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Review Article

Iodized Milk Proteins - Natural Iodotyrosine Essential Nutraceuticals

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Abstract

In the original review article, the authors discuss the view on the low efficiency of salt iodization by additives of inorganic forms (iodide and potassium Iodate) in the prevention and treatment of iodine deficiency conditions. Through analysis of literary and own data on the mechanisms of biogenesis, transformation and organification iodine in the thyroid gland of the person advances, the concept of nature natural iodine nutraceutical, adequate iodine the metabolism of the body in the form of iodized milk proteins. The authors propose to use as the main source of iodine in human nutrition its organic form - iodotyrosines- in the form of iodinated tyrosine-rich milk proteins, which reduces the cost of production, technological application in the food industry, iodine-containing nutraceuticals and increases their biological efficacy, stability, safety.

Previously, the authors investigated features of tissue metabolism of exogenous iodotyrosines, including the background of the different states of iodine status, showing their biological effectiveness as the main source of iodine in animal (mice, rats). Studied in detail and found that the active form of iodine in the body after absorption of molecular iodine and iodide ions, in fact, are iodotyrosines and to a lesser extent iodinated lipids. Investigated the structure of iodized dairy whey proteins, confirming the presence of iodinated amino acids, in particular mono-, di - and triiodo-containing milk protein alpha-lactalbumin. Measurement of the mass fraction of iodination based on the enzymatic hydrolysis of the sample food product, the extraction and purification of target substances from the sample by the method of solid-phase extraction (SPE), and subsequent derivatization of the extract and HPLC-MS/MS analysis. Verification of the covalent organic nature of iodine in iodized milk proteins was performed by high-resolution HPLC-MS native-ESI chromatography-mass spectrometry to detect the native bond of iodine with the protein matrix.

Keywords: *Milk Proteins; Iodotyrosine; Nutraceuticals*

Introduction

The aggravation of the issue of insufficient iodine consumption due to the development of new technogenic and urban living conditions, population growth and increased nutrition issues, changes in the nature and composition of the diet, urgent preventive measures (in particular, artificial enrichment of basic food products with inorganic iodine (mainly in the form of iodide), chosen about a century ago).

Statistical studies raise various doubts concerning effectiveness of the iodization and universality of salt as a food product. Individual tolerance to excess of iodine is highly variable. Most people can be exposed to large amounts of iodine without obvious consequences. Important exceptions from this assumption exist consist in the previous prolonged iodine deficiency, autoimmune thyroiditis, and papillary thyroid cancer. In subpopulations where recent iodine deficiency has been rapidly corrected, iodine-induced hyperthyroidism is a predictable phenomenon [1]. Mostly, older individuals with autonomic ganglions suffer because they are unable to properly regulate the

intake of iodine having recently become available and produce an excessive amount of thyroid hormone. The incidence and complications of iodine-induced hyperthyroidism increase in case of poor control over the concentration of iodine in the salt, which allows for a high and uneven content of iodine, as well as of inadequate medical care, which complicates the diagnosis and the relevant treatment. Without simplifying the severity of the issue, most researchers agree that the risk related to iodine-induced hyperthyroidism shall not obscure the benefits of the balanced iodine diet for women and children and should not slow down the pace of correction of the iodine deficiency in the society.

In the current strategy of carrying out the mass iodine deficiency prevention, it is quite appropriate to use other fillers for iodine-containing component that are less harmful and indifferent to the consumer taste preferences in comparison with sodium chloride.

The intensive introduction of iodate, instead of iodide, to enrich the salt does not change the whole situation [2,3]. Iodate provides for a greater stability and preservation of iodine in foods, expands the technological possibilities of using iodine-containing nutraceuticals, but it does not differ from iodide absorbed in the intestine in accordance with the biochemical criteria, if it is represented in physiological amounts. However, the greater chemical stability of iodate compared to iodide will bring additional dissonance to the iodine nutrition, since the rate of enrichment with this compound is not corrected, considering the relevant decrease in iodine loss due to the compound, in comparison with the situation with iodide.

Iodized salt is ineffective in reducing goiters (enlarged thyroid glands) in children [4]. The structure of thyroid diseases has changed significantly towards increased hyperthyroidism, simple goiters and thyroid nodules. The level of iodized salt intake and the manifestation of hyperthyroidism showed a significant correlation [5].

Despite the increasing degree of salt iodization, there is a decrease in iodine intake in Switzerland, Austria and several other countries, which is associated with a decrease in the consumption of salt by populations [6].

The excess of iodide enhances oxidation, which leads to the increased level of lipid peroxides and the increase in the catalase activity within the target tissues and blood, as well as the decrease in H+ of donor serum. Any negative changes due to iodide excess in the thyroid gland, liver tissue and blood are mediated through oxidative stress [7,8]. As it turns out, the antioxidant protection of the thyroid glands, which are non-deficient in iodine, can also be weakened by a moderate excess of iodide in Wistar rats [9].

Numerous studies confirm significant differences in the behavior of elemental iodine and iodide among humans and animals [10,11].

Iodized milk proteins - Natural iodotyrosine and nutraceuticals

Currently, there is no generally accepted view of the origin of a natural iodine nutraceutical adequate to the iodine metabolism of the person.

According to the reconstructed structure of the human diet, it becomes clear that organic compounds with the covalently bound form of this halogen, primarily iodotyrosines, degradation products of thyroid hormones, thyroxine and iodine-containing lipids are evolutionarily selected as the main source of dietary iodine for the body. Inorganic iodine (iodide) is also a component of alimentary iodine in the human diet, but never serves as its main source in the evolutionary aspect. It is due to its low content in animal tissues. Thus, the concentration of free iodide in the plasma of mammals, the main deposit of iodide in the body, is 50 - 300 nM [12,13]. Water and plant products cannot be used as the natural sources of iodine for humans due to their low content in such halogen [14]. In addition, plant tissues also have the powerful system ensuring the organification of iodine, especially with tyrosine-containing compounds.

Despite the intensive implementation of the practice of universal salt iodization, the use of iodide (iodate) as a food source of iodine still raises various doubts [15].

The passive prevention of the iodine deficiency in industrialized countries is ensured, first of all, by the high level of consumption of milk and dairy products, eggs, meat, and (to a lesser degree) seafood [16,17].

The iodine nutrition in these countries undergoes seasonal fluctuations associated with a variation in the content of iodine in milk during winter and summer. Until recent times, a significant contribution to the total content of iodine in milk was made by iodophors (antiseptics with organically bound iodine) used to process animals and dairy equipment. The refusal of such drugs reduced the level of iodine consumption by the populations of several countries. However, in the developed countries, instead of salt iodization, national programs are being developed to increase the iodine content in dairy, meat products, poultry eggs by increasing the iodine consumption by farm animals [18-21] or enriching plants with iodine (for example, in China) [22,23].

While considering the increased importance of milk as the equivalent of animal proteins, and other animal products, eggs and fish, it is important to note that organic and non-hormonal iodine prevails in them, mainly in the form of iodized proteins (iodotyrosines) [24-26].

Therefore, in maintaining the iodine balance of our body during the process of iodine absorption in the intestine, at least three main transport systems with different biochemical parameters and properties are involved, where iodotyrosines, iodide, iodine-containing fatty acids and their derivatives are substrates. Unlike the thyroid gland, the iodide transport system (Na^+/I^- symporter) cannot be dominant in the intestine since the evolutionarily relevant human diet lacks in iodide.

Since 1998, we have been proposing to use its organic form, iodotyrosines, in the form of tyrosine-rich iodinated proteins as the main source of iodine in the human nutrition, which currently reduces the cost of production and technological use of iodinated nutraceuticals in the food industry and increases their stability and preservation (Figure 1).

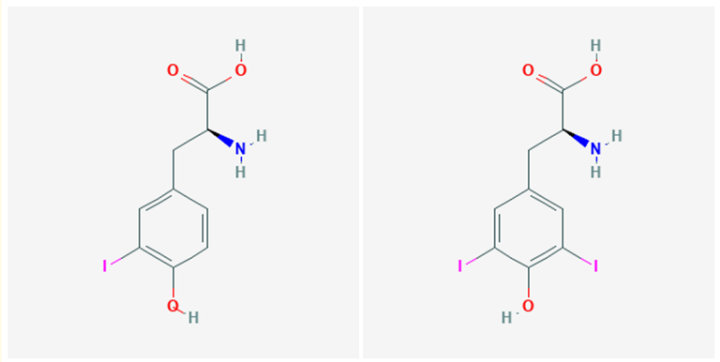

Figure 1: *Structural Formulas of the Active Components of Natural Nutraceuticals Based on Iodized Milk Proteins: Monoiodotyrosine (MIT) and Diiodotyrosine (DIT). Gross formula MIT - C9H10NO3J and DIT - C9H9NO3J2 (from https://pubchem.ncbi.nlm.nih.gov/compound/439744#section=2D-Structure).*

Our earlier studies have been aimed at studying the tissue metabolism of exogenous iodotyrosines (in particular, considering different iodine status) and showing their biological effectiveness as the main source of iodine among animals (mice, rats) [27].

Substitution therapy with various forms of iodine turns out to be tissue-specific. A moderate reduction in the size of the thyroid gland in rats during the iodine deficiency control was noted after a short-term exposure to both iodide and molecular iodine.

We have studied and established in detail that, after the absorption of the molecular iodine and iodide ions, the active form of iodine in the body was represented by iodotyrosines and (to a lesser degree) iodized lipids. Elemental iodine is a highly reactive compound,

and chemical conditions of the body, high concentration of phenolic compounds, compounds with unsaturated bond, and the pH of the medium contribute to its rapid chemical interaction. The result of such chemical reactions in the body was not only covalently bound iodine, but also iodide released in equimolar amounts. In fact, the iodide output was much greater due to the interaction with various reducing elements. All iodotyrosines formed during iodization blocked the entry of iodide to the thyroid gland. This represents the effect of a greater safety of large doses of iodine compared to iodide, and the possibility of oral therapeutic use of the amyloid iodine complex as an antiseptic.

The catalytic activity of peroxidase enzymes was reduced to generation of the active form of iodine from iodide represented in the form of iodonium ion (I_3^+). The iodization of phenolic derivatives proceeded spontaneously and freely without the participation of enzymes. The same process took place with the participation of hydrogen peroxide and plant peroxidases in plant tissues with the organification of iodide. According to the same scheme, but with less efficiency, the enzymatic iodization of chemical compounds with unsaturated double bond (for example, lipids) was observed.

Thus, the unusual behavior of elemental iodine and iodide ions was represented in the synthesis occurring in the body of iodized proteins containing iodotyrosines, and possibly iodized lipids or their structural analogues, the metabolism of which lead to the observed differences in the biological effects of iodotyrosines.

The presence of iodized components in the structure of whey milk proteins, which are iodotyrosines, can explain the positive effects of feeding animals with them during various model experiments and in the development of pathologies associated with the iodine deficiency. However, earlier the researchers did not associate own experimental results with iodotyrosines [28]. Therefore, it is necessary to pay close attention to iodotyrosines not only in the processes occurring in the thyroid gland, but also in the extra-thyroidal iodine metabolism. The systematic studies of mice provided with the excess content of iodine in water, with the monitoring of changes in the thyroid mass, iodine content, thyroid histology, thyroid peroxidase activity, thyroid hormone concentration and thyroid stimulating hormone, show that the diet with 50% of iodized milk proteins significantly inhibits the development of iodide goiter.

The effect of the diet with 50% of iodized milk proteins on iodide goiter, as suggested by the authors, is that the intake of excess iodine in thyrocytes decreases and the formation of excess colloid in the follicular cavity decreases [28]. To evaluate the effectiveness of iodized milk proteins in prevention of iodine deficiency in humans, the following parameters were studied: iodine intake adequacy determined through the median of ioduria, thyroid size measured in accordance with the relevant American standards, functional state of the hypothalamic-pituitary-thyroid system evaluated by the level of thyroid hormones and free thyroxine (FT4) in the blood, physical activity evaluated by performing the cycle ergometric test, memory status, and change in attention. The iodine deficiency is initially detected in 88.9% of the main group and in 94.4% of the control group. The median of ioduria corresponds to the moderate iodine deficiency in both groups. After the initial examination, all people from the main group are given iodized bread for 9 months.

Control results confirm the effectiveness of the iodine deficiency prevention while using iodized milk proteins [29]. It is also experimentally established that the provision of rats with iodized milk proteins reduces the activity of antithyroid agents, such as thiourea, in a dose-dependent manner, which is represented in the decreased thyroid absorption ^{131}I and the concentration of stable iodine in the thyroid, as well as the increase in the mass of the thyroid gland [30]. However, such dose-dependent effect of iodized milk proteins is erroneous [31] and related to high protein nutrition, but not to iodine-containing amino acids of iodotyrosines in the iodized milk proteins. The fact that feeding animals with iodized milk proteins weakens the negative effects of iodine deficiency has not been paid the appropriate attention in relation to the study of the mechanism of such phenomenon. Undoubtedly, iodotyrosines, as its usual structural components, are the active substance.

Iodized proteins as the source of iodotyrosines

The most famous endogenous iodized protein is thyroglobulin, specialized thyroid protein, which is the precursor to thyroid hormones. Thyroglobulin is the main reserve form of iodine in the thyroid gland. Iodized thyroglobulin in the follicle cavity deposits not

only thyroid hormones, but also iodine, which is among iodotyrosine residues [32]. The hydrolysis of thyroglobulin occurs in lysosomes, while its synthesis takes place in the cytosol. In thyroid cells, tyrosine residues of thyroglobulin, were iodized in situ and 660-kDa of glycoproteins were sent to the lysosome, where thyroglobulin was hydrolyzed to its constituent amino acids represented by the following: thyroxine (T4), triiodothyronine (T3), diiodotyrosine (DIT) and diiodotyrosine (DIT) [33]. Iodotyrosines represent two thirds of iodine in thyroglobulin and serve as the precursors in the formation of thyroid hormones of thyroxine (T4) and triiodothyronine (T3). T4 and T3 leave the cell, enter the blood circulation cycle and perform their endocrine functions in distant target cells. MIT and DIT remain within the cell, while providing iodine for possible re-incorporation into newly synthesized thyroglobulin. Such events occur within the cytoplasm of thyroid cell and require the movement of iodotyrosines from lysosomes. The existence of the transporter of lysosomal MIT in thyroid cells may explain the way this product of thyroglobulin catabolism can be transported into the cytosol for the reuse of iodine. The proteolysis of thyroglobulin in lysosomes is accompanied by the release of significant amounts of iodine: 11 atoms per thyroglobulin molecule in the form of monoiodotyrosine, which is 1/3 of the amount of iodine, and diiodotyrosine being equivalent to 2/3 of the amount of iodine. The compounds enter the cytoplasm and are deiodized there and then the released iodide undergoes reutilization.

About 10 - 20% of serum organic iodine is represented in the form of iodized proteins, primarily iodalbumin, which is at least partially formed during the peripheral metabolism of thyroid hormones. Previously, the distribution and chemical forms of iodine in various subcellular fractions of the human liver were studied by performing the epithermal radioactive analysis with slow neutron irradiation in combination with chemical and biochemical methods of separation, such as density gradient centrifugation and gel chromatography. It was found out that the total amount of iodine was located in various subcellular fractions as follows: nucleus > cytosol > mitochondria > lysosome > microsome. In the lysosomal fraction, iodine mainly binds to macromolecules, while the nuclear and mitochondrial fractions it binds mainly to various organic compounds with low molecular weight. In the cytosolic fraction, iodine was found mainly in the composition of three proteins [34]. Iodine proteins were formed in the subcellular organelles of various tissues. The metabolism of thyroid hormone lead to the formation of structural and soluble tissue iodized proteins in addition to circulating iodized proteins. The rate of formation of such compounds in the liver and plasma was probably related to the rate of hormonal metabolism. Lower, but significant, concentrations of iodized proteins were seen in the skeletal and cardiac muscles. The study with subcellular fractionation in the liver showed that the main part of iodized proteins in the liver was in the microsomal fraction, and lower concentrations were present in the nucleus, mitochondrial and soluble fractions.

As for the human serum, after the treatment with protease, it was previously detected by reverse phase liquid chromatography with the mass spectrometric detector: iodide - 1.1, diiodotyrosine - 2.1, monoiodotyrosine - 1.6, reverse triiodothyronine - 3.9, triiodothyronine - 5.9, thyroxine - 60 μg of iodine per liter of serum in patients with hypothyroiditis [35]. The main form of serum iodine compounds was predominantly represented by organic iodine compounds, including iodotyrosines [13].

Diiodotyrosine (DIT) and monoiodotyrosine (MIT) in the normal human serum circulate with the albumin fraction. DIT and MIT are not bound to other proteins and together represent 19-25% of extracted serum iodine [36].

The identification of diiodotyrosine in the human serum by the sensitive RIA method, excluding any interference from T4 and its other analogs by isolation of DIT from serum by preparative immunoprecipitation, gave the following average (+/-SD) serum levels of DIT: 161 +/- 133 pM/l (7.0 ng/100 ml) in healthy persons, 64 +/- 30 pM in pregnant women, 241 +/- 83 pM in the umbilical cord serum of newborn infants, 542 +/- 494 pM in hyperthyroid patients, and 101 +/- 71 pM in hypothyroid patients. The average values in pregnant women, newborns and hyperthyroid individuals were significantly different from the average values of healthy people. The data show that circulating diiodotyrosine is produced mainly by the thyroid gland, and the peripheral formation of diiodotyrosine is a secondary metabolic pathway in humans [37].

The peroxidase-mediated pathway for deiodization of thyroxine involves the cleavage of the ether bond of the T4 molecule resulting in the formation of 3.5-diiodotyrosine from a part of the amino related to the molecule and the rapid deiodization of the outer ring residue.

As for the livers of normal rats and people, the pathway for generating DIT from T4 via the cleavage of the ether bond is inactive, while awaiting the activation by the factors inhibiting the monodeiodization of T4 into T3 [38].

It specifies the participation of tissue peroxidases in the synthesis of iodotyrosines through the iodization of tissue proteins. Lactoperoxidase is capable of catalyzing the iodization and subsequent coupling of tyrosine residues with the efficiency comparable to that of thyroid peroxidase [39]. Non-enzymatic (I_2-mediated) and lactoperoxidase-catalyzed iodization of tyrosine is inhibited by excess iodide (I^-) and/or hydrogen peroxide (H_2O_2). This phenomenon is a consequence of the concentration-dependent dual role of iodide and H_2O_2 in the iodization system.

Iodotyrosines in milk

The role of lactoperoxidase in the formation of the iodine profile of milk in women is undeniable. The mammary gland has a high ability of concentrating iodide and forming iodized proteins through breast peroxidase exclusively during pregnancy and lactation, which is considered a protective condition against breast cancer [40]. Mature breast milk of healthy young women on a balanced diet contains averagely 81 ng/ml of the total amount of iodine. Iodide represents averagely 77% of the total amount of iodine. Approximately 1 ng from 22 ng/ml of organic iodine related to thyroxin and triiodothyronine, as well as up to 40% of organic iodine was in the form of monoiodotyrosine after hydrolysis with pepsin [41].

The ability of the mammary gland to capture and organically bind radioactive iodine was studied in non-pregnant, pregnant and lactating rats. The iodization was not found in the mammary glands of non-pregnant rats, but was observed at the end of the twelfth day of pregnancy and continues throughout pregnancy and lactation.

Protein-containing vacuoles in alveolar cells and caseins in milk were the main areas of iodization within the gland. Milk proteins in the cavity of the tubules adjacent to the alveoli were also iodized. In contrast, passages, myoepithelial cells, lipocytes, blood vessels, and other histological components of the gland did not show any iodizing ability. The cytochemical analysis showed that the presence and localization of peroxidase correlated with the iodizing ability [42].

The evaluation of milk and iodine-containing compounds in rats balanced with ^{125}I on the 10^{th} day showed that milk contained 195 ± 65 ng/ml of iodine consisting of 43 ng/ml of iodide, 148 ng/ml of non-hormonal organic iodine, and included only 3 ng/ml of hormonal iodine. After the enzymatic proteolysis of milk 80-90% of the total amount of non-hormonal organic iodine turned out to be monoiodotyrosine, while the rest of such iodine was identified as diiodotyrosine. The high level of iodotyrosines contained in milk proteins was an indicator of high peroxidase activity in milk and mammary gland. Thus, in rats, mother's milk was fed to young animals essentially in the form of iodide and non-hormonal protein-bound iodine [43].

Metabolism of iodotyrosines

Most of DIT, administered orally or intravenously, was accessible to animal tissues. The intake of 1.57 μM of diiodotyrosine, which corresponded to 400 micrograms of iodine, rapidly increased the serum concentration of DIT from the average endogenous baseline level of 0.23 nM to the maximum values between 6.0 and 20 nM within 30 - 60 minutes. The level of diiodotyrosine decreased in all animals in 2 hours after the intake of DIT. The urinary excretion of intact diiodotyrosine was low and amounted to less than 1% of the administered dose of exogenous DIT for 2 days. In contrast, 52% of iodine obtained in the form of diiodotyrosine was excreted with the urine over the same period of time. The intake of 200 micrograms of iodine in the form of diiodotyrosine for eight weeks (7 x 200 μg of iodine per week) resulted in 67% excretion with the urine of the dose of iodine taken [44]. The urinary excretion of 3.5-diiodothyrosine in euthyroid, hypothyroid and hyperthyroid individuals measured by sensitive gas chromatography and mass spectrometry showed that the average excretion values in two pathological conditions significantly differed from those of the euthyroid individuals ($p < 0.01$ in both cases), while the significant overlap with the normal range was observed [45]. The average urinary excretion of diiodotyrosine measured by the RIA

method with the immunoprecipitation is 1.23 +/- 0.43 (+/- SD) nM/24 hours (533 ng/24 hours) or 0.108 +/- 0.048 nM/nM of creatinine in healthy people. The absence of any effect of acid hydrolysis on the measured urinary concentrations of diiodotyrosine ensures the presence of predominantly unconjugated DIT in the urine. In patients with defective thyroid metabolism of iodine, the urinary diiodotyrosine extremely increased in the range from 1.2 - 17.7 nM/mM of creatinine.

The comparison of normal production and excretion rate suggested that approximately 5% of the daily extra thyroid turnover of DIT was excreted in the urine unchanged or in the form similar to DIT [46,47]. The urinary excretion of diiodotyrosine was significantly increased in patients with any chronic renal disease compared to healthy individuals.

Iodotyrosines in foods

Iodotyrosines and their proteins are safe and effective nutraceuticals. Long-term feeding of rats with the feeds containing meat of young bulls and pigs with monoiodotyrosine and diiodotyrosine did not affect the content of free and bound cholesterol and the total amount of phospholipids in the liver, as well as the intensity of oxidative phosphorylation in mitochondria, which was proved by the results of morphological, histochemical and ultrastructural studies. The biological value of beef and pork obtained from animals with antithyroid drugs did not differ from that of the control group in various trials [48].

The studies performed in relation to the structure of iodized milk whey proteins obtained by the method developed by us confirmed the presence of iodinated amino acids, in particular, mono-, di-, and triiodo-containing milk protein of alpha-lactoalbumin. The actual binding of iodine atoms in the molecule of milk whey proteins (alpha-lactoalbumin, lactoglobulin, casein, etc.) was determined by the conditions of enzymatic iodization, availability of protein molecule in the solution and the characteristics of the product. The experimentally confirmed iodine content in iodinated lactoalbumin reached 3 iodine atoms per molecule, which is 2.62 wt.%. The measurement of the mass fraction of iodotyrosine was based on the enzymatic hydrolysis of food sample, extraction and purification of target substances from the sample by solid-phase extraction (SPE), subsequent derivatization of the extract and the HPLC-MS/MS analysis. The identification of analytes was performed by the absolute retention time of the chromatographic peaks of the target substances recorded in the multiple-reaction monitoring mode (MRM) [26].

To verify the covalent organic nature of iodine in iodized milk proteins, we used the high-resolution HPLC-MS to identify the native bond between iodine and the protein matrix. The analysis was performed using Agilent Technologies 1290 Infinity II liquid chromatograph with native mass spectrometry (QTOF 6545XT). AdvanceBio Peptide Mapping chromatography column, ZORBAX Extend-C18 Narrow-Bore Guard Column. The elution was performed with the mixture of components (A and B) in the gradient mode: up to 0.5 minutes - 5% of B, from 0.5 to 15.0 minutes with the increase to 35% of B, from 17.0 - 95% and held for 13 minutes, from 30.01 minutes - with the return to the initial conditions. The column equilibration time under the initial conditions was 5 minutes. Component A was represented by 0.1% formic acid solution in deionized water, component B - by 0.1% formic acid solution and 10% deionized water in acetonitrile. The flow rate was 400 µl/min, the period of the analysis was 30 min. As an example, the data of the high resolution ESI mass spectrometry for the iodized alpha-lactalbumin fraction obtained by reverse phase chromatography are given below. The non-iodized initial protein had the mass of 14176 Da. After iodization, the significant change occurred in the mass spectrum, namely, the signals corresponding to proteins containing 1, 2 and 3 iodine atoms in the molecule with the masses in the spectrum of 14303.9, 14429.8 and 14555.7 Da, respectively, appeared.

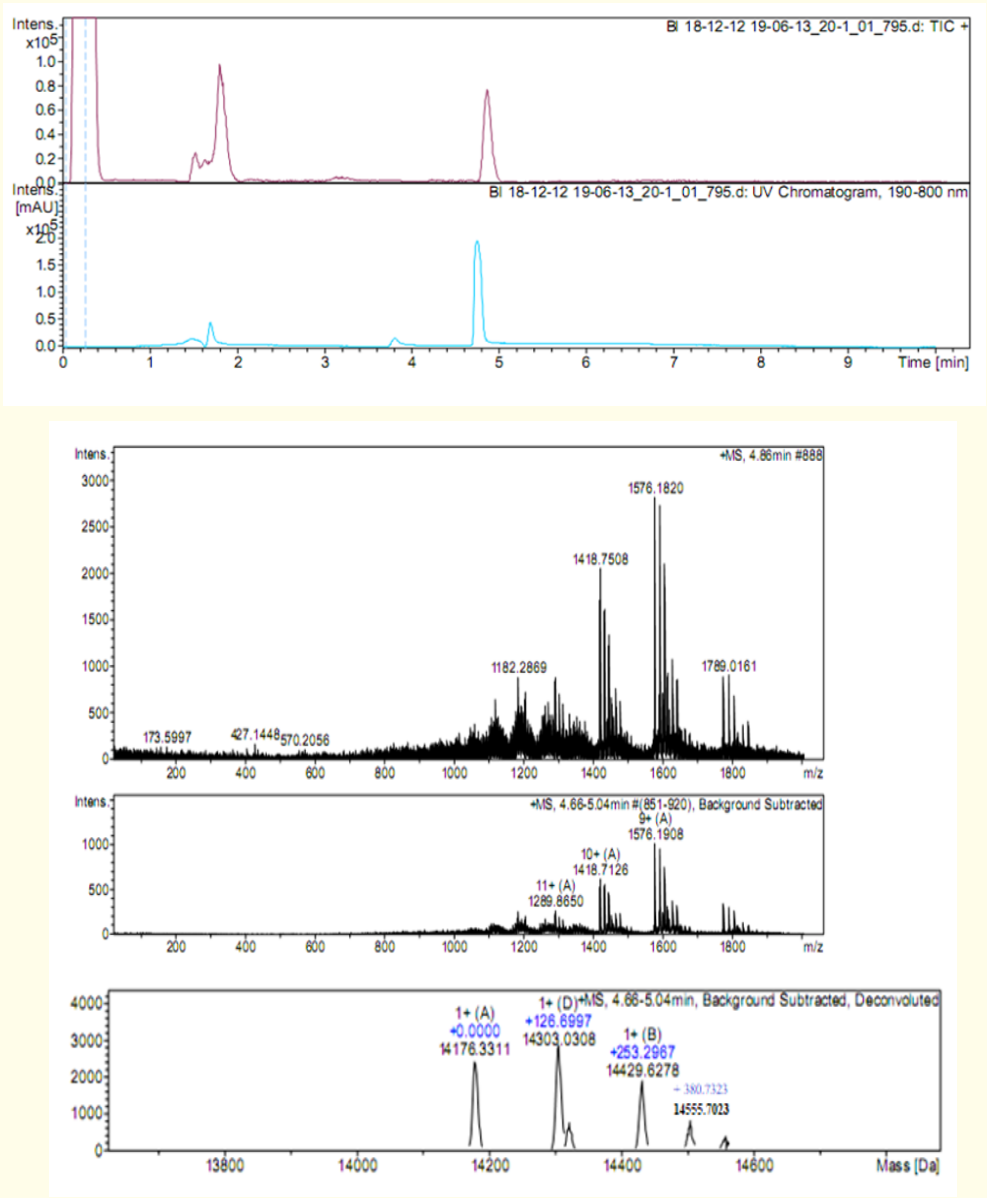

Figure 2: *Chromatogram of Iodized Milk Protein Sample Solution (UV detection). The peaks in the chromatogram corresponded to iodized areas in the alpha-lactalbumin protein molecule, while containing respectively 1, 2, and 3 odine atoms per alpha-lactalbumin molecule, after its deconvolution.*

Conclusion

Thus, iodotyrosines represented in the form of mono- and diiodotyrosines are an effective source of alimentary iodine for humans and animals. Iodized proteins undergo proteolysis in the gastrointestinal tract and, in an intact state, are absorbed in the gastrointestinal tract by any alternative iodide transport mechanism. Iodotyrosines under the conditions of metabolism enter the majority of body tissues with

blood flow, where, unlike iodide, they undergo the intracellular metabolism and complete intracellular and enzymatic deiodization. The thyroid gland, in our opinion, does not belong to the organs that actively deposit iodotyrosines. The iodide form released as a result of the extra-thyroidal and intracellular dehalogenation of iodotyrosines is actively concentrated by the thyroid gland. According to the results of our studies, iodotyrosines inhibit the transportation of iodide in the thyroid gland and possibly in the intestine, while the extra-thyroidal iodine metabolism has a significant effect on the iodine metabolism as a whole.

Iodotyrosine nutraceuticals based on iodized milk proteins ensure the effective functioning of all autoregulatory mechanisms of thyroid status. The ability of iodotyrosines concerning the intracellular accumulation significantly expands the functionality of the therapy with iodine-containing drugs. The covalently bound and organic form of dietary iodine in the form of iodized milk protein enhances the differentiation of the iodine metabolism and its regulation. The transmembrane transfer of iodotyrosines is specific and alternative to the transport mechanism of iodide. We confirm that there are alternative mechanisms for the absorption of iodine-containing compounds, and the metabolism of iodotyrosines is to be determined by the iodine status of the body. Moreover, various chemical forms of iodine have a differentiated influence on the iodine metabolism.

We propose to apply a new scientific paradigm in the metabolism and biogenesis of iodine in the body based on the scientific data on the importance of the organification of iodine and the high role of iodotyrosines as an essential and identical to natural form of iodine. Iodized milk proteins represent an alternative and effective way of preventing and correcting the iodine deficiency conditions concerning a wide range of populations, all age groups and various iodine status.

Bibliography

1. Stanbury JB., *et al*. "Iodine-induced hyperthyroidism: occurrence and epidemiology". *Thyroid* (1998) 8: 83-100.
2. Burgi H and Zimmermann MB. "Salt as a carrier of iodine in iodine deficient areas". *Schweizer Monatsschrift für Zahnmedizin* 115.8 (2005): 648-650.
3. Burgi H., *et al*. "The toxicology of iodate: a review of the literature". *Thyroid* 11.5 (2001): 449-456.
4. Aghini-Lombardi F., *et al*. "Effect of iodized salt on thyroid of children living in an area previously characterized by moderate iodine deficiency". *The Journal of Clinical Endocrinology and Metabolism* 82.4 (1997): 1136-1139.
5. Huang Q., *et al*. "Study on the effects of increased iodized salt intake on the incidence of thyroid diseases". *Zhonghua Liu Xing Bing Xue Za Zhi* 22.6 (2001): 455-458.
6. Als C., *et al*. "Why is iodine deficiency once again present in the Berne region? Schweiz Med Wochenschr". 127.1-2 (1997): 18-22.
7. Joanta AE., *et al*. "Iodide excess exerts oxidative stress in some target tissues of the thyroid hormones". *Acta Physiologica Hungarica* 93.4 (2006): 347-359.
8. Nadolnik LI. "Free-radical processes and iodine metabolism in thyroid cells". Minsk: Belaruskaya Navuka (2014): 275s.
9. Zhang N., *et al*. "Effect of chronic mild and moderate iodine excess on thyroid anti-oxidative ability of iodine deficiency and non-iodine deficiency Wistar rats]". *Zhonghua Yi Xue Za Zhi* 86.18 (2006): 1274-1278.
10. Dunn John T. "What's Happening to Our Iodine?". *The Journal of Clinical Endocrinology and Metabolism* 83.10 (1998): 3398-3400.
11. Robison LM., et al. "Comparison of the effects of iodine and iodide on thyroid function in humans". *Journal of Toxicology and Environmental Health A* 555.2 (1998): 93-106.
12. Michalke B and Schramel P. "Iodine speciation in biological samples by capillary electrophoresis-inductively coupled plasma mass spectrometry". *Electrophoresis* 20.12 (1999): 2547-2553.

13. Michalke B., *et al*. "Iodine speciation in human serum by reversed-phase liquid chromatography-ICP-mass spectrometry". *Biological Trace Element Research Winter* 78.1-3 (2000): 81-91.

14. Krajcovicova-Kudlackova M., *et al*. "Iodine deficiency in vegetarians and vegans". *Annals of Nutrition and Metabolism* 47.5 (2003): 183-185.

15. Wu T., *et al*. "Iodised salt for preventing iodine deficiency disorders". *Cochrane Database of Systematic Reviews* 3 (2004): CD003204.

16. Bader N., *et al*. "Pilot study: tendency of increasing iodine content in human milk and cow's milk". *Experimental and Clinical Endocrinology and Diabetes* 113.1 (2005): 8-12.

17. Girelli ME., *et al*. "Milk represents an important source of iodine in schoolchildren of the Veneto region, Italy". *Journal of Endocrinological Investigation* 27.8 (2004): 709-713.

18. Launer P and Richter O. "Investigations for iodine concentration in the blood serum of milk cows". *Berliner und Münchener tierärztliche Wochenschrift* 118.11-12 (2005): 502-508.

19. He ML., *et al*. "Supplementation of algae to the diet of pigs: a new possibili to improve the iodine content in the meat". *Journal of Animal Physiology and Animal Nutrition* 86.3-4 (2002): 97-104.

20. Hemken RW. "Milk and meat iodine content: relation to human health". *Journal of the American Veterinary Medical Association* 176. 10 (1980): 1119-1121.

21. ГОСТ 33422-2015. Meat and meat products. Determination of the mass fraction of iodotyrosines by high performance liquid chromatography with a mass spectrometric detector.

22. Zhu YG., *et al*. "Iodine uptake by spinach (Spinacia oleracea L.) plants grown in solution culture: effects of iodine species and solution concentrations". *Environment International* 2003 29.1 : 33-37.

23. Weng HX., *et al*. "Capacity and degree of iodine absorbed and enriched by vegetable from soil". *J Environ Sci (China)* 15.1 (2003) : 107-111.

24. Inikhov GS. Biochemistry of milk. Pishchepromizdat M (1956).

25. Zhukov G.F. *et al*. "Methods for the quantitative determination of iodine in foodstuffs and food raw materials". *Voprosy pitaniia* 5 (2004): 42-48.

26. Method of determination organic iodine (iodotyrosines) in food. A.V. Kulikovskiy. Voprosy pitaniia [Problems of Nutrition]. 85 (2016): №4. (in Russian).

27. Bolshakova LS., *et al*. "The study of the metabolism of iodotyrosines included in the iodized milk protein in rats". *Voprosy pitaniia* [Problems of Nutrition]. 2018 87: №3. (in Russian).

28. Qin G. "An experimental study on casein in the prevention of excessive iodide induced goiter". Zhonghua Yi Xue Za Zhi. 73.8 (1993) : 471-510.

29. Dyulya MS., et al. "Fortification of flour and bakery products with iodine". *Bakery Products* 5 (2014): 52-55.

30. Gaitan E Merino H. "Antigoitrogenic effect of casein". *Acta Endocrinologica (Copenhagen)* 83.4 (1976): 763-771.

31. Yu B. "Experimental study on the antigoiter effect of high casein nutrition". *Zhonghua Yi Xue Za Zhi* 69.7 (1989): 378-381.

32. Nilsson M. "Iodide handling by the thyroid epithelial cell". *Experimental and Clinical Endocrinology and Diabetes* 109.1 (2001) : 13-17.

33. Taurog A. "The Thyroid Werner's". 5th Edition, JB. Li pencott C6., Philadelphia (1986): 53-97.

34. Hou X., *et al*. "Study of chemical species of iodine in human liver". *Biological Trace Element Research* 69.1 (1999) : 69-76.

35. Gijs Afink *et al*. "Molecular Characterization of Iodotyrosine Dehalogenase Deficiency in Patients with Hypothyroidism". *The Journal of Clinical Endocrinology and Metabolism* 93.12 (2008): 4894 – 4901.

36. Materials Indistinguishable from Iodotyrosines in Normal Human Serum and Human Serum Albumin Journal of Clinical Investigation 46.7 (1967): 264- 1273.

37. Meinhold H., *et al*. "Circulating diiodotyrosine: studies of its serum concentration, source, and turnover using radioimmunoassay after immunoextraction". *The Journal of Clinical Endocrinology and Metabolism* 53.6 (1981): 1171-1178.

38. Balsam A., *et al*. "Formation of diiodotyrosine from thyroxine. Ether-link cleavage, an alternate pathway of thyroxine metabolism". *Journal of Clinical Investigation* 72.4 (1983) : 1234-1245.

39. Taurog A., *et al*. "Comparison of lactoperoxidase- and thyroid peroxidase-catalyzed iodination and coupling". *Endocrinology* 94.5 (1974): 1286-1294.

40. Eskin BA. "Iodine metabolism and breast cancer". *Annals of the New York Academy of Sciences* (1970) 32: 911-947.

41. Etling N and Gehin-Fouque F. "Iodinated compounds and thyroxine binding to albumin in human breast milk". *Pediatric Research* 18.9 (1984): 901-903.

42. Strum JM. "Effect of iodide-deficiency on rat mammary gland". *Virchows Archiv B Cell Pathology Including Molecular Pathology* 30 (1979): 209-220.

43. Vigouroux E., *et al*. "Estimation of hormonal and non-hormonal iodine uptake from maternal milk in suckling rats". *Acta Endocrinologica* 93 (1980): 332-338.

44. Wahl R., *et al*. "Iodide supplementation: 200 micrograms daily or 1,500 micrograms weekly?" *Zeitschrift für Ernährungswissenschaft* 37.1 (1998): 18-22.

45. Mohammed MN., *et al*. "3,5-Diiodotyrosine excretion in human urine in individuals of differing thyroidal status". *Biomedical Mass Spectrometry* 1983 10(9): 507-511.

46. Meinhold H., *et al*. "Pharmacokinetics and urinary excretion of orally administered diiodotyrosine". *Acta Endocrinologica (Copenhagen)* 116.3 (1987): 395-398.

47. Meinhold H., *et al*. "Turnover and urinary excretion of circulating diiodotyrosine". *The Journal of Clinical Endocrinology and Metabolism* 64.4 (1987): 794-800.

48. Zhirnov VV., *et al*. [Biological value of the meat from animals implanted with betazine and diiodotyrosine and its effect on the body]. *Vopr Pitan* 4 (1986): 60-62.

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