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Seasonal variations of total and free thyroid hormones in healthy men: a chronobiological study

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Abstract. The seasonal variations of iodothyronines and TSH serum concentrations were evaluated in 24 healthy subjects of both sexes. Using a 12 month cosine function, a significant circannual rhythm was found in T_3, T_4 and free T_4 whose maximal values were found in September for T_3, in August for T_4 and in October for free T_4. No significant seasonal variations in free T_3 or TSH were found. Some circannual but not sinusoidal changes were found for reverse T_3 and TBG. A positive linear correlation between T_4, T_3 and reverse T_3 changes was observed, indicating that the sinusoidal oscillations of these hormones were in phase with each other. Relative weight was significantly lower in summer than in winter, though spontaneous caloric intake and physical activity did not change. A slight but significant correlation was found between relative weight and T_4, free T_4 and reverse T_3 values measured during the period of study.

Acclimatization to different environmental temperatures involves several hormonal and metabolic adaptive phenomena. Studies in animals and in man have revealed increasing evidence as to the primary role of thyroid hormones. Both short- and long-term exposure to heat or to cold induces several changes in thyroid hormone and thyrotrophin (TSH) blood concentrations (Collins & Weiner 1968; Epstein et al. 1979; Chen & Meites 1975; Wilson et al. 1970; Ingram & Kaciuba-Uscilko 1977; Sellers & Yon 1950; Eastman et al. 1974; Evans & Ingram 1977; Van Hardeveld et al. 1979; Tuomisto et al. 1976; Fisher & Odell 1971; Jobin et al. 1975; Raud & Odell 1969).

In several countries, environmental temperatures may show marked variations during the year: e.g. in Italy, temperatures below 0°C may frequently be observed in winter, while in summer their values may rise above 30°C. Although acclimatization is difficult to demonstrate in man, due in part to his success in avoiding temperature stress by behavioural mechanisms, several investigators have demonstrated seasonal variations of peripheral thyroid hormone and TSH blood concentrations (Smals et al. 1977; Osiba 1957; Du Ruisseau 1965; Lewitus et al. 1964; Rogowski et al. 1974; Oddie et al. 1979; Konno & Morikawa 1982; Rastogi & Sawhney 1976), though conflicting results have been reported (Rastogi & Sawhney 1976; Hedstrand & Wide 1973; Postmes et al. 1974; Konno 1976). Except in a study performed in thyroxine-replaced hypothyroid patients (Konno & Morikawa 1982), no data are available on seasonal variations of free thyroid hormones.

Nutritional intake may affect thyroid hormone peripheral metabolism (Yung et al. 1980; Danforth et al. 1979). Moreover, food intake and ambient temperature interaction in governing the utilization of thyroxine have been demonstrated in laboratory animals (Ingram & Kaciuba-Uscilko 1977). Therefore, in chronobiological studies, the interrelationships between dietary intake, ambient temperatures and thyroid hormone concentrations should be investigated. In this regard, data in human population are lacking. The present study was therefore undertaken in a group of healthy volunteers in order to evaluate: 1) the seasonal variations in total and free thyroid hormones and TSH blood concentrations; 2) the seasonal variability of body weight and spontaneous dietary intake.

Material and Methods

Subjects

Twenty-four healthy volunteers participated in the study which began in September 1979 and lasted until July 1980. All subjects were selected from doctors, students, biochemists or nurses working in or attending our institute. All were living in Bologna during the entire period of study. There were 14 males and 10 females, aged 28.2 ± 1.2 years (range 24–48) when the study started; their body weight was 61.9 ± 1.9 kg and relative weight was 100.7 ± 1.8 kg (range 89–120) (ideal body weight was obtained from the tables of the Metropolitan Life Insurance Co. 1960). Drugs were carefully avoided during the study period, nor did any subject undergo X-ray examinations using iodine compounds in the 12 months before the study was started.

Protocol study

Starting in September 1979, all subjects were examined every 2 months (November, January, March, May and July). A complete examination of each participant was performed in 2–4 days at each control visit, during which the following parameters were measured:

1) Body weight (always using the same scales).

2) Spontaneous nutritional intake, by means of 1 day questionnaires and interviews, performed by a long-term experienced dietician of our staff.

3) The usual physical activity. A conventional score was used (on the basis of a subjective evaluation expressed by each subject) as follows: sedentary activity (1 point), moderate (walking, etc.) (2 points), good (jogging, gymnastics, etc.) (3 points) and sporting (4 points).

4) The lowest and the highest environmental temperatures on the day of the control visit and the day before, were recorded according to the Meteorological Service at the Airport of Bologna, Borgo Panigale.

5) After an overnight fast (08.00–09.00 h), blood samples were drawn from an antecubital vein for hormonal determinations. After centrifugation, serum samples were stored at −20°C until analysis.

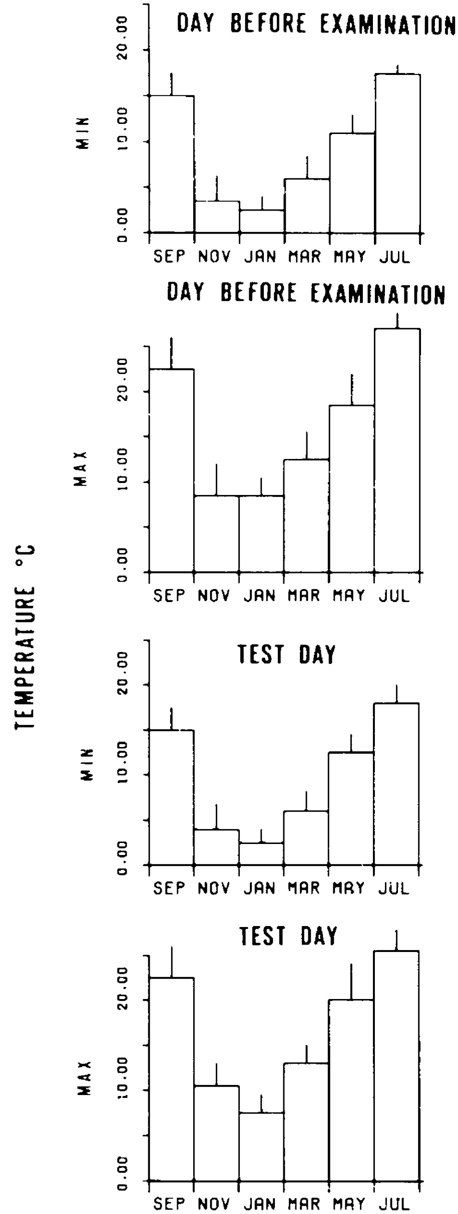

Fig. 1.
Minimum and maximum temperatures (T°C) (Borgo Panigale Airport, Bologna, Italy – Meteorological Service), recorded the day before and the day of the test during the period September 1979 to July 1980.

Hormonal assays

All determinations were made in duplicate. We used commercially prepared kits for 3,5,3'-triiodothyronine (T_3), thyroxine (T_4) and TSH (Diagnostic Products Co., Medfield, USA), 3,3',5'-triiodothyronine (reverse T_3, rT_3) (Abbott, Rome, Italy), thyroid binding globulin (TBG) (Corning Med., Medfield, USA); free T_4 (fT_4) and free T_3 (fT_3) were measured according to the method of

Table 1.
Body weight, relative weight, spontaneous caloric intake and physical activity in 24 healthy subjects who participated in the study. All parameters were evaluated every 2 months for 1 year. Physical activity was evaluated by means of a score ranging from 1 to 4 (see text for explanation).

	1979		1980			
	September	November	January	March	May	July
Body weight (kg)	61.9 ± 1.9	61.6 ± 1.8	61.7 ± 1.9	61.9 ± 1.9	61.8 ± 1.9	60.7 ± 1.7
Relative weight	100.7 ± 1.8	100.4 ± 1.8	100.3 ± 1.8	100.5 ± 1.8	100.1 ± 1.8	99.5 ± 1.8
Calories	2251 ± 106	2182 ± 105	2210 ± 112	2235 ± 118	2273 ± 120	2193 ± 121
Carbohydrates (g)	253 ± 15	240 ± 16	247 ± 17	248 ± 18	258 ± 17	242 ± 16
Proteins (g)	92 ± 3	90 ± 4	89 ± 4	90 ± 3	90 ± 4	87 ± 5
Lipids (g)	77 ± 4	74 ± 3	74 ± 3	76 ± 4	76 ± 4	80 ± 4
Physical activity	1.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1

Romelli et al. (1979) with reagents supplied by Lepetit Diag. Products, Milan, Italy. In our laboratory, inter- and intra-assay variation was respectively: 8.6 and 5.2% for T_3, 8.9 and 6.1% for T_4, 5.4 and 4.4% for TSH, 12.8 and 7.5% for rT_3, 3.9 and 3.8% for fT_3, 4.9 and 4.1% for fT_4, 5.9 and 4.8% for TBG.

Statistical analysis

All data are expressed in mean ± SEM of absolute values and their relative changes. Student's *t*-test was used when indicated. The possible periodicities of each hormone were determined by the cosine function (Koukkari et al. 1974). This provide an estimate of the mesor, the amplitude and acrophase by least squares fit of a cosine function. The mesor represents the rhythm-determined average value (midway between the highest and the lowest point of the cosine function used to approximate the rhythm). The amplitude represents the difference between the maximal value (or the minimal) and the mesor value of the cosine function. The acrophase is the lag from 0 time of the peak in the cosine function, expressed in degrees (360° = 12 months). In addition, the relative change of individual values were calculated in order to cancel the individual variability of mesors and the mean (± SEM) of relative changes were computed. This computation makes it possible to observe the occurrence of mean values for each hormone, which, after the addition or the subtraction of 2 standard errors, still fall respectively under or over the mesor (the value of which was expressed as being equal to 100). In this case, it is reasonable to presume that these represent statistically different modifications ($P < 0.05$) with respect to the mean of the parameter for the whole year.

Results

Environmental temperature, body weight and physical activity

Fig. 1 depicts the mean lowest and highest values of environmental temperaures recorded in Bologna during the period of study. Respectively, no differences were found between values recorded the day before each visit and those of the data collection day. January proved to be the coldest month and July the hottest. Table 1 reports mean values of body weight, nutritional intake and physical activity: no significant variations were found in the last two parameters while relative weight was slightly but significantly lower in July (99.5 ± 1.8) than in January (100.3 ± 1.8; $P < 0.03$).

Thyroid hormones and TSH

The patterns of absolute values for thyroid hormones, TSH and TBG serum concentrations during the study are shown in Table 2, while their relative changes are depicted in Fig. 2. T_3, T_4 and fT_4 serum concentrations showed a statistical circannual rhythm ($P < 0.05$), as shown by the cosine function with acrophase in September for T_3, in August for T_4 and in October for fT_4. Furthermore, some points in the patterns of rT_3 and TBG showed a significant difference from the mesors (2 standard errors added to or subtracted from mean values falling respectively over or under the

Table 2.
Absolute values of total and free thyroid hormones, TSH and TBG in healthy subjects evaluated in this study (m ± SEM). The Obs. column refers to the number of subjects who had all the hormones measured each time.

Hormones	Obs. No.	1979		1980			
		September	November	January	March	May	July
T_3 nmol/l	21	1.92 ± 0.11	1.86 ± 0.09	1.67 ± 0.08	1.70 ± 0.08	1.75 ± 0.08	1.83 ± 0.08
T_4 nmol/l	22	144.92 ± 6.18	148.00 ± 4.50	136.42 ± 6.05	133.85 ± 5.15	144.14 ± 6.95	148.00 ± 5.15
rT_3 nmol/l	19	0.32 ± 0.02	0.33 ± 0.02	0.30 ± 0.01	0.25 ± 0.01	0.33 ± 0.02	0.35 ± 0.02
TSH µU/ml	21	3.04 ± 0.47	2.78 ± 0.38	4.02 ± 0.37	2.81 ± 0.39	3.72 ± 0.39	2.98 ± 0.40
fT_3 pmol/l	22	7.08 ± 0.27	6.87 ± 0.37	7.08 ± 0.37	6.48 ± 0.31	7.04 ± 0.20	6.45 ± 0.28
fT_4 pmol/l	20	14.39 ± 0.67	13.79 ± 0.86	13.40 ± 0.62	12.45 ± 0.50	12.24 ± 0.49	13.29 ± 0.61
TBG nmol/l	22	345.7 ± 13.2	335.7 ± 15.4	288.1 ± 16.5	283.2 ± 11.1	294.5 ± 14.1	260.0 ± 11.1

mesors). In detail: for rT_3 the values of January and March fall under the mesor; for TBG the values for September and November fall over the mesors, while the values for March and July fall under the mesor (Fig. 2). This means that some circannual but not significantly sinusoidal changes were found for rT_3 and TBG.

In order to establish whether circannual changes would correlate to each other, a linear regression analysis was performed between the relative changes of all variables for each month and the respective correlation coefficient compound. A positive linear correlation was found between T_3 and T_4 ($r = 0.91$; $P < 0.01$) and between T_4 and rT_3 ($r = 0.81$; $P < 0.05$). This means that the sinusoidal oscillations for these hormones are in phase with each other during the period of study.

Moreover, a slight but highly significant negative correlation was detectable between the values of relative weight, measured during the period of the study and T_4 ($r = -0.27$; $P < 0.01$), fT_4 ($r = -0.30$; $P < 0.01$) and rT_3 ($r = -0.33$; $P < 0.01$) serum concentrations. On the contrary, no correlation was found between nutritional intake, physical activity and thyroid hormone or TSH values, nor between hormone levels and environmental temperatures.

Discussion

Studies in man have revealed that blood thyroid hormone patterns may differ during the year. Seasonal variations of thyroidal radioiodine uptake (Lewitus et al. 1964), protein binding bound iodine levels (Osiba 1957; Du Ruisseau 1965), serum (Smals et al. 1977; Rogowski et al. 1974; Oddie et al. 1979) and urine (Rastogi & Sawhney 1976) T_3 and T_4, and TSH (Rogowski et al. 1974; Konno & Morikawa 1982) have been documented by several investigators, though others did not confirm these findings (Hedstrand & Wide 1973; Postmes et al. 1974; Konno 1976). Taken together, these studies indicate that the coldest and the hottest period of the year (i.e. winter and summer) are associated respectively with greater or lesser blood concentrations of the thyroid hormones. Moreover, some studies revealed a positive correlation between the values of the environmental temperature and the serum concentrations of iodothyronines (T_3 and T_4) (Smals et al. 1977; Konno & Morikawa 1982). Finally, some studies have shown a periodical variation of serum TSH according to a circannual rhythm (Halberg et al. 1977).

In our study, we reported that the serum concentrations of TSH and fT_3 showed no significant variations during the various seasons. On the other hand, we found a significant variation in the concentrations of T_3, T_4 and fT_4 and the cosine function revealed that their acrophase was respectively August/September for T_3 and T_4 and October for fT_4. Moreover, although a significant periodical function was not found for TBG levels, the values of September and November were significantly higher than the mesor just as the values of March and July were significantly lower. Relative changes of T_4, T_3 and rT_3 showed a highly significant positive correlation with each other, suggesting that their sinusoidal oscillations during the period of study were in phase.

It is of interest to show that the zenith value (acrophase) of each hormone which revealed a periodical function was detectable well in advance

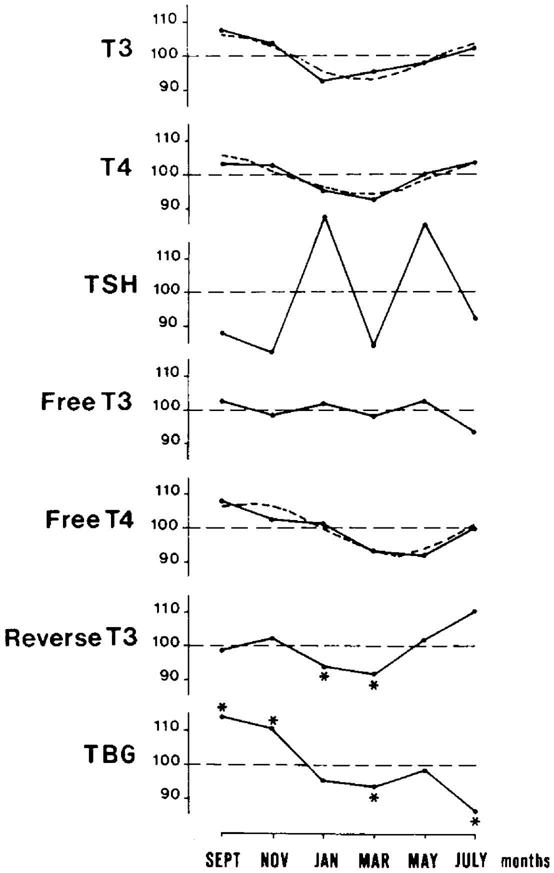

Fig. 2.
Circannual relative changes of total and free thyroid hormones, TSH and TBG; 100 per cent represents the mesor at each point. The dashed lines represent the theoretical values of the sinusoidal function relative to the hormones (T_3, T_4 and fT_4) for which a significant circannual periodicity was observed. Asterisks indicate values which, on the addition or subtraction of 2 SE do not reach the level of 100. Therefore, for these hormones some circannual but not sinusoidal changes were observed.

of the beginning of the cold season, while the lowest temperatures during the year were observed in January. This could mean that the start of the cold season is immediately associated with modifications in the concentrations of thyroid hormones and that hormone acrophase does not necessarily coincide with the coldest month. Relatively similar hormonal adaptations were also found in hibernating animals, such as as the woodchuck (Young et al. 1979). This could therefore explain why we found no correlation between the values of environmental temperature and those of iodothyronine during the study period. Comparisons with similar studies in humans (Smals et al. 1977; Du Ruisseau 1965; Rastogi & Sawhney 1976) are difficult since none of them used chronobiological methods in analysing the results.

The factors which may influence these variations are at present unknown. Changes in T_4, T_3 and fT_4 levels could be consistent with thyroid hyperactivity, probably as a consequence of hypothalamo-pituitary function (Jordan et al. 1980; Young et al. 1979; Hoffman & Barrow 1958). In fact, in rats, a fall in ambient temperature causes an increase in the rate of secretion and utilization of thyroid hormones (Ingram & Kaciuba-Uscilko 1977) and a similar increase in secretion from the thyroid gland can be elicited by cooling the hypothalamus (Gale et al. 1970; Reichlin 1964).

In this study, we evaluated the possibility that nutritional intake may be a factor affecting hormone blood concentrations. Both overnutrition (Danforth et al. 1979) as well as undernutrition (Yung et al. 1980; Danforth et al. 1979) are known to substantially affect iodothyronine peripheral metabolism. Moreover, an increase in food intake is usually observed in animals exposed to cold environments, while high temperatures may be associated with low nutritional requirements (Collins & Weiner 1968). In young growing pigs, a direct influence of food intake and ambient temperature on the rate of T_4 utilization was also found (Ingram & Kaciuba-Uscilko 1977). We found no variation in nutritional intake during the year, nor did we detect any correlation with thyroid hormone modifications. Our results could be in agreement with recent studies which found no evidence that increased food intake is a contributory factor in the development leading to increased iodothyronine plasma levels during cold exposure in rats (Van Hardeveld et al. 1979). On the other hand, we observed a slight but significant variation in relative weight, whose values were lower in summer and higher in winter. Values of relative weight were also slightly but significantly correlated to thyroid hormone serum concentrations. Therefore, since interaction between diet, environmental temperature and thermogenic hormones (such as iodothyronines) in the control of the whole body metabolism may be suggested, we believe that more extensive studies should be performed in this field in humans.

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References

Chen H J & Meites J (1975): Effects of TRH and somatostatin (SRIF) on TSH and prolactin release at high and low temperatures. Fed Proc 34: 342.

Collins K J & Weiner J S (1968): Endocrinological aspects of exposure to high environmental temperatures. Physiol Rev 48: 785–839.

Danforth E Jr, Horton E S, O'Connel M, Sims E A H, Burger A G, Ingbar S H, Braverman L & Vagenakis A G (1979): Dietary-induced alterations in thyroid hormone metabolism during overnutrition. J Clin Invest 64: 1336–1347.

Du Ruisseau J P (1965): Seasonal variation of PBI in healthy Montrealers. J Clin Endocrinol Metab 25: 1513–1515.

Eastman C J, Ekins R P, Leith I M & Williams E S (1974): Thyroid hormone response to prolonged cold exposure in man. J Physiol (Lond) 241: 175–181.

Epstein Y, Udassin R & Sack J (1979): Serum 3,5,3'-triiodothyroine and 3,3'5'-triiodothyronine concentrations during acute heat load. J Clin Endocrinol Metab 49: 677–678.

Evans S E & Ingram D L (1977): The effect of ambient temperature upon the secretion of thyroxine in the young pig. J Physiol (Lond) 264: 511–521.

Fisher D A & Odell W D (1971): Effect of cold on TSH secretion in man. J Clin Endocrinol Metab 33: 859–862.

Gale C C, Jobin M, Proppe D W, Notter D & Fox H (1970): Endocrine thermoregulatory responses to local hypothalamic cooling in unanaesthetized baboons. Am J Physiol 219: 193–201.

Halberg F, Caradente F, Cornelissen G & Katina G S (1977): Glossary of chronobiology. Chronobiologia 4, Suppl 1: 1–189.

Hedstrand H & Wide L (1973): Serum thyrotropin and lipid levels in summer and winter. Br Med J 1: 420.

Hoffman R A & Barrow M W (1958): Seasonal changes in the basophilic cells of the pituitary gland of the ground squirrel (Citellus tridecemlineatus). Anat Rec 131: 727–735.

Ingram D L & Kaciuba-Uscilko H (1977): The influence of food intake and ambient temperature on the rat of thyroxine utilization. J Physiol (Lond) 270: 431–438.

Jobin M, Ferland L, Cole J & Labrie F (1975): Effect of exposure to cold on hypothalamic TRH activity and plasma levels of TSH and prolactin in the rat. Neuroendocrinology 18: 204–212.

Jordan D, Rousset B, Perrin F, Fournier M & Orgiazzi J (1980): Evidence for circadian variations in serum thyrotropin, 3,5,3'-triiodothyronine and thyroxine in the rat. Endocrinology 107: 1245–1248.

Konno N (1976): Comparison between the thyrotropin response to thyrotropin-releasing hormone in summer and that in winter in normal subjects. Endocrinol Jpn 25: 635–640.

Konno N & Morikawa K (1982): Seasonal variation of serum thyrotropin concentration and thyrotropin response to thyrotropin-releasing hormone in patients with primary hypothyroidism on constant replacement dosage of thyroxine. J Clin Endocrinol Metab 54: 1118–1124.

Koukkari W L, Duke S H, Halberg F & Lee J K (1974): Circadian rhythmic leaflet movements: student exercise in chronobiology. Chronobiologia 1: 281–302.

Lewitus Z, Hasenfratz J, Toor M, Massry S & Rabinowitch E I (1964): Uptake studies under hot climate conditions. J Clin Endocrinol Metab 24: 1084–1086.

Metropolitan Life Insurance Company (1960): Frequency of overweight and underweight. Stat Bull Metropol Life Insur Co 41: 4.

Oddie T H, Klein A H, Foley T P & Fisher D A (1979): Variation in values for iodothyronine hormones, thyrotropin and thyroxine-binding globulin in normal umbilical-cord serum with season and duration of storage. Clin Chem 25: 1251–1253.

Osiba S (1957): The seasonal variation of basal metabolism and activity of thyroid gland in man. Jap J Physiol 7: 355–365.

Postmes Th J, Van Hout J C, Saat G, Willems P & Coenegracht J (1974): A radioimmunoassay study and comparison of seasonal variation in plasma triiodothyronine and thyroxine concentrations in normal healthy persons. Clin Chim Acta 50: 189–195.

Rastogi G K & Sawhney R C (1976): Thyroid function in changing weather in a subtropical region. Metabolism 25: 903–908.

Raud H R & Odell W D (1969): The radioimmunoassay of human thyrotropin. Br J Hosp Med 2: 1366–1376.

Reichlin S (1964): Function of the hypothalamus in regulation of pituitary-thyroid activity. In: Cameron M P & O'Connor M (eds). Brain-Thyroid Relationships, p 17–32. Little & Brown, Boston.

Rogowski P, Siersbæk-Nielsen K & Hansen M (1974): Seasonal variation in neonatal thyroid function. J Clin Endocrinol Metab 39: 919–922.

Romelli P B, Pennisi F & Vancheri L (1979): Measurement of free thyroid hormones in serum by column absorption chromatography and radioimmunoassay. J Endocrinol Invest 2: 25–32.

Sellers E A & Yon S S (1950): Role of the thyroid in metabolic responses to a cold environment. Am J Physiol 163: 81–92.

Smals A G H, Ross H A & Kloppenborg P W C (1977): Seasonal variation in serum T_3 and T_4 in man. J Clin Endocrinol Metab 44: 998–1001.

Tuomisto J, Männistö P, Lamberg B A & Linnoila M (1976): Effect of cold exposure on serum thyrotrophin levels in man. Acta Endocrinol (Copenh) 83: 522–527.

Van Hardeveld C, Zuidwijk M J & Kassenaar A A H (1979): Studies on the origin of altered thyroid hormone levels in the blood of rats during cold exposure. Effect of iodine intake and food consumption. Acta Endocrinol (Copenh) 91: 473–483.

Wilson O, Hedner P, Laurel S, Nosslin B, Rerup C & Rosengren E (1970): Thyroid and adrenal response to acute cold exposure in man. J Appl Physiol 28: 543–548.

Young R, Danforth E, Vagenakis A G, Krupp P P, Frink R & Sims A H (1979): Seasonal variation and the influence of body temperatures on plasma concentrations and binding of thyroxine and triiodothyronine in the woodchuck. Endocrinology 104: 996–999.

Yung R T, Shetty P S & James W P T (1980): Nutritional effects on thyroid and catecholamines metabolism. Clin Sci 58: 183–191.

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