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Effect of Vitamin A and Iodine Status on Thyroid Gland Functions in Rats

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Abstract: Vitamin A (VA) deficiency (VAD) and iodine deficiency (ID) disorders (IDD) are major global public health problems, mostly affecting women of reproductive age and young children. The present study aims to investigate the effect of iodine and VA (deficient, sufficient or supplement) alone and in combination on of thyroid gland functions in hypothyroid rat model. 60 weaning male albino rats were fed on VA and iodine deficient diet for 4 weeks to induce hypothyroidism. Then they were divided into six groups, 10 rats per group. Each group received different diet which differs in their content of iodine and VA for a consecutive 2 weeks. These groups were compared with 20 control rats. Serum TSH, TT4, FT4, TT3, and FT3 were measured to estimate the efficiency of each diet on treatment of hypothyroidism. Also, serum retinol was measured to determine the influence of VA in replenishing VA deficiency status. Moreover, hepatic and serum lipid profiles, serum glucose, and hepatic glycogen were estimated to detect the effect of thyroid gland functions on cardiovascular disease and carbohydrate metabolism. The results showed that, however VA alone was effective in improvement TSH level; it had no effect on thyroid gland function. Whereas, when VA was in combination with iodine in the sufficient diet, they significantly promoted the normalization of thyroid gland functions than iodine alone. Moreover, treatment of hypothyroidism could reduce the serum and hepatic lipid profiles with no significant effect on both of serum glucose and hepatic glycogen values. Hence, the treatment of hypothyroidism by iodine supplementation would be a risk factor for hyperthyroidism, therefore this study suggested a better treatment for hypothyroidism by the standard sufficient iodine diet in relation to VA status. Also, the current treatment of hypothyroidism showed beneficial effects in reducing the risk for cardiovascular disease and carbohydrate metabolic disorders.

Key words: vitamin A, iodine, hypothyroidism, lipids, rats.

INTRODUCTION

Vitamin A (VA) is the generic name for a group of compounds having similar biological activity. These compounds are retinol, retinal, and retinoic acid (RA). RA an oxidation product in the physiological metabolism of retinol which can support most of the physiological functions attributed to VA. This active metabolite of retinol regulates numerous processes, including cell proliferation and differentiation, embryonic development, and immune function (Cifelli and Ross, 2007).Additionally isomers of RA are used therapeutically in the treatment of skin disorders, acute promyelocytic leukemia (Jimenez-Lara *et al*., 2004 and Ortega *et al*., 2005) and other cancers (Okuno *et al*., 2004). Moreover, RA may possess immune adjuvant properties (Ma and Ross, 2005 and Ma *et al*., 2005). Cifelli and Ross (2007) suggested that treatment with RA might reduce the severity of certain infectious disease.

VA deficiencies include tissue depletion below functional impairment. Mild pathophysiological change may occur in parallel with or may follow measurable depletion in circulation or in tissues (Congdon and Ross, 2002). A series of disorders were described in animals, including impaired growth, reproduction, epithelial integrity and disease resistances that were relieved by consumption of both animal and plant sources of vitamin (Underwood, 2004).

Iodine was first described as a constituent of burned seaweed although its major role in the human thyroid was not identified until 1927 (Smyth, 2003). One of the most importances of iodine is its function as an antioxidant in human systems including eye, thyroid and breast (Venturi *et al*., 2000). The antioxidant properties of dietary iodine depend on a series of redox reactions underlying the iodination of tyrosine leading to the formation of thyroid hormone (Smyth, 2003).

Iodine deficiency can cause thyroid dysfunction including hypothyroidism, impaired mental and physical development, loss of energy, and increased prenatal and infant mortality (Clar *et al*., 2002).

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Vitamin A (VA) deficiency (VAD) and the iodine deficiency (ID) disorders (IDD) are major global public health problems, affecting >30% of the population world wide. The most vulnerable groups are women of reproductive age and young children (WHO, 2001). In many regions of the developing world, young women, infants, and children suffer from both VAD and IDD (Zimmermann *et al*., 2004, Zimmermann *et al*., 2007). VAD has multiple effects on thyroid metabolism that may be dependant on iodine status. In severely VA deficient rats, thyroidal iodine intake is decreased, thyroglobulin synthesis is impaired and thyroid size is increased. Peripherally, VAD increase free and total circulating thyroid hormone and binding of transthyretin to retinol-binding protein decreases VA turnover and enhances VA delivery (Robbins, 2000). In addition, VAD in goitrous children was associated with increased TSH stimulation and thyroid size and reduced risk for hypothyroidism that may be reflected on the whole metabolism in the body (Zimmermann *et al*., 2004).

Periodic high dose oral VA supplementation (VAS) is the recommended strategy to control VAD in affected population, many of them are also iodine deficient. It has been demonstrated that, VAS given with iodized salt reduced the goiter rate compared with iodized salt given alone (Biebnger *et al*., 2007).

The present study was undertaken to investigate the effect of iodine and VA (deficient, sufficient or supplement) alone and in combination on modulation of thyroid gland functions in hypothyroid rats.

MATERIALS AND METHODS

Experimental design of this study was divided into two parts as follows:

Part (1):

Deals with the determination of iodine concentration in both of VAS and VAD diets by using titermetric method according to Bohn, (1917).

Part (2):

Deals with both of biological and biochemical examinations of the relationship between different ratios of iodine and vitamin A feeding on thyroid gland function. Eighty weaning male albino "Sprague-Dawely" rats 21±3 day of age weighing 50-70 g were divided into 8 groups with similar average weights. The animals were housed individually in wire cages. Diet and water were provided *ad libitum* for 6 weeks. The diets were prepared according to AIN-93G purified rodent diet guidelines with variations in their content of VA and iodine (Reeves *et al*., 1993) as shown in figure (1).

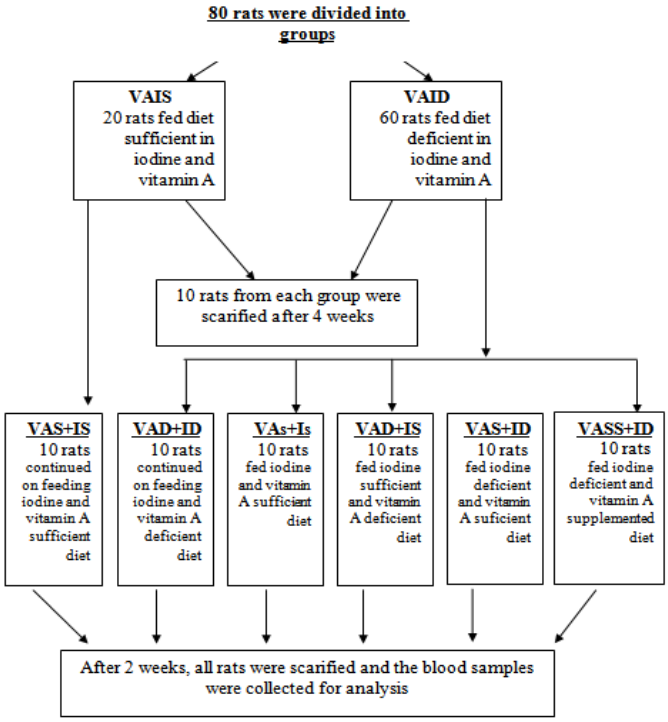

Fig. 1: Dietary design with variations in the contents of VA and iodine

Food intake was measured daily and body weight was recorded twice a week. At the end of the experimental duration, animals were scarified using diethyl ether. Blood samples were collected from hepatic portal vein for serum separation by centrifugation at 3000 rpm for 10 min and liver tissues were immediately removed. Then, samples were stored at -20 C˚ untill analysis.

Glucose concentration was measured immediately after serum separation by enzymatic method using kit according to Barham and Trinder (1972).Hepatic glycogen content was determined chemically according to Corroll *et al*. (1955).

Serum TSH, total T_3 and T_4 levels were determined by (DRG) kit according to Wada *et al*. (1982), Walkerl (1977) and Wistom (1976), respectively.Free T_3 and T_4 levels were determined using (Acculite) kit by competitive chemiluminscence immunoassay analog method according to Verheecke (1997) and Midgalsy (2001), respectively.

Serum VA content was determined by HPLC according to Coral *et al*. (1997).Serum total lipids and phospholipids concentrations were determined colorimetrically using kit according to Knight *et al*. (1972) and Connerty *et al*. (1961), respectively.Serum triacylglycerides, total cholesterol and HDL – cholesterol concentrations were determined by enzymatic colorimetric method using kit according to Fossati and Prencipe (1982), Allain *et al*. (1974)and Lopez-Virella *et al*. (1977), respectively Serum LDL- cholesterol concentration was calculated by an equation of Friedewald *et al*. (1972). Hepatic lipids were extracted by the chloroform / methanol method of Folch *et al*. (1957) for the determination of lipids profile content as described in serum.

Statistical Analysis:

The statistical analysis for the biochemical data was performed using SPSS version 9.0. Data were expresed as mean ±S.D. statistical differences between groups were performed using student t-test, differences considered significant when $P<0.05$, $P<0.01$.

RESULTS AND DISCUSSION

1-Iodine in diet:

Iodine content in sufficient diet (contain sufficient iodine) was 0.846mg/kg diet while in deficient diet (diet deficient in iodine) was 0.423 mg/kg diet.

2-Effect of feeding weaning male albino rats on either VA and iodine sufficient (VAIS) or VA and iodine deficient (VAID) diet for four weeks:

Table 1: Effect of vitamin A and iodine sufficient or vitamin A and iodine deficient diet on body weight, food intake, liver weight, serum retinol, glucose and hepatic glycogen

Parameters	VAIS	VAID
Body weight gain (g)	121.02±23.31	124.30±13.71
Food intake (g)	411.00±40.99	387.13±33.46
Liver weight (g)	6.68±0.93	6.68±0.93
Relative liver weight (g %)	3.52±.39	3.87±0.42
Serum retinol (mg/dl)	110.3±2.96[a]	38.24±2.97[a]
Serum glucose (mg/dl)	108.06±3.83[a]	76.17±3.47[a]
Hepatic glycogen (mg/g tissue)	5.58±1.08[1]	3.84±0.23[1]

Values are means ± S.D. ; n = 10.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.

The current results showed that, each of body weight gain, liver weight, and relative liver weight values were higher in VAID than VAIS, while, the measured food intake was lower in VAID than VAIS (table 1). Also the present data indicated that, rats fed sufficient diet VAIS has a higher glucose concentration and hepatic glycogen content compared with rats fed deficient diet VAID.

Table 2: Effect of vitamin A and iodine sufficient or vitamin A and iodine deficient diet on serum TSH, TT4, FT4, TT3 and FT3 levels for 4 weeks.

Parameters		VAIS	VAID
TSH	(µIU/ml)	0.101±0.021[a]	0.355±0.032[a]
TT_4	(µg/ml)	8.57±0.84a	6.44±0.80[a]
FT_4	(ng/dl)	1.70±0.56	1.41±0.58
TT_3	(ng/ml)	1.72±0.75[a]	0.73±0.11[a]
FT_3	(Pg/ml)	0.954±0.42[a]	0.292±0.19[a]

Values are means ± S.D. ; n = 10.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.

The data in table (2) showed that ,there was a general decrease in all thyroid hormones levels in weaning male albino rats fed deficient diet VAID compared with those fed the sufficient diet VAIS.

Table 3: Effect of vitamin A and iodine sufficient or vitamin A and iodine deficient diet on serum lipids profile for 4 weeks.

Parameters	VAIS	VAID
Total lipid (mg/dl)	111.15±3.26[a]	390.32±3.28[a]
Phospholipids (mg/dl)	27.84±3.04[a]	126.59±3.19[a]
triacylglycerol (mg/dl)	28.30±2.64[a]	94.42±2.46[a]
total cholesterol (mg/dl)	70.37±2.81[a]	223.75±2.433[a]
HDL-C (mg/dl)	5.59±2.13[a]	29.89±3.25[a]
LDL-C (mg/dl)	63.92±2.28[a]	166.55±10.19[a]

Values are means ± S.D. ; n = 10.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.

Table 4: Effect of vitamin A and iodine sufficient or vitamin A and iodine deficient diet on hepatic lipids profile for 4 weeks.

Parameters	VAIS	VAID
Total lipid (mg/g)	284.99±4.18[a]	835.84±3.43[a]
Phospholipids (mg/g)	81.62±3.57[a]	421.11±9.03[a]
Triglyceride (mg/g)	7.46±1.52[a]	31.92±3.57[a]
T-Ch (mg/g)	28.81±3.59[a]	166.11±3.33[a]
HDL-C (mg/g)	3.69±0.30[a]	16.97±2.22[a]
LDL-C (mg/g)	23.74±3.75[a]	142.39±3.10[a]

Values are means ± S.D. ; n = 10.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.

The result in table (3) and (4) showed that there was a remarkable increase in both serum and hepatic lipids profile values of the weaning male albino rats fed deficient diet VAID than the rats fed the sufficient diet VAIS.

3-effect of Feeding Weaning Male Albino Rats on Different Diets Varying in Their Iodine and Vitamin a Content for 6 Weeks:

Table 5: Effect of varying vitamin A and iodine status on physiological parameters for 6 weeks.

Groups	Body weight gain (g)	Food intake (g)	Liver weight (g)	Relative liver weight (g %)
VAS+IS	158.50±21.10[a2]	609.86±45.75[a]	7.97±1.62	3.60±0.48
VAD+ID	193.62±22.22[a1]	600.86±37.43[13]	8.98±1.30[a]	3.62±0.65
VAs+Is	178.62±25.11	588.75±72.85[2]	7.96±0.69	3.27±0.32
VAD+IS	183.36±22.81[2]	607.62±72.84[b]	8.09±1.19	3.24±0.432
VAS+ID	173.50±21.56	588.00±34.94[3]	8.12±1.39	3.41±0.46
VASS+ID	165.86±36.821	537.74±60.04[ab12]	7.46±1.26[a]	3.21±0.32

Values are means ± S.D. ; n = 10.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.

Table (5) shows that, at the end of 6 weeks it was observed that there was a highly significant difference ($p<0.01$) between the body weight gain values of weaning male albino rats of VAS+IS and VAD+ID groups. The weaning male albino rats fed VASS+ID diet had a highly significant difference ($p<0.01$) in food intake values when compared with rats fed either VAS+IS or VAD+IS diets. Also, there was a highly significant difference ($p<0.01$) between liver weight values of VAD+ID and VASS+ID group of rats. However, there was no significant difference among the relative liver weight values of the different experimental groups.

The data in table (6) indicated that, serum retinol level of weaning male albino rats of VAD+ID group showed highly significant changes ($p<0.01$) with all the other experimental group except VAD+IS group. There was a highly significant difference ($p<0.01$) between the serum glucose level of the weaning male albino rats fed VAD+ID diet and all of the other experimental groups. Weaning male albino rats fed VAD+ID diet had a highly significant difference ($p<0.01$) in hepatic glycogen content with all the other experimental groups except the VASS+ID group.

Table (7) showed that, there was a highly significant difference ($p<0.01$) between the serum TSH, TT_4 and TT_3 levels of the weaning male albino rats fed the VAD+ID diet and all of the other experimental groups. For both serum FT_3 and FT_4 levels , there was a highly significant difference ($p<0.01$) between the VAD+ID and the VAS+IS groups.

Table 6: Effect of varying vitamin A and iodine status on serum retinol, glucose and hepatic glycogen levels for 6 weeks.

Groups	Rretinol (mg/dl)	Glucose (mg/dl)	Glycogen (mg/g tissue)
VAS+IS	111.52±2.59^{ab}	102.31±3.29^{a}	5.35±0.41$^{a\ N.S}$
VAD+ID	28.88±4.41$^{ab\ N.S*}$	73.25±3.52^{a}	3.16±0.24^{a1}
VAs+Is	91.23±2.87$^{ab\ N.S}$	90.78±3.30^{a}	5.28±0.33$^{a\ N.S}$
VAD+IS	27.96±3.66$^{b\ N.S*}$	87.49±3.40^{a12}	4.40±0.33^{a}
VAS+ID	88.51±2.95$^{ab\ N.S}$	81.71±3.16$^{a\ N.S}$	3.83±0.23^{a}
VASS+ID	98.47±3.90^{ab}	83.76±3.69$^{a2\ N.S}$	3.04±0.27^{1}

Values are means ± S.D.; n = 10.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01
N.S means insignificant

Table 7: Effect of varying vitamin A and iodine status on serum TSH, TT4, FT4, TT3 and FT3 levels for 6 weeks.

Group	TSH (µIU/ml)	TT_4 (µg/ml)	FT_4 (ng/dl)	TT_3 (ng/ml)	FT_3 (pg/ml)
VAS+IS	0.111±2.41^{a}	8.45±0.73^{ab}	1.93±0.65^{a}	1.76±0.54^{ab}	0.875±0.43^{ac34}
VAD+ID	0.765±4.76^{a}	3.04±0.86^{a}	1.16±0.28^{a}	0.699±0.11^{a}	0.214±0.17^{ab12}
VAs+Is	0.171±3.11^{a}	8.18±1.26^{a1}	1.62±0.78	1.50±0.56^{a}	0.682±0.316^{b}
VAD+IS	0.277±3.57^{a}	8.01±0.80^{a}	1.69±0.71	1.58±0.45^{a1}	0.561±0.30^{3}
VAS+ID	0.281±3.29^{a}	7.21±1.17^{ab1}	1.69±0.58	1.21±0.11^{ab1}	0.418±0.24^{c1}
VASS+ID	0.198±2.88^{a}	7.26±1.10^{a}	1.45±0.36	1.51±0.25^{a}	0.546±0.32^{24}

Values are means ± S.D. ; n = 10.
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05

Table 8: Effect of varying vitamin A and iodine status on serum lipids profile for 6 weeks.

Group	Total lipid (mg/dl)	Phospholipids (mg/dl)	Triglyceride (mg/dl)	T-Ch (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
VAS+IS	123.77±3.62^{a}	23.63±3.28^{a}	26.06±2.18^{a}	94.32±3.16^{a}	5.14±1.83^{a1}	83.96±3.90^{a}
VAD+ID	438.41±3.22^{a}	145.69±3.40^{a}	96.53±2.34^{a}	225.49±11.53^{a}	39.92±2.89^{a}	177.86±6.7^{a}
VAs+Is	187.75±3.18^{a}	46.26±3.30^{a}	56.63±2.70^{a}	126.83±2.9^{a}	7.78±2.06^{a1}	107.71±4.26^{a}
VAD+IS	230.47±3.50^{a}	78.91±3.08^{a}	62.59±2.09$^{a\ N.S}$	146.09±2.82^{a}	15.53±2.46^{a}	117.92±4.43^{a}
VAS+ID	321.21±3.27^{a}	100.20±3.35^{a}	90.23±2.77^{a}	201.78±11.67^{a}	25.00±2.30^{a}	158.38±11.88^{a}
VASS+ID	271.32±3.56^{a}	60.25±3.27^{a}	62.93±2.18$^{a\ N.S}$	181.09±3.4^{a}	11.33±2.59^{a}	157.24±3.49^{a}

Values are means ± S.D.; n = 10.
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05
N.S means insignificant

Table 9: Effect of varying vitamin A and iodine status on hepatic lipids profile for 6 weeks.

Group	Total lipid (mg/g)	Phospholipids (mg/g)	Triglyceride (mg/g)	T-Ch (mg/g)	HDL-C (mg/g)	LDL-C (mg/g)
VAS+IS	307.24±3.96^{a}	81.22±2.72^{a}	9.61±1.99^{a}	36.90±2.71^{a}	3.55±0.30^{a1}	30.38±2.68^{a}
VAD+ID	893.40±4.17^{a}	536.59±3.07^{a}	35.43±3.48^{a}	184.76±3.69^{a}	25.20±2.17^{a}	152.54±3.40^{a}
VAs+Is	384.39±3.44^{a}	136.44±3.57^{a}	17.02±2.79^{a}	78.70±6.18^{a}	4.87±0.35^{a1}	71.02±3.50^{a}
VAD+IS	599.60±4.00^{a}	191.22±4.26^{a}	21.75±2.52^{a}	110.81±3.41^{a}	6.69±0.354^{a}	96.48±3.67^{a1}
VAS+ID	680.57±4.23^{a}	236.27±3.27^{a}	26.20±3.67^{a1}	112.82±3.29$_{a}$	7.46±0.36^{a}	100.33±3.87^{a1}
VASS+ID	715.34±3.57^{a}	267.80±4.00^{a}	29.29±3.48^{a1}	126.12±3.98^{a}	10.16±2.22^{a}	112.30±3.98^{a}

Values are means ± S.D.; n = 10.
Data within the same column bearing similar alphabetic superscripts are significant different at P< 0.01.
Data within the same column bearing similar numerical superscripts are significant different at P<0.05

The data in tables (8) and (9) indicated that all the serum and hepatic lipids profile showed highly significant (P< 0.01) differences among all of the different experimental groups fed the different diets for 6 weeks.

Discussion

1- Iodine in diet:

Iodine in sufficient diet (VAIS) was added to the mineral mixture according to AIN-93 in the form of potassium iodide. Casein may be contaminated with iodine (Biebnger *et al*, 2007); and therefore the iodine deficient diet (VAID) had a trace amount of iodine. This indicated that the group of rats fed VAID were iodine deficient than those fed the VAIS.

2- Biochemical section:

VAID group of weaning male albino rats becomes hypothyroidism by iodine and VA depletion and the rats showed slight increase in body weight than those in VAIS group, even the food intake of weaning male albino rats of group VAID was lower than that of weaning male albino rats of group VAIS. Body weight was related to both food intake and the physiological condition of rats. It's noteworthy that thyroid dysfunction is

well recognized as a cause of weight change and weight loss is a frequent manifestation of hyperthyroidism, and hyperthyroidism patients who are treated gain nearly 4 kg/year (Dale *et al.,* 2001).Conversely weight gain is common complaint in patients with hypothyroidism and treatment with thyroid hormone is associated with modest weight loss (Tzotzas *et al*, 2000).

After 2 weeks on feeding different diets varying in iodine and vitamin A content, weaning male albino rats of VAD+ID, VASS+ID, and VAS+ID groups (groups with low thyroid hormone) were increased in gaining weight more than the weaning male albino rats of VAS+IS, VAs+Is, and VAD+IS groups (groups with normal thyroid function) with regard to food intake. The gain in weight in rats of VASS+ID group was lower than VAS+ID group because the food intake of weaning male albino rats of VASS+ID group was lower than the other rats of VAS+ID group. This result was in agreement with the other human studies of Tzotzas et.al. (2000) in which, patients with overt hypothyroidism lose weight when treated.

Also the absolute and relative liver weights were not significantly differing; they were changed according to the change in body weight. Parallel changes to the body weight were found for both the absolute and relative liver weights in the study of Aranda *et al*. (1972), but as the thyroidectomized rats treated with different doses of thyroxine were losing weight, their absolute and relative liver weights were also decreased.

At the end of 4 weeks of depletion period combined dietary deficiency of VA and iodine results in VA deficiency with serum retinol concentration reduced by more than 50% in group VAID than group VAIS. After another 2 weeks of repletion with different diet varying in VA content it was observed that weaning male albino rats of VAD+ID, VAD+IS groups still having low serum retinol concentration indicating sever VA deficiency. While weaning male albino rats of VAS+IS, VAs+Is, VAS+ID groups were started to return serum retinol concentration, but the high dose of VA of weaning male albino rats of VASS+ID group was sufficient to return serum retinol concentration to normal, regardless of iodine status. Normalization of serum retinol suggests VA repletion, these data suggest that high dose of VA treatment likely be effective in humans, even in areas of ID and endemic goiter. These data were agreed with previous animal study of Biebnger *et al*. (2007) who concluded that, iodine deficiency did not reduce the efficiency of high doses of oral VA to increase serum retinol in rats with primary hypothyroidism due to VA deficiency and iodine deficiency. Thus ID and hypothyroidism do not impair VA repletion with high doses of VA.

After four weeks on feeding deficient diet (VA and iodine deficient diet), serum TSH was highly increased in group VAID than group VAIS. However the other thyroid hormones were decreased, indicating the presence of clinical hypothyroidism due to VA and iodine depletion. After two weeks of feeding different diet with varying iodine and VA content; TSH reaches the highest value in weaning male albino rats of VAD+ID group. TSH Value was nearly return to normal value in the weaning male albino rats of VAs+Is group. Neither the weaning rats of VAS+ID group nor VAD+IS group can normalized TSH value while high dose of VA in the diet of rats of VASS+ID group can slightly normalized TSH value which indicated that VA have a high effect on TSH regardless iodine status. Thyroid hormones concentrations reach their minimum value in the weaning male albino rats of VAD+ID group which indicate sever clinical hypothyroidism. All thyroid hormones of weaning male albino rats of VAs+Is, and VAD+IS groups was increased and reach the normal value but the diet of the weaning male albino rats of VAs+Is group was more efficient. Either VA sufficient or high dose of VA in diet of weaning male albino rats of VAS+ID, VASS+ID groups with iodine deficient diet had no discernible effect on circulating thyroid hormone concentration (as there was no significant difference between either VAS or VASS).

This indicates that iodine sufficient diet entirely reversed the abnormalities of the pituitary-thyroid axis produced by VA and iodine depletion. Also, VA has a high effect on TSH, even with iodine deficient diet. There are several potential explanations for this effect. Control of TSH production by the pituitary depends on 2 main factors; the binding of the thyroid hormone receptor, which is activated by T_3 and T_4, and the binding of the RXR, which is activated by retinoic acid (Wolf 2002). Both receptors suppress transcription of the pituitary TSH β gene by occupying half-sites on the promoter region of the gene, thus, VA status modulates TSH production (Brown *et al*, 2000).

The current study VAS may suppress TSH β m RNA expression, decrease serum TSH concentrations, and reduce thyroid stimulation. Reduced TSH stimulation might have been expected to reduce thyroid hormone production and thereby reduce circulating level of thyroid hormone, but circulating level of thyroid hormones did not fall. This implies that either the sensitivity of the thyroid to TSH improved with VA repletion or metabolism of circulating thyroid hormone was altered to maintain thyroid hormones levels. Our finding was agreed with previous study of Zimmerman *et al*. (2004) who suggest that VA supplementation improves the efficiency of iodine sufficient to control goiter children with moderate VA deficiency.

After four weeks of depletion with iodine and VA deficient diet, hypothyroidism was observed in rats of group VAID which indicated by reduced concentration of thyroid hormones. These what exactly occur in rats of group VAID after they become hypothyroidism.

There were a general elevation in the lipids profile in both serum and liver. Overt hypothyroidism is characterized by a marked increase in low density lipoprotein (LDL) and apolipoprotein B (Klein and Danzi, 2007). So, cardiovascular disease (CVD) risk factors are known to be more prevalent in hypothyroid subjects (Hoption-cann, 2006).

After other 2 weeks of feeding diet with different diet varying in their VA and iodine content, all lipid fractions were changed depending on the changes occur in thyroid hormones. As thyroid hormone increased (normalization of hypothyroidism) the total lipid decreased specially cholesterol, LDL-cholesterol.

The nature and degree of dyslipidemia in overt hypothyroidism has been demonstrated in many other studies, and no doubt about the beneficial effects of thyroid substitution on serum lipids and on the risk for CVD. The results obtained by Klaus and Stangi (2000) that pigs with hypothyroidism and elevated cholesterol, HDL, and LDL showed a reduction in lipid profile by T_4 replacement. In this study, there was a significant negative correlation between the plasma concentration of cholesterol and T_4, which may be explained by the difference in the experimental animals used in the study. Also in other human studies of (Efstathiadou *et al*, 2001) the total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride were significantly elevated in subclinical hypothyroid patients compared with the control. In this study subjects with subclinical hypothyroidism had significantly higher levels of total cholesterol, LDL-cholesterol, apolipoprotein B, thus displaying a more atherogenic lipid profile when compared with healthy individuals. But the treatment by L-thyroxine substitution causes a decrease in total cholesterol and LDL- cholesterol. It appears that the degree of change depends on two parameters, the initial levels of cholesterol and the degree of thyroid dysfunction. Although rats become hypothyroidism after 4 weeks of iodine and VA depletion, serum glucose not significantly changed either in hypothyroid period or after treatment with different diet for other two weeks. This may related to the severity of hypothyroidism or because of in hypothyroidism, (1) the metabolic rate is fall, (2) insulin secretion in response to oral glucose is appropriate for the slightly flattened oral glucose tolerance curve, (3) hepatic gluconeogenesis and glucose utilization usually remain normal and blood glucose level are maintained within normal limits (Degroot *et al*, 2001). Also glucose utilization in some tissues is diminished, although in others, such as in adipose tissue glucose utilization does not seem to differ from that in normal subject (Aronda *et al*, 1972).

For liver glycogen, it was decreased in hypothyroid rats of VAID. This may be explained by that the hypothyroidism reduces the capacity for glucogensis, in part by lowering hepatic glycogen synthesis (Fowden *et al*, 2001). In adult rats, thyroid hormones enhance total glycogen synthase activity in the liver and the percentage of synthase in the active form (Bollan and stalman, 1988).

After two weeks of feeding rats with different diets varying in their vitamin A and iodine content, liver glycogen was normalized in groups of rats which repletion their thyroid hormones level (weaning male albino rats of VAS+IS, VAs+Is, VAD+IS groups). While liver glycogen in weaning male albino rats of VAD+ID group was diminished to minimal value. Also the weaning male albino rats of VASS+ID, VAS+ID groups were still have low glycogen as rats of this groups were still hypothyroid even with low TSH level. These results were correlated to that of Aronda *et al*. (1972), who treated both of the thyroidectomized and thyroidectomized rats with different doses of L-thyroxine. They found that all of the treated rats had normal blood sugar level similar to control group while the liver glycogen content was slightly reduced in thyroidectomized rats. Whereas, when rats treated with different doses of L-thyroxine, the liver glycogen content was returned to normal level depending on the dose of hormone.

Conclusion:

This study exerted a beneficial treatment for hypothyroidism by the standard sufficient iodine diet in relation to VA status and in reducing the risk of cardiovascular disease and carbohydrate metabolic disorders.

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