salt, reclaimed to be iodized, being consumed in home either in the country and the urban areas were tested and showed different concentrations of iodine ranging from 21 to 42 ppm. Two samples were below the legal limit of 30-60 ppm and none had less than 20 ppm (Table 1).

Glucose metabolism

Generally, body weights (Table 2a) of the groups were significantly increased after 1, 2, and 3 months' as compared to the control group ($p \leq 0.05$). However, plasma fructosamine level showed significant decrease in all treated groups as compared to control rats (Table 2b).

Concerning plasma insulin levels, it showed significant decrease in all the treated groups after 3 months of iodized salt intake although it was marked in. Group II after 2 months of daily oral intake'.

Thyroid metabolism

Plasma TSH level (Table 2d) showed increased level after daily oral administration of iodized table salt which was significant after 1 and 2 months in group 11 but group (III) showed this increase after one month while it was marked in group IV after 3 months of daily intake ($p \leq 0.05$).

FT3 levels were significantly decreased in group II after 1 and 2 months and also in group IV ($p \leq 0.05$) in a stepwise manner through the time interval of the experiment (1 month) as compared to the initial state (Table 2e).

FT4 plasma levels (Table 2f) showed significant decrease after 2 months of oral salt administration in group II but no significant in group (IV). In group II the increase in fT4 plasma level was significant after 1 month (- 31 % from initial level) and markedly significant after 3-months'.

Inflammation

Qualitative CRP test was positive in group IV which received high iodine intake after 2 and 3 months.

Histopathology

Thyroid gland of the control group showed normal architecture where the follicles were filled with moderate amount of colloid in the lumen and being lined with cuboids epifollicular cells (Fig. 1a). However, the follicles showed homogenous deep eosinophilic colloid material in the wide lumen with flattened lining epithelium of most thyroid gland of rats received low iodide level (Fig. 1b). Moderate-dose-level group showed mild blurred gland follicles without colloids with proliferation producing smaller islets dividing the gland (Fig. 1c). Intake of higher iodide level resulted in mild inflammatory cells infiltration and focal atrophic changes with scanty colloid with cuboidal lining epithelium and narrow lumen (Fig. 1d).

2. Materials and Methods

Experimental design

The study was divided into two parts.

First, screening some fine and coarse retailed iodized table salt samples enriched in iodine for their iodine content in terms of iodated, according to **Diosady *et al.*** [26].

Second, administration of one of these salts orally by gastric gavages once daily for 3 months'.

Animals

Male Wistar rats weighing 150–170 g were obtained from the animal facility of El-Nasr Co. for Pharmaceutical and Chemical Industries (Cairo, Egypt). Handling and experimental procedures were conducted in accordance with the international ethical guidelines concerning the care and use of laboratory animals.

Animals were kept acclimatized in our animal house at Faculty of Pharmacy, Ain Shams University, Abassia, Cairo, Egypt, in a constant controlled environment of temperature, humidity, and illumination (24–25°C, 50–60% relative humidity, 12 h light/dark cycle) for at least 5 days prior to the start of the experiments. Animals were put on basal diet and water supply *ad libitum*. The day before the experiment, animals were arranged in groups (10 rats /group). Group I served as control and received no salts, while groups II–IV were given for 90 days the assigned retail iodized table salt orally at dose levels 36, 108, and 216 mg/day, respectively, calculated according to be corresponding to the equivalent human dose, referred to as low, moderate, and high iodine intake.

Body weights were recorded; blood samples were collected from each rat through retro-orbital bleeding after 1, 2, and 3 months', and processed directly for plasma preparation and finally kept at -20ºC for subsequent analysis.

Biochemical Measurements

Plasma fT3 and fT4 determination were done by radioimmunoassay technique using Coat-A-count T3 and T4 kits purchased from Diagnostic Products Corporation (DPC), Los Angeles. Plasma TSH level was determined by chemiluminometric technique using ACS: 180 Automated Chemiluminescence's system.

Plasma fructosamine was determined by nitro blue tetrazolium method, plasma insulin using enzyme linked immunosorbent assay (ELISA) technique using commercially available kit (Nova Tec Immundiagnostica GmbH, Germany).

Latex agglutination slide test was used for the qualitative estimation of CRP in plasma. Presence of agglutination indicates a level of CRP in the sample equal or > 6 mg/L. The lack of agglutination indicates a CRP level < 6 mg/L in the sample. The elevation of CRP levels above normal indicates tissue damage, inflammation, or both. Normal values were derived from the control group.

Histopathology

At the end of experiment all rats were scarified and thyroid glands were removed carefully dissected free of other tissues, fixed in 10% aqueous neutral-buffered formalin for 24 hr, washed in tap water, then serial dilutions of alcohol (methyl, ethyl, and absolute ethyl) for dehydration process. Specimens were cleared in xylene and embedded in paraffin at 56° in hot air oven for 24 hrs. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by *slidge* microtome. The prepared sections were collected on glass slides, deparaffinized, and stained by hematoxylin and eosin (H&E) stains [27], and processed for histopathological examination to illustrate any cellular alterations, being examined by a light microscope by a professional histopathologist unaware of the groups. Alterations from the normal structure were registered.

Statistical Analysis

All statistical analysis was performed using SPSS Version 17 software package (USA). Data are presented as mean ± S.D, range, with analysis of variance (ANOVA) followed by Bonferroni's post hoc analysis for multiple comparisons between different groups. Student's paired *t* test was used for comparison of two related samples, when comparing inclusion, one-month, 2-month, and 3 months' samples within same animal. Correlations coefficient was evaluated by Spearmans' rank correlation (*r*). The level of statistical significance was set at $P \leq 0.05$.

3. Results

Table 1. Iodine species content in retail iodized table salt determined with iodometric titration

Retail table salt	Iodide content (ppm)
1	49
2 fine	49
2 coarse	42
3	42
4	24.5
5	21
6	42
7	35
8	35

N.B. theoretical iodate content is 30-60 ppm

Table 2a. Effect of iodized table salt oral daily administration on rats body weight (gm)

Groups	Initial B.Wt. (gm)	% change from initial			*P* value		
		After 1 month	After 2 months	After 3 months	1,2	1,3	2,3
I	159 ± 8.4 (150-170)	0	0	0	NS	NS	NS
II	158 ± 6 (150-165)	+7	+12	+27	NS	0.001	0.001
III	160 ± 5 (155-165)	+16	+21	+36	0.002	0.02	NS
IV	160 ± 5 (155-165)	+19	+18	+29	0.04	NS	NS

Data are mean ± S.D, range (n= 10)

Table 2 b. Effect of iodized table salt oral daily administration on rats plasma fructosamine level

Groups	Initial fructosamine (µmol/L)	% change from initial			*P* value		
		After 1 month	After 2 months	After 3 months	1,2	1,3	2,3
I	237 ± 15 (217-256)	0	-0.85	-1.2	NS	NS	NS
II	236 ± 22 (208-277)	-12.3	-19.5	-15	0.001	0.001	0.001
III	236 ± 17 (208-258)	-27	-35	-29	0.001	0.001	0.001
IV	236 ± 21 (208-258)	-20	-24	-21	0.001	NS	0.001

Data are mean ± S.D, range (n= 10)

Table 2 c. Effect of iodized table salt oral daily administration on rats plasma insulin level

Groups	Initial plasma insulin (µIU/mL)	% change from initial			*P* value		
		After 1 month	After 2 months	After 3 months	1,2	1,3	2,3
I	3 ± 0.1 (2.9-3.1)	0	0	0	NS	NS	NS
II	2.85±0.05(2.8-2.95)	0	-10	-17	0.008	0.005	0.01
III	2.9 ± 0.05 (2.9-3)	0	0	-33	NS	0.001	0.001
IV	2.7 ± 0.1 (2.6-2.8)	0	0	-28	NS	0.001	0.001

Data are mean ± S.D, range (n= 10)

Table 2 d. Effect of iodized table salt oral daily administration on rats plasma TSH level

Groups	Initial TSH (µIU/mL)	% change from initial			*P* value		
		After 1 month	After 2 months	After 3 months	1,2	1,3	2,3
I	3 ± 0.3 (2.7-3.6)	0	0	0	NS	NS	NS
II	3 ± 0.1 (2.7-3)	+10	+30	+40	0.008	0.015	NS
III	3 ± 0.1 (2.7-3)	+40	+33	+33	NS	NS	NS
IV	3 ± 0.3 (2.7-3.6)	+17	+30	+40	NS	0.001	NS

Data are mean ± S.D, range (n= 10)

Table 2 e. Effect of iodized table salt oral daily administration on rats plasma fT3 level

Groups	Initial fT3 (ng/dL)	% change from initial			*P* value		
		After 1 month	After 2 months	After 3 months	1,2	1,3	2,3
I	4.4 ± 0.2 (4.15-4.8)	0	0	0	NS	NS	NS
II	4.45 ± 0.2(4.25-5.1)	+1.1	+15	-17	0.005	0.006	0.001
III	4.46 ± 0.2 (4.2-4.9)	-10	-8	-19	0.001	0.008	0.001
IV	4.5 ± 0.1 (4.45-4.6)	-11	-9	-2.2	NS	0.001	NS

Data are mean ± S.D, range (n= 10)

Table 2 f. Effect of iodized table salt oral daily administration on rats plasma fT4 level

Groups	Initial fT4 (µg/dL)	% change from initial			*P* value		
		After 1 month	After 2 months	After 3 months	1,2	1,3	2,3
I	1.3 ± 0.13 (1-1.5)	0	0	0	NS	NS	NS
II	1.3 ± 0.1 (1.25-1.5)	0	-54	-38	0.001	0.006	0.05
III	1.3 ± 0.24 (1-1.6)	-31	-15	-38	0.004	NS	0.001
IV	1.4 ± 0.1 (1.3-1.5)	-14	-18	-14	NS	NS	NS

Data are mean ± S.D, range (n= 10)

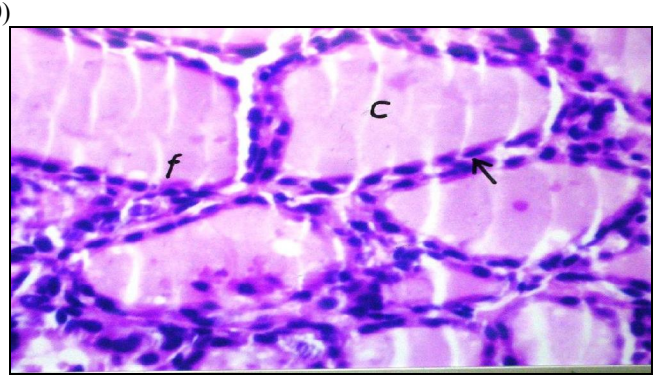

Figure 1a. Sections of rat thyroid (H&E); high-power view from control rat shows normal morphology. Showing normal histological structure identifying thyroid follicles (f) with homogenous deep eosinophilic colloid (c) material in the wide lumen with flattened lining epithelium (arrow) of most active gland (original magnification x64)

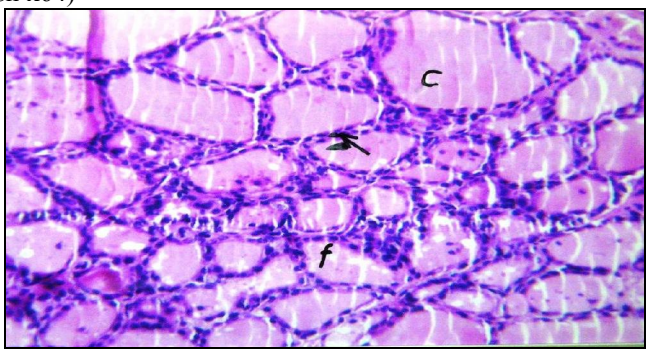

Figure 1b. Sections of rat thyroid (H&E); high-power view from rat treated odine (low dose; 36 mg/kg, orally).
Identifying the eosinophilic colloid (c) in the lumen of the follicle (f) with flattened

lium (arrow) in the inactive thyroid follicle (original magnification x160)

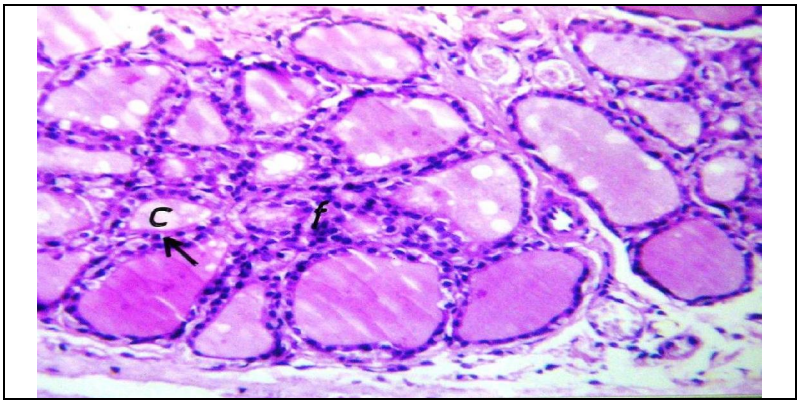

Figure 1c. Sections of rat thyroid (H&E); high-power view from rat treated with iodine (moderate dose; 108 mg/kg, orally).
Thyroid showing scant colloid (c) with narrow follicular lumen (arrow) and cuboidal lining epithelium of the follicles (f) (original magnification x64)

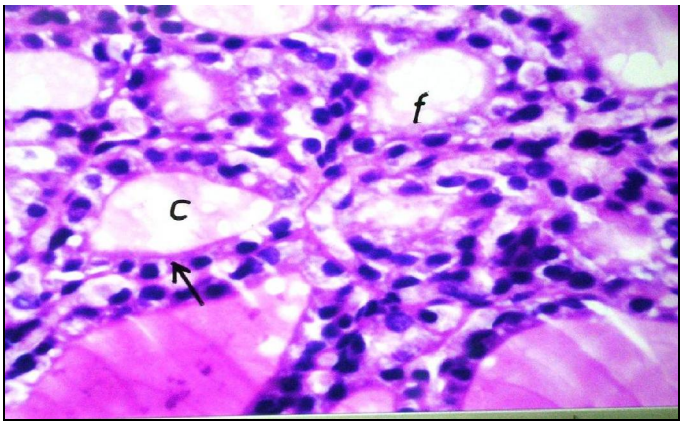

Figure 1d. Sections of rat thyroid (H&E); high-power view from rat treated with iodine (high dose; 216 mg/kg, orally).
Thyroid showing hyperactive gland with scant colloid, narrow lumen, and cuboidal lining epithelium (original magnification x160)

4. Discussion

The US institute of medicine recommended dietary allowance (RDA) 150 mg/day of iodine for adults and adolescents [20]. Only few vegetables are rich in iodine such as baked peeled potato, cooked beans, boiled eggs and tuna canned in oil are expressed as rich sources.

After the introduction of mandatory iodization, increased iodine concentrations were reported in retailed salt, where the iodine status of primary-school children should be improved [21].

The present study demonstrated significant decrease in plasma fT3 and fT4 levels, especially in groups received low and moderate iodide levels and non significant in the group that received higher dose, as an evidenced by hypothyroidism.

Zois *et al.* [22] reported the impact of increased nutritional iodine in 3,000 schoolchildren in Northern Greece, being chronic autoimmune thyroiditis associated with positive anti-TPO antibodies whereas 2.5% had laboratory evidence of subclinical hypothyroidism. However, in a recent study by **Teng *et al.*** [23] conducted in three areas of China with different levels of iodine intake (low, median urinary iodine excretion = 84 µg/L; more than adequate, 243 µg/L; and excessive, 651 µg/L), the authors demonstrated that patients from the area with excessive iodine intake had 5.6 times more chronic

autoimmune thyroiditis and 6.6 times more hypothyroidism (subclinical and overt) as compared with patients from the area with low iodine intake. The authors concluded that excessive iodine intake may lead to autoimmune thyroiditis and hypothyroidism.

When dietary iodine intake is inadequate for thyroid hormone synthesis, plasma fT_4 level initially falls and a number of processes ensue to restore adequate thyroid hormone production. The pituitary gland senses low levels of circulating T_4 and releases more TSH. TSH stimulates the growth and metabolic activity of thyroid follicular cells. TSH stimulates each cell to increase iodine uptake and thyroid hormone synthesis and secretion. Increased TSH levels and reduction of iodine stores within the thyroid result in increased fT_3 production relative to T_4 production [24].

Iodides in general can induce free radicals and oxidative stress in thyroid cells, and possibly in immunocompetent cells. This may be in agreement with the reported hypothesis regarding the pathogenesis of lymphocitic thyroiditis since environmental factors, iodides alone or combined with increased TSH level may damage the thyroid [25]. Numerous phenomenons may offer additional support, eg. monocytes secrete IL-1 which exerts a direct cytotoxic effect on thyrocytes and thereby provide signals to CD4 cells. T and B lymphocytes may migrate into the damaged thyroid, recognize sequestered antigen thyroglobulin as foreign, activate complement and elicit inflammation – thyroiditis [25]. This may be clear in the present study where group of rats, received higher iodine intake showed hyperactive gland with scant colloid, narrow lumen and cuboidal lining epithelium (Fig. 1d and 1c) as compared to control group (Figure 1a). Evident of inflammation was also demonstrated as provoked by CRP positive result (although it is qualitative and not quantitative) in rats received higher iodide intake and in agreements with the aforementioned studies [22, 23].

The homeostasis of glucose metabolism is maintained by many factors, including intestinal glucose absorption, liver gluconeogenesis, glycogenolysis muscle glucose uptake, insulin secretion from pancreatic cells and anti – insulin hormones ex, cortical and thyroid hormones.

It is well known that an impaired glucose tolerance is induced by the excessive hormone secretion seen in various endocrinopathies [19]. This is frequently observed in hyperthyroid state, being caused by abnormal glucose metabolism in muscle, fat, and liver or by the sluggish secretion of insulin, accelerated degradation of plasma insulin and/or by increased glucose absorption by a hyperkinetic GIT [19]. This may explain that too much thyroid hormone speeds up metabolism and tends to raise blood sugar, indeed more requirements to insulin or other antidiabetic medications. Consequently those individuals with a low thyroid hormones level, less insulin might be required. Accordingly decreased insulin, fructosamine, and increased TSH levels might be meaningful and indicative to certain disturbances in thyroid hormones profile in subsequent to intake of iodized salt of different iodine levels. Moreover, the effect of thyroid hormones on glucose metabolism may be attributed to induced changes in glucose transporter levels.

In Conclusion, iodide effects on thyroid gland are complex according to the thyroid states in the body. Histopathological pattern of thyroid gland demonstrated different inflammatory pattern according to dosage levels of iodide intake as compared to normal. Hormonal states of TSH, fT3, and fT4 in the treated groups demonstrated in consequence positive response. This was also reflected on glucose homeostatic alterations, being represented by insulin and fructosamine.

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Authors' contributions

MMS invented concepts, design, carried out data acquisition, and manuscript review. NMH participated in sample design, sample analysis, data acquisition, data analysis, statistical analysis, manuscript preparation, manuscript editing, and manuscript review. OE shared sample design and samples analysis.

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