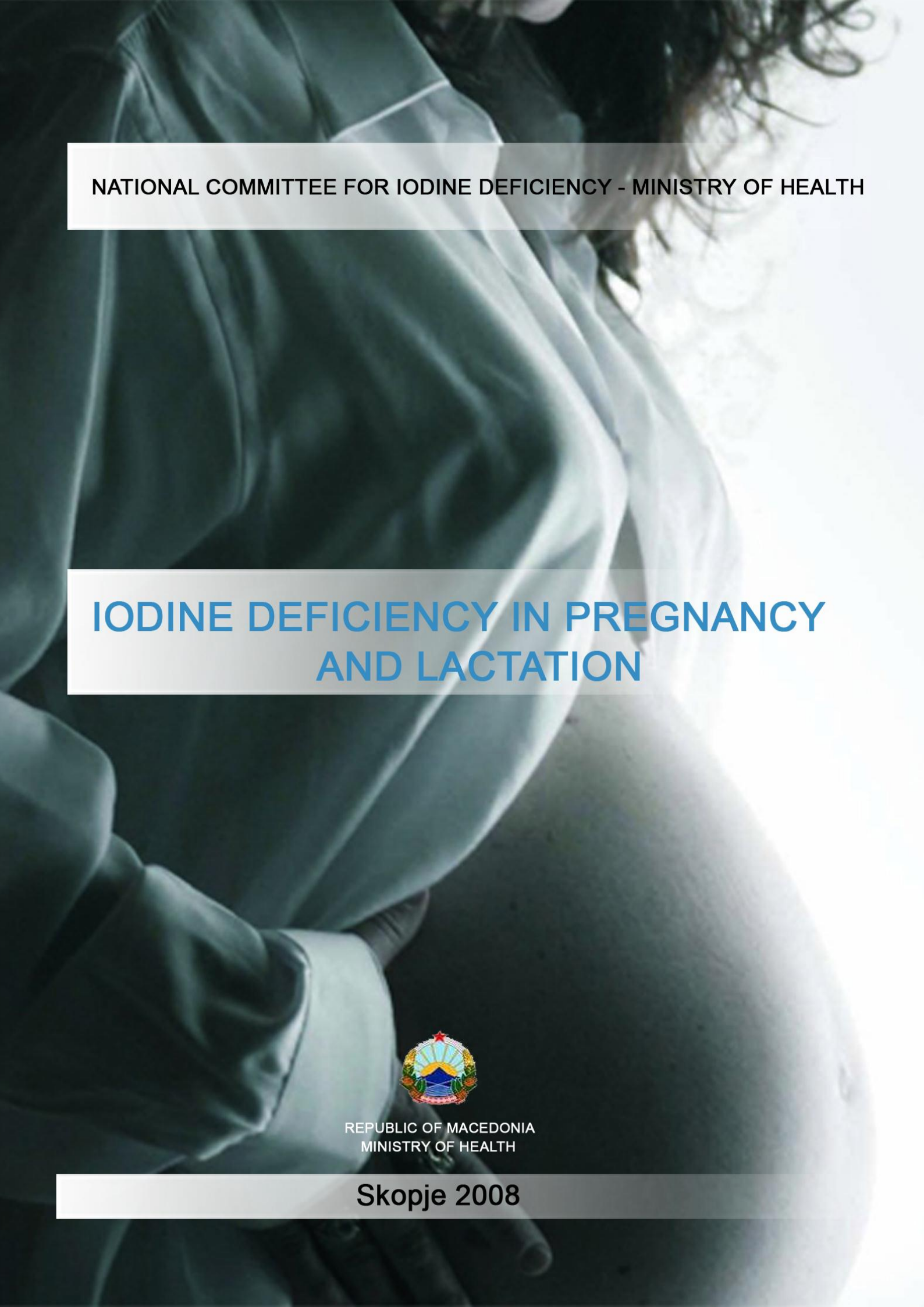

NATIONAL COMMITTEE FOR IODINE DEFICIENCY - MINISTRY OF HEALTH

IODINE DEFICIENCY IN PREGNANCY AND LACTATION

REPUBLIC OF MACEDONIA
MINISTRY OF HEALTH

Skopje 2008

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CONTENT

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Frequently used abbreviations:

CNS – Central Nervous System
DIT – diiodotyrosine
DNA – Deoxyribonucleic acid
H_2O_2 – Hydrogen peroxide
hCG - human chorionic gonadotropin
ICCIDD – International Council for Control of Iodine Deficiency Disorders
MIT - monoiodotyrosine
NIS – sodium – iodide co-transporter
rT_3 - reversed - triiodothyronine
T_2 - diiodothyronine
T_3 - triiodothyronine
T_4 - tetraiodothyronine
TBG – thyroxin binding globulin
TG - thyroglobulin
TPO – thyroid peroxidase
TRH – Thyrotropin Releasing Hormone
TSH – thyroid stimulating hormone (thyreothropin)
TSI – thyroid stimulating immunoglobulin
UNICEF – United Nations Children's Fund
WHO – World Health Organization

FOREWORD

In the past, the Republic of Macedonia was an iodine deficient area with frequent incidences of goiter which, in some regions was endemic. Through a long lasting well programmed activity of all relevant societal factors in the country, coordinated by the National Committee for Iodine Deficiency , the iodine deficiency in the country was corrected, as confirmed also by the document of the expert group of the WHO,UNICEF and ICCIDD.

However, this improvement of the situation with iodine deficiency can not be applied to pregnant and lactating women whose thyroid hormone requirements are almost doubled in comparison with the rest of the population. In order to the thyroid gland to be able to satisfy the increased requirements of the organism during pregnancy and lactation, the iodine intake must be increased. Iodized salt used for the rest of the population cannot satisfy the iodine requirements of pregnant and lactating women.

The iodine deficiency in the organism during pregnancy causes hypothyroxinemia of the mother, with serious disorders in the development of the brain of the fetus, as well as mental retardation and reduction in the IQ coefficient of the newborns. During the period of lactation, the infant receives iodine through the breast milk of the mother which provides iodine for normal synthesis of thyroid hormones and normal psychophysical development of the infant. In the event of iodine deficiency of the mother, the breast milk of the mother cannot transfer the required quantity of iodine in the organism of the infant.

In the world today there is consensus about offsetting the iodine deficiency of pregnant and lactating women by iodine supplementation. Many developed countries already apply the principle of giving daily iodine dosages, usually in the form of tablets. The iodine supplementation of the pregnant and lactating women is used in many countries in Europe, and the dosages are determined for each country separately.

Our research aimed at detecting and studying the extent of the iodine deficiency in pregnant and lactating women in Macedonia in order to assess the need, as well as the dosage of iodine necessary for the iodine supplementation.

The overcoming of the problem with the iodine deficiency in pregnant and lactating women requires a wider societal involvement. The findings obtained from the research initiatives should be conveyed to the health professionals in order to motivate them to implement the iodine supplementation. The involvement of education organizations, societal and nongovernmental organizations should provide to the pregnant and lactating women the possibility to accept and use the iodine supplementation as an especially beneficial preventive measure, which will ensure a normal psychophysical development of newborn babies.

Borislav Karanfilski

THE ROLE OF IODINE IN PREGNANCY

Olivija Vaskova

Pregnancy is a complex physiological process accompanied by a series of hormonal and metabolic changes aiming at adapting of the endocrine system of the mother. The hormonal processes, in harmony with the metabolic requirements, cause changes of the biochemical parameters of the thyroid status (1), thereby changing the metabolism of iodine, which is an essential component of the thyroid function. They occur sequentially during the gestation stages, they reflect on the thyroid of the fetus, and may be present reversibly even after the birth (2). These processes have stimulatory effect on the function of the thyroidal gland of the mother (1).

Iodine is the main substrate of the specific metabolism of the thyrocytes. It is the key structural component of the thyroid hormones, L – thyroxin, (tetraiodothyronine, T_4) and L-triiodothyronine (T_3), which are essential for the differentiation, the growth, metabolism, and physiological functions of all tissues (3). The thyroid hormones are necessary for the normal maturation of the central nervous system (4) and the development of the brain of the fetus, i.e. the processes of terminal brain differentiation including the growth of the dendrites and axons, synaptogenesis, neural migration and mielinization (5).

Iodine as an essential micronutrient, is introduced in the organism through food (in the form of iodide or iodate salt, by using iodinated salt and/or other iodinated additives used in the food industry), the drinking water, but also accidentally through medical preparation, in the form of therapeutic or contrast diagnostic agents.

Most of the iodide in the gastrointestinal tract is reduced and almost completely absorbed in the small intestine. The iodinated amino acids, the iodopeptides with short chains, as well as the radiographic iodinated dyes pass intact through the wall of the intestine. The iodide absorbed in the blood binds to the serum proteins, mostly albumin, while the free iodide is excreted through the kidneys with the urine. The concentration of iodide in the plasma, with the exception of the period of absorption, is 10 μg/L. It is distributed in a volume which corresponds to approximately 38% of the body weight of an adult. The biggest portion is located in the extracellular compartment and only small quantities are located within the cells.

About 80% of the iodide in the body is used by the thyrocytes, and the rest is transported in the salivary glands, gastric mucosa, chorioid plexus, placenta and the breast of lactating women. Small quantities of iodine can be found in sweat and exhaled air.

Breast milk contains a significant quantity of iodine, mainly during 24 hours after intake of iodine in the organism (6) and is the only source of iodine for the newborn. The content of iodine in breast milk is proportional to dietary iodine (6, 7).

Most of the iodide from the plasma goes in the thyroid gland and the kidneys. The renal iodide clearance is 30-50 ml per minute (8) and mainly depends on the glomerular filtration. The increase of the renal blood flow and the glomerular filtration in pregnancy cause an increased excretion of iodide in urine. This reduction of the available iodide is emphasized during the second half of the pregnancy because the iodide is targeted to the fetoplacentary unit (9). The concentrations of serum anorganic iodide fluctuate during pregnancy and lactation; however, with the adaptation of the organism of the mother with sufficient iodine intake they do not vary significantly (10).

According to the recommendations of numerous research studies, as well as the World Health Organization, the iodine requirement for maintaining the euthyreotic state of pregnant and lactating women grows from 150 μg/day to about 250 μg/day. The increased

iodine needs lead to vulnerability of the thyroid gland of the mother, especially in the regions with borderline iodine intake (11, 12).

The thyrocytes extract the iodide from plasma and normally maintains 20-50 times greater concentration of free iodide in the gland, in comparison to the concentration in the plasma. The concentration gradient varies along a very wide range depending on the functional status of the gland, but also on the available quantity of iodide.

The active accumulation of iodide and synthesis of thyroid hormones in the fetal thyroid gland is detectable on the period between the tenth and twelfth week of gestation. However, in human fetuses, active receptors for thyroid hormones are found even before the active iodine uptake and secretion of the thyroid hormones from the fetal thyroid gland (13). Prior to this, the fetus is completely dependent on the very significant (14) placental diffusion of the thyroid hormones of the mother (15). During that period the factor determining the thyroid status of the fetus is the mother – fetus transmission of the thyroid hormones. Although the fetal hypothalamic -pituitary axis develops later on and functions independently, beyond the first trimester of pregnancy, the thyroid hormones of the mother contribute to maintaining the fetal thyroid status (16, 17). Thus, the human utero-placental unit is a sophisticated system that can regulate the amounts of T_4 and T_3 transferred from the mother in relation to the age of the developing fetus and the production capacity of the fetal thyroid (16).

The synthesis of T_3 and T_4, the most significant segment of iodine metabolism in the thyroid occurs in several successive steps:

1) Active transport of the iodide across the basal membrane of the thyrocytes;
2) Oxidation of the iodide and iodination of the tyrosine residues in the thyreogrlobulin (TG);
3) Linking pairs of molecules within TG to form iodothyronines T_3 and T_4;
4) Proteolysis of TG and secretion of the thyroid hormones;
5) Deiodination of the iodothyrosines within the thyroid cells;
6) Conversion of T_4 in T_3 and transport of thyroid hormones and
7) The last, very important segment for the tissue effect of the thyroid hormones is their peripheral metabolism.

Transport of the iodide through the membrane of the thyreocytes

The iodide accumulation is the first critical step in the synthesis of thyroid hormones. The process of transport of the iodide through the basolateral plasmatic membrane of the thyreocytes depends on the availability of energy and oxygen and is under the influence of the membrane Na^+-K^+ ATP- ase. The active transport mechanism uses the energy released with the intake of sodium at a lower electrochemical gradient, and a simultaneous intracellular translocation of the iodide, opposite from the electrochemical gradient. The protein that enables this function, sodium – iodide co-transporter (NIS) is abundantly exprimated on the basolateral membrane of the thyroid follicular cells. Human NIS has 643 amino acid sequences, and the gene coding its synthesis is localized on the 19^{th} chromosome (18). Many studies have clearly shown that NIS is responsible for most of the events for iodide concentration by thyroid gland (19, 20, 21, 22).

A physiological stimulator of the function of the NIS is the thyroid stimulating hormone (TSH). The pathological thyroid stimulating immunoglobulin (TSI) is present in the Graves- Basedow disease. The salivary glands, the mucosa of the gaster, the tissue of the mammary gland, as well as the placenta do not express NIS so strongly; however they also, although to a lesser extent, concentrate iodide. However they do not organify or store iodide and their NIS activities are not stimulated by TSH.

The iodide transport mechanism is highly regulated, allowing adaptation to variations in dietary supply. Large quantities of iodide suppress not only the NIS activity, but also its genetic expression, whereas low levels of iodide increase the expression of NIS and iodide accumulation (iodide auto-regulation mechanism).

After entering the thyrocyte, the iodide is transported to the apical membrane of the thyroid follicular cell, where the second iodide protein transporter pendrin is located. It facilitates the transport of the iodide in the follicular lumen. The passive transport through the apical plasmatic membrane of the thyrocyte, aside of the pendrin, is enabled by another protein, discovered in recent times, i.e. the so called apical iodide transporter (23). The pendrin codes the PDS gene. It comprises 780 amino acids, and it functions as chlor/iodide co-transporter (24, 25). It is expressed in different organs including kidneys, the inner ear and the thyroid gland (26, 27). The mutation of the PDS gene causes the Pendred syndrome characterized by defective organification of iodine, goiter and sensoneural hearing loss. The apical iodide transporter has 610 amino acids, of which 46% are identical to those of NIS (23). The iodide that enters the thyroid gland is free for about 10 to 20 minutes before metabolizing and it accounts for less than 1% of the total iodine pool of the gland.

Oxidation of the iodide and incorporation in the organism

After the transport the iodide quickly oxidizes with the locally produced H_2O_2, in a reaction catalyzed by the enzyme thyroid peroxidase (TPO). The oxidation helps create an active iodide intermediate which is incorporated (in part of the tyrosine residuals) in the thyroglobulin molecule, process known as organification of iodine. The products of organification are iodothyrosines: monoiodothyrosine (MIT), and diiodothyrosine (DIT) inactive products of iodine metabolism. The assumption is that H_2O_2 is created with the help of NADPH-oxidase and calcium. At the same time, the necessarily high concentrations of H_2O_2, are potentially harmful for the thyrocytes, and its production is a limiting factor in the synthesis of thyroid hormones. This process is regulated by TSH through a complex network of interactive systems of secondary messengers (28). The iodination of TG and the production of H_2O_2 occur on the luminal surface of the apical membrane of the thyrocytes. Such structural organization of the processes makes the surface H_2O_2 immediately available for the iodination reactions, while the excess harmful H_2O_2 diffuses in the thyrocytes, where it is degraded with glutathione peroxidase, catalases and thyroxin reductases (29).

TPO is a membrane glycoprotein. It is synthesized in the rough endoplasmic reticulum of the thyrocytes. The expression of the gene coding the synthesis of TPO, depends on the level of TSH, and the function of enzyme depends on the share in the hem of its structure (30). In addition to TG, TPO catalyzes the iodination of the tyrosine molecules in other proteins as well, such as albumin or thyroglobulin fragments but this does not lead to the production of products with hormonal activity (31).

TG is the most abundant protein in the thyroid gland and is the main ingredient of the colloid. The concentration in the follicular lumen may reach up to 200-300 g/L. Its biochemical characteristics have been defined in great detail (32). It is a large homodimeric glycoprotein. Carbohydrates account of 10% of its weight. The N-terminal segment, marked as “TG type 1 domain” acts as a potent inhibitor of the cysteine proteases active in its proteolysis. The TG molecule can, through this segment modulate its own degradation and the hormone release (33, 34). The S-terminal segment of the molecule is homologous with the acetylcholinesterases and by analogy it is presumed that it may be responsible for binding with the cell membrane (35).

The gene that codes the synthesis of TG is localized on the long arm of the eighth chromosome. The expression of the gene is regulated by TSH, but also by certain transcription factors specific for the thyroid, which also regulate the synthesis of TPO (36). TG is synthesized by the thyrocytes, and the polypeptide chain starts maturing in the rough endoplasmic reticulum. After the glycosylation of the nucleus, monomers fold into dimers.This process is very significant for the appropriate synthesis of the new TG molecules and largely depends on the main molecular chaperones BiP, GRP 94, ERP 72 and calexin (37, 38).

TG also provides the peptide backbone for synthesis and storage of thyroid hormones (39). At the same time it also offers a convenient depot for iodine storage which is then made available for hormonal synthesis in the event of an external deficiency.

The thyroglobulin molecule has about 140 thyrosine residues, but only 4 thyrosine have the appropriate steric (spatial) orientation for an effective hormonogenesis. Isolated from the thyroid gland it rarely contains more than 1% iodide, i.e. the thyroglobulin iodine content varies from 0,1%-1%.

Coupling of the iodothyrosines and formation of iodothyronines

The next step in the hormonal synthesis, also catalyzed by TPO, is the coupling of the iodothyrosines and the formation of the iodothyronines. After the oxidation of the iodide with H_2O_2 and TPO it is transferred to the thyrosine residues in TG. These process is preceded by a reaction between H_2O_2 and TPO which form an oxidizing “Component-I” which participates in the formation of iodothyrozines. This is followed by the formation of “component-II” which is necessary for the coupling reaction and formation of thyroid hormones. The excess iodide inhibits the formation of the second component, and thus the formation of thyroid hormones. The coupling of the two DIT molecules results in the formation of T_4, and the coupling of MIT and DIT results T_3 and small quantities of biologically inactive rT_3. The reaction depends on H_2O_2, but also on the TG structure.

The usual distribution of products of hormone synthesis in TG which contains 0.5% iodine for an iodine sufficient person includes 5 residues of MIT and DIT 2.5 T_4 and 0.7 T_3. The increase of iodine available to the gland increases the ratio DIT/MIT and T_4/T_3, and the iodine deficit has the opposite effect. The iodinated TG is deposited extracellulary in the lumen of the thyroid follicles. Physiologically, the organic iodine pool, localized mainly in the intrafollicular material changes with a rate of 1% per day (8).

Proteolysis of the thyroglobulin and secretion of thyroid hormones

The prohormone TG of the apical thyrocyte membrane, with mechanisms of macro and micropinocytosis is absorbed in the thyroid polarized cell. The internalization begins with organizing micro domains on the apical membrane. After this process, some TG molecules are transferred to the lysosome compartments. Part of the molecules is recycled directly or indirectly into the follicular lumen (40), and the rest are transported and released through the basolateral membrane of the thyrocytes in the plasma (41). The TG molecules in the lysosomes are exposed to hydrolytic reactions that create free thyroid hormones and completely degrade TG.

The disintegration of TG begins with the action of endopeptidases, in particular the cathepsins D, H and L, and then the products react with the exopeptidases. It is assumed that the proteolysis of TG occurs in two sequential steps: 1) early and selective disintegration with release of T_3 and T_4 and 2) late complete proteolysis. After the digestion of TG, the T_3 and T_4 move from the lysosomes to the cytoplasm, and from their they go in the circulation through the basolateral plasmatic membrane by way of simple diffusion.

Inactive iodothyrosines, peptides and free amino acids are also released in the thyrocyte. The existence of a lysosome iodothyrozine membrane transporter has been proven.

Other components, such as serum albumin (thyroalbumin), present during a disease of the gland, or lipids that reduce the production of H_2O_2 and in turn reduce any further iodination of TG, are rarely iodinated in the gland (42).

The secretion of the thyroid hormones is stimulated by TSH and inhibited by excess iodine. Usually the thyrocytes also release small quantities of TG.

Deiodination of the iodothyrosines in the thyrocytes

The unbound and hormonally inactive MIT and DIT are deiodinated in the thyrocytes and thus facilitate recycling of the iodide which has not been converted in thyroid hormones. The reaction is specifically catalyzed by the enzyme iodothyrozine dehalogenase, NADPH dependent flavoprotein isolated from the mitochondria and microsomes of the thyrocytes. Usually only a small portion of the iodine is lost from the gland in circulation. The loss increases as the iodine intake increases, and this is assumed to be a possible auto regulatory mechanism for prevention of excessive iodination of TG. Although the values of the non-hormonal iodine lost from the gland vary, for a daily intake of about 100 μg, a normal gland may release up to 50 μg iodide.

Conversion of T_4 into T_3 and transport of the thyroid hormones

In the peripheral tissues, the 5' type I deiodinase converts T_4 into T_3 and the same process happens within the thyroid. However, T_4 is secreted from the thyroid in quantities twenty times bigger than those of T_3.

In circulation thyroid hormones bind to the plasma proteins: globulin, transthyretin and albumin. These proteins influence the pool of circulating hormones, slow down the hormonal clearance and modulate the delivery of the hormones in the tissues. The concentration of the carrying globulin (TBG) is relatively low, but due to its large affinity, mainly for T_4, and less so for T_3, it carries about 80% of the binding hormonal fraction. The transthyretin binds 10% of T_4 and very little T_3, while the albumin, which has a relatively low affinity, but a higher concentration in the plasma, binds 10% of the T_4 and 30% of the T_3. The free concentrations of the hormones are approximately $2x10^{-11}$M for T_4 and $6x10^{-12}$M for T_3, which corresponds to their receptor constants for tissue binding.

The most frequently reported change in the thyroid physiology of the mother during pregnancy is the increase of the level of TBG (43). This increase begins in the first trimester of the pregnancy, levels off in the midgestation and remains approximately double the level during non-pregnancy. Quickly after delivery, during 4 to 6 weeks, the level normalizes and returns to the value before the pregnancy. The increase in TBG is also attributable to the reduction of the liver clearance because of the induced syalisation with estrogen hormones (44). The increased concentration of TBG expands the extra thyroidal pool, triggering a concomitant increase in maternal thyroid hormone synthesis and elevation of total T_4 and T_3 levels.

Regarding the levels of total T_4, T_3 and TSH during pregnancy, the literature data are consistent, which is not the case with the level of the free T_4, especially during the first trimester, where the referent literature reports higher, lower or same concentrations as before the conception (45). The different values can be partly attributed to the differences in iodine intake by the observed individuals (46). Still, it is presumed that the concentrations of the free fractions of the thyroid hormones easily increase during the first trimester, as a response to the increased human chorionic gonadotropin (hCG). It has an intrinsic thyrotropic activity due the homology between the hormone specific beta – sub unit and the extracellular receptor binding domains of hCG and TSH (47, 48). It is

assumed that the high serum concentrations of hCG in early pregnancy directly trigger the TSH receptor. This claim is corroborated by the negative correlation between the values of the serum concentrations of hCG and TSH, and positive correlation with the concentrations of free T_4 (45, 49).

Peripheral metabolism of thyroid hormones

The peripheral metabolism of thyroid hormones is a critical step for their intracellular activity. They metabolize in the peripheral tissues, with enzyme catalyzed deiodination, conjugation, deamination and decabroxilation.

The significance of the deiodination arises from the fact the activation of the thyroid hormones require the activation of the long-lived prohormone molecule T_4 (T/2~7 days) with deiodination in the short-lived biologically active T_3 (T/2~1 day). T_3 modulates the genetic expression in almost all of the tissues of vertebrae, by interacting with ligand dependant transcription factors – thyroid hormone receptors. The deiodination of T_4 intoT_3 occurs in the phenol, external or 5' ring and it is catalyzed by two iodothyroxin deiodinases D_1 and D_2. On the other hand, inactive rT_3 occurs when inhibiting the activation with deiodination of the inner ring in position 5 and the reaction is catalyzed with D_3 or D_1 deiodinase.

Three iodothyronine deiodinases have been identified until today (D1- type I, D2- type II and D3- type III). All of them are integral proteins from 29-33 kDa and have a 50% identical sequence. Each of them has a selenocysteine residue in the active center which facilitated the highly catalytic activity of the enzyme (50). They have different substrate specific and tissue distribution (16). The coordinated changes of the expression and activity of these enzymes provide the homeostasis of the thyroid hormones and appropriate formation of T_3. Thus they are a part of the very important mechanism of adaptation of the organism to the quantitative variations of the iodine intake.

The deiodinase D1 is the most frequent isoform in the liver, kidneys and the pituitary gland. It catalyzes the 5 or 5' monodeiodination. The significant physiological role of D1 involves the provision of most of the peripheral 3,5,3'-T_3. The active place of the enzyme contains selenocysteine and its activity depends on selenium.

The pituitary gland, the nervous system and the dark adipose tissue with 5'-type II deiodinase are also capable of converting T_4 into T_3. These tissues are relatively independent from the circulating levels of T_3 for their metabolic requirements. In normal serum concentrations of T_3, the thyroid receptors in most tissues are about 50% saturated. Additional quantities of T_3 are formed in the tissues that excrete the D_2 deiodinase with conversion of the intracellular thyroxin, and therefore the saturation of the receptors may reach even 100% (and half of the T_3 is produced locally). Type III deiodinase is also isolated in the central nervous system and it catalyses the deiodination of T_4 in position 5, and therefore rT_3 is formed in this tissue as well (51, 52).

The products of the further transformation of the thyroid hormones, the isomers of T_2 (3,5-diiodothyronin, 3,3'-diiodothyronin and 3',5'- diiodothyronin) and T_1 are formed only in the peripheral tissues (53). All transformations are catalyzed with deiodinase enzymes which remove the iodine atoms from the inner or external phenol benzene rings in the hormones.

The deiodinases have a significant tissue and time specific expression during the fetal period and they are important regulators of the processes of maturation of the fetus, modifying the delivery of T_3 to the T_3 – responsible genes (54, 55).

The reactions of conjugation, through glucuronidation or sulfation result in an irreversible elimination of the thyroid hormones and they are primarily catalyzed with the microsomal enzymes of the liver. The main substrate for this type of reactions are the

partially deiodinated metabolites of the thyroid hormones (56). The liver decarboxilation and deamination also, although to a lesser degree, participate in the formation and elimination of the specific metabolites of the thyroid hormones (57).

Pregnancy is a complex physiological state which causes a series of hormonal and metabolic adaptation mechanisms in the endocrine system of the mother. The thyroid hormones, their precursors and degradation products are the only natural bodily components which contain iodine and the processes of their synthesis and degradation, and closely linked with the iodine metabolism. The emphasized alterations in the thyroid economy result in a complex combination of factors which stimulate the thyroid gland of the mother and increase the iodine requirements to about 200 micrograms per day, providing euthyroid state of the mother and normal development of the fetus.

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IODINE DEFICIENCY AND PREGNANCY

Borislav Karanfilski

Function of the thyroid gland during pregnancy

The basic characteristics of the thyroid function of the mother during pregnancy depend on the increased thyroid hormones requirements during the first, early stage of gestation. During that time, the thyroid gland of the mother must provide optimal quantities of thyroid hormones that will ensure normal growth of the fetus. These optimal quantities are 1.5 to 2 times greater in comparison to the period before pregnancy. The increased hormone requirements can be satisfied only by an increased hormone production which, for a healthy woman, depends on the appropriate iodine intake. In case of an insufficient iodine intake, instead of a normal physiological adaptation, a pathological alteration occurs as a result of the chronically increases thyroid stimulation. This stimulation occurs as a consequence of several factors acting independently on the thyroid gland during pregnancy.

The first factor is the adaptation of the thyroid gland to the significant increase in the level of protein serum carrier of the thyroid hormones – thyroxin binding globulin (TBG), which occurs as a result of the increased level of estrogen hormones during pregnancy. The estrogen hormones stimulate the production of TBG with a heavier molecular form which has prolonged T ½. In order to cause such an effect the level of estrogens in the blood should reach a concentration of 500-1,000 ng/L. During normal pregnancy, the estrogen hormones reach this level between the 6^{th} and 12^{th} weeks after conception, which can explain the significant individual differences between TBG levels. In addition to this main effect of the estrogen hormones, one should also have in mind the other, more subtle effects. Thus, some professional publications suggest that the estrogen hormones can have a direct impact on the thyrocytes and the secretion of TSH. However, up to now there are no reliable data suggesting that these effects of the estrogen hormones have a significant role during normal pregnancy (1).

For a non-pregnant adult woman, TBG transports about 2/3 of the thyroxin in the blood, while 1/3 is bound to the other two protein carriers of thyroxin. When the level of TBG increases proportionally, most of the thyroxin binds to TBG (>75%), and the bonds are tighter since the binding affinity of TBG with thyroxin increases (2). The increase of TBG temporarily reduces the free serum fraction of the thyroid hormones which through the normal pituitary-thyroid feedback mechanisms , stimulates the secretion of TSH from the pituitary gland. The increase of serum concentrations of TSH causes an increase of the production of thyroid hormones and the reestablishment of homeostasis of their free fractions. The faster the TBG blood levels increase, the quicker the system must adapt. These changes in the adaptation occur easily in the organism has sufficient quantities of iodine available and if the functional capacity of the thyroid gland is preserved (for example: this capacity may be reduced if the woman suffers from autoimmune thyroiditis) (3). The concentrations of TBG in the extracellular space increase, during the first half of the pregnancy, from about 2,700 to 7,400 nmol. Since the changes in TBG occur in a period of three months, adaptation means that the secretion of T_4 from the gland, during the first month will increase by 40% in comparison to the period before pregnancy, by 60% during the second month, and by 75% during the third month of pregnancy. During this time, this represents an increase of 1% to 3% per day. Such an increase is relatively small for the thyroid gland with adequate iodine intake. However, when the iodine intake during pregnancy is limited, this adaptation mechanism of the thyroid gland cannot be realized which leads to a an insufficient thyroxin saturation of the increased TBG level, while, at the

same time, the serum concentration of the free thyroxin and triiodothyronine reduces, the serum TSH increases and goiter occurs (4).

The second factor is the human chorionic gonadotropin which, during pregnancy, forms in large quantities and reaches its highest levels by the end of the third month. According to the molecular structure the human chorionic gonadotropin is very similar to the TSH. It, although to a much lesser extent, directly stimulates the thyroid gland of the mother. Presently, it is unclear whether the thyromimetic effect of the gonadotropin has a physiological role or if it is just a result of the molecular similarity with TSH.

The third factor is the increased deiodination of the placenta which can have an important role in the acceleration of the peripheral metabolism of thyroxin. Three enzymes have been identified (selenoproteins according to their structure), as integral membrane proteins that catalyze the deiodination of the thyroid hormones in tissues. The deiodinase of type I acts primarily in the liver, kidney or the thyroid gland. It causes deiodination of the external T_4 ring, thus becoming the main source of T_3 in the blood. Therefore, this enzyme provides T_3 not only to the organs where it is formed, but also to other tissues. The activity of this deiodinaze does not change during pregnancy. Deiodenase of type II acts primarily on the brain and anterior pituitary, but also on the placenta. It acts on the external T_4 ring. It provides T_3 locally to the organ itself, in addition to the T_3 already present in the blood, previously created by the diodenase type I. The activity of this type of diodenase is strengthened with the reduction of the T_4 level, during hypothyroidism or iodine deficiency. It was already mentioned that the activity of the deiodinase in the placenta is greatest during the first three months of the pregnancy, while this is hard to prove for the last phase of the pregnancy. Therefore, the relatively high activity of the diodenase type II in the placenta can provide for sufficient local production of T_3 to help the differentiation of the trophoblastic cells. In addition, the activity of this deiodenase is important for the maintenance of homeostasis in the production of T_3 in the placenta, when the concentration of T_4 in the blood of the mother is reduced (5). The deiodenase of type III catalyzes the conversion of T_4 and T_3 in their inactive metabolites rT_3 and T_2. It acts predominantly on the brain, the placenta and the tissues of the fetus (for example: the liver). It has been demonstrated that the placenta contains large quantities of this deiodenase. Although its precise location in the placenta membrane cannot be determined, the enzyme is mostly present in the trophoblasts (6). It is presumed that the physiological role of this deiodenase is to prevent the unlimited transfer of thyroid hormones of the mother in the fetal area and to block the excessive formation of T_3 in the fetus. The activity of this deiodenase reduces during the gestation, however since the total surface area of the placenta is much larger during the second part of the gestation, the total activity per placenta of the deiodenase increases significantly as the gestation period progresses. Therefore the physiological role of this deiodenase is to reduce the toxic level of T_3 in the fetus, to inactivate the mother T_4 and to provide iodine to the fetus, as well as to regulate the metabolism of the thyroid hormones originating from the fetus (7).

In addition, during the pregnancy there is an increased elimination of iodine from the organism. The renal clearance of iodine significantly increases from 1.3 to 1.5 times its normal values (which are 30-50 ml plasma per minute), as a result of the increased glomerular filtration. The renal clearance of iodine starts to increase in the first weeks of the gestation and continues all the way to delivery (8). The loss of iodine through the kidneys causes a reduction of the plasmatic inorganic iodine, and thereby causes a compensatory increase of the thyroid iodine clearance which can reach values of 60-100 ml plasma per minute (normal values are 15-25 ml).

These mechanisms increase the thyroid activity during pregnancy. In circumstances of iodine sufficiency, the pregnancy does not have any significant influence on the iodine

concentration in the blood, while if there is an iodine deficiency, the concentrations of inorganic iodine in the blood are reduced. During pregnancy, with an iodine intake of 70 μg per day, in spite of its increased fixation in the gland, the equilibrium becomes unbalanced due to the high loss of iodine through the urine. In order to maintain the production of hormones, the thyroid iodine reserves are reduced, which causes a progressive closed cycle which leads to an increased stimulation of the gland and the occurrence of goiter (9).

Later during gestation, further iodine depletion of the mother occurs because part of the iodine from the circulation of the mother gets transferred to the fetal area. The iodine transfer is not precisely quantified, but, in the middle of the gestation period, the fetal thyroid gland starts to produce thyroid hormones necessary for adequate development of the fetus. Therefore, the iodine depletion which occurred after conception deepens during the first half of the gestation period and becomes emphasized in the final stage of the pregnancy. During lactation, significant quantities of iodine are eliminated through the milk.

For pregnant women in regions with iodine sufficiency, such as the Scandinavian countries, USA and Japan, these changes of the iodine metabolism during pregnancy remain without consequences. However is case of an iodine deficiency, the pregnancy causes hypothyroxinemia and thyroid stimulation with the occurrence of goiter. If pregnant women do not get iodine supplementation, their iodine balance becomes negative, with significant negative repercussions for the mother and the fetus or the newborn.

Detection of increased thyroid stimulation during pregnancy

The question being asked is how to detect increased thyroid stimulation in regions with insufficient iodine intake during pregnancy. Presently, biochemical parameters are used. The first is relative hypothyroxinemia, or determination of the level of TBG saturation using the ration of total T_4 to TBG which can indicate inadequate increase of total T_4 during the first trimester in relation to the serum TBG. When directly determining the concentrations of free T_4, they are close to the bottom limit of the range of normal values.

The second indicator is the preferential secretion of T_3 in comparison to T_4. The stimulation of the thyroid gland in case of iodine deficiency causes increase of T_3 production with an increase of the ration between T_3 / T_4 , usually above 0.025 (molar values).

The third parameter is the serum TSH. From the beginning of pregnancy and almost to the end of the first trimester is the stage of low TSH values, due to the high level of human gonadotropin. However, the level of serum TSH increases progressively and almost doubles by the middle of the gestation period, in comparison to the initial values. This increase of TSH remans within the boundaries of the referent values for (< 5.0 mU/L).

The fourth parameter is the change of the serum concentrations of the thyroglobulin. For some pregnant women, the level of thyreogloblin increases already in the first trimester and it grows progressively until the end of pregnancy.

We can conclude that in case of normal pregnancy we can employ relatively simple methods to detect the increase thyroid stimulation by determining the total T_4 and T_3, TBG, as well as free thyroglobulin in the serum. However it is necessary to stress that the discovered changes must be interpreted correctly in all of the stages of the gestation period, having in mind the various mechanisms of thyroid adaptation during pregnancy.

Several studies conducted in Europe between 1989 and 1994 clearly indicate that the volume of the thyroid gland of some healthy women significantly increases during pregnancy. The comparison of the values for the thyroid volume in the first and in the last trimester of the pregnancy, in regions without iodine deficiency suggested an increase in

volume by to 10 to 15%. However, in regions with iodine deficiency the results showed significant increase of the thyroid volume (by 16% to 31% on average). The above mentioned biochemical parameters indicating increased thyroid stimulation were also positive in pregnant women with increase volume of the thyroid gland (10).

Thyroid gland of the fetus and the newborn

The presence of thyroid hormones and the significant number of hormone receptors in the tissues of the fetus before the twelfth week of pregnancy indicate that the thyroid hormones of the mother cross the placental barrier, as well as that the fetus is dependent from the thyroid hormones of the mother in this, earliest, period of gestation, when the fetal thyroid gland is not capable to produce hormones. Therefore, the hypothyroxinemia of the mother, or the iodine deficiency in this stage has catastrophic consequences on the fetus.

The fetal thyroid gland becomes capable of concentrating iodine and synthesizing hormones between the tenth and the twelfth week of gestation, and after the 20^{th} week of gestation it is under the control of the fetal pituitary TSH. The secretion activity of the fetal thyroid starts to increase by the middle of the gestation period, and the values of the total thyroxin in the blood of the fetus increase continuously until delivery. The generally accepted opinion is that the thyroid function of the mother and the fetus are regulated autonomously, but not entirely independently from one another (11). TSH does not pass through the placenta. Only TRH and iodine pass through the placenta.

It has been shown that mothers with normal thyroid function can provide to the fetus 25% to 50% of the normal thyroxin needs until birth. Therefore, in comparison to children whose mothers have iodine deficiency, the children with congenital hypothyroidism, such as the congenital agenesia, or total iodine organification defect, and who are not capable of production of any amounts of thyroid hormones, from conception until birth, have a milder form of mental and motor retardation if treated adequately immediately after birth.

In the next stage of gestation, when fetal thyroid activity starts, it will depend on the transfer of iodine from the circulation of the mother. In case of moderate iodine deficiency, the fetus, during this stage, can be protected against hypothyroxinemia by stimulating the hormone synthesis in its thyroid gland by increasing the serum TSH thus causing glandular hyperplasia or goiter. However, in case of severe iodine deficiency, this compensatory mechanism is not sufficient and cannot provide an optimal production of thyroid hormones, which leads to a combined disorder of the thyroid hormone production of both the mother and the fetus, i.e. a state which threatens the life of the newborn with an severe physical and mental retardation and even cretinism in spite of any treatment with thyroid hormones immediately after birth.

If the mother has a relative postpartal hypothyroxinemia because of iodine deficiency, the newborn does not necessarily have to have it too, since the newborn can have significantly higher values of total T_4, free T_4 and better TBG saturation than the mother. In case of a moderate iodine deficiency, the newborns are protected against hypothyroxinemia by an emphasized stimulation of their thyroid gland, by the significantly higher level of TSH and thyroglobulin in comparison to the mother (11).

Iodine deficiency and the development of the brain

The results of numerous researches suggest that the thyroid hormones are a very important regulator of the development of the central nervous system. Thus thyroid hormone deficiency in the prenatal and perinatal period slows the normal development of the brain. The extent of the brain damage depends on the time and intensity of the thyroid hormone deficiency.

The relation between the hypothyroxinemia of the mother in the early stage of pregnancy and the damage to the CNS of humans, due to ethical considerations, cannot be directly verified. However, this can be presented in experiments conducted on rats (12).

Rats put of a low iodine intake diet become, like humans, hypothyroxinemic without signs of hypothyroidism, due to their normal serum T_3. However, clear abnormalities were found in the cell migration in various parts of the somatosensory cortex and hippocampus. This cellular change which end their migration before the fetal thyroid function start can be a consequence of the hypothyroxinemia of the mother even without the signs of hypothyroidism. The previously mentioned alterations in the cellular architecture of the brain have been confirmed in various experimental models, such as treating pregnant rats with strumogenic substances for only three days. After this treatment, the hypothyroxinemia of the mother is significantly less and lasts for a shorter period in comparison to the animals placed on a diet without any iodine in the food. Abnormalities in the cellular migration and cellular architecture were also discovered. These findings clearly confirm that even a short period of a relatively minor thyroid hormone insufficiency of the mother is enough to cause negative effects in the early development of the brain of the fetus.

The deficiency of the thyroid hormones also has negative effects on the multiplication of the neuroblasts, causes reduction of the weight of the brain and DNA content as a result of the reduction of the number of cells. The thyroid hormones regulate the synthesis of the numerous proteins in the brain. One of them, known as RC-3, or neurogranin, is important for the development and remodeling of the synapses.

Epidemiological studies suggest that the relative hypothyroxinemia in the first trimester of the pregnancy is a potential risk for the development of the CNS of children. Most of the data come from regions with iodine deficiency. The study conducted by Man et al. (13) with pregnant women in the USA showed defects in the CNS of children of mothers with low values of T_4 in the blood during the period of pregnancy and increased values of TSH by the middle of the gestation period.

In his research Haddow (14) shows an increased risk for CNS development in children of mothers which, during the first trimester, were found to have low values of free T_4 which are more discriminating than the elevated TSH during the second trimester. The children of one of every two women with low values of free T_4, during the first trimester have CNS development risks. However, these risks are not so pronounced as the risks in children with untreated congenital hypothyroidism or neurological cretins.

Until now, normal values for the free T_4 during the first trimester and thyroglobulin have not been determined, mostly because of the use of different analytical procedures. The increased level of circulating thyroglobulin is a more sensitive parameter of the iodine deficiency than TSH.

Endemic cretinism

Endemic cretinism has been recognized in Europe, in the villages in the Alps, several centuries ago. A total of 4.000 cretins were registered in the area known today as canton Valais in Switzerland, with a population of 70,000 inhabitants. The census in Switzerland from 1870 identified 24.5 deaf-mutes persons per 10,000 people, i.e. about three times more than in other countries in Europe. There was a strong correlation between the frequency of deaf-muteness and the incidence of goiter and cretinism in the different areas (15).

The use of the general term cretinism does not reveal the very significant differences in the clinical presentation between the two different types of cretinism – neurological and myxedematous, shown in the table below:

Clinical phenomena	Neurological type of cretinism	Myxedematous type of cretinism
Mental retardation	Present, usually emphasize	Present, less emphasize
Deaf-muteness	Usually present	Absent
Cerebral diplegia	Frequently present	Absent
Growth	Normal	Largely reduced
General characteristics	No physical signs of hypothyroidism	Rough and dry skin, coarse voice
Reflexes	Particularly vigorous	Slow relaxation
ECG	Normal	Low voltage on the QRS complex
X-ray of legs and arms	Normal finding	Dysgenesis of the epiphyses
Thyroid hormone effects	None	Clinical effects present

The neurological type of cretinism was first described in the first decade of the twentieth century (16). This type of disorder is dominated by neuromotor defects, including strabismus, deaf-mutism, spastic diplegia and other walking and coordination related disorders. 50% of people suffering from this type of cretinism have complete deafness, but vision in not affected.. The mental retardation is characterised by a marced impairment of the capacity for abstract thought, whereas the autonomic, vegetative function and the memory appear to be relatively well preserved. The neurological cretins do not hypothyroid and their thyroid function is similar to that of the rest of the population in the same area exposed to some degreel of iodine deficiency. They have low values for serum T_4, but normal or slightly increased values of T_3.

From the knowledge that the thyroid hormones of the mother are transferred through the placenta, it is assumed that the neurological type of cretinism is caused by the hypothyroidism of the mother, due to the iodine deficiency. Epidemiologic research suggest that period between the 12^{th} and 14^{th} and between the 20^{th} and 30^{th} week of gestation, when neurons of the coirtex and striatum proliferate, may be critical. In order to prevent cretinism, the iodine deficiency should be corrected by the hird month of pregnancy at the latest (17).

A typical myxedematous cretin is severely hypothyroid. Such a patient exhibits severe mental and physical retardation, delayed bone and sexual maturation, as well as severe growth retardation (18).The movements are torpid, and the reflex relaxation is usually much prolonged, the voice is coarse, the skin is dry and rough. These cretins are rarely deaf. Radiological studies show severe skeletal immaturity with dysgenesis of the epiphyses and metaphyses. The ECG result corresponds to a myxedema. The thyroid gland is in the normal position, can be of normal size, often atrophic. The concentrations of the serum T_4 and T_3 are low, and TSH concentrations are significantly increased (19). The fixation of the radioactive iodine is somewhat lower than the values in normal individuals from the same community. On a scanogram the thyroid gland presents with normal size, and in normal position, but severely hypothyroid cretins exibit atrophic changes.

A myxedematous type of cretinism occurs due to the hypothyroidism of the fetus in late pregnancy and/or postnatal hypothyroidism (20). The myxedematous cretinism is similar to the sporadic or congenital hypothyroidism, which can be seen in all parts of the world and which occurs due to anatomic or biochemical anomalies of the thyroid gland,

unlike he neurological cretinism which can be found only in areas with severe iodine deficiency. The myxedematous cretinism is especially common in areas where is used abundantly in nutrition. Two different mechanisms can explain the pathogenesis of this type of cretinism: simultaneous presence of iodine and selenium deficiency. The selenium deficiency decreases the level of glutathione peroxidase in the thyroid gland which detoxifies H_2O_2. The iodine deficiency causes thyroid hyperstimulation thorugh TSH which leads to hyperproduction of H_2O_2 with destruction of thyroid cells (21). The second reason can be the autoimmunity with the thyroid blocking immunoglobulins.

However, there is no clear distinction between these two types of cretinism because the myxedematous cretins can also exhibit neurological defects.

In spite of the different pathogenesis, the clinical picture of cretinism depends on the time and extent of the iodine deficiency. Fetal hypothyroidism causes neurological damage and the postnatal hypothyroidism causes myxedematous manifestations.

It my be concluded that brain demage and mental retardation in case of congenital hypothyroidism can be overcome with adequate therapy, which should be started as soon as possible after birth, while in case of a neurological cretin, the damage is already irreversible by the middle of the gestation . In case of a neurological cretin, the prevention of iodine deficiency must begin before the middle of the gestation period, because the damage to the CNS due hypothyroxinemia occurs in the early phase of pregnancy.

Even untreated newborns with congenital hypothyroidism do not have such severe neurological manifestations, typical for neurological cretinism, such as bilateral deafness, spastic diplegia etc. The different periods in which the brain damage occurs in case of iodine deficiency and congenital hypothyroidism and the entirely different period in which the respective disorders must be prevented, clearly indicate the different etiology of these two conditions.

Level of iodine deficiency and CNS damage

During the last decade there were many epidemiological studies that clarified the issues related to the level of iodine deficiency at which CNS development defects can occur.

While children born as neurological cretins can be found in regions with severe iodine deficiency, where the urinary iodine excretion is 20 or less micrograms per day, population with reduced intelligence quotient (IQ) or with neurological disorders (such as bilateral hearing impairment or loss) can be also found in areas with moderate or even mild level of iodine deficiency.

Classification	Intelligence Quotient
Exceptionally (very) superior	> 130
Superior	120 - 129
Above average	110 - 119
Average	90 - 109
Below average	80 - 89
Border line	70 - 79
Mental retardation	< 69

Currently the generally accepted knowledge is that the mental retardation in case of untreated congenital hypothyroidism is caused by inadequate thyroid function of the fetus, whose brain is not exposed to high hormone deficiency until delivery because of the transfer of thyroid hormones of the mother through the placenta, mainly T_4. During the fetal

life T_4 is a necessary precursor for the intracillular generation of T_3 in the brain cells, created through the effect of the enzyme deiodenase II (22).

The normal thyroxinemia of the mother and the normal activity of the type II cerebral deiodenase have a protective effet on the fetal brain in utero. This hypothesis explains the good results of the early treatment with T_4 after the birth of children with congenital hypothyroidism, if the mother has a normal thyroid function.

The situation is quite opposite in the case of CNS damage and a milder level of iodine deficiency accompanied by hypothyroxinemia of the mother in the early stages of pregnancy (23).

The mothers in this case are not hypothyrotic because of the compensatory mechanism triggered by the thyroid gland in case of iodine deficiency – preferential synthesis of T_3, which leads to a reduction of T_4, but with normal or slightly increased values of T_3 in the blood. Therefore, many tissues that can use T_3 directly from the blood for their own purposes, like the liver, kidneys, muscles, pituitary gland etc, are in an euthyreotic state and do not show signs of hypothyroidism. Therefore the values for blood TSH are not increased. However, some parts of the CNS of the fetus have receptors only for T_4, and the cells themselves can generate metabolically active T_3. Due to the reduced secretion, the quantity of serum T_4 is not sufficient to saturate the receptors in some parts of the brain and to provide for intracellular T_3 formation which is necessary for normal development of the brain. In that case the brain suffers damages which are irreversible, known as cerebral hypothyroidism (24).

Therefore, cerebral hypothyroidism occurs because of selective deficiency of active T_3 in the brain, unlike the other tissues that can use T_3 from the blood and bring it within the cells. Thus, these pregnant women and their newborns do not exhibit clinical signs of hypothyroidism nor increased TSH values. This information must be insisted upon since the pediatricians and endocrinologists do not manage to find the usual indicators of hypothyroidism and therefore conclude that the problem of iodine deficiency has been overcome.

Assessment of the level of iodine deficiency

The level of iodine deficiency is assessed using the values of the urinary iodine excretion and the incidence of goiter among school children. However, these criteria do not present entirely clearly the level of iodine deficiency of pregnant and lactating women, where the iodine requirements are about twice the requirements of school children and adults.

The following table shows the daily iodine requirements by age, determined in 1992 (25).

Age	Daily iodine needs in micrograms
Premature births	32
Children 0-5 months	90
Children 6-12 months	90
Children 1-3 years	90
Children 4-6 years	90
Children 7-10 years	120
Adults	150

According to the recommendations of Delange (26) from 2004, the daily iodine requirements of pregnant women are 250 to 300 μg, 225 to 350 μg for lactating mother, and newborns require 90 μg of iodine per day. The optimal values of iodine urinary

excretion of pregnant and lactating women should be 150 to 230 μg, and for newborns 180 to 225 μg iodine per liter of urine.

According to the latest recommendations of the WHO from 2007 (27), the daily needs of the pregnant and lactating women are 250 μg, and the needs of newborns and children younger than two years are 90 μg iodine per day. The median of the urinary iodine excretion of pregnant women should be between 150 μg/L and 249 μg/L, > 100 μg/L for lactating women, while the median for newborns and infants younger than two years should be > 100 μg/L urine.

High levels of iodine deficiency that can cause the birth of cretins are not present in Europe and other developed countries. However, the areas with moderate and mild levels of iodine deficiency, with hypothyroxinema of the mothers, are not limited only to the undeveloped countries. Such places may be found in Europe, even in areas where the results from examinations of school children provide the basis for concluding that the iodine deficiency has been corrected, such as Macedonia. In these areas, pregnant women have much higher iodine deficiency than that determined with the examinations of school children. The iodine intake of pregnant and lactating women through iodine rich food and iodinated salt cannot increase to the level corresponding to their increased iodine requirements, which may lead to negative effects on the development of the brain of their children (28). In that case, we face a very significant problem, whether the control of the iodine deficiency in this population group, assessed by determining the incidence of goiter and urinary iodine excretion among school children, permits us to conclude that the iodine deficiency has been corrected also for pregnant and lactating women, especially considering the consequences to the development of the CNS.

Even in countries where, in the past decades the iodine intake has been more the adequate, such as the USA, the relative iodine deficiency during pregnancy became a reason for concern, since in the last few years the iodine intake is reduced (29).

In several European countries there are no assessments of the true situation with the iodine deficiency among pregnant and lactating women (30).

Correction of the iodine deficiency among pregnant and lactating women

Large efforts have been made throughout the whole world, especially in the developing countries to correct the iodine deficiency. As a result of the measures undertaken, the birth of cretins and the incidence of goiter among newborns have been reduced. However, to conclude that serious results in the protection against mental retardation of large number of children throughout the world have been achieved would be unrealistic and overly optimistic. There are still not reliable, direct scientific evidence that corroborate this statement.

The examinations conducted by Glinoer et al. (31) 6 months after delivery, on women having an increased value of the index T_3/T_4 and elevated serum values of thyreogloblin during pregnancy, have shown that for some of them the iodine deficiency extends also in the lactation period.

Some of these women, after twelve months after delivery, the values of the thyroid volume still remained elevated in comparison to the beginning of the gestation. This study showed that the changes which have occurred during gestation are not limited only to the pregnancy period and that they are only partially reversible (31). In case of iodine deficiency, the pregnancy is a risk for the thyroid gland of the mother and goiter can occur which will persist even after delivery. This stress of the thyroid gland during pregnancy may be one of the reasons for the high prevalence of thyroid disorders among women. The results from these research efforts represent an additional argument in favor of the

need to increase the iodine intake during pregnancy, as well as during the period of lactation.

There are several studies that show that many pregnant women in European countries still have iodine intake which becomes inadequate even during the first half of gestation. Therefore, many such women cannot increase the free T_4 in the early stages of pregnancy to the level reached by women with adequate iodine intake. This causes the volume of the thyroid gland to increase, as well as the ratio T_3/T_4 and the values of thyroglobulin in the serum. The thyroid gland reacts to the reduced quantity of iodine available by triggering adaptation mechanisms which do not increase the serum TSH. Iodine supplementation during pregnancy results in an increase of the free T_4, reduction of the ratio between T_3/T_4, reduction of the thyroglobulin in circulation and reduction of the volume of the thyroid gland of both the mother and the newborn. Iodine supplementation given to pregnant women in larger quantities of iodine than those applied in the European studies has proven to be safe, i.e. without any negative consequences.

The effects of iodine supplementation during pregnancy on the thyroid functionality of the newborns were examined in the studies of Glinoer et al. (32) and Pedersen et al. (33). In the study conducted by Pedersen et al. the first group of healthy pregnant women received 200 micrograms of iodine per day, and the second group was the control group. In the study conducted by Glinoer et al. the first group of healthy women received 100 micrograms iodine per day and the second group was the control group – women who did not receive iodine. The parameters of the neonatal thyroid functionality were evaluated in the umbilical blood. Both studies demonstrated very similar results. The iodine supplementation does not change significantly the level of TSH, which is below 10 mU/L on average. However, it highly significantly reduces the level of thyroglobulin which is 50% lower in comparison with the thyroglobulin of the pregnant women in the control groups.

The volumes of the thyroid gland of the newborns were measured in the first week after birth using an ultrasound method. The thyroid volumes are significantly larger among newborns of the mothers that did not receive iodine supplementation, and they are 39% smaller among the newborns of mothers that did receive the iodine supplementation. These results clearly suggest that in case of iodine deficiency the process of fetal strumogenesis occurs in the early stage of fetal development (34).

The retardation in the development of CNS and the consecutive permanent neurologic damage can be prevented with an adequate iodine prophylaxis. The examinations made in the Chinese province Xinjiang showed that iodine administration during the period of pregnancy causes improvement of the microcephalia of the newborns. Microcephalia is an objective consequence of iodine deficiency which is measurable and is directly correlated with the neurological damage.

How large should the iodine supplementation is a question open for discussion. This primarily depends on the iodine reserves in the thyroid gland before pregnancy. The purpose of the substitution should be renewal and maintenance of the iodine status balance which can be achieved with 100 to 200 micrograms of iodine per day, for the entire duration of the pregnancy and lactation. In case of a long lasting iodine deficiency before pregnancy, a relatively long period must elapse (about one trimester) before the iodine supplementation can achieve favorable results regarding the thyroid gland functionality. In addition, Glinoer et al. (34) showed that pregnant women with highly expressed thyroid stimulation or occurrence of goiter achieve better results if, in addition to the iodine supplementation, they also receive thyroxin. The combined treatment is much more efficient in normalizing the thyroid function, but, also, in preventing the occurrence of goiter during pregnancy.

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SELECTION OF MATERIALS, METHODS AND STATISTICAL APPROACH TO THE RESEARCH

Vukosava Bogdanova

One of the important issues when planning a research is the size and location of the sample that will be used in order to ensure that it is representative of the envisioned aim of the research (1).

The aim of the research was to determine how pregnancy and lactation influence the thyroid function, by determining the iodine in urine, the size of the thyroid gland as determined by palpation (qualitative parameter) as well as using ultrasound (quantitative parameter). The quantitative indicators (iodine excretion and ultrasound) can also show the discrete changes, and patterns can be discovered using statistical methods. In addition, quantities of iodine in the milk of the lactating women were determined, as well as the biochemical indicators used to assess the function of the thyroid gland during pregnancy and lactation: serum TSH, free thyroxin, thyroglobulin and thyroid antibodies (anti -TRO and anti TG) (2).

Since the iodine requirements increase during pregnancy and lactation, the urine iodine and the size of the thyroid gland were monitoring in the different stages of pregnancy (I, II and III trimester), as well as in the pospartal period (lactation). For part of the pregnant women this examination was done only in one stage, for another part it was done in two stages and for some it was done in three stages, while for part of the subjects the examination was performed during lactation as well. We tried to have as many subjects as possible in order to be able to form a representative group suitable for the statistical methods (3.4).

We covered the territory of the Republic of Macedonia by conducting examination in five cities: Skopje (70 subjects and 128 examinations), Gostivar (20 subjects and 34 examinations), Shtip (46 subjects and 75 examinations), Kichevo (16 subjects and 32 examinations) and Berovo (6 subjects and 17 examinations). A total of 286 tests were performed on 158 patients.

The age range of the patients was from 17 to 42 year old, and the average age was 26 years.

For 74 patients this was their first pregnancy, for 55 patients it was their second, for 20 it was their third, for 4 patients it was their fourth, for three patients it was their fifth and for one patient it was her seventh pregnancy.

According to the ethnicity of the patients, the group included 91 Macedonians, 49 Albanians, 6 Turkish, 5 Bosnian, 5 Roma, 1 Croatian and 1 Serbian patient.

The size of the thyroid gland was determined using palpation in 158 cases, during all stages of pregnancy and lactation.

The size of the thyroid was determined using ultrasound in 158 cases, and a total of 286 measurements were made: 100 in the first trimester, 54 in the second trimester, 74 in the third trimester and 58 during lactation.

Iodine in urine was determined in 157 cases, and 283 analyses were performed: 99 in the first trimester, 54, in the second trimester, 74 in the third trimester and 56 during lactation. In addition 36 analysis of iodine in the breast milk were performed.

Biochemical tests of the thyroid function: FT4, TSH, thyroglobulin and thyroid antibodies were performed for all patients in the different stages of pregnancy as well as during lactation: FT4 - 246 analyses, TSH - 246 analyses, thyroglobulin - 226 analyses, thyroid antibodies - 185 analyses.

The following table provides the number of subjects and the analysis for all methods used to examine the thyroid gland:

Palpation

Subjects	Tests	Pregnant	Lactating
158	286	228	56

Size of the thyroid gland using ultrasound

Subjects	Tests	Pregnant			Lactating
158	286	228			56
		I trimester	II trimester	III trimester	
		100	54	74	

Urinary iodine excretion

Subjects	Tests	Pregnant			Lactating
157	283	227			56
		I trimester	II trimester	III trimester	
		99	54	74	
Without substitution with KI		99	46	46	49
With substitution with KI		0	8	28	7

Iodine in the breast milk of the lactating women

Subjects	Tests
36	36

Biochemical tests

Tests	Pregnant			Lactating
TSH and FT4	190			56
	I trimester	II trimester	III trimester	
	98	46	46	
Thyroglobulin	226			
Antibodies	122	54	9	

Statistical analysis

Standard statistical methods were used to process the gathered data. These include: median, mean, standard deviation and statistical significance. The Student t-test was used to test the differences between the independent samples. The statistical significance of the differences between the results was assessed using Fisher distribution, taking the value $p < 0.05$ to indicate statistical significance.

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BIOCHEMICAL INDICATORS FOR ASSESSMENT OF THE IODINE AND THYROID STATUS OF PREGNANT AND LACTATING WOMEN

Sonja Kuzmanovska

In epidemiologic studies iodine intake is usually monitored among school children, since this group is most suitable and easily accessible representative of the general population. For this purpose WHO/UNICEF/ICCIDD (1) recommend using the concentration of iodine in urine as the most suitable biochemical indicator.

In the case of pregnant and lactating women, the iodine intake affects the health of both the mother and her offspring. These last few years, many authors in literature express an opinion that pregnant and lactating women need iodine supplementation in their nourishment, with quantities ranging from 100 μg and 200 μg per day (2,3,4), even in regions with iodine sufficiency at the general population level. This is justified by the fact that prenatal prevention of the iodine deficiency is the most efficient way to achieve optimal neurological development of the child. According to a study conducted by Paul (5), the lowest quantity of iodine, which exceeds the quantity usually taken in through the food and which does not affect the thyroid function is 500 μg day. This implies that a slightly elevated quantity of iodine, assessed as excessive among the general population (>300 μg/L), should not cause any significant changes in the thyroid function of pregnant women. On the other hand, it is known that iodine excess of the mother may lead to blockage of the thyroid function of the fetus, which would result in hypothyroidism and goiter (6, 7). Therefore, each additional iodine substitution given to this population must be monitored through biochemical and clinical indicators during the period of pregnancy and lactation.

The selection of biochemical indicators in this case needs to be expanded with additional tests. Measuring only the urinary iodine excretion may lead to wrong conclusions and to overestimating the realistically available iodine quantities of pregnant women because of the increased renal iodide clearance, which is a consequence of the increase of the glomerular filtration rata (GFR) by about 50% (8, 9). In addition, significant quantities of iodine are excreted through the breast milk during lactation, which should be taken into consideration when determining the adequate iodine intake limits. According to recent data, the optimal quantities of iodine in the breast milk of the mother range from 150 to 180 μg/L (10), while iodine concentration in the breast milk lower than 50 μg/L indicates iodine deficiency of the mother (11).

Finally, regarding the assessment of the level of success of iodine substitution in pregnant women and monitoring of the possibility of iodine induced autoimmune disorders of the thyroid gland, different authors (12, 13) recommend determining a wide variety of markers including TSH, Tg, FT4 and thyroid antibodies (anti-TPO i anti-Tg). The thyroid markers do not only represent additional indicators of the consequences from the iodine intake, but they are also a real indicator of the thyroid function, whose normalization is the ultimate aim of these epidemiological studies.

Determination of thyroid stimulating hormone (TSH)

The thyroid stimulating hormone is a glycoprotein secreted by the thyrotropic cells from the anterior pituitary gland, as a reaction to the level of thyroid hormones in the bloodstream. TSH acts by binding to the TSH specific receptors on the basal membrane of the thyrocyte which leads to an increased iodine reception, synthesis of TPO and Tg and excretion of thyroid hormones. TSH also stimulates the formation of H_2O_2, iodination and the synthesis of thyroid hormones (14). The long lasting effect of TSH on the thyrocytes leads to an increased vascularization and hyperplasia of the thyrocytres which results in goiter.

Several authors (15, 16) recommend that TSH should be determined as a first screening test for pregnant women. Elevated values during the second trimester are an indication of subclinical hypothyroidism. This condition is especially unfavorable for the development of the offspring because of the unsuitable production of the necessary thyroid hormones. On the other hand, in the first trimester of a normal pregnancy, the level of TSH in the serum transitorily reduces due to the increased secretion of hCG, which to some extent is TSH-mimetic. Therefore it is necessary to determine trimester-specific referent values in order to be able to correctly interpret the results of this test.

Determination of thyroglobulin (Tg)

Thyroglobulin is the most frequently present protein in the thyroid gland. It plays the role of a precursor in the synthesis of the thyroid hormones and, at the same time, plays a role in synthesizing hormone and iodine reserve in the follicular lumen. The concentration of thyroglobulin in the serum depends on the level of stimulation of the TSH receptor on the basal membrane of the thyrocyte, irrespective of whether it is caused by the TSH itself, by hCG or by the receptor antibodies (TR-Ab). Any injury or inflammation of the thyroid gland can increase their secretion in the bloodstream, and this applies especially to the differentiated thyroid carcinoma. The normal secretion of thyroglobulin largely depends on the iodine intake in a specific geographic area. Therefore, many authors recommend it as a marker used to monitor iodine deficiency in epidemiological studies. For this purpose, thyroglobulin has been recommended as a more sensitive indicator than TSH (17, 18, and 19). It is one of the mandatory tests in recent studies, which study the thyroid function during pregnancy (20, 21, and 22). Although it does not play the role of a hormone, the level of thyroglobulin in the circulation is a sensitive biochemical indicator of thyroid stimulation during pregnancy which strongly correlates to the other gestation goiter generating factors. It's especially strong correlation with the volume of the thyroid gland makes in a good prognostic marker for the occurrence of goiter in case of iodine deficiency, as well as for monitoring the effects of iodine supplementation.

Determination of thyroxin (FT4)

Thyroxin during early pregnancy is exceptionally important for the neurological and physical development of the child. Low concentrations of thyroxin may lead to disruption of the cognitive and motor functions even in cases when the FT3 and TSH levels are not pathological. During pregnancy, it is especially important to monitor the thyroxin level, especially in the first trimester, because thyroxin can indicate the presence of possible hypothyroxinemia (23, 24, and 25). This condition is defined as levels of thyroxin lower than the normal value for pregnant women during the trimester, irrespective of whether there are clinical signs of hypothyroidism. One of the basic reasons for hypothyroxinemia may be iodine deficiency (26), which, if compensated in time with adequate quantities, may prevent goiter of pregnant women and provide for sufficient substrate for normal differentiation of the tissues of the fetus. It is known that during pregnancy, especially from the 16^{th} to the 20^{th} gestation week, the level of thyroxin binding globulin doubles and thereby the concentration of total thyroxin in the blood increases as well. Therefore, the free thyroxin fraction is a more adequate marker for assessment of the thyroid function, although it is only 0.03% of the total circulating hormone quantity.

From an analytical point of view it is very difficult to determine FT4, because the concentrations in question are of the order of magnitude of picomoles. In addition the resulting values as well as the referent ranges depend on type of the techniques used as well as the method standardization.

Determination of anti-TPO antybodies

The thyroid peroxidase is one of three thyroid antigens to which the organism develops autoimmunity. The prevalence of these antibodies in Hashimoto thyroiditis is about 90%, and in Grave's disorder it is 80%. The increased concentration of circulating a-TPO antibodies is related to infertility, and increased incidence of spontaneous abortions among pregnant women and it is a predictor for development of autoimmune thyroid disorder postpartally (26). The determination of anti-TPO is recommended by many authors (27, 28, and 29) as part of the variety of screening tests for the pregnant women. The high concentration of these antibodies suggests not only high risk of abortion, but also inappropriate neurological and physiological development of the fetus. On the other hand, the additional iodine intake can contribute to increasing the incidence of autoimmune disorders (30). All of this suggests the necessity to include anti-TPO antibodies in the variety of mandatory tests for pregnant women, starting in the first trimester, for the entire duration of the pregnancy and postpartally.

Determination of anti-Tg antibodies

The thyroglobulin antibodies are an atypical indicator for monitoring thyroid autoimmunity, because their isolated presence in the bloodstream is exceptionally rare. The detected concentrations of these antibodies are also high in case of Hashimoto thyroiditis (70 to 80%), only 30% in case of Grave's disorder, whereas there prevalence in endemic goiter is 10% to 15% (31). The clinical significance of this test is that it provides for the possibility to quantify the anti-Tg antibodies and thus determines the level of analytical interference when determining the levels of thyroglobulin. This interference can cause falsely lower or higher results depending on the method used and represents the biggest analytical problem for determining the levels of thyroglobulin (32, 33).

In our study, the iodine intake of pregnant women is assessed by the iodine concentration in urine, while in case of lactating women, in addition to this parameter we also determine the level of iodine excreted in the breast milk.

In order to assess the thyroid status we determine the serum quantities of TSH, Tg, FT4, anti-Tg and anti -TPO antibodies.

METHODS FOR DETERMINING IODINE IN BIOLOGICAL MATERIALS

The basic conditions for the application of these methods include an isolated, air-conditioned laboratory, glassware and tools intended specifically for this purpose. The reagents, as well as the standard iodine solutions which we used were prepared in the National Laboratory for Determining Iodine in Urine. The solvent used was deionizated water obtained by treatment through an ion-exchange resin (U-01503-30, Cole-Parmer, USA). Every new series of reagents was subjected to a control test to determine possible iodine contamination (34).

The materials used for the purposes of the study were random urine samples, poured in plastic test-tubes and sealed. Until the analysis, the materials were kept either at +4ºC or frozen at -20ºC. The milk from the lactating women was treated similarly.

We used the following basic equipment in order to assess iodine in biological material:

1) Aluminum thermostatic digestion heat block (GEBR-LIEBISH, Germany)
2) Spectrophotometer UNICO UV-2102 (USA), with continuous flow cuvette and an acido-resistant tubing system
3) Peristaltic pump UNICO AS-21P (USA)

The analyses were performed with two manual colorimetric methods, based on the Sandell-Kolthoff reaction where iodine, in the form of iodide catalyses the reduction of ceric (Ce^{+4}) in cerous (Ce^{+3}) ions in the presence of arsenic (As^{+3}), which oxidizes during the reaction. The differences in the methods are related to the pre-analytical procedure, the composition of the reagents used and the reaction conditions.

Method for assessment of iodine in urine (Method A)

This method uses Ammonium persulphate solution for oxidizing digestion in the pre-analytical procedure which removes all interfering and organic substances from the urine. It is classified by WHO/UNICEF/ICCIDD as Method A, and for epidemiological purposes it has been introduced in our laboratory in 2000. The principle and procedures are described in detail in the monograph “Correction of Iodine Deficiency in Macedonia” (35).

The method achieves analytical sensitivity of 7 μg/L, the precision within a series ranges from 2.6 – 7.1 CV%, and the day to day precision ranges between 2.9 and 6.6 CV% (good precision criterion is < 10 CV% (coefficient of variation)).

Method for assessing iodine in milk

Due to the high content of proteins and fats, the assessment of iodine in breast milk from lactating women employs methods which, in the pre-analytical procedure utilize chloric acid as a more efficient oxidation agent. The Dunn method (36), classified as Method B, which is usually applied for assessing iodine in urine, was adopted for this purpose (37). By increasing the volume of chloric acid from 750 μl to 3 ml we achieved a more efficient mineralization of the fatty ingredients.

The optimized protocol in its final form is as follows:

1) The milk samples, the working standard iodine solution and the control samples are left to reach room temperature.
2) The milk is mixed until it becomes fully homogeneous and than 250 μl samples are placed in test - tubes.
3) The standard iodine samples are prepared by adding appropriate volumes of the working solution and deionizated water according to the provided scheme.
4) 3 ml of the chloric acid solution is added to each of the test-tubes mixing carefully.
5) The test-tubes are covered with plastic caps (not fully) and are placed in a thermostatic block. They are heated for 1 hour at a temperature of 110ºC, and the working area is well ventilated.
6) The test – tubes are removed from the thermostatic block and are cooled to room temperature.
7) 3.5 ml arsenic reagent is added to each test-tube, the tube is mixed with a Vortex mixer and is then left to react for about 15 minutes.
8) 800 μl ceriammonium sulphate is added to each tube in 20 second intervals (to be controlled by a stopwatch), and the tube is mixed with a Vortex mixer every time the solution is added.
9) After 15 minutes have elapsed from the time the ceriammonium sulphate solution was added to the first test-tube, the absorbance (at 405 nm) for each sample in the interval according to the order in which the solution was added.

The values of the absorbencies were processes using the MultiCalk software and the final iodine concentrations in the samples were read from the standard curve.

The analytical characteristics of the method, obtained in accordance with standard procedures are as follows: analytical sensitivity is 8,2 μg/L, the precision within a series

ranges from 1.7 – 5.8 CV%, the day to day precision ranges from 3.6 to 7.7 CV% (the criterion for good precision is < 10 CV% (coefficient of variation)).

METHODS FOR DETERMINING THE THYROID STATUS

The thyroid status of pregnant and lactating women can be determined using serum and routine immunological analyses, or using full blood obtained as a dry stain on filter paper as a dry stain, which is then appropriately processed and analyzed using specially designed immunological tests for this purpose. For the purposes of our study we used serum, which, after several hours after acquiring the blood samples from the field, was extracted using centrifuge, aliquoted in three test-tubes in 1 ml volumes and kept at a temperature of - 20ºC, until the time of the analysis.

We determined the thyroid markers using competitive and noncompetitive immunological tests, selected from the variety of routine tests using availability, reliability, and economic criteria.

For the immunological analyses using flurorphore as a label, we used the following equipment:

1) Fluorometer 1234 DELFIA-Wallak (Finland), linked to a personal computer for data processing
2) Automatic plate shaker for incubation with various fixed and variable speeds
3) Automatic washing device, with programmed single or multiple washing cycles for washing the microtitration plates.

For the immunological analyses based on a chemoluminescent label we used the following equipment:

1) Chemoluminometer IMMULITE DPC (SAD) automatic analyzer type connected to personal computer for data processing.

Method for determining TSH

We determine TSH using noncompetitive floroimmunometric test and a commercial, ultrasensitive kit of the type DELFIA hTSH Ultra, produced by Perkin Elmer, Finland. The kit comprises a microtitrate reaction plate with 96 wells and a set of reagents.

The principle of the method involves the formation of immunocomplex on the walls and hollows of the microtitration reaction plate. The walls are coated with monoclonal antibodies with affinities towards various antigen determinants of the TSH molecule. After adding the test serum and a specific anti-TSH antibody labeled with the fluophore europium, a double immobilization of TSH occurs between the solid phase antibodies and the labeled ones. After the time envisaged for incubation elapses, the excess reagents are removed by multiple washing and a reagent for europium dissociation is added. A strongly fluorescent chelate compound is formed. The intensity of this compound is proportional with the concentration of the analyte. The analyses are performed in series always using standard solutions and control serums. The analytical sensitivity of the kit is 0.05 IE/L.

Method for determining thyroglobulin (Tg)

We determine thyroglobulin using a noncompetitive, flurorimmunometric method and a commercial DELFIA hTg kit produced by the company Perkin Elmer, Finland. The kit comprises a microtitrate reaction plate with 96 wells and a set of reagents.

The analysis involves two-stage incubation, formation of an immunocomplex between the thyroglobulin from the serum and an appropriate antibody, coated on the

bottom of the reaction plate, during the first incubation. After the addition of the second antibody, labeled with europium, double immobilization of the thyroglobulin ("sandwich technique") occurs during the second incubation. After the separation of the fractions, a reagent is added, causing the dissociation of the fluophore europium and the formation of a strongly fluorescent chelate compound. The intensity of this compound is read out using the so called time-resolved technique of the flurorometer. The data are processed using a personal computer and the MultiCalc software package. The analytical sensitivity of this kit is 0.2 ng/ml.

Method for determining FT4

We determine the free fraction of thyroxin using a competitive fluoroimmunological analysis and a commercial kit type DELFIA FT4 produced by the company Perkin Elmer, Finland. The kit comprises a microtitration reaction plate with 96 wells and a set of reagents.

The principle involves a three-stage incubation. In the first stage, the anti-T4 monoclonal antibody builds an immunocomplex with the secondary antibody compound coated on the bottom of the reaction plate. During the second incubation FT4 from the analysis serum reacts with the anti-T4 antibody and forms an immunocomplex, while the serum and the buffer solution are removed from the reaction mixture. The third stage involves the addition of europium labeled T4 that reacts with the remaining antigen binding places of the antibody. Finally a reagent is added that causes the dissociation of the fluorophore europium, by forming a strongly fluorescent chelate compound. The intensity is read out using the time-resolved technique of the fluorometer and is inversely proportional to the concentration of the analyte. The data are processed using a personal computer and the MultiCalc software package. The analytical sensitivity of this kit is 2 pmol/L.

Method for determining thyroid antibodies: a-TPO and a-Tg

The two thyroid antibodies of interest for our study were determined using a sequential, chemolumino-immunometric method and commercial kits from the company IMMULITE DPC, USA. One commercial set contains 100 test-tubes with polystyrene beads for the immunologic reaction as well as appropriate reagents.

Before the analysis the serum samples are diluted in a 1:101 ratio, and then at least 100 µl of the diluted solution is transferred using a pipette into specially designed caps, placed in a carrier with a bar code reader. The analysis sample, together with the buffered serum from reagent A, is automatically transferred into a test-tube containing a bead lined with highly purified treated thyroid peroxidase as the solid phase. During this immunological reaction, the entire antibody quantity binds to the solid phase, during the first incubation, in 30 minutes and a temperature of 37ºC. The excess serum is removed with centrifuging. Then a secondary antibody is added, labeled with alkaline phosphatase, which during the second incubation cycle of 30 minutes binds with the immobilized analyte. After the completion of the immunological reaction, the excess label is removed from the test-tubes and a substrate, which reacts with the alkaline phosphatase is added. The luminescence is intensive and proportional to the quantity of the appropriate antibody in the serum. The analytical sensitivity of the method to determine a-TPO e 10 kU/L, while for a-Tg it is 20 kU/L

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RESULTS FROM THE BIOCHEMICAL TESTS FOR ASSESSMENT OF IODINE AND THYROID STATUS OF PREGNANT AND LACTATING WOMEN

Sonja Kuzmanovska and Gjorgi Shestakov

1. RESULTS FROM THE ASSESSMENT OF URINARY IODINE EXCRETION

The results related with the iodine excretion of the subjects considered in our study were interpreted in accordance with the latest recommendations of the WHO (1), presented in the following table:

Table 1. Classification of iodine intake of pregnant and lactating women

Group	Urinary iodine concentration median (µg/L)	Iodine intake category
Pregnant women	<150 150 -249 250-499 ≥ 500	Insufficient Adequate More than adequate Excessive
Lactating women	< 100 ≥ 100	Insufficient Adequate

The pregnant women were divided by the urine sampling time during the gestation period, starting from 2005 until 2007. The results are selected according to the proportions of the values for the given concentration intervals and they are presented in table 2. 98 samples were tested during the first trimester, 45 during the second and 46 during the third trimester.

Table 2. Urinary iodine excretion of pregnant women (2005 -2007)

	no.	Median µg/L	0-149	150-249	250-499	>500	<20	20-49	50-100
Pregnancy 1st trimester	98	199.7	29.6%	39.8%	28.6%	2%	0%	3.1%	12.2%
Pregnancy 2nd trimester	45	199.7	37%	28.3%	30.4%	2.2%	0%	0%	13%
Pregnancy 3rd trimester	46	174.9	39.1%	32.6%	26.1%	2.2%	0%	2.2%	19.6%

The table shows that the median value for the iodine concentration in urine, for all trimesters is within the interval from 150 µg/L to 249 µg/L, which corresponds to an adequate iodine intake. In the first and the second trimester, the median is 199.7 µg/L, while the value for the third trimester is slightly lower (174.9 µg/L). These results, when compared to the results from our previous study from 2001 (149.7 µg/L for the first, 157.6 µg/L for the second and 130.4 µg/L for the third trimester) indicate an increase in the iodine intake of pregnant women, in a situation when the general population has a confirmed iodine sufficiency (2). However, the distribution of the individual values, presented on the histograms on chart 1 below, shows that for a significant number of subjects the results are lower than 150µg/L, which is the lower limit for the optimal iodine intake range.

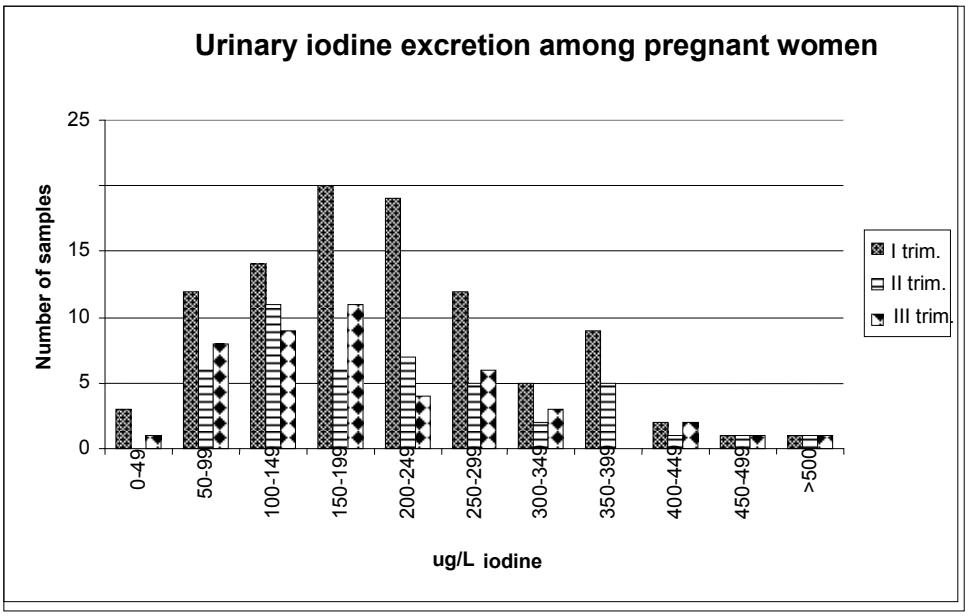

Chart 1. Urinary iodine excretion of pregnant women (2005-2007)

In this context, we determined an insufficient iodine intake in 29.6% of the samples in the first trimester, 37% in the second and 39.1% in the third trimester. We did not find exceptionally low urinary iodine excretion values (<20 µg/L) in any of the urine samples. The proportion of samples within the 20µg/L to 49 µg/L range was also low (2.2% to 3.1%). Values ranging from 50µg/L to 100µg/L were found in 12.2% of the samples in the first trimester, 13% in the second and 19.6% in the third trimester. This last criterion was also used in the study of pregnant women from 2001, when this category was populated by 21.8% of the samples from the first trimester, 17.3% from the second and 23.2% from the third trimester. The results imply that even in case of iodine sufficiency, a significant portion of the pregnant women intake inadequate quantities of iodine with their food. These findings are also confirmed by other authors (3, 4, and 5).

In a more recent study, conducted in Iran, a country with confirmed iodine sufficiency, the authors (6) publish successive reduction of the median values for urinary iodine excretion as the pregnancy progresses. According to other authors, these median values are not significantly different than the values for non-pregnant women or the general population (7, 8, and 9).

Iodine excretion of delivered women and lactating women is determined from the urine and the breast milk of the mothers. The distribution of the values is presented in charts 2 and 3.

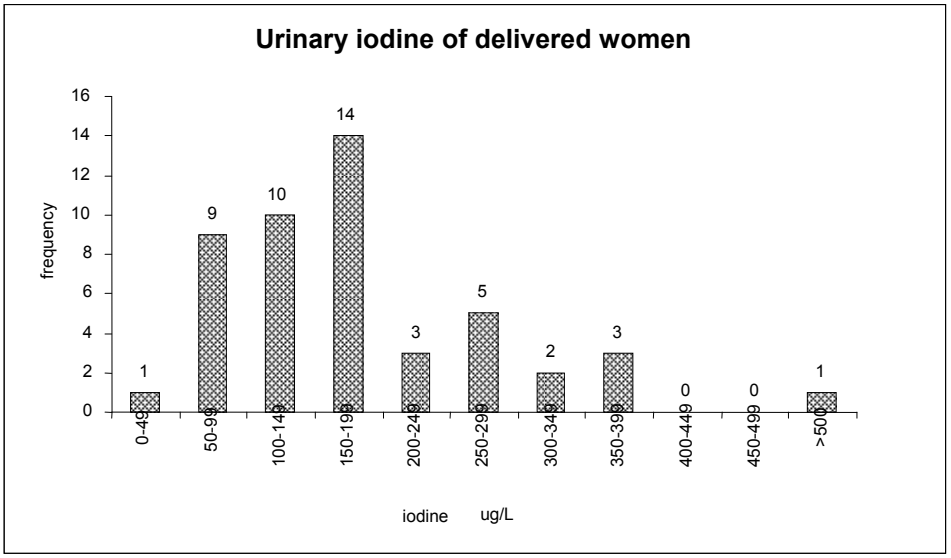

Chart 2. Urinary iodine excretion among delivered women and lactating women (2005-2007)

The urinary excretion data for this group of subjects are presented in the following table:

Table 3. Overview of the results for urinary iodine after delivery

Tested parameter	Resultant value n = 49
Median	157.9 µg/L
Values < 50 µg/L	2 %
Values < 100 µg/L	20.4%
Values from 100 - 199 µg/L	49%
Values from 200 - 300 µg/L	16.3 %
Values > 500 µg/L	2%

One notes that the urinary iodine excretion median is 157.9 µg/L which is lower than the median obtained during pregnancy. It is known that after delivery the glomerular filtration rate stabilizes and therefore the urinary iodine excretion results are interpreted using the criteria applicable to the general population. Having these criteria in mind, our study determined that 20.4% of the test subjects had insufficient iodine intake, 49% had adequate iodine intake, and 16.3% had more than adequate iodine intake. The proportion of the values classified as iodine excess (2%) was not different than the value obtained for pregnant women.

The iodine excretion through the breast milk of the mothers is presented on the histogram on the chart below.

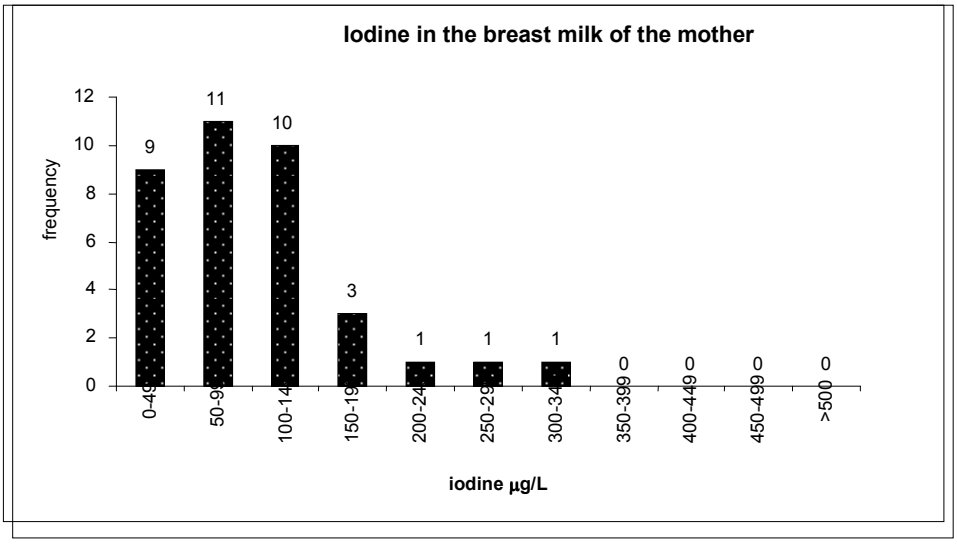

Chart 3. Excretion of iodine in breast milk

The chart shows convergence of the values toward the lower concentrations and high proportion of values indicating inadequate iodine excretion (<150µg/L). Table 4 provides an overview of the results.

Table 4. Overview of the results for iodine in the breast milk of the mothers

Tested parameter	Resultant value n = 36
Median	89.7 µg/L
Values < 50 µg/L	25%
Values < 150 µg/L	83%
Values from 150 - 180 µg/L	8.3%
Values > 200 µg/L	8.3%

The median is 89.7µg/L, and the concentration range is between 11.3 µg/L and 333.1µg/L. The proportion of values with inadequate iodine intake is quite high at 83%.

2. RESULTS FROM THE DETERMINATION OF THYROID PARAMTERS

2.1 Assessment of TSH of pregnant and lactating women

We determined the TSH of pregnant women in all trimesters, and the distribution of values is presented on the following chart:

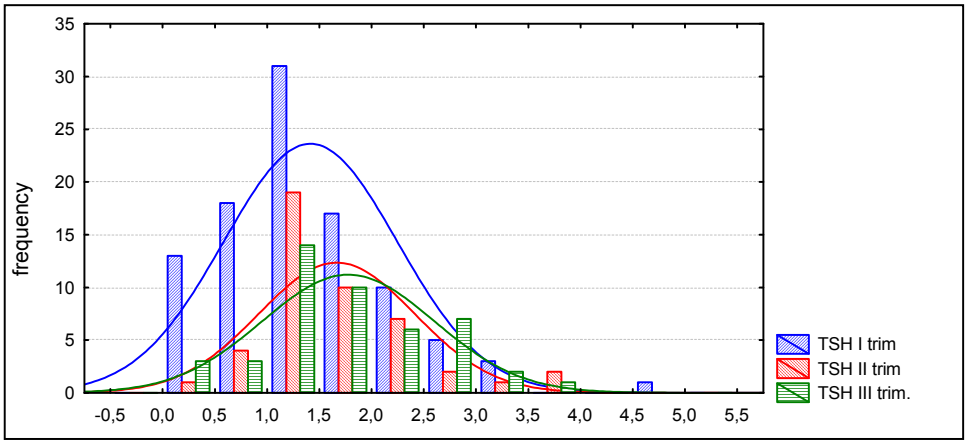

Chart 4. Distribution of TSH values from all trimesters

The graph shows that the values are distributed asymmetrically and therefore the evaluation is made by using the median and non-parametric statistical tests. The statistical analysis showed an insignificant difference in the value distribution between the second and the third trimester, which enabled us to group the values from the second and the third trimester. The results are presented in table 5.

The obtained TSH values differ from the TSH values for the general population, analyzed using the same method during the same time interval, where the median is 1.3 mIE/L, while the intervals for the appropriate percentiles (2.5 and 97.5 percentile) range from 0.5 – 3.3 mIE/L. For the first trimester we obtained a lower median value (1.22 mIE/L) and decreased lower limit from the central 95% interval (0.2 mIE/L), which is in accordance with the transitory physiological changes during this period.

Table 5. TSH Median and limit values among pregnant women

Trimester	Tester parameter	Obtained values (mIE/L)
First n = 98	Median	1.22
	2.5 percentile	0.21
	97.5 percentile	3.3
Second/third n = 92	Median	1.6
	2.5 percentile	0.3
	97.5 percentile	3.6

During the second and the third trimester the lower limit value increases from the central 95% interval (3.6 mIE/L), and this value is somewhat higher than the respective value obtained for the general population. Here we have also determined the higher value for the median (1.6 mIE/L), which indicates a slightly increased thyroid stimulation during this period.

After delivery, the TSH values return to the level of the general population, with a median of 1.4 mIE/L and interval from 0.6mIE/L – 2.8mIE/L. This can be seen on chart 5.

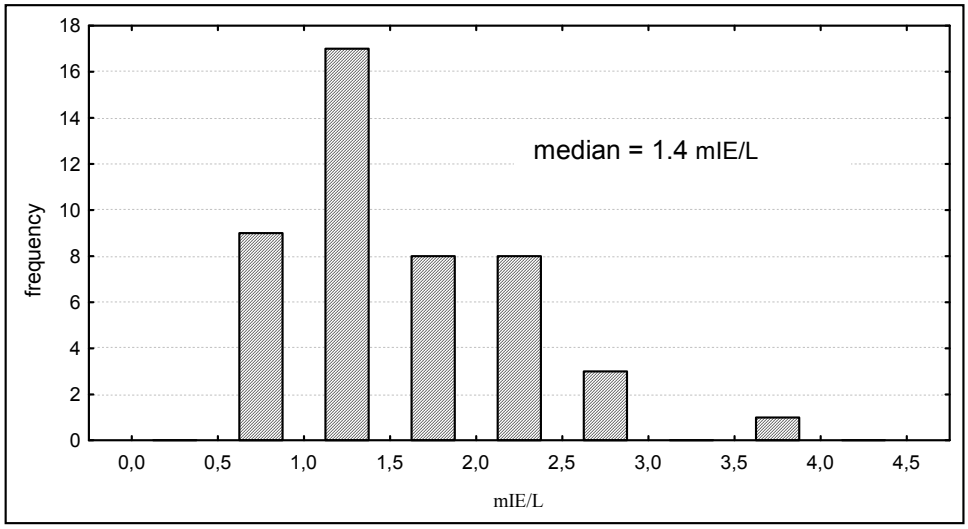

Chart 5. Distribution of values for TSH after delivery

2.2 Determination of FT4 of pregnant and lactating women

The free fraction of thyroxin was determined among pregnant women in all trimesters as well as after delivery. We presented the results of the appropriate groups graphically and we analyzed them statistically. When interpreting the results we used the reference values for the general population (8.4 pmol/L – 18pmol/L) and the respective median of 12.6pmol/L. The histograms presented on chart 6 show a normal distribution of the values in all trimesters, while there is a significant statistical difference in their distribution. Therefore, the median and the limits are presented for each trimester separately. From the chart, one can clearly see a shift of the values towards the lower concentrations as the gestation period progresses, as can be also seen from table 6.

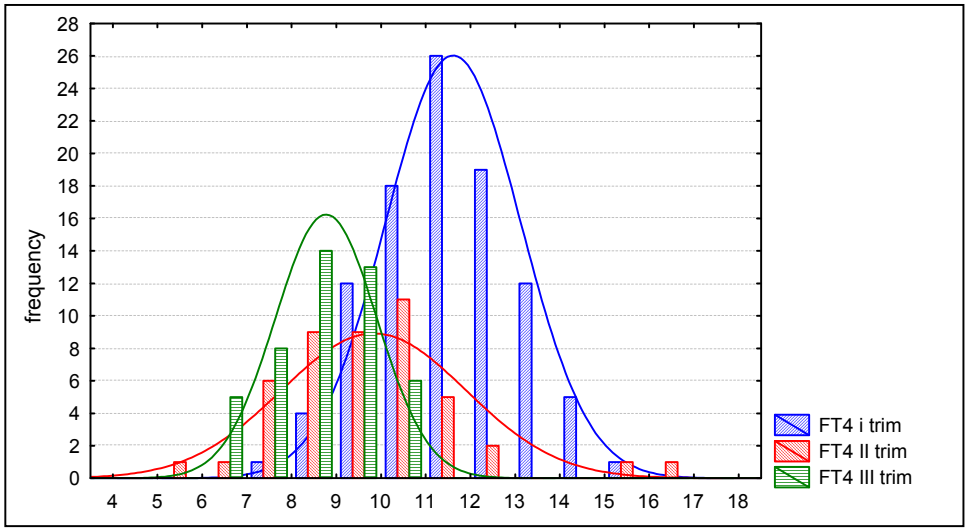

Chart 6. Distribution of FT4 values FT4 from all trimesters

Table 6. FT4 median and limits for pregnant women

Trimester	n	median (pmol/L)	95% interval (pmol/L)
first	98	11.6	8.8 – 14.7
second	46	9.6	6.0 – 16.0
third	46	8.7	6.6 – 10.7

The table also shows a gradual reduction of the median value as the pregnancy progresses reaching the lowest value (8.7pmol/L) in the third trimester. The lowest bottom limit of 6.0pmol/L is obtained in the second trimester.

Unlike these results, the median for the FT4 values obtained after delivery (12.4pmol/L) coincides with the median for the general population (12.6pmol/L), and the limits are 8.1pmol/L and 15.7pmol/L. The distribution of the values is presented on chart 7.

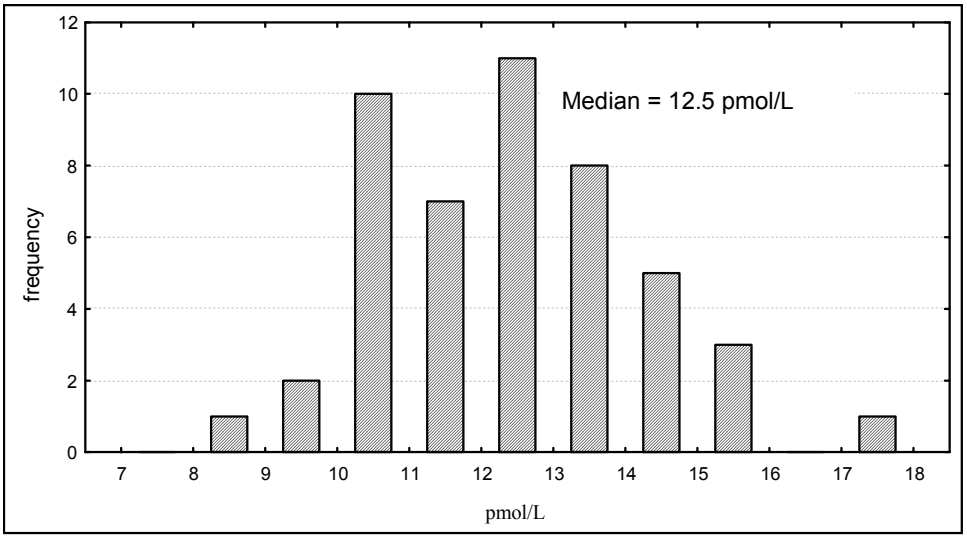

Chart 7. Distribution of the FT4 values after delivery

2.3 Determination of Tg of pregnant and lactating women

We determined thyrglobulin levels of pregnant women in all gestation periods. The values were graphically presented and statistically analyzed. Chart 8 shows an almost identical distribution of Tg values for all trimesters, and there are no statistically significant differences between the different trimesters.

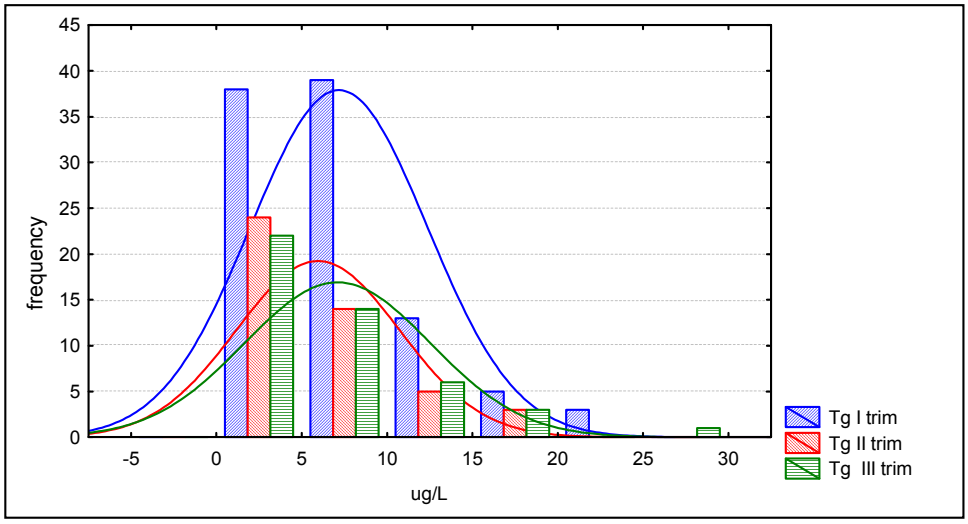

Chart 8. Distribution of Tg values from all trimesters

Therefore we could group the values and determine single limits and median for the entire duration of the pregnancy. The results obtained from a total of 226 samples are presented in table 7.

Table 7. Median and limits for Tg

Tested parameter	Obtained value (µg /L) n = 226
Median	6.05
2.5 percentile	0.2
97.5 percentile	20.6

When interpreting the results we followed the guidelines provided by WHO/UNICEF/ICCIDD (10). In the assessment of Tg as an indicator of iodine deficiency in epidemiological studies, the declared value for the median is 10µg/L. This implies that the values for a portion of the population with adequate iodine intake should nevertheless be below this declared median value. The median for Tg in our study is 6.05µg/L. It is somewhat higher than the median for the general population (5.55µg/L). However, both values indicate a state of adequate iodine intake.

The values for Tg obtained after delivery do not deviate from the values during pregnancy. The same interval from 0.2µg/L -20.6µg/L is obtained, while only the value of the median is slightly higher with 6.55µg/L. The chart below shows the distribution of the values and median obtained for Tg after delivery.

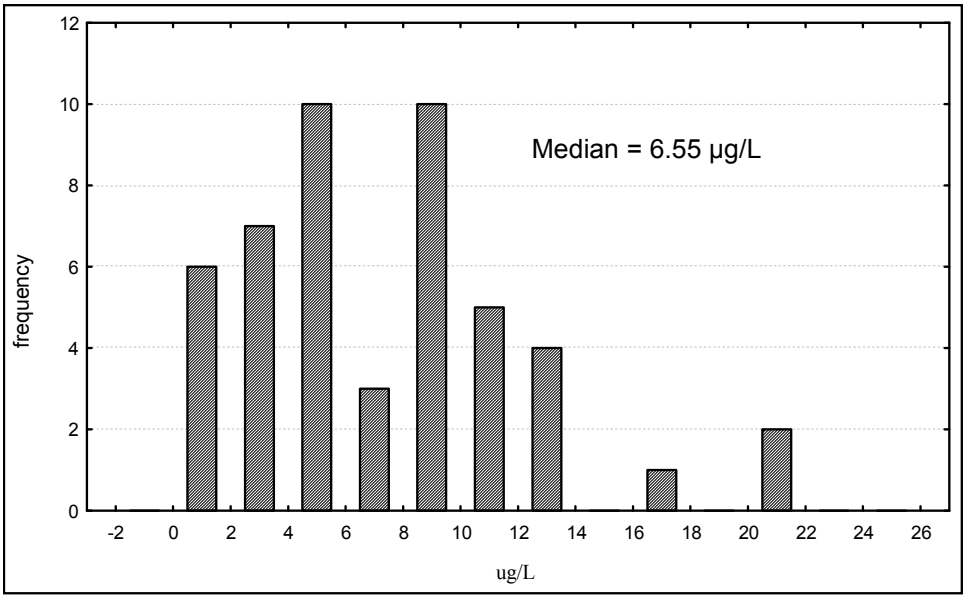

Chart 9. Distribution of Tg values after delivery

2.4 Determining of thyroid antibodies of pregnant women

We determined thyroid antibodies (a-TPO and a-Tg) in all subjects that were examined for the first time, irrespective of the gestation period. The concentration of thyroid antibodies was an exclusive criterion. We considered values for a-TPO > 35kIU/L, and values for a-Tg > 40kIU/L to indicate a positive finding according to the declaration of the kit producer. Antibodies were determined with each examination of the subjects involved in the study. This applied especially to the subjects receiving iodine substitution, for the purposes of monitoring possible iodine induced thyroid disorders. Table 8 provides the prevalence of both thyroid antibodies according to the gestation period.

Table 8. Prevalence of positive thyroid antibody finding among pregnant women

Trimester	First	Second	Third
No. of subjects	122	54	9
a-TPO (+)	12 (9.8%)	4 (7.4%)	0
a-Tg (+)	6 (4.9%)	1(1.8%)	0

The results show that the pregnant women from the first trimester have the highest prevalence of a-TPO antibodies (9.8%), while the prevalence for a-Tg is half of this value. Among the subjects examined for the first time during the second trimester, the percentage of positive antibodies is 7.4% for a-TPO and 1.8% for a-Tg. No positive antibodies have been determined among the pregnant women which have been examined for the first time during the third trimester. Finally, for all 185 pregnant subjects, the antibody prevalence is 8.6% for a-TPO and 3.8% for a-Tg antibodies.

These results are consistent with the data quoted in literature (11, 12) where the prevalence of a-TPO during pregnancy ranges between 8% and 11.3%.

3. RESULTS FROM THE DETERMINATION OF BIOCHEMICAL INDICATORS OF SUBJECT RECEIVING IODINE SUBSTITUTION

From the subjects that agreed to take iodine substitution in the form of iodine tablets of 100μg per day, for the purposes of our study we received only 36 samples during pregnancy and 7 samples after delivery. The results of the pregnant women are presented through the median value for each parameter separately and these values are compared with the respective values of the subjects without iodine substitution.

Since for certain biochemical parameters there were no statistically significant differences between the trimesters, table 9 contains the median values of the grouped data.

Table 9. Iodine substitution and biochemical markers among pregnant women

Tested parameter Median	Without KI n = 91	With KI n = 36	p
Urinary iodine (II and III trim.)	182.0 μg/L	220.5 μg/L	n.s
TSH (II and III trim.)	1.6 mIE/L	1.6 mIE/L	n.s
Tg (II and III trim.)	5.1 μg/L	6.3 μg/L	n.s
FT4 (II trim.)	n = 64 9.56 pmol/L	n = 8 10.65 pmol/L	n.s
FT4 (III trim.)	n = 46 8.7 pmol/L	n = 28 9.25 pmol/L	n.s

n.s ($p > 0.05$)

The table shows an increase of the median value for urinary excretion, Tg and FT4 among pregnant women with iodine substitution. For TSH, the value of the median does not change as the iodine intake increases. On the other hand, the statistical analysis of the data did not show any statistically significant differences in the distribution of the values between the examined groups of subjects.

In the group of delivered women, the statistical sample of the subjects that received iodine substitution was very small (7) and therefore we present the results only through histograms in order to obtain initial findings regarding the value distribution.

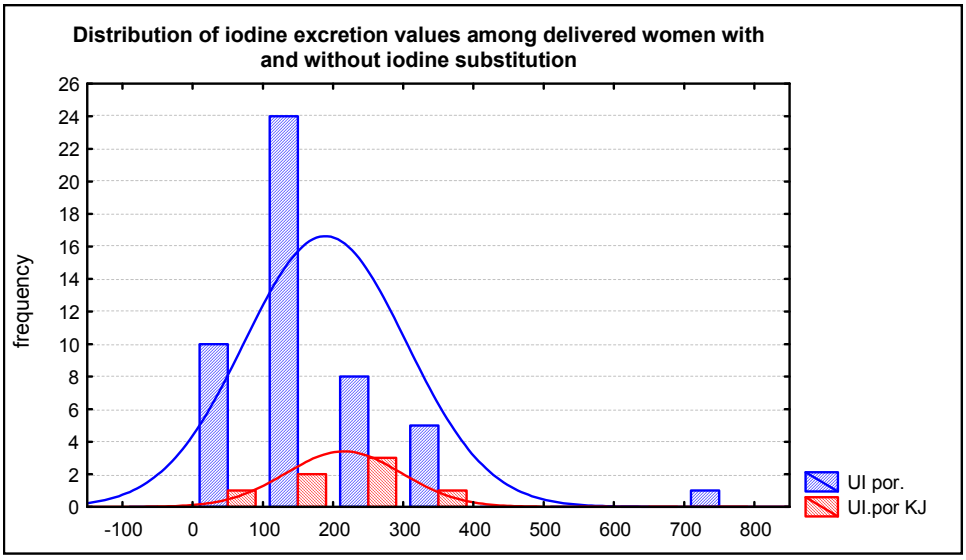

Chart 10. Iodine excretion among delivered women with and without iodine substitution

Regarding the results from the urinary iodine excretion, the values are shifted towards the higher concentrations, which is confirmed by the median value of 213.3µg/L in comparison to the median value of 157.9µg/L for the subjects without iodine substitution.

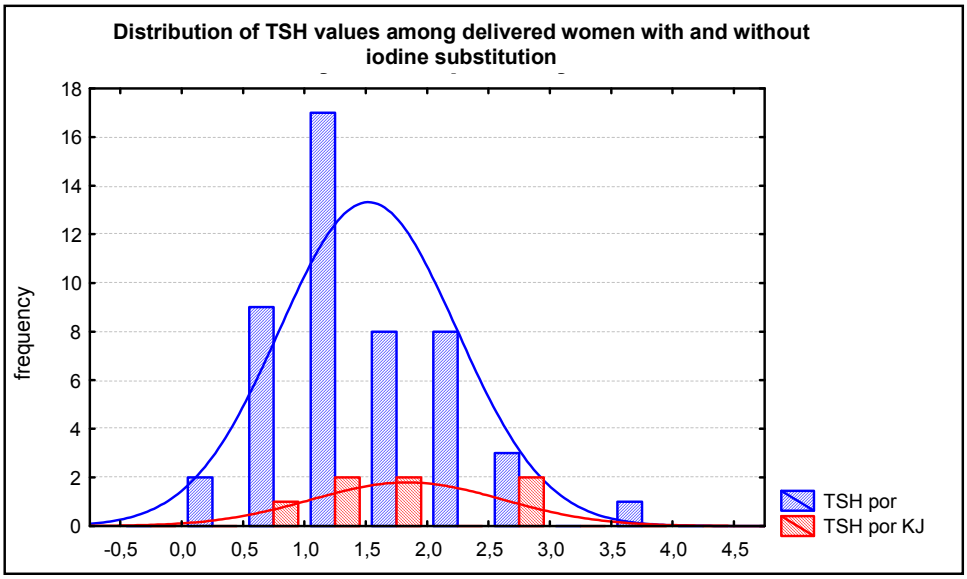

Chart 11. TSH among delivered women with and without iodine substitution

The values for TSH among the subjects not receiving iodine substitution are asymmetrically distributed towards the left. However, there are some values above the reference interval (0.6mIE/L to 2.8mIE/L). For the subjects with iodine substitution, the distribution is more homogeneous and the values do not deviate from the reference interval for delivered women.

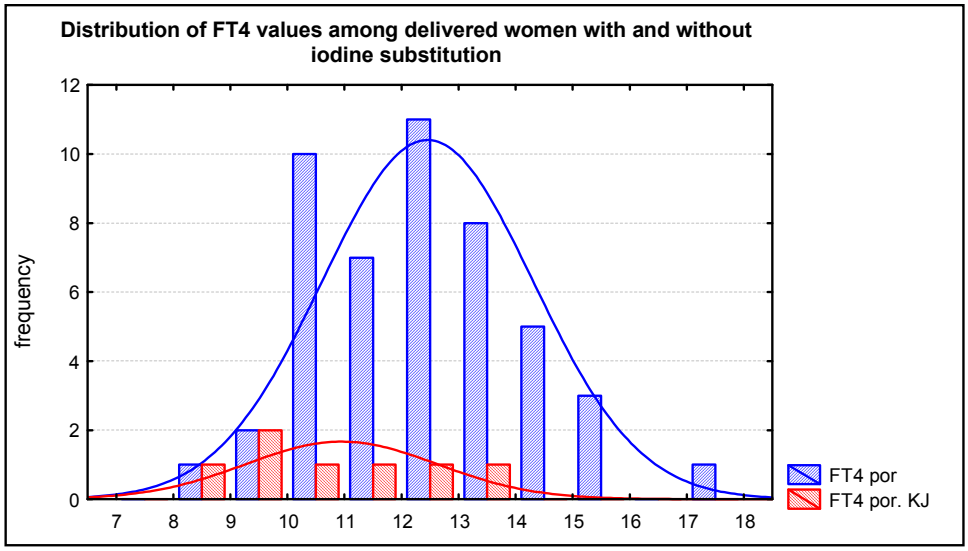

Chart 12. FT4 among delivered women with and without iodine substitution

The results presented on chart 12 show a wider distribution of the FT4 values obtained from the delivered women without iodine substitution. These results are consistent with the limits of the reference interval. The results from the delivered women with iodine substitution show higher values grouped towards lower concentrations, but still within the limits of the referent interval. The values for Tg determined among delivered women with iodine substitution also have this tendency (chart 13). This indicates a reduced thyroid substitution among these subjects, compared to the values of the subjects without iodine substitution. However, we did not note any values in any of the groups that exceed the referent interval.

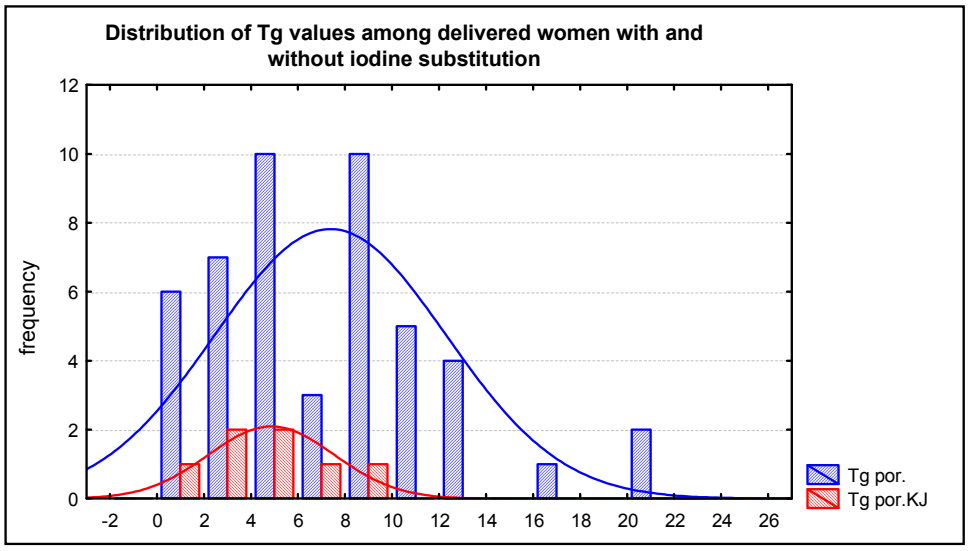

Chart 13. Tg among delivered women with and without iodine substitution

The results presented so far lead to a conclusion that in cases of iodine sufficiency among the general population, additional iodine intake does not have a significant impact on the function of the thyroid gland during pregnancy and lactation. The evaluated thyroid markers stay within the referent intervals in both the group of subjects receiving iodine substitution as well as in the group of subjects without additional iodine substitution.

The only notable difference is related to the proportion of subjects with insufficient iodine intake between the two groups of subjects (chart 14). This difference is mostly expressed in the second trimester with high 37% among pregnant women without iodine substitution compared to 12.5% among those receiving iodine substitution. In the third trimester, 39.1% of the pregnant women without iodine substitution had insufficient intake, compared to 21.4% of the pregnant women with substitution. After delivery, the differences between the respective proportions are not so big (20.4% without substitution and 14.3% with substitution).

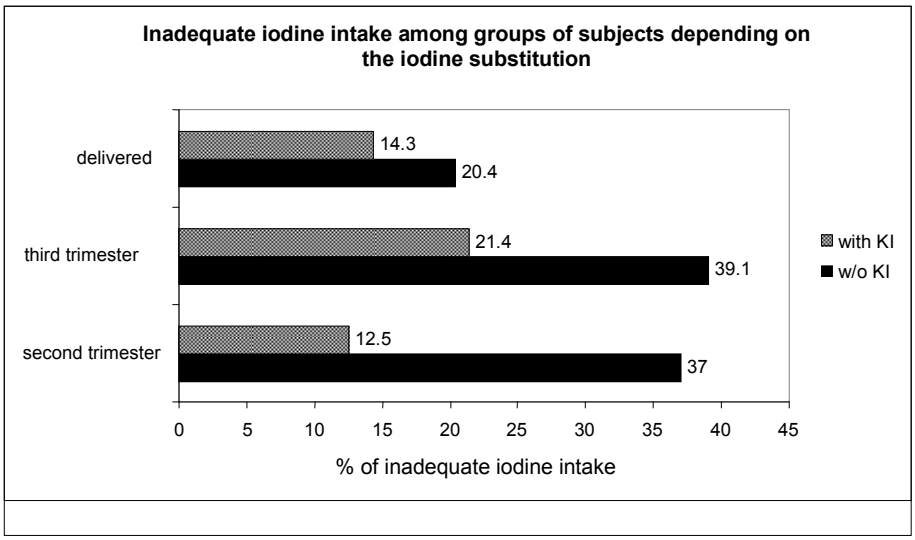

Chart 14. Proportion of subjects with insufficient iodine intake depending on the iodine substitution

The influence of the iodine substitution is also high when comparing the results for iodine excretion through the breast milk of the mothers. The histogram is presented on chart 15, which suggests an almost central distribution of the iodine values in the milk of lactating women receiving substitution, compared to the accumulation of the values towards the low concentrations for the samples from lactating women without iodine substitution. The iodine substitution during lactation impacts the provision of sufficient iodine quantities to meet the needs of the infant.

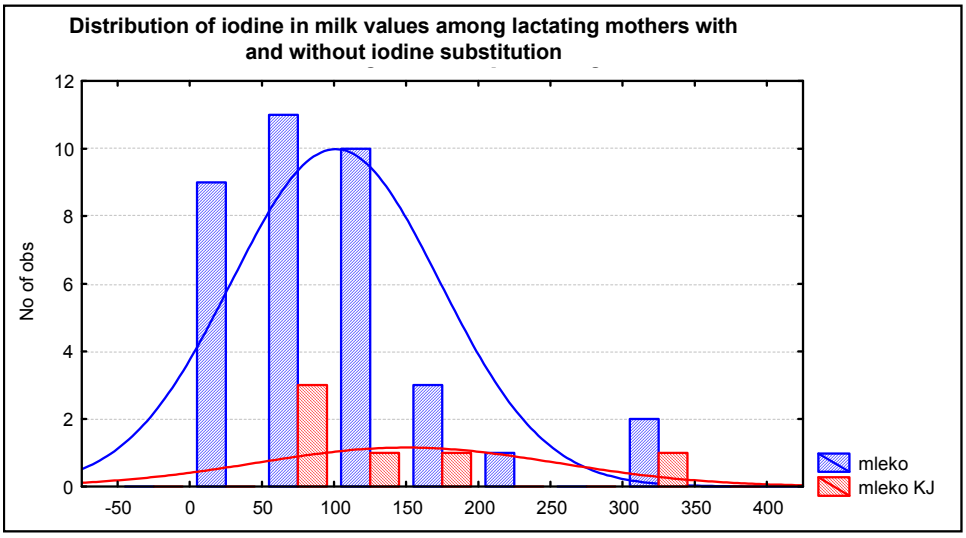

Chart 15. Iodine in milk among lactating women with our without iodine substitution

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PALPATION OF THE THYROID GLAND

Svetlana Miceva – Ristevska

The thyroid gland is located on the front side of the neck, in front of the trachea and is easily accessible for examination. There are several in vivo and in vitro methods for diagnosing its status. One of those methods is palpation.

Palpation is one of the oldest methods for examining the thyroid gland and it is mainly related to the change in size of the thyroid gland. The value of this method for assessing the status of the thyroid gland is not the same as it was at the beginning, but it is still used, especially for assessing the size of the gland.

Palpation is simple and widely accessible; it does not depend on the conditions where it is performed because it does not require any special equipment or any addition tools. It is sufficient to have experienced people, i.e. persons with long term experience with examining the thyroid gland and diagnosing its disorders.

There are several rules for this method: the person performing the palpation must stand behind the sitting patient or the person performing the palpation can sit in front of the patient, who is also seating. Then the person performing the palpation assesses the size of the thyroid gland using his/her fingers. The experience of the examiner should surpass the subjectivity related to this method.

According to WHO, UNICEF and ICCIDD, there are several classifications for determining the grade of goiter of patients providing for either 3 or 5 grades of goiter (1, 2). There is also an additional criterion that determines whether the patient's thyroid gland is increased at all, i.e. whether there is any goiter. Studies have confirmed that if, while palpating the gland, the examiner concludes that the size of each lobe of the thyroid gland is equal or smaller than the size of the terminal phalanx of the thumb of the examiner, then the subject does not have any goiter. However, if the finding is different, then there is goiter (enlarged thyroid gland) which can be classified in one of the grades of goiter provided in accordance with the WHO criteria.

In our study we used the new classification proposed on the consultation meeting in Geneva (3, 4). According to this classification there are three grades of goiter:

- grade 0 (0°) - the thyroid gland cannot be seen or palpated (the patient does not have goiter);
- grade 1 (1°) - the thyroid gland can be palpated, but cannot be seen when the neck is in the normal position;
- grade 2 (2°) - the thyroid gland is enlarged and can be seen even if the neck is in normal position.

PALPATORY FINDINGS OF THE PREGNANT WOMEN

The examination of the iodine intake status and the status of the thyroid gland of pregnant and lactating women was organized as a prospective study of the research project in cooperation with UNICEF.

The study included 203 pregnant women between 17 and 42 years of age (average age 26.6 ± 4.7 years.). We excluded 13 pregnant women from the study because they had previous thyroid gland disorders (hypothyroidism during therapy, Hashimoto thyroiditis with hypothyroidism and nodular goiter).

Therefore the study involved a total of 190 pregnant women. Out of the total pregnancies, in 86 cases it was their first pregnancy, in 63 cases it was their second pregnancy, in 28 cases it was their third pregnancy, in 6 cases it was their fourth

pregnancy, in 4 cases it was their fifth pregnancy, in 1 case it was her sixth pregnancy and in 2 cases it was their seventh pregnancy.

The examination of the thyroid status of the pregnant women included in the study involved determining the size of the thyroid gland using the palpation method (according to the existing current classification criteria of the WHO), determining the size of the thyroid gland using ultrasound methods, determining the values for FT_4, TSH, TAT, Tg and determining the urinary excretion of iodine. All of these tests were envisaged to be done during the first and the third trimesters of the pregnancy, as well as during the first 6 months after delivery (the lactation period).

The results from the palpation examinations of the 190 subjects were presented by trimesters as well as the period after delivery.

Of the total number of subjects, in 117 cases palpation was performed during the first month of pregnancy (period from conception to the end of the 13^{th} week); in 58 cases palpation was performed in the second trimester (from the 14^{th} to the 26^{th} week of pregnancy) and in 43 cases palpation was performed in the third trimester (from the 26^{th} to the 40^{th} week of pregnancy). In 52 cases the palpation was performed after delivery, 7 of them were examined for the first time after delivery, while the rest were monitored from the time of pregnancy until delivery.

In 112 out of the 117 cases palpated during the first trimester, the finding is normal, i.e. goiter level 0 (according to the classification proposed by the WHO which we used); the remaining 5 women had goiter level 1.

In the second trimester, 47 of the 58 women had a normal palpatory finding of the gland; 7 women had grade 1 goiter and 2 women had grade 2 goiter.

In the group of 43 pregnant women palpated during the third trimester of pregnancy, 39 women had a normal finding (grade 0), and 4 women had grade 1 goiter.

Out of the 52 women examine after delivery, the 7 women examined for the first time after the delivery had a normal finding. In 43 cases, out of the remaining 45 women that were monitored from the start of pregnancy until the delivery and in the postpartal period, the fining was normal (both during the first examination as well as after the delivery), while 2 patients had nodular goiter (both during the first examination as well as after the delivery).

The results from the palpation are presented in table 1 below.

Table 1

Grade of goiter	**0^0**	**1^0**	**2^0**	**Total number of subjects**
I trimester (no. of subj.)	112	5	/	117
%	95.7%	4.3%	/	100%
II trimester (no. of subj.)	47	9	2*	58
%	81%	15.5%	3.5%	100%
III trimester (no. of subj.)	39	4	/	43
%	90.7%	9.3%	/	100%
First examination after delivery (no. of subj.)	7	/	/	7
%	100%	/	/	100%
Monitored until delivery (no. of subj.)	43	/	2*	45
%	95.5%	/	4.5%	100%

The results show that in spite that in most cases the findings from the palpation of the thyroid gland of the pregnant women were normal, there is still a small percentage of subjects which have goiter, mostly during the second trimester of pregnancy and slightly less during the third trimester. This finding relates to minor goiter of grade 1.

The subjects (*) which were found to have a higher grade of goiter (level 2), in fact had a small nodule which was discovered using ultrasound method and the subjects themselves were unaware of the nodule. This nodule had not been discovered with palpation, and in fact it had persisted during the control tests as well as after the delivery.

One part of the examined pregnant women (40) involved in the study also underwent an additional iodine therapy (KI tablets or a different type of combined vitamin – mineral tablets containing iodine). Seven of these pregnant women had a positive palpation finding during the first examination, and the other 33 had normal findings. The results from the palpation examination of these women are presented in table 2:

Table 2.

Examined pregnant women with a positive palpation finding, receiving additional iodine supplements (total 7 subjects.) (°)			
Grade of goiter	I control (I trimester)	II control (III trimester)	III control (after delivery)
1^0	5/7 were 1^0	1/5 remained 1^0 4/5 become 0^0	2/5 remained 0^0
2^0	2/7 were 2^0	2/7 remained 2^0.	2/7 remained 2^0
Examined pregnant women with normal palpation findings, receiving additional iodine supplements (33 subjects)			
Goiter grade	I control (I trimester) 33 subjects	II control (III trimester) 33 subjects	III control (after delivery) 23 subjects
0^0	33 were 0^0	33/40 remained 0^0	23/40 remained 0^0
Examined pregnant women with a positive palpation finding, without additional iodine supplements (43 subjects)			
Goiter grade	I control (I trimester)	II control (III trimester)	III control (after delivery)
0^0	43	43	38

We can conclude that the examined pregnant women which had a positive palpation finding during the first examination (mostly goiter a grade 1), after receiving the iodine supplementation experienced a notable withdrawal of the goiter; however the number of these subjects is small to provide for any definitive, significant conclusion.

The group of subjects that had a normal palpation finding of the thyroid gland (grade zero) during the first examination (first control) and who received additional iodine therapy, as well as the group with normal finding, but without additional iodine supplementation did not experience any notable change in the palpation finding during the subsequent controls performed as part of this study.

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VOLUME OF THE THYROID GLAND OF PREGNANT WOMEN, DETERMINED USING ULTRASOUND METHODS

Suzana Loparska

Due to hormonal changes, increased renal iodine clearance and augmented needs for iodine, pregnancy can lead to an increase of the volume of the thyroid gland, especially in the iodine deficient regions. The introduction of the ultrasound technique as a method to determine the volume of the thyroid gland in epidemiologic studies by Gutenkust (1) provided for a more objective approach for assessing the size of the thyroid gland. This method is noninvasive and there are no contraindications to its application in the delicate period of pregnancy and lactation. However, unlike the numerous studies assessing the thyroid gland volume of school children from iodine deficient or iodine sufficient regions, there are far less studies of this kind involving pregnant women (2, 3, 4).

In the Republic of Macedonia, in 1995, ultrasound methods were used for the first time to assess the thyroid gland volume of pregnant women. That study involved 36 subjects from the capital Skopje (5). Again in 2000, there was another study involving a small group of 34 pregnant women from Skopje, where the thyroid gland volume was assessed using an ultrasound method (5). This study is the first study involving a large number of pregnant women from different regions of the country.

We have examined 202 women, both pregnant and lactating women, from several cities in the Republic of Macedonia (Shtip and Berovo in eastern Macedonia, Kichevo and Gostivar in western Macedonia and the Capital Skopje). The numbers of examined subjects by cities are provided in the next table.

	Skopje	Shtip	Berovo	Kichevo	Gostivar
Examined	85	66	7	19	25

The study involved a total of 360 tests. Out of the total number of tests 86 patients received one examination each, 74 patients received two examinations each and 42 patients received three examinations each. A total of 44 patients (77 tests) were excluded from the study because they were found to have thyroid disease, noduls larger than 6 mm, in the biggest diameter, or positive anti thyroid antibodies. The definitive number of healthy pregnant and lactating women included in the study is 158, with 286 performed tests. Depending on the time when the tests were performed, the patients were classified as follows:

1) first trimester of pregnancy (1^{st} to 13^{th} week of pregnancy)
2) second trimester of pregnancy (14^{th} to 26^{th} week of pregnancy)
3) third trimester of pregnancy (27^{th} to 40^{th} week of pregnancy)
4) lactating women (1 to 6.5 months after delivery)

The ultrasound examination was performed using ultrasound equipment of the type Aloka SSD 500 with a 7.5 MHz linear probe. The patients were examined while they were lying down and had their neck slightly extended. The transversal section was used to determine the width and the thickness, and the sagittal section was used to determine the length of the thyroid lobe (figure 1). All the tests were done by one examiner (6). The lobe volume was determined using the formula for a rotating ellipsoid, a modification of Brunn (7).

V (lobe) = a x b x c x 0.479

(a = width; b = thickness; c = length of the lobe)

The volume of the thyroid gland was calculated as a sum of the volumes of the right and the left thyroid lobe.

V (thyroid gland) = V (right lobe) + V (left lobe)

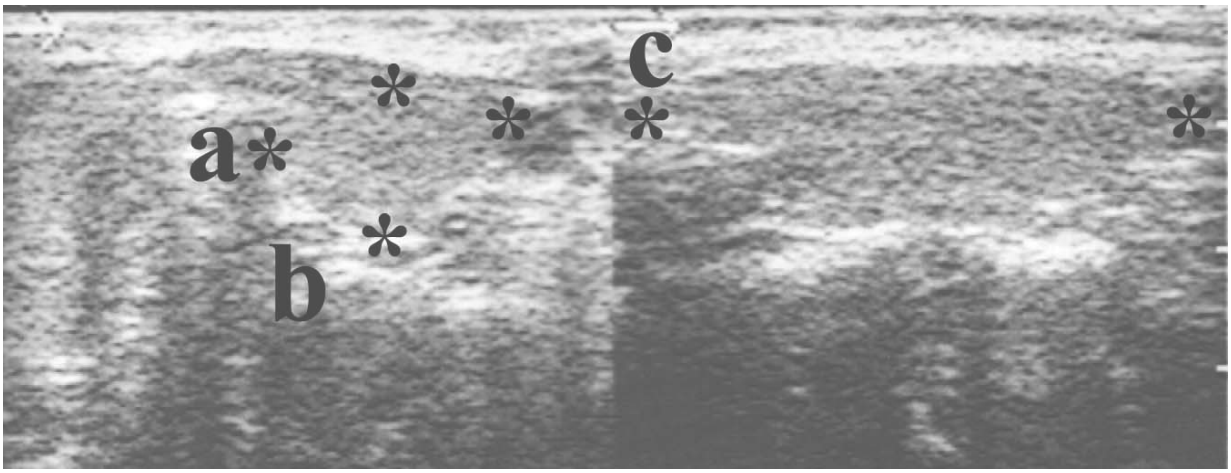

Figure 1. Transversal and sagittal section of a thyroid lobe
(a - width; b - thickness; c - length)

In addition the structure of the thyroid gland was analyzed in comparison with the echogenicity of the sternocleidomastoid muscle.

Table 1 and chart 1 show the volumes of the thyroid gland of the examined pregnant women.

	Number of tests	Median P50	P97	Average value	Standard deviation
all	286	8.04	15.72	8.39	2.95
All pregnant	228	8.1	16.00	8.55	3.13
I trimester	100	7.8	15.96	8.16	3.05
II trimester	53	7.86	15.47	8.45	3.08
III trimester	74	8.75	16.78	9.16	3.23
lactation	58	7..5	11.94	7.75	2.01

Table 1 Ultrasound volume of the thyroid gland (ml)

The thyroid volume, expresses as a median (P50) increases as the pregnancy progresses from 7.8 ml in the first trimester to 7.86 ml in the second trimester to 8.75 ml in the third trimester of pregnancy. After delivery, during the period of lactation the thyroid volume decreases to 7.5 ml.

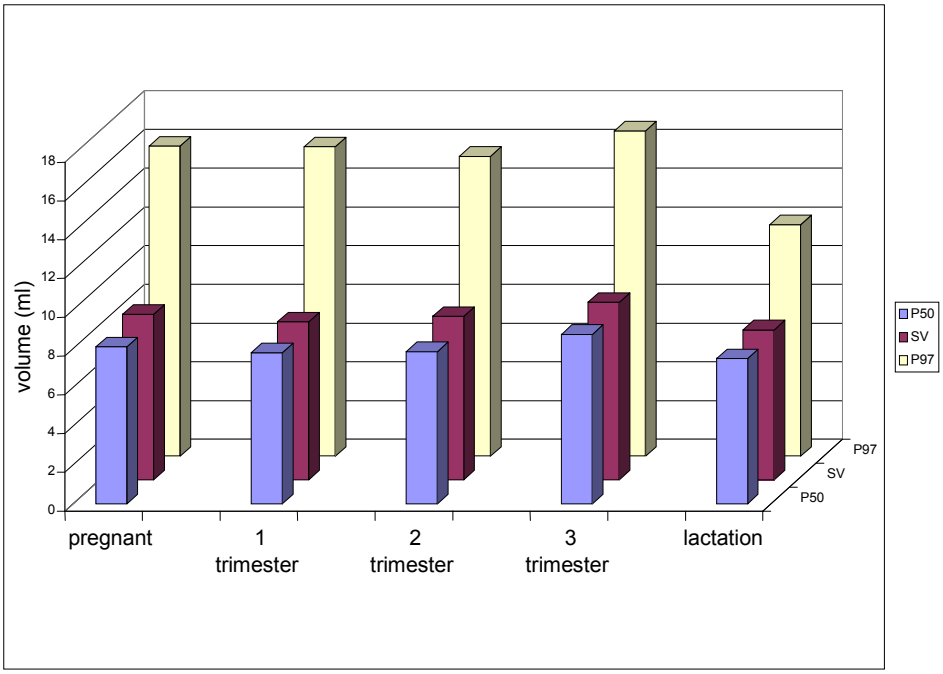

Chart 1 Ultrasound volume of the thyroid gland

According to the criteria of WHO, UNICEF and ICCIDD (8, and 9) the normal volume of the thyroid gland of an adult women, expressed through the median (P50) is 8 ml. Only in the third trimester of the pregnancy we received value larger than that (8.75 ml).

The volume differences are significant ($p<0.05$) between the first and the third, the second and the third trimester of pregnancy, as well as between the third trimester of pregnancy and the lactation period ($p <0.01$). There are no significant volume differences between the first and the second trimester, the first trimester of pregnancy and the lactation period, as well as between the second trimester of the pregnancy and the lactation period.

The thyroid volume expressed as P97 is 15.96 ml in the first trimester, 15.47 ml in the second trimester and in the third trimester it increases to 16.78 ml. During the lactation period this volume decreases significantly to 11.94 ml. According to the criteria of WHO, UNICEF, ICCIDD (7) the P97 (upper limit of the normal range) of the thyroid volume of adult women is 18 ml. The volumes of the thyroid gland do not exceed this value during the entire period of pregnancy as well as during the lactation period, i.e. no goiter was identified in any of these periods.

Individually speaking, we registered 2 cases (0.7%) where the thyroid volume was greater than 18 ml. The first case was identified during the first trimester of pregnancy (18.17 ml) and no further monitoring was performed. The second case was identified during the second trimester (19.16 ml), but this patient during the first trimester of pregnancy had a thyroid gland volume of 16.01 ml.

Regarding the ultrasound structure of the thyroid glands, we have discovered a diffuse inhomogeneous structure without changes of the functional status in two cases. In 16 cases we have discovered impalpable nodule. In addition we discovered two cases of hemithyroidism, one of which had hypothyroidism and therefore was placed on a thyroxin substitution therapy.

Longitudinal examination was performed in 32 cases. The thyroid volume was determined three times, twice during pregnancy (different periods) and once during the period of lactation (Table 2 and chart 2).

	Median (P50)	Mean	Standard deviation
Pregnancy (first test)	7.17	7.69	2.68
Pregnancy (second test)	7.66	8.31	3.34
lactation	7.22	7.74	2.23

Table 2 Longitudinal examination of the ultrasound volume of the thyroid gland (ml)

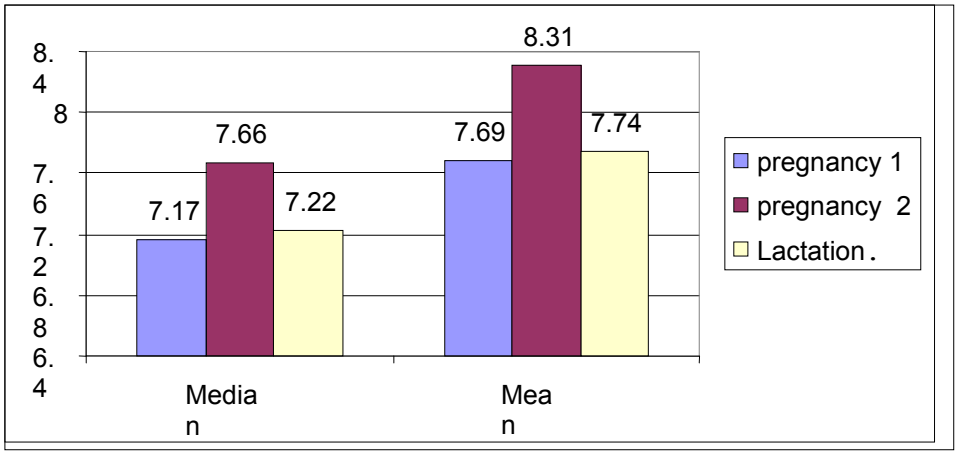

Chart 2 Longitudinal monitoring of the ultrasound volume of the thyroid gland (ml)

In the case of longitudinal monitoring of the thyroid volume, we can also note a slight increase of the volume as the pregnancy progresses, as well as a reduction of the volume after the delivery and during the period of lactation. However, these volume differences are not significant.

Some of the pregnant women received 100 micrograms per day of iodine in the form of KI tablets. The therapy started after the first examination of the pregnant women and was administered until the end of pregnancy and after the delivery until the last examination.

Table 3 and chart 3 present the results. The women receiving 100 micrograms of iodine per day have slightly smaller thyroid volumes in the third trimester and during lactation in comparison to the women who did not receive any iodine substitutions. This difference is not significant.

	Number of tests	Median	Mean	Standard deviation
Thyroid volumes of women receiving KI				
III trimester	28	8.67	9.01	3.39
lactation	6	6.92	6.9	1.44
Thyroid volumes of women not receiving KI				
III trimester	46	8.80	9.25	3.17
lactation	52	7.50	7.84	2.05

Table 3 Comparison of thyroid volumes of women with and without KI

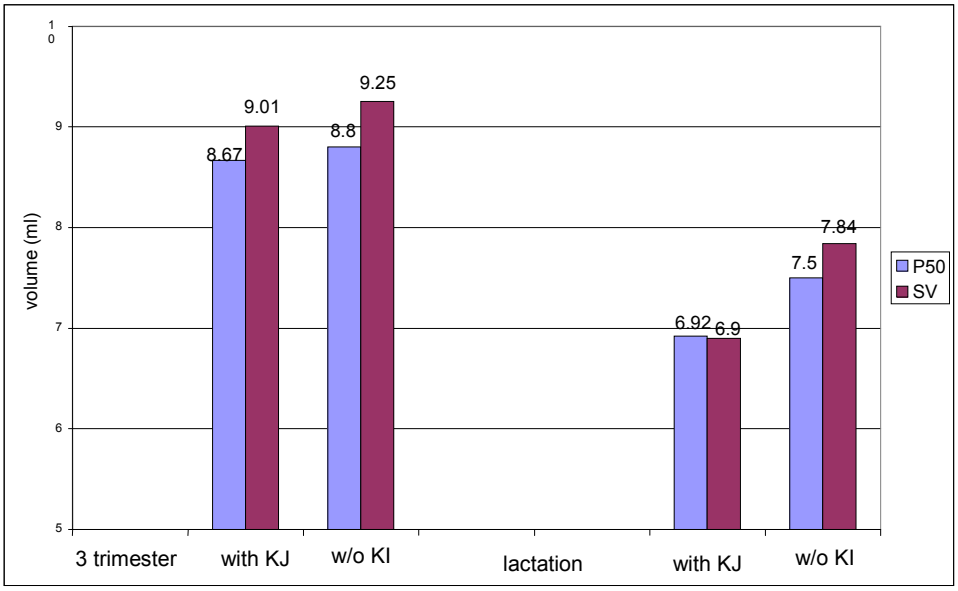

Chart 3 Comparison of thyroid volumes of women with and without KI

The thyroid volumes obtained with this examination were compared to the volumes obtained in the previous studies conducted in the Republic of Macedonia (table 4).

Pregnant women	Median (P50)	Mean	P97
Pregnant women (1995)	13.58	14.06	22.25
Pregnant women (2000)	11.65	11.16	16.92
Pregnant women (2006/2007)	8.1	8.55	16.0

Table 4 Comparison of the thyroid gland volumes among pregnant women in different years.

The obvious reduction of the thyroid gland volume of the pregnant women points to the efficiency of the application of the new measures for salt iodination with 20 to 30mg iodine per kg of salt in the form of KJO_3 in comparison to the previous for salt iodination with 10mg KI per kg of salt (chart 4). The new Rule Book on Salt Iodination in the Republic of Macedonia was enacted in 1999. When examining school children we, also registered a reduction of the thyroid volume after the implementation of the new legislation on salt iodination (10).

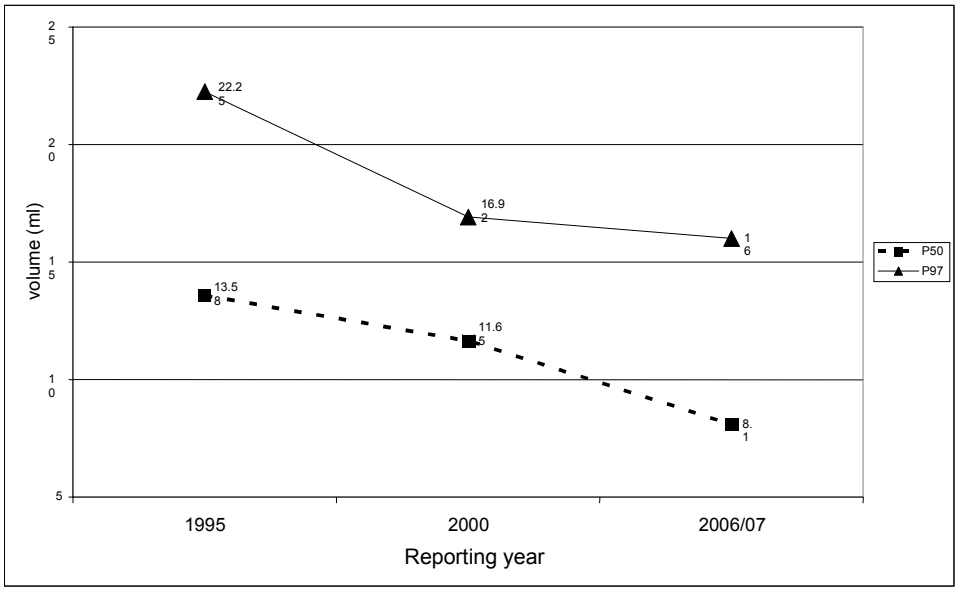

Chart 4 Comparison of thyroid volumes of pregnant women in different reporting years.

Our results shows that among pregnant and lactating women in the Republic of Macedonia goiter was registered only in 0.7% of the cases (volume greater than 18 ml). Authors from other countries register larger percentages of goiter: Tajtakova, Slovakia 10.1% (volume greater than 22 ml) (3); Smyth, Ireland, 19.5% to 32% (4); Caron, France 3.5% to 15.4% (in 11% of the cases the volume was greater than 22 ml) (11); Mezosi, Hungary, 19% (12) (table 5).

	Country	Num	T1	T2	T3	After delivery	goiter % (V>18ml)
Brander (2)	Finland	21	11,5	12,8	13,7		
Smyth (4)	Ireland	115	13,9		16,0	16.0	19.5-32
Tajtakova (3)	Slovakia	258				14,9	10,1 (>22ml)
Berghout (10)	Netherlands	10	10,6	9,6	9,4		
Caron (11)	France	246					3,5-15,4 11 (>22ml)
Mezosi (12)	Hungary	313					19
Karanfilski (this study)	Macedonia	158	8,16	8,45	9,16	7,75	0.7

Table 5: Comparison between our findings and the findings of other authors.

(T1, T2 and T3 = the three trimesters of pregnancy; mean values are provided for the thyroid volumes in ml)

In our study, the thyroid volume increases slightly as the pregnancy increases which is consistent with the findings of other authors (2, and 4). However, there are studies (10) where the thyroid volume remains constant throughout the pregnancy. This refers particularly to the Netherlands, a land with a long term iodine sufficiency.

The values for the thyroid volume during the three trimesters of pregnancy and after delivery are smaller in our study in comparison with other studies. However, urinary iodine excretion of the women involved in these studies is low, under 90 μg/L, while our pregnant and lactating women have urinary excretion above 180 μg/L.

In our study we have a small difference between the thyroid volumes of pregnant and lactating women who received and those who did not receive KI tablets, which is not the case in the study of Glinoer (13) from Belgium, which registers minimal changes of the thyroid volume of the pregnant women receiving 100μg KI per day, and significant increase of the thyroid volume of pregnant women without iodine therapy.

The normal thyroid volume and the low percentage of goiter of pregnant and lactating women in our study confirms the efficiency of the Iodine Deficiency Prevention Program in the Republic of Macedonia.

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REVIEW OF THE STUDY RESULTS

Borislav Karanfilski

A lot of data clearly indicate that, in the past Macedonia was a iodine deficient area, with high incidence of goiter, which, in certain regions, was endemic (1). Because the other republics of former Yugoslavia were also iodine deficient areas, a law was passed in 1956 which stipulated that all salt for human use shall be iodinated with 10mg potassium iodide (7mg iodine) per kilogram of salt. The enforcement of this law resulted into slight correction of iodine deficiency.

However, even after many years of such a iodine prophylaxis in Macedonia, the thyroid pathology still had all the characteristics of an iodine deficient area, with a large number of goiter cases among the population born after the introduction of the iodine prophylaxis. Every year Macedonia registered more than 1000 new goiter patients (2). Preliminary studies showed that goiter is present among more than 60% of the primary school children in certain villages in Macedonia (3).

All of these indicators clearly suggested that the iodine deficiency prevention measures undertaken in Macedonia are not sufficiently efficient. This led to the conclusion that a complete study and assessment of the iodine deficiency in Macedonia was needed. The research initiatives conducted in 1995/96 encompassed the whole territory of the country and they applied the iodine deficiency assessment methods recommended by WHO, UNICEF and ICCIDD, which included palpation of the thyroid gland, determination of the thyroid gland volume by ultrasound methods and measurement of the urinary iodine excretion (4).

The analysis of the results from the palpation method showed that the general prevalence of goiter among the examined children was 18.7%, the values related to the thyroid gland volume were higher than the norms established by WHO, UNICEF and ICCIDD, and the median value of the urinary iodine excretion was 117μg/L. The overall research results showed that Macedonia was an iodine deficient area and that the measures that had previously been implemented are not sufficiently efficient to correct the iodine deficiency (5).

We conveyed these conclusions to the Ministry of Health and proposed that it should form a National Committee for Iodine Deficiency comprising representatives from all relevant institutions that can contribute to the correction of the iodine deficiency and its consequences in Macedonia. The Minister of Health accepted our proposals and enacted a decision (on 26.12.1997) establishing the Committee.

After the National Committee for Iodine Deficiency in Macedonia was formed, many well conceived, organized, continuous and coordinated activities aimed at correcting the iodine deficiency were initiated.

The annual programs of the Committee, which were fully and successfully implemented, envisaged monitoring activities of the iodine deficiency situation of the population in Macedonia, activities in the field of monitoring and control of the salt iodination, as well as activities aimed at educating and informing the population about the buying, storage and utilization of salt in the households.

A new rule book on mandatory iodination of salt for human use was enacted. It stipulates that the salt for human use, including the food industry must be iodinated with 20 to 30 milligrams of iodine per kilogram of salt using only the more stable compound potassium iodate.

The incidence of the goiter went from 18.7% before the effectuation of the new measures to a normal level of 5% in 2000, 5.8% in 2002, 4.7% in 2003, 2.05% in 2005 and 0.99% in 2007.

The assessments of the volume of the thyroid gland using ultrasound methods continuously show a reduction of the gland volume after the enforcement of the new salt iodination regulations.

The median for urinary iodine excretion which was 117μg/L in 1999, before the enactment of the new regulations, increased to 154.1 μg/L in 2000, 164.5μg/L in 2001, 198.5μg/L in 2002, 191μg/L in 2003, 228μg/L in 2005 and 241μg/L in 2007. Neonatal TSH screening in Macedonia was first introduced in 2002 on the Clinic for Disorders of Children at the Medical Faculty in Skopje (prof. Dr. Mirjana Kochova). The screening is used for early detection of hypothyroidism among newborns. However, in the event of iodine deficiency, more than 5% of the tested newborns have TSH values bigger than 5mU/L. The surveys implemented by the clinic found this percentage to be 4.3% in 2002, 5.9 in 2003, 2.8% in 2004, 0.8% in 2005, 1.8% in 2006 and 1.5% in 2007 of the tested newborn children. The number of screened children gradually increased and in 2007 this number reached 21,090 newborn children.

In 2001, the National Committee assessed that the iodine deficiency was corrected and asked the WHO, UNICEF and ICCIDD for an international expert evaluation of the achieved results related to the correction of iodine deficiency in Macedonia. The team of experts visited Macedonia from 19th to 23rd of March 2003 and performed a thorough assessment of the implemented activities and the results achieved to correct the iodine deficiency in Macedonia. The team prepared a report with quite a positive assessment of the achieved results in correcting iodine deficiency in Macedonia.

Summarizing the results achieved in the prevention of the consequences of iodine deficiency for the general population in Macedonia, we can conclude that they have been overcome. However, the control of the iodine deficiency among the general population, assessed by the frequency of the goiter and by the level of urinary iodine excretion of school children, does not permit us to conclude that iodine deficiency has been corrected for pregnant and lactating women as well.

During pregnancy the thyroid hormone requirements increase significantly. During this period, the thyroid gland of the mother should provide for optimal quantities of thyroid hormones to ensure normal growth of the fetus. These requirements are 1.5 to 2 times greater that the requirements before the pregnancy. The increased hormone requirements can be satisfied only by an increased hormone production which, for a healthy pregnant woman, depends on the appropriate iodine intake. If the iodine intake is insufficient, instead of a physiological adaptation, a pathological alteration occurs as a result of the chronically increased thyroid stimulation. This stimulation occurs as a consequence of several factors that have mutually independent impacts on the thyroid gland during pregnancy and increase its activity.

In the event of iodine sufficiency pregnancy does not have any significant impact on the iodine concentration in the blood, whereas in case of iodine deficiency the inorganic iodine concentrations in the blood are reduced. In order to maintain the hormone production, the level of thyroid iodine reserves reduces, and this causes a progressively closed cycle that leads to an increased thyroid gland stimulation which in turn causes goiter (6).

Later, during the gestation period, the iodine reserves of the mother are further depleted because part of the iodine transfers from the bloodstream of the mother into the fetal space. Therefore, the iodine depletion occurring at conception deepens during the first half of the gestation period and becomes highly emphasized in the final stage of pregnancy. During the lactation period, significant quantities of iodine are eliminated through the milk.

The results from numerous studies suggest that the thyroid hormones are a very significant regulator of the development of the central nervous system. The deficiency of the thyroid hormones also negatively influences the multiplication of neuroblasts, causes reduction of the weight of the brain and the DNA content as a result of the cell number reduction. The thyroid hormones also regulate the synthesis of numerous proteins in the brain. One of them, known as RC-3, or neurogranin, is important for the development and remodeling of the synapses. The extent of this damage depends on the duration and intensity of the thyroid hormone deficiency.

High levels of iodine deficiency that can cause the birth of cretins are not present in Europe and other developed countries. However, the areas with moderate and mild levels of iodine deficiency, with hypothyroxinema of the mothers, are not limited only to the undeveloped countries. Such places may be found in Europe, even in areas where the results from examinations of school children provide the basis for concluding that the iodine deficiency has been corrected, such as Macedonia. In these areas, pregnant and lactating women have much higher iodine deficiency than that determined with the examinations of school children. The iodine intake of pregnant and lactating women through iodine rich food and iodinated salt cannot increase to the level corresponding to their increased iodine requirements, which may lead to negative effects on the development of the brain of their children (7).

The mothers are not clinically hypothyrotic because of the compensatory mechanism triggered by the thyroid gland in case of iodine deficiency – preferential synthesis of T_3, which leads to a reduction of T_4, but with normal or slightly increased values of T_3 in the blood. Therefore, many tissues that can use T_3 directly from the blood for their own purposes, like the liver, kidneys, muscles, pituitary gland etc, are in an euthyreotic state and do not show signs of hypothyroidism. Therefore the values for blood TSH are not increased. However, some parts of the CNS of the fetus have receptors only for T_4, and the cells themselves can generate metabolically active T_3. Due to the reduced secretion, the quantity of serum T_4 is not sufficient to saturate the receptors in some parts of the brain and to provide for intracellular T_3 formation which is necessary for normal development of the brain. In that case the brain suffers damages which are irreversible, known as cerebral hypothyroidism (8).

There are several studies that show that many pregnant women in European countries still have iodine intake which becomes inadequate even during the first half of gestation. Therefore, many such women cannot increase the free T_4 in the early stages of pregnancy to the level reached by women with adequate iodine intake. This causes the volume of the thyroid gland to increase, as well as the ratio T_3/T_4 and the values of thyroglobulin in the serum. Iodine supplementation during pregnancy results in an increase of the free T_4, reduction of the ratio between T_3/T_4, reduction of the thyroglobulin in circulation and reduction of the volume of the thyroid gland of both the mother and the newborn.

The first studies of iodine status of pregnant and lactating women in Macedonia were performed in 2001. They included 382 pregnant and 110 lactating women (9).

The median values for the urinary iodine excretion for all pregnant and lactating women were 140.4 μg/L or 140.7 μg/L for pregnant and 139.2μg/L for lactating women. For pregnant women in the first trimester of pregnancy the value of the median is 149.7 μg/L, in the second 157.6 μg/L, and in the third trimester this value is lowest or 130.4μg/L.

Low values for urinary iodine excretion of 0 μg/L - 100 μg/L were found for 17 women in the first trimester (21.8%), 33 women in the second trimester (23.5%) and 4 women in the third trimester (28.1%), or 96 (25.1%) of al examined women had low values for urinary iodine excretion.

Urinary iodine excretion values between 100μg/L and 200μg/L were found for 47 pregnant women in the first trimester (60.2%), 69 women (49.3%) in the second trimester, 87 women (53%) in the third trimester or a total of 203 women (53.2%).

Only 11 women (14.2%) in the first trimester, 19 women (13.6%) in the second and 24 women (14.6%) in the third trimester of pregnancy, or a total of 54 pregnant women (14.1%) had optimal urinary iodine excretion values.

Three women (3.8%) in the first trimester, 19 women (13.6% in the second and 7 women (4.3%) in the third trimester of pregnancy, or a total of 29 pregnant women (7.6%) had urinary iodine excretion values of more than 300μg/L.

The median value for urinary iodine excretion of 110 lactating women was 139.2μg/L.

Low values of under 100μg/L were found in 29 lactating women (26.9%). Fifty one lactating women (47.2%) had optimal median values from 100μg/L to 200μg/L, while 20 lactating women (18.6%) had values between 200 μg/L and 300 μg/L, and 8 lactating women (7.3%) had values bigger than 300 μg/L.

The following table compared the values for urinary iodine excretion between school children, pregnant and lactating women, obtained in 2001.

Urinary iodine excretion (median μg/L) in 2001

	Median	>100	<100	<50	>300
School children	164.5	84%	16%	3.2%	8.5%
Pregnant women	140.7	67.3%	25.1%	4.4%	7.6%
Lactating women	139.2	65.8%	26.9%	4.7%	7.3%
Pregnant and lactating vomen	140.4	67%	25.5%	4.5%	7.5%

The results from the research involving pregnant and lactating women in 2001 show that even the new regime of iodinating the salt by adding 20 – 30 mg iodine to a kilogram of salt, applied since October 1999, is insufficient to satisfy the optimal iodine requirements of pregnant and lactating women, which are greater than the iodine requirements of the rest of the population.

This new study, conducted in 2006/2007, used a wider spectrum of parameters which provide for a more comprehensive picture of the iodine status of pregnant and lactating women. One of them is the urinary iodine excretion. The results show that the median value the concentration of iodine in urine in all trimesters of pregnancy ranges between 150 μg/L – 249 μg/L, which corresponds to an adequate iodine intake. For the first and second trimester it is 199.7μg/L, while the value for the third trimester is slightly lower (174.9μg/L). These results, compared to the results from our previous study conducted in 2001 (149.7μg/L for the first, 157.6μg/L for the second and 130.4μg/L for the third trimester) suggest an increase in the iodine intake among pregnant women in a situation where the general population has a confirmed iodine suficiency. However, the distribution of the individual values, presented on the histograms on chart 1 below, shows that for a significant number of subjects the results are lower than 150μg/L, which is the lower limit for the optimal iodine intake range. Hence, we determined an insufficient iodine intake in 29.6% of the samples in the first trimester, 37% in the second and 39.1% in the third trimester. We did not find severe iodine deficiency in any of the samples, and the share of samples within the 20 μg/L to 49 μg/L range was also low (2.2% to 3.1%). Values ranging from 50 μg/L to 100 μg/L were found in 12.2% of the samples in the first trimester,

13% in the second and 19.6% in the third trimester. This last criterion was also used in the study of pregnant women from 2001, when this category was populated by 21.8% of the samples from the first trimester, 17.3% from the second and 23.2% from the third trimester. The results imply that even in case of iodine suficiency, a significant portion of the pregnant women intake non adequate quantities of iodine with their food.

Iodine excretion of lactating women is determined in the urine and in the breast milk. We concluded that the urinary iodine excretion median is 157.9μg/L which is lower than the median obtained during pregnancy. It is known that after delivery the renal iodine clearance rate stabilizes and therefore the urinary iodine excretion results are interpreted using the criteria applicable to the general population. Having these criteria in mind, our study showed that 20.4% of the test subjects had insufficient iodine intake, 49% had adequate iodine intake, and 16.3% had more than adequate iodine intake.

The median value for iodine excretion through the breast milk is 89.7μg/L, and as much as 83% of the tested subjects have values lower than optimal.

The obtained TSH values differ from the TSH values for the general population, analyzed using the same method during the same time interval. The median was determined to be 1.3 mU/L. For the first trimester we obtained a smaller median (1.22 mU/L) which is in accordance with the transitory changes during this period. In the second and the third trimester the value of the median increases (1.6 mU/L) to a level higher than the respective average value for the general population which indicates increased thyroid stimulation during this period. After delivery, the TSH values return to the level of the general population, with a median of 1.4 mU/L.

The free fraction of thyroxin was determined among pregnant women in all trimesters as well as after delivery, and the results were compared to the reference values for the general population (8.4 pmol/L – 18 pmol/L) and median (12.6 pmol/L). The results show a gradual reduction of the median value as the pregnancy progresses. The median for free thyroxin after delivery is identical to the median for the general population.

The thyroglobulin values of pregnant women were determined for each of the trimesters separately and there are no statistically significant differences between them. The thyroglobulin median is 6.05 μg/L. It is somewhat higher than the median for the general population (5.55μg/L). The thyroglobulin median values obtained after delivery are somewhat higher (6.55μg/L).

We determined thyroid antibodies (anti-TRO and anti-TG) in subjects during their first examination as well as the subsequent examinations throughout the gestation periods. According to the declaration of the producer of the kits, the normal value range for a-TPO is <35 kIU/L, and for a-Tg <40 kIU/L. The highest prevalence of a-TRO antibodies was found among pregnant women in the first trimester (9.8%), while for a-TG the prevalence is half that value. For the subjects examined for the first time in the second trimester the percentage of positive antibodies is 7.4% for a-TRO and 1.8% for a-TG. No positive thyroid antibodies were found among pregnant women that were examined for the first time during the third trimester. Overall, for all 185 pregnant women, the prevalence of a-TRO antibodies is 8.6% and the prevalence of a-TG antibodies is 3.8%. According to data from the literature, the prevalence of TRO in pregnancy is between 8% and 11.3%.

Among some of the pregnant women receiving 100 μg iodine tablets per day, we found an increase of the urinary iodine excretion median, but no changes of the TSH and FT_4 medians. The TG values have a tendency to be lower which suggests reduced thyroid stimulation. These results suggest that, according to the biochemical indicators the iodine substitution influences the presence of low iodine intake levels of pregnant and the lactating women. This influence can be seen in the second trimester, when 37% of the pregnant women without iodine substitution were found to have low iodine intake levels,

compared to 12.5% of women who received iodine substitution. This difference is mostly expressed in the third trimester, where 39.1% of pregnant women without substitution had low iodine intake compared to 21.4% of pregnant women with substitution. After the delivery, the differences are not so intense (20.4%, without substitution and 14.3% with substitution).

Iodine substitution also influences the breast milk iodine excretion levels. Iodine substitution during lactation influences the provision of adequate iodine quantities for the needs of the infant.

The results of the palpation of the thyroid gland indicate that most of the subjects have a normal palpation finding. Goiter grade one was discovered in 4.3% of the women in their first trimester of pregnancy, 15.5% in the second trimester and 9.3% in the third trimester of pregnancy. The goiter discovered during the first examination at the beginning of the pregnancy recedes as the pregnancy progresses after application of iodine supplementation.

The volume of the thyroid gland determine using ultrasound methods, expressed as a median, increases as the pregnancy progresses from 7.8 ml in the first trimester, to 7.86 in the second trimester and 8.75 ml in the third trimester and then reduces to 7.5 ml after delivery. The women receiving iodine supplementation have smaller median values for the thyroid gland volume in comparison to the women who did not receive iodine supplementation.

Conclusion remarks

These conclusions derive from the results from the conducted research involving pregnant and lactating women in Macedonia during 2001 and 2006/2007 and the latest recommendations of the expert group comprising representatives from The Endocrine Society, American Thyroid Association, Association of American Clinical Endocrinologists, European Thyroid Association, Asia and Oceania Thyroid Association i Latin American Thyroid Society from 2007 (11).

The daily iodine needs of adults and children older than 12 are 150μg of iodine per day. The pregnancy causes changes of the thyroid economy of the mother, which in turn leads to an increase of the thyroid hormone production by 50% do 100% compared to the production before conception. In order to achieve the required increase of the thyroid hormone production, the iodine intake of the organism during pregnancy should increase (12).

Due to many health related reasons pregnant and lactating women must not be advised to increase the iodine intake by consuming larger quantities of iodinated salt.

In the past people wrongly thought that the thyroid gland of a healthy pregnant woman will physiologically adapt to the state of pregnancy, without significant changes. Today this standpoint is completely modified (13).

Women having iodine suficiency have little consequence from the loss of iodine through the kidneys since the iodine reserves in the thyroid at conception are large and they remain unchanged during gestation (14). The situation is very much different for pregnant women that have limited or deficient iodine intake, which can be seen from the low urinary iodine excretion values (15).

Iodine deficiency during pregnancy, even if it is mild or moderate, leads yo a hypothyroxinemia of the mother, which causes increased stimulation of the thyroid gland through the pituitary TSH, and goiter of the mother and the fetus (16). Application of iodine supplementation early during gestation may prevent the occurrence of goiter (17).

The iodine intake before pregnancy should be at least 150 μg per day in order to ensure sufficient iodine reserves within the thyroid gland.

According to the recommendations of the WHO from 2007 (10), the daily needs of the pregnant and lactating women are 250 μg, and the needs of newborns and children younger than two years are 90 μg iodine per day. The median of the urinary iodine excretion of pregnant women should be between 150 μg/L and 249 μg/L, while for lactating women, newborns and infants younger than two years it should be greater than 100 μg/L.

Several studies conducted between 1981 and 2002 showed that iodine implementation ensures a normal thyroid gland function, removes hypothyroxinemia of the mothers and prevents goiter of the mother and the fetus (18).

Excessive iodine intake may cause disturbance of the thyroid function. In addition, it is necessary to identify the persons that could have adverse effects from an excessive iodine intake, such as patients suffering from autoimmune disorders or autonomous thyroid tissue (19). Since there are no clearly defined criteria for determining iodine quantities that would be excessive, it is assumed that it would not be beneficial to give pregnant women iodine quantities twice as large as the daily needs of 250 μg.

During lactation, the production of thyroid hormones and the urinary iodine excretion normalize. However, iodine is concentrated in the mammary gland and is lost through the milk. One liter of milk normally contains from 150 μg to 180 μg iodine. Therefore, the appropriate iodine intake level for lactating women is considered to be 250 μg per day.

Clearly, the faster the iodine requirements are fulfilled (ideally during the first trimester), the easier the thyroid function will adapt to the conditions of pregnancy. When implementing the recommendations for iodine intake during pregnancy, one should consider the normal iodine intake levels of the population in order to undertake adequate measures to ensure an optimal iodine intake.

In a situation of general iodination of the salt, as in Macedonia, the pregnant women at risk of iodine insufficiency are advised to use a peroral iodine supplementation of 100 μg iodine per day (in the form of tablets) or 100-150 μg iodine, in the form of multivitamin or multimineral tablets specially prepared for the purposes of pregnant women, since it is well known that even if the iodine intake of the general population is satisfactory, the iodine intake of pregnant women may still be insufficient (20). In our situation, this can be the case with the pregnant and lactating women for whom the salt consumption is restricted for some reasons, or who have insufficient salt intake through their daily diet. It can also be the case with the pregnant and lactating women who have hypothyroidism in their family anamnesis, who due to a social or other reasons use mainly food products from Macedonia that are iodine deficient (mainly population living in the rural areas).

The pregnant women are advised to check whether the multivitamin or mineral tablets contain the required iodine quantity.

The best parameter used for evaluation of the adequacy of the iodine intake of the population is the urinary iodine excretion. In case of adequate iodine intake during pregnancy, the values of urinary iodine excretion should be between 150μg and 250μg. (21). However, while urinary iodine excretion is especially important for evaluating the iodine intake of the entire population, it is not a valid criterion for separate individuals. For example current national studies in the USA indicate that in spite of a urinary iodine excretion median of about 150 μg for the general population, 5% to 10% of the pregnant women still do not intake sufficient quantities of iodine (20). Although the country does not need measures for correction of iodine deficiency of the general population, it is nevertheless necessary to increase the iodine intake of the pregnant women.

In order to evaluate the adequacy of iodine nutrition of an individual, the best parameter is to determine the iodine reserves in the thyroid gland which, in case of iodine

sufficiency should be 10 to 20 mg. However, it is impractical to determine this parameter. Therefore, the best option would be to evaluate the parameters that change due to iodine deficiency. These are reduction of the serum free T_4, increase of the TSH level, progressive increase of the serum thyroglobulin, increase of the molar T_3 /T_4 ratio, and an increase of the thyroid volume and occurrence of goiter of the mother and the fetus. The results from the biochemical tests should be interpreted according to the determined trimester specific referent values.

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