Hypothyroidism

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Hypothyroidism is when someone's thyroid gland does not produce enough thyroid hormones to meet the body's needs. Hypothyroidism can also be referred to as an underactive thyroid. The thyroid hormone is a small gland that is shaped like a butterfly in the front of the neck.[1] Thyroid hormones are important to the body because they control the way the body uses energy. Basically they affect nearly every organ in the body, from your metabolism to the way your heart beats. If you do not have enough thyroid hormone, a lot of the body's functions slow down.[2]

Symptoms of hypothyroidism can include the following: fatigue, weight gain, a puffy face, trouble tolerating the cold, dry skin, irregular menstrual periods, fertility problems, depression, etc. Hypothyroidism can also develop slowly, so a lot of people rarely notice the symptoms of this disease for many months or years. A lot of the symptoms, especially fatigue and weight gain, are commonly found in people and do not always signify that a person has a thyroid problem.[1,2]

There are many causes for hypothyroidism, such as Hashimoto's disease, thyroiditis, congenital hypothyroidism, radiation treatment of the thyroid, etc. Hashimoto's Disease is the most frequent cause of hypothyroidism.[1] With Hashimoto's disease, the immune system attacks the thyroid. The thyroid then becomes inflamed and is unable to produce enough thyroid hormones. Thyroiditis is when the thyroid becomes inflamed that it results in thyroid hormone leaking out of the thyroid gland. Initially, this leaking elevates the hormone levels in the blood, which then results in hyperthyroidism. Hyperthyroidism is the opposite of hypothyroidism, and it is when the levels of thyroid hormone are too high. Hyperthyroidism may last up to 3 months, but then the thyroid becomes underactive. After, hypothyroidism occurs and can usually last from a year to a year and a half, sometimes becoming permanent.[1]

Another cause of hypothyroidism is congenital hypothyroidism. This is when babies are born with a thyroid that is not developed to its full extent or does not function efficiently. If this is left untreated, it causes intellectual disability and growth failure. If this condition is caught early, then treatment can be given as soon as possible, decreasing the chance of a worsened thyroid condition. Last but not least, when the thyroid gets treated with radiation, like radioactive iodine, the cells of the thyroid gradually get destroyed. The humans who get treated with radioactive iodine treatment eventually end up with hypothyroidism.[1]

Acronym	Meaning
T_4	Thyroxine
T_3	Triiodothyronine
TBG	Thyroxine-binding globulin
TSH	Thyroid-stimulating hormone
TRH	Thyrotropin-releasing hormone
TG	Thyroglobulin
MIT	Monoiodotyrosine
DIT	Diiodotyrosine
TSHR	Thyroid stimulating hormone receptor
NIS	Sodium-iodide symporter
SMCT1	Sodium-dependent cotransporter
vSGLT	Sodium/galactose transport

Table 1: List of acronyms frequently used in this paper

The mechanism involved in thyroid-related conditions begins with the thyroid hormone. The thyroid hormone is essential for the regular functions of numerous tissues in the body. In a healthy individual, the thyroid gland mainly secretes thyroxine (T_4), which then is converted into triiodothyronine (T_3) in different organs by the selenium-dependent enzyme iodothyronine deiodinase.[3] Iodothyronine deiodinases are part of a family of deiodinase enzymes that are crucial in the activation and deactivation of thyroid hormones.[3]

T_4 can be seen as a prohormone for the more stronger hormone, T_3. In addition, the hormone binds to integrin $\alpha v\beta 3$ on the cell membrane.[3] The mechanism first begins with triiodothyronine binding (T_3) to the thyroid hormone receptor in the nucleus of cells. A lot of the thyroid hormones that are bound to receptors are in the T_3 form, either discharged into the circulation by the thyroid gland or derived from T_4 to T_3 conversion by 5' monodeiodinases.[4] There are three different monodeiodinases- type I, type II, and type III. The dispersal and regulation of these enzymes are important for thyroid hormone action. An example is that Type II deiodinase has a high affinity for T_4 , and is mainly found in the pituitary gland, brain, and brown fat where T_4 gets converted to T_3, causing a modulation of the intracellular concentration of T_3. So, tissues that have type II deiodinase can respond differently to a given circulating concentration of T_4 (by intracellular conversion to T_3) than organs that are only able to respond

to T_3 . In addition, both type I and and type II deiodinases control the circulation of T_4 and T_3 levels.[4]

After T_3 binds to the thyroid hormone receptor, it causes a stimulation of the sodium-hydrogen antiporter and other processes, such as blood vessel formation and cell growth. It is important to address that the majority of thyroid hormones are bound to plasma proteins, most commonly known as the thyroxine-binding globulin.[5] The thyroxine-binding globulin (TBG) is a globulin protein that binds thyroid hormones that are being circulated. It is one of three transport proteins (in addition to transthyretin and serum albumin) that are important for carrying the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) in the bloodstream.[5] From the three proteins, TBG has the most affinity for T_4 than for T_3, but is found in lower concentrations, in comparison to the concentrations of transthyretin and albumin, which also bind T_3 and T_4 when circulated. Since there is very low concentrations of T_4 and T_3 in the blood, the thyroxine-binding globulin (TBG) is rarely more than 25% saturated with its ligand. TBG differs from transthyretin and albumin by having a single binding site for T_4/T_3. [5]

The thyroid gland is the only source where thyroid hormone is found in the body. This process needs iodine and the amino acid tyrosine. Thyroglobulin molecules incorporate iodine from the bloodstream that has been taken up by the thyroid gland. This iodine can come from the environment, such as the food digested in humans. Once thyroglobulin molecules incorporate iodine in the bloodstream, the process is regulated by the thyroid-stimulating hormone (TSH), which is discharged by the pituitary. When there is not enough iodine or TSH, there is a decreased production of thyroid hormone.[6]

Just to provide more background information on the thyroid stimulating hormone, this hormone, which can also be known as thyrotropin, is a pituitary hormone that stimulates the thyroid gland to make thyroxine (T_4), then triiodothyronine (T_3), which revitalizes the metabolism of most tissues in the body.[7]

TSH is a glycoprotein hormone produced in the pituitary gland. One of the pituitary gland's roles is to control the endocrine function of the thyroid. The exact mechanism of TSH begins when TSH stimulates the thyroid gland to discharge the hormone thyroxine (T_4), which plays a minor part in our metabolism. T_4 is converted to triiodothyronine (T_3), which is the main active hormone that "turns on" our metabolism. It was mentioned that about 80% of this conversion is in the liver and other organs, while the remaining 20% is inside the thyroid. The hypothalamus, located in the brain, produces thyrotropin-releasing hormone (TRH), which then stimulates the pituitary gland to make TSH. The concentration of thyroid hormones (T_3 and T_4) in the blood controls the pituitary release of TSH. For example, when the concentration levels of T_3 and T_4 are low, TSH production is elevated, and vice versa.[7]

Another important molecule involved is thyroglobulin (TG). Thyroglobulin is used entirely inside the thyroid gland. TG is the predecessor to thyroid hormones, which are made when thyroglobulin's tyrosine residues are mixed with iodine and the protein is repeatedly cleaved.[6] TG serves as a substrate for the production of thyroid hormones T_4 and T_3, as well as

the storage of the inactive forms of thyroid hormone and iodine. When T_3 and T_4 are first made, they attach to TG. When TSH stimulates TG, the colloid it comprises is endocytosed from the follicular lumen into the surrounding thyroid cells. The colloid is repeatedly cleaved by proteases to free TG from its T_3 and T_4 attachments. After, T_3 and T_4 are now considered active and are released into circulation where they can be unbound or bound to plasma proteins. TG is then recycled back into the follicular lumen where it serves as a substrate for thyroid hormone synthesis again.[7]

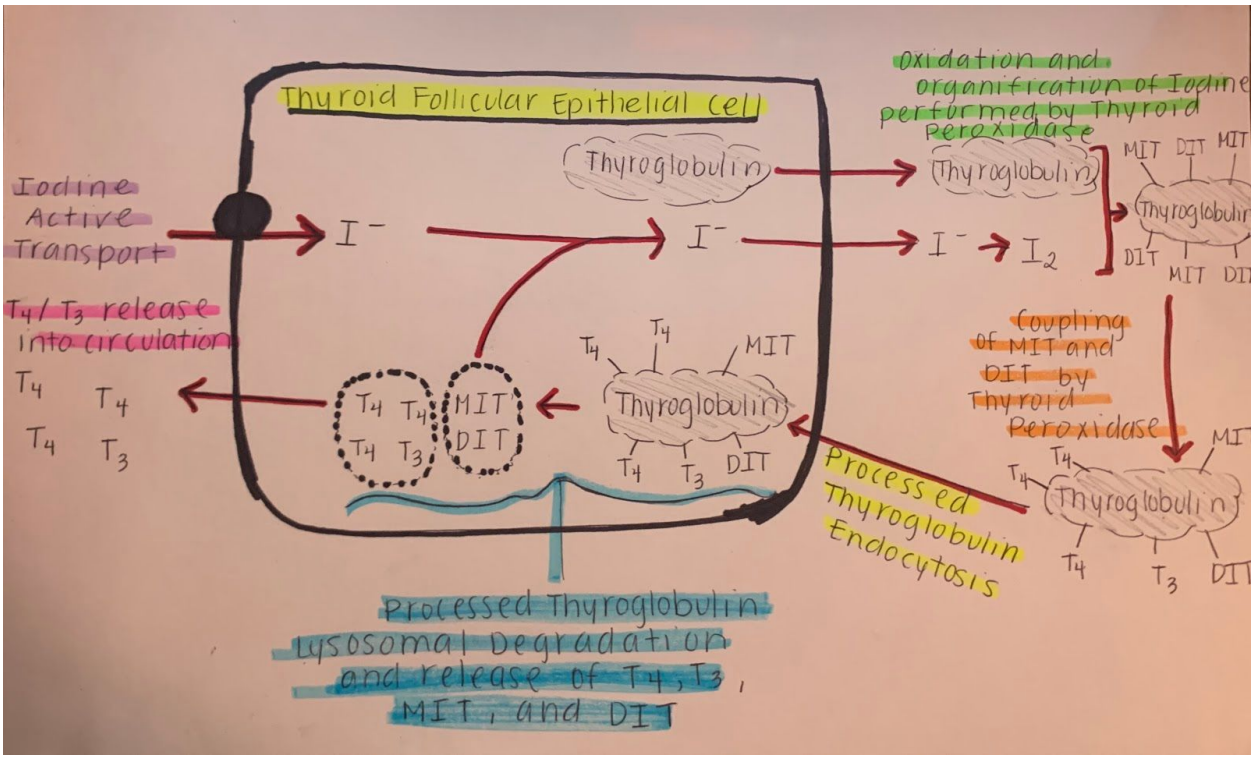

Figure 1: Mechanism for thyroid hormone synthesis.[8]

The figure above (figure 1) demonstrates how thyroid hormones are synthesized. Thyroid hormones are amino hormones, which means their synthesis is based on the amino acid tyrosine. As mentioned earlier, the main synthetic organ of thyroid hormones is the thyroid gland which makes about twenty times more T_4 when compared to T_3. T_4 is then converted to either T_3 or rT_3 by an enzyme, iodinase, which is found throughout the body's tissues.[8]

To further explain figure 1, the thyroid gland is full of thyroid follicles, which are the basic units of thyroid hormone synthesis. The thyroid follicles are surrounded by a lining of follicular epithelial cells and have an acellular lumen that contains a lot of proteinaceous material known as the thyroid colloid. Thyroid hormone synthesis is a complex multi-step process that has steps that occur inside the follicular epithelial cells, as demonstrated in the figure, and also in the acellular follicular lumen.[8]

So, the thyroid hormone synthesis has four important parts: iodine transport, thyroglobulin synthesis, thyroid peroxidase acting as an enzyme, endocytosis of peroxidase-processed thyroglobulin, and the release of T_4 and T_3 from thyroglobulin. In iodine transport, there is a significant amount of iodine that is needed for synthesis of levels of thyroid hormones. To make enough concentrations of iodide, the ionic form of the atom, iodide (I^-), is actively taken from the blood stream into the follicular lumen by the follicular epithelial cells. As a consequence, iodide becomes highly concentrated in the thyroid gland when comparing to the rest of the body. In regards to thyroglobulin synthesis, thyroglobulin is a protein that has large amounts of tyrosine amino acids that eventually become individual thyroid hormone molecules. Thyroglobulin becomes synthesized inside the follicular epithelial cell and gets discharged into the follicular lumen.[8]

Thyroid peroxidase is an enzyme that is found in the acellular colloid of the follicular lumen and is involved in many important reactions. One of these important reactions is that thyroid peroxidase first makes I_2 by oxidizing I^- ions that are found in the follicular lumen. Thyroid peroxidase then performs organification on the generated I_2 by covalently linking it with the tyrosine residues that are present in thyroglobulin. Organification is a process that occurs in the thyroid gland, and it involves incorporating iodine into thyroglobulin for the production of thyroid hormone.[9] This step is done after iodide gets oxidized by thyroid peroxidase.[8]

Once I_2 is covalently linked with tyrosine residues, this makes either single or doubly-iodinated species of tyrosine, written as “monoiodotyrosine (MIT)” and “diiodotyrosine (DIT)”, respectively. Thyroid peroxidase also combines MIT and DIT residues to make T_4 and T_3 species inside the thyroglobulin protein, this is known as coupling. T_4 is made by combining two DIT residues while T_3 is made by combining one DIT residue with one MIT residue. Importantly, thyroid peroxidase is more efficient at combining two DIT residues, so this results in T_4 being generated more faster, which explains why the thyroid gland mainly makes T_4 rather than T_3. Some MIT and DIT residues do not become coupled, so thyroid peroxidase-processed thyroglobulin will keep some MIT and DIT residues.[8]

Thyroid peroxidase-processed thyroglobulin is then endocytosed by follicular epithelial cells regularly, whenever the thyroid gland is stimulated to let out thyroid hormone into circulation. In addition, thyroid peroxidase-processed thyroglobulin inside the follicle can act as a reservoir for thyroid hormones when there is no sign of stimulation for hormone release. Also, the reservoir of thyroid peroxidase-processed thyroglobulin is usually sufficient for months of use, which explains why defects in the synthesis of thyroid hormones takes month to become diagnosed.[8]

Once endocytosed into the follicular epithelial cell, thyroglobulin is degraded by lysosomes, which releases attached T_4, T_3, MIT, and DIT. T_4 and T_3 are taken out of the follicular epithelial cells and are circulated. The iodine atoms of MIT and DIT are saved and brought back into the follicular lumen as I^-.[8]

There are many articles in hypothyroidism research where authors experiment with TSH, T_4, T_3, and TG, which serves as a demonstration that these molecules are important factors with hypothyroidism. One article titled "*De novo* triiodothyronine formation from thyrocytes activated by thyroid-stimulating hormone" focused on demonstrating that within the carboxyl terminus of mouse TG, T_3 is made *de novo* independently of deiodination from T_4.[11] It was observed that upon iodination of TG *in vitro, de novo* T_3 formation in TG was decreased in mice lacking TSHRs (thyroid stimulating hormone receptors). In addition, *de novo* T_3 that was secreted from PCCL3 (rat thyrocyte) cells, increased in cells that were exposed to elevated levels of TSH. What this paper succeeded in demonstrating is that TSH stimulates TG phosphorylation and plays a big role in increasing *de novo* T_3 formation.[10]

The importance of TG phosphorylation first begins with the fact that TG (thyroglobulin) is the principle hormone product that is secreted and stored in the outside of the thyroid cell. An article titled "Intrinsic Regulation of Thyroid Function by Thyroglobulin", proposed a mechanism, based on experimental observations of TG effects on thyroid cell behavior, that could explain for the phenomenon of follicular heterogeneity as a cycle that is highly regulated in increasing and decreasing colloidal TG concentration. This functions to enhance thyroid hormone production through the transcriptional activation or suppression of specific genes, like thyroglobulin or TSHR (thyroid stimulating hormone receptors).[10]

In another article titled "Relationship between Dimerization of Thyroglobulin and Its Ability to form Triiodothyronine", it mentioned about how the authors used a novel, convenient immunoblotting assay to find T_3 formation after protein iodination *in vitro,* which facilitated the study of T_3 formation in recombinant TG which is secreted from thyrocytes or heterologous cells.[11] What this assay showed was that the antepenultimate residue of TG is a major T_3-making site. The assay also demonstrated that the side chain of this residue interacts with the same residue in the opposed monomer of the TG dimer. What this paper concluded is that TG dimerization brings neighboring unique tail sequences into much closer distance, where a coupling reaction begins to take place. The longer and more stable the interaction is between the tail sequence, the better the chances for coupling. The authors also added a Cys^{2744} missense mutation which eliminated the important Tyr residue at this position and significantly altered the formation of T_3 upon iodination of either tail sequence.[11]

In "Hypothyroidism Induced by Loss of the Manganese Efflux Transporter SLC30A10 May be Explained by Reduced Thyroxine Production", the authors focused on determining whether hypothyroidism is caused by a reduction in thyroxine production due to the loss of function in manganese efflux transporter SLC30A10.[12] The authors mentioned that a deficiency in manganese could cause or contribute to a hypothyroid condition. With this in mind, the contribution of protein transporters SLC30A10 and SLC39A14 were experimented with. SLC30A10 transporter is a manganese efflux transports and highly present in the liver and can be activated by the presence of manganese. SLC39A14 transporter also transport manganese across cell membranes, but it is also known for transporting any manganese that is in abundance

from the blood into liver cells. Once in the liver cells, the manganese is removed from the body in the form of bile.[12]

The main conclusions of this paper is that SLC39A14 transporter is the transporter that predominantly transports manganese into cells. The authors also demonstrated that the loss of SLC39A14 transporter in mice prevents an increase in liver manganese levels in the liver, which differs from the mice lacking SLC30A10 transporter. This led to the conclusion that manganese levels were elevated in the blood and brain, but not in the liver, in the SLC39A14 transport single knockout mice and SLC30A10/SLC39A14 transporters double knockouts mice.[12]

Another conclusion was that SLC39A14 and SLC30A10 transporters work accordingly with one another to maintain manganese homeostasis and detoxification. In addition, it was proved that manganese toxicity in the thyroid gland directly causes hypothyroidism in the SLC30A10 single knockout mice. The role of manganese in the body is very important for many functions at the cellular level. If we are exposed to high levels of this metal, it accumulates in the brain and causes neurotoxicity. Neurotoxicity is when damage is done to the brain when it has been introduced to high levels of a natural or human-made toxic substance. These toxins can then gravely affect the activities done by the nervous system that can eventually disrupt or destroy nerves.[12]

Last but not least, the research concluded that manganese toxicity in SLC30A10 transporter single knockout mice inhibited thyroxine production in the thyroid gland, which as a result, lowered serum thyroxine levels and caused hypothyroidism. The exact step that goes into biosynthesizing thyroxine when it is impacted by manganese levels is still to be determined, but this paper contributes information that can potentially lead to discovering the exact step of thyroxine biosynthesis when manganese is involved.[12]

In “Iodide Binding in Sodium-Coupled Cotransporters”, the authors evaluated the structural and functional similarities between SMCT1 (sodium-coupled monocarboxylate transporter 1) and NIS (sodium-iodide symporter) that serves as a medium in the primary step of iodide entry into the thyroid.[13]

Background information that was crucial to this paper was the iodide transport into the thyroid gland is very important for synthesizing thyroid hormone. So, when defective iodide is built up, it disturbs thyroid hormone status and negatively affects metabolism, growth, and maturation of a variety of organ systems. The sodium-iodide symporter (NIS) is the mediator in the uptake of iodide from blood plasma to the thyroid follicular cells. The sodium-dependent cotransporter (SMCT1) is a protein that acts as an apical iodide efflux mediator in the thyroid. Since it is observed that NIS and SMCT1 localize to opposing sides of thyroid epithelial cells, a role for SMCT1 in iodide efflux was put into question.[13]

The first experiment of this paper aimed to see how similar NIS and SMCT1 were at the structural and sequence level. The authors made models of both NIS and SMCT1 proteins while in an inward facing conformation. The template that was used for the two target proteins was the structure of sodium/galactose transport (vSGLT), which was also in an inward facing

conformation. The reason why vSGLT was chosen has to do with the fact that it belongs to the SSS (sodium-solute symporter) protein family, along with NIS and SMCT1. During this experiment, vSGLT was captured in an inward-facing conformation with its galactose binding site blocked from the outside solution by hydrophobic residues. In order to prepare the models, the authors aligned the proteins' sequences using the AlignMe server v1.1 in PS mode.[13]

The authors aimed to find the residues that are involved in sodium and iodide binding in SMCT1 and hNIS, they used PyMOL v1.77 and compared the models, while in their inward facing conformation. When comparing the models, the authors found that vSGLT had 14 TM segments, and had both of the N- and C- terminus facing the extracellular milieu. On the other hand, the SSS family share the feature that they have a core of 13 TM segments, which is a feature both NIS and SMCT1 shared. NIS and SMCT1 had 13 **α**-helical TM segments, with their N-terminus facing an extracellular medium and the C-terminus facing the cytoplasm. Since the three proteins were not all related by function, the model did demonstrate that vSGLT, NIS, and SMCT1, did share a mechanism where there is tight coupling between sodium and its substrate transport.[13]

The authors did a sequence similarity analysis on all three proteins, and the results showed that NIS and SMCT1 showed a 20.1% and 22.1%, respectively, similarity to vSGLT. These percentages were low, but the authors just aimed to have models that were correct at the large-scale structure. Also, when comparing NIS and SMCT1 with each other, there was a 51.1% sequence identity and 71.4% similarity.[13]

In another experiment, the authors noticed that there was a conserved binding site in NIS and SMCT1. Based on previous studies, iodide uptake in NIS show that five polar residues are located in the TM9 area, which is an area that plays an important function in Na^+/iodide cotransport.[13-15]

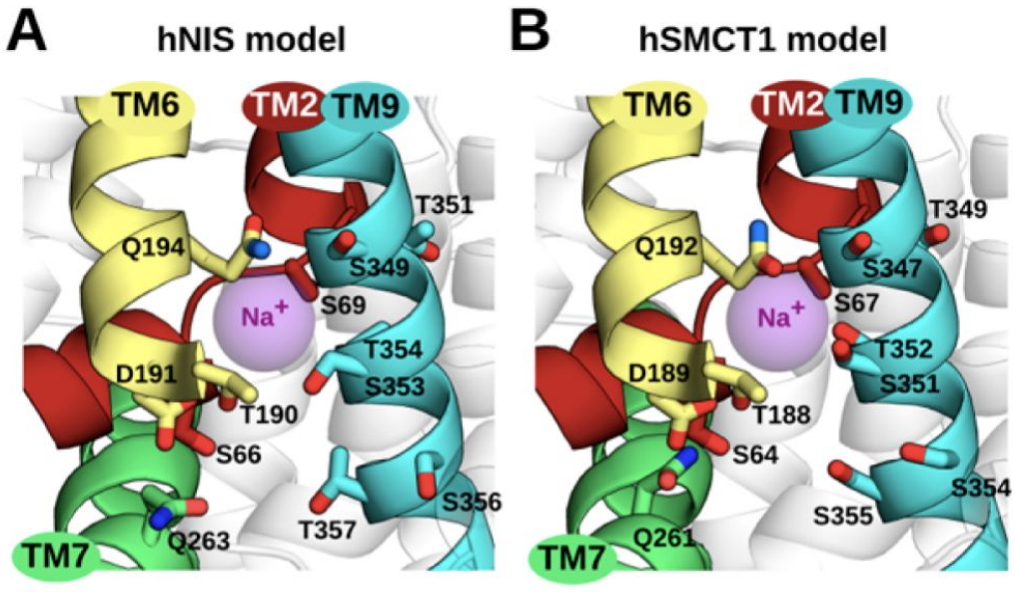

Figure 2: Predicted sodium-binding sites in NIS and SMCT1 that shows the conserved residues. When comparing the model with the vSGLT crystal structure, a putative sodium-binding site was predicted for NIS and SMCT1. The purple sphere is the sodium ion that is apparent in vSGLT, which was used as reference for the analysis.[13]

In addition, the five polar residues that were mentioned earlier were located in a similar area as the sodium binding site that was demonstrated by the structure of vSGLT. Other residues were then identified that were in close proximity in the models of NIS and SMCT1 that could contribute to sodium binding. NIS and SMCT1 were reported to share important residues that coordinate sodium transport.[14] The figure above (figure 2) shows the predicted sodium-binding site that is conserved in both models and uses residues in TM2, TM6, TM7, and TM9 segments. The putative sodium-coordinating residues are shown as sticks colored by atom type (oxygen is shown in red and nitrogen is showed in blue). The figure demonstrates the authors' assertations that there was conservation of important residues. The authors observed these residues at their specific positions, and it was seen that there was an excess of polar or acidic residues, specifically those of threonine, aspartic acid, or serine. Serine was very highly conserved. Glutamine residues were mainly conserved in the TM7 segments.[13]

Another observation was that of the putative residues in iodide binding in NIS and SMCT1. The model of NIS showed an inward facing conformation of their target protein, which allowed authors to find a presumed iodide-binding pocket. The SMCT1 model was compared to NIS to determine whether there is a similar substrate-binding cavity that is present in the former. What the results demonstrated is that SMCT1 had a cavity that is formed by nine specific residues, and four of these residues were conserved between NIS and SMCT1. The authors

hypothesized that the difference between a residue (G93 in hNIS) and the homologous residue T91 in SMCT1 play an important role in iodide transport in both proteins. To tie in all the experiments of this paper together and its value to this paper, it demonstrates that there was much lower iodide affinity in the pocket of SMCT1 when comparing to the iodide-binding pocket of NIS. The data demonstrated that SMCT1 is very unlikely to work as an iodide transporter in comparison with NIS. This study provided a useful and better understanding of iodide binding and transport by sodium-coupled cotransporters.[13]

In the paper "Analysis of the Protective Effects of γ-Aminobutyric Acid during Fluoride-Induced Hypothyroidism in Male Kunming Mice", the authors explored the γ-Aminobutyric Acid (GABA) to improve fluoride-induced thyroid injury in mice.[16] The mechanism(s) associated with GABA induced protection was investigated and what this study aimed to do was to further understand the protective effects of GABA on thyroid tissue through an investigation of its effects on the synthesis of thyroid hormones, oxidative stress in hypothyroidism mice, and when thyroid function-associated genes are expressed.[16]

Some helpful background information in relation to this paper begins with the thyroid being pointed out as a crucial endocrine gland. As I mentioned in the beginning of this paper, the thyroid discharges thyroid hormones, including triiodothyronine (T_3) and thyroxine (T_4). These hormones control our growth and development, metabolism, and other processes. Throughout the years, there has been an increase in EEDs (environmental endocrine receptors) that can alter the endocrine system. The thyroid is sensitive to EEDs, specifically fluoride. Fluorine is found in nature and is absorbed by the human body through food, water, and air.[16]

When we are exposed to high levels of fluoride, our thyroid can be gravely damaged. Also, fluoride impedes the activity of adenylate cyclase and decreases cyclic adenosine monophosphate (cAMP) formation, which leads to an increase in thyroid iodine clearance. Adenylate cyclase (AC) is an enzyme with important roles in essentially all cells.[17] It is an enzyme that catalyzes the cyclization of ATP into cAMP, where PPi is cleaved by pyrophosphatase. Once cAMP is produced by AC, it functions as a regulatory signal through specific cAMP-binding proteins, such as transcription factors, cAMP-dependent kinases, or ion transporters.[16, 17] As a result, surrounding T_3 and T_4 are decreased and thyroid hormone transport/function are inhibited.[16]

This source relates to the topic of this paper because it mentions that patients with hypothyroidism need thyroid hormone therapy throughout their lives, which usually is when they recieve synthetic T_4. Accompanied with taking synthetic T_4 long term, there are adverse side effects such as coronary heart disease, heart failure, and arrhythmia.[16] Finding new compounds to treat hypothyroidism without side effects are required.

γ-Aminobutyric acid (GABA) is an important inhibitor neurotransmitter. Applications of GABA have became popular since it is anti-anxiolytic (anti-anxiety), antihypertensive, and growth promoting. There have been previous works where authors have demonstrated that when treating fluoride-exposed mice with GABA, there is a decrease in metabolic toxicity caused by

fluoride and the microstructural and ultrastructural organization of the thyroid gland becomes fixed. As a result, there was an upregulation of T_4 and T_3 and TSH levels also increased in the thyroid follicular epithelial cells. On the other hand, the mechanism that explains the protective effects of GABA against fluoride-induced thyroid damage were not further looked in to.[16]

Mice were used as test subjects for the experiments in this paper and they were divided into two groups of equal average body weight. The experimental mice were given a daily dose of NaF (50 mg/kg) for 30 days and were models of fluoride-induced hypothyroidism. Negative control mice were just given pure water. To see the effects of GABA administration on hypothyroidism, the mice that were exposed to fluoride were divided into eight groups. The groups received their treatments for 14 days.[16] The groups were as follows:

- Group 1: Negative Control Group (Mice only received pure water)
- Group 2: Positive Control Group (Mice received oral thyroid tablets with a daily dose of 50 mg/kg)
- Group 3: Low concentration of pure GABA ("G1", Mice received pure GABA orally at a daily dose of 50 mg/kg)
- Group 4: Medium concentration of pure GABA ("G2", Mice received pure GABA orally at a daily dose of 75 mg/kg)
- Group 5: High concentration of pure GABA ("G3", Mice received pure GABA orally with a dose of 100 mg/kg)
- Group 6: Low concentration of laboratory-separated GABA ("LSG1", Mice received laboratory-separated GABA orally with a dose of 50 mg/kg)
- Group 7: Medium concentration of laboratory-separated GABA ("LSG2", Mice received laboratory-separated GABA orally with a dose of 75 mg/kg)
- Group 8: High concentration of laboratory-separated GABA ("LSG3", Mice received laboratory-separated GABA orally at a daily dose of 100 mg/kg).

Again, these mice in the groups listed above were exposed to NaF, except for the negative control group. The authors treated the mice with GABA for 14 days, sacrificed them and their thyroid, blood, liver, and heart were collected immediately. Before sacrificing the animals, their thyroid samples were collected when they were under anesthesia.[16]

The effects of GABA on blood T_4, T_3, and TR**β** levels were monitored as the authors' first experiment. The levels of the blood T_3, T_4 , and liver TR**β** were monitored after exposure to NaF for 30 days and after 14 days of being treated with GABA (groups 3-8) and thyroid tablets (group #2). The results were that T_4, T_3, and TR**β** levels were much lower in the negative control group (NCG) than in the positive control group (PCG). When the doses of GABA changed, there was an increase in T_4, T_3, and TR**β** that was significantly higher than that in the NCG. The authors stated that the effect of GABA on increasing levels of T_4 was inversely related to the dose, meaning that as the dosage increase, the effects of GABA decreased. The reason for this was not explained. In the positive control group (PCG), thyroid tablets positively affect T_4, T_3, and TR**β** levels, but demonstrated no noticeable differences to the low-dose GABA groups

(groups 3 and 6). On the other hand, the T_4 and T_3 levels in the PCG and GABA treatment groups (groups 3-8) stayed lower than those of the positive control group.[16]

To continue on, the authors also observed the effects of GABA on thyroid hormone synthesis. Proteins that are related with thyroid hormone synthesis (TG, TPO, and NIS) were measured after 30 days after being exposed to NaF (groups 2-8) and 14 days of GABA exposure (groups 3-8) and thyroid treatment (group 2). TG stands for thyroglobulin, which I briefly explained its significance in the beginning of this paper. TPO stands for human thyroid peroxidase, which is an important enzyme that is responsible for the synthesis of hormones done by the thyroid gland. TPO catalyzes iodination and couples tyrosine residues in thyroglobulin, which then results in the making of triiodothyronine (T_3) and thyroxine (T_4).[16]

NIS, which was also mentioned in an earlier paper that was referred to, stands for sodium-iodide symporter which acts as a medium in the first step of iodide entry into the thyroid. The results were that in the negative control group (NCG), the thyroid levels of TG, TPO, and NIS were much lower than the positive control group, low-dose pure GABA(group 3) and laboratory-separated GABA groups (groups 6-8). The group that was given the low concentration of laboratory-separated GABA groups (group 6) did not demonstrate an increase in levels of thyroid TG, TPO, and NIS, compared to the positive control group. So, the GABA-induced increase in TG, TPO, and NIS were inversely related with the GABA dose. When comparing to thyroid tablets, the effect of low-concentration pure GABA (group 3)and laboratory-separated GABA (group 6-8) on the levels of thyroid TG, TPO, and NIS were much stronger.[16]

Another aspect this paper on was the protective effect of GABA on the heart in hypothyroidic mice. Heart HDL levels and myocardial cell microstructure were monitored after 30 days of NaF exposure (groups 2-8) and 14 days of GABA (groups 3-8) and thyroid tablet (group 2) treatments. What was seen is that the levels of HDL went down significantly in NCG compared with the positive control group. HDL levels also went up significantly in the mice that were given different concentrations of GABA (groups 3-8) compared with NCG mice, but not the positive control mice. In the positive control group, the arrangement of myocardial fibers was regular and dense; the fibers were in place with no fractures. In NCG and PCG mice, myocardial fibers were neatly aligned, and the swelling and pyknosis of nuclei went down significantly in GABA-treated mice (groups 3-8). This led to the conclusion that the myocardial morphology showed an improvement due to GABA treatment.[16]

The conclusions of this paper was that both pure and laboratory-separated GABA, at same GABA concentrations, cause protective effects against fluoride-induced hypothyroidism. An experiment the authors did that that was not fully explain concluded that when there are low T_4 levels, GABA treatment kept the stability of lipid and glucose metabolism *in vivo*, which causes a loss of T_4. When comparing the results to the results from thyroid tablets, GABA demonstrated fewer cardiac side effects. To some level, it detained the stress caused by hormone replacement therapy on the heart. The importance of this paper to the subject of hypothyroidism

is that it supports that statement that GABA could exert a great therapeutic capability in hypothyroidism.[16]

While a lot of progress has been made in learning about hypothyroidism, or anything about the thyroid in general, there is always room for more knowledge about this condition that affects several people around the world. While there is no cure for this condition, the studies mentioned in this paper did demonstrate that there are experiments done to pinpoint specific problems and provide solutions to these problems. Currently, the standard treatment for hypothyroidism involves patients taking a daily prescribed dosage of the synthetic thyroid hormone, levothyroxine. This oral medication restores a sufficient amount of hormone levels, reversing the signs and symptoms of hypothyroidism. Typically blood tests are made to diagnose hypothyroidism. Low levels of thyroxine and high levels of TSH (thyroid stimulating hormone) signify an underactive thyroid. The reasoning is because the pituitary produces more TSH in an attempt to stimulate the thyroid gland into producing more thyroid hormone. TSH tests also play an important function in managing hypothyroidism. It assists doctors in determining the correct dosage of medication.[18]

To find out the right dosage of levothyroxine in the beginning, the doctor typically checks the level of TSH after six to eight weeks. After that, blood levels are checked after six months. When there is a great amount of hormone, side effects such as increased appetite, insomnia, heart palpitations, and shakiness can occur. The medication is best taken on an empty stomach at the same time every day. Doctors strongly recommend to take the hormone in the morning and wait an hour before eating. If taken at bedtime, the patient has to wait four hours after their last meal or snack.[18]

Treatment with levothyroxine is a lifelong process, and the dosage may change as years pass by so doctors will continue checking your TSH levels every year. As was demonstrated in this paper, there have already been studies that have focused on specific steps in thyroid hormone synthesis, such as determining whether hypothyroidism is caused by a reduction in thyroxine production due to the loss of function in manganese transporters[12], while other papers have studied the role of iodide in the thyroid hormone[13]. In conclusion, with further development of experiments and research on hypothyroidism, there will be better treatment plans and increased knowledge that will positively alter the lives of many people around the world in the future.

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