GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

New endocrine concepts - between hormones and nosological entities

- HABILITATION THESIS -

PREDA CRISTINA, MD, PhD

- 2019 -

CONTENTS

ABBREBIATIONS
Autism spectrum disorders - ASD

computed tomography – CD
Autoimmune Polyglandular Syndrome – APS
Symptomatic pituitary adenomas – Pas
pheochromocytomas/paragangliomas - pheo/PGL
fumarate hydratase – FH
aryl hydrocarbon receptor interacting protein – AIP
multiple endocrine neoplasia – MEN
pituitary adenomas – PA
magnetic resonance imaging – MRI
endocrine disrupting chemicals - EDCs
Environmental Protection Agency – EPA
growth hormone – GH
quality of life – QoL
oral glucose-tolerance test – OGTT
adrenocorticotropic hormone – ACTH
thyroid-stimulating hormone – TSH
follicle-stimulating hormone – FSH
luteinizing hormone – LH
Acromegaly Quality of Life Questionnaire – AcroQoL
Trail making test – TMT
Dichlorodiphenyltrichloroethane – DDT
estrogen receptors – ER
selective estrogen receptor modulators – SERM
sex hormone binding globulin – SHBG
Bisphenol A – BPA
Diethylstilbestrol – DES
testicular dysgenesis syndrome – TDS
tetrachlorodibenzeno-p-dioxin – TCDD
aryl hydrocarbon receptor – AhR
Bone mineral density – BMD
bone mineral content – BMC
body composition – BC
excess weight – EW
waist circumference – WC
body mass index – BMI
Society for Clinical Densitometry – ISCD
laparoscopic sleeve gastrectomy – LSG
Food and Drug Administration – FDA
idiopathic short stature – ISS
Urinary iodine concentration – UIC
iodine deficiency disorders – IDDs
Polyglandular autoimmune syndromes – PAS

THESIS SUMMARY

The academic profession assumes a permanent formation and development of the teaching staff. The teacher evolves in ordertotransmit to the student the knowledge needed to develop its personality, professional training, and proficiency in the future profession.

The current professional requirements aim primarily to increase the quality of the educational process, augmenting the level of competence and performance of students and making education more flexible. In this context of university education, the general objectives and the role of the teaching staff is fundamental.

A real professionist must sustain continuous training and involvement in the contemporary scientific world. It is not possible to achieve a higher quality of teaching and to improve the learning process without connecting the didactic experience with the achievements in research and in the medical career.

The success of an academic profession is given by perseverance in self-denial, in connection with permiability and flexibility to new ideas and availability and ability to communicate in teamworks.

All of theseare a necessary amount of skills that are meaningless and out of context without love and respect for the chosen profession. I have always been convinced that experience in medical practice has to be found both in the didactic act and in the research projects.

By meeting current academic standards, the results obtained throughout anuniversity career have a significant impact on the entire academic community.Thus, in an academic career it must reflect the ability to identify and motivate the human resource in order to give incentive and coordinate functional working teams.

This thesis is structured into three main sections, according to the criteria recommended and approved by the National Council for Attestation of Titles, Diplomas and Certificates (CNATDCU). The thesis summarizes the postdoctoral, professional, academic and scientific activity and presents my concerns in the areas of interest and related issues.

Following these recommendations, my habilitation thesis summarizes my research, didactic and medical work from the post-doctoral study,since 1999. Based on the results and experience gained throughout my career, I also include in this paper a series of future research directions that I want to follow in a short term. In the corresponding section of the thesis these projects are detailed as current knowledge and implementation plan.

The first part (SECTION I) titled "Scientific achievements from the postdoctoral period" presents the professional, scientific and academic results obtained throughout my career in three chapters. In this section are presented the main personal contributions in the field of endocrinology, with direct applicability in the future selected and presented research directions.

These three components are:

1. New theories on endocrinological disorders
2. Growth hormone pathology between excess and deficiency
3. The alchemy of the thyroid gland.

Various environmental factors can influence the normal functioning of the endocrine system and trigger their pathologies. Part of my research on endocrinology is heading in this direction and is the subject of the first part of Section I. In the field of endocrinology there is an important need for integration studies combining several measures covering a broad range

of social behaviors and links these aspects of the epigenetic and environmental genetic profile.The purpose of this study is to take part in this effort to describe intrinsic and extrinsic risk factors for endocrine disorders.

In the second part I present the results of my personal studies on the growth hormone (GH) associated pathology. The high diversity of GH actions can only be explained by the fact that the hormone plays different roles by activating a large number of proteins involved in cell signaling and by different mechanisms of action.

From these considerations, researching the actions and roles of the GH are far from the end. Exploring them requires multiple trials on significant batches of patients and is one of my directions for future study.

In the third part of first section, I present the results of the research on the chemical compounds underlying the normal functioning of the thyroid gland: iodine and selenium. Metabolism of iodine, selenium and interrelation with vitamin D metabolism are not yet fully elucidated.

The etiopathogenicity of the metabolic changes of these compounds following the appearance of a thyroid malignancy is also a direction of research that I will deepen in the near future.

In the second part of the thesis (SECTION II) titled "Scientific, professional and academic development",I summarize the most significant aspects of my personal career and those which add value to my ability to achieve the ultimate goal of putting into practice the medical research results in endocrinology.

In achieving this goal, I rely on a series of attestations obtained from the continuing medical training courses I have followed and on increasing national and international visibility. This was and it is possible by publishing books, book chapters and articles, as well as by active participations at congresses. The dissemination of academic research results hides behind a team-led work and, implicitly, it increases the prestige of the university I represent.

The subject of my doctoral research was idiopathic virilization syndrome, namely hirsutism. The action of binding protein (SHBG) in idiopathic hirsutism as well as studying the efficacy of oral contraceptives in combination with cyproterone acetate have been the objectivethe main thesis. The studies mentioned in the addition of corporeal composition research in correlation with androgen levels and SHBG in women with idiopathic hirsutism have opened the way for opening the other mentioned directions of study.

The last chapter of the thesis (SECTION III) presents the main publications that are the basis of my professional training and the determination of the current state of knowledge in the fields of interest.

REZUMATUL TEZEI

Profesia academică presupune opermanentă formare şi dezvoltare a cadrului didactic. Acesta evoluează astfel încât să poată transmite către student cunoștințele necesare dezvoltării personalității, formării profesionale și asigurării competenței în viitoarea profesie.

Cerințele profesionale actualevizează, în principal creșterea calității procesului educațional, creșterea nivelului de competență și performanță al absolvenților și flexibilizarea educației. În acest context al învățământului universitar obiectivele generale și rolul cadrului didactic este unul esențial.

Un adevărat profesionist trebuie să fie capabil de o pregătire continuă și o implicare în lumea științifică contemporană. Nu se poate ajunge la o calitate superioară a predării și la îmbunătățirea procesului de învățare fără a fuziona experiența didactică cu realizările pe care le avem în cercetare și în cariera medicală.

Reușita unei profesii academice este dată de perseverența în a te autodepăși, în corelație cu deschiderea și flexibilitatea către idei noi și disponibilitatea și capacitatea de a comunica în echipe de lucru.

Toate acestea reprezintă un cumul necesar de abilități care sunt lipsite de sens și scoase din context fără dragostea și respectul față de profesia aleasă. Am avut dintotdeauna convingerea că experiența din practica medicală trebuie să se regăsească atât în actul didactic cât și în proiectele de cercetare inițiate.

Prin atingerea standardelor academice actuale, rezultatele obținute de către un profesor universitar de-a lungul carierei sale au un impact semnificativ asupra întregii comunități academice. Astfel, într-o carieră academică trebuie să reflecte capacitatea de a identifica și motiva resursa umană în scopul motivării și coordonării unor echipe de lucru funcționale.

Manuscrisul de față este structurat în trei secțiuni principale, conform criteriilor recomandate și aprobate de către Consiliul Naţional de Atestare a Titlurilor, Diplomelor si Certificatelor Universitare (CNATDCU). Lucrarea sintetizează activitatea postdoctorală, profesională, academică și științifică și prezintă preocupările mele în domeniile de interes și cele conexe.

Urmând aceste recomandări, teza mea de abilitare rezumă activitatea mea de cercetare, didactică și medicală, din perioada de după finalizarea studiului doctoral în anul 1999 și până în prezent.Pe baza rezultatelor și experienței obținute de-a lungul carierei mele, includ în această lucrare și o serie de direcții de cercetare viitoare pe care îmi doresc să le urmez în viitorul apropiat.În secțiunea corespunzătoare a tezei sunt detaliate aceste proiecte ca moment actual al cunoașterii și plan de implementare.

În prima parte (SECTION I) intitulată "Scientific achievements from the postdoctoral period" prezint rezultatele profesionale, științifice și academice obținute de-a lungul carierei mele în trei capitole.În această secțiune sunt prezentate principalele contribuții personale în domeniul endocrinologiei, cu aplicabilitate directă în viitoarele direcții de cercetare selectate și prezentate.

Aceste trei părți componente sunt:

1. New theories on endocrinological disorders
2. Growth hormone pathology between excess and deficiency
3. The alchemy of the thyroid gland.

Diverși factori de mediu pot influența funcționarea normală a sistemului endocrin și declanșează patologii ale acestora. O parte a activității mele de cercetare în domeniul endocrinologiei se orientează în această direcție și face subiectul primei părți a secțiunii I. În domeniul endocrinologiei există o necesitate importantă de studii de integrare care combină mai multe măsuri care acoperă o gamă largă de comportamente sociale și leagă aceste aspecte de profilul genetic, epigenetic și de mediu.

Scopul acestui studiu este de a lua parte la acest efort pentru a descrie factorii de risc intrinseci și extrinseci pentru tulburările endocrinologice.

În cea de a doua parte prezint rezultatele studiilor personale în ceea ce privește patologia asociată hormonului de creștere. Diversitatea ridicată a acțiunilor GH poate fi explicată doar prin faptul că hormonul joacă roluri diferite prin activarea unui număr mare de proteine implicate în semnalizarea celulară și prin mecanisme diferite de acțiune.

Din aceste consideratii cercetările asupra acțiunilor și rolurilor hormonului de creștere sunt departe de final. Explorarea acestora necesitate studii multiple, pe loturi semnificative de pacienți și reprezintă una dintre direcțiile mele de studiu în viitor.

În partea a treia a secțiunii I prezint rezultatele cercetărilor în domeniul compușilor chimici care stau la baza funcționării normale a glandei tiroide: iodul și seleniul. Metabolismul iodului, seleniului și interrelația cu metabolismul vitaminei D nu sunt încă pe deplin elucidate.

Etiopatogenia modificărilor metabolice ale acestor compuși în urma apariției unei formațiune maligne tiroidiene reprezintă, de asemenea o direcție de cercetare pe care o voi aprofunda în viitorul apropiat.

În partea a douaa tezei (SECTION II) rezum aspectele cele mai semnificative ale carierei personale și care aduc un plus de valoare abilitării mele în vederea atingerii scopului final de a pune în practica curentă medicală rezultatelecercetării științifice din domeniul endocrinologiei.

În atingerea acestui scop mă sprijin pe o serie de atestate obținute în urma stagiilor de perfecționare medicală continuă pe care le-am urmat și pe creșterea vizibilității naționale și internaționașle. Acest lucru a fost și este posibil prin publicare de cărți, capitole de carte și articole precum și realizarea de comunicări la congresele de profil. Diseminarea rezultatelor cercetării academice ascunde în spatele acestora o muncă asiduă de echipă și care concură, implicit la creșterea prestigiului Universității pe care o reprezint.

Subiectul cercetării mele doctorale a fostsindromul de virilizare idiopatic, mai precis hirsutismul. Acțiunea proteinei de legare (SHBG) în hirsutismul idiopatic precum și studierea eficacității contraceptivelor orale în combinație cu ciproteronul acetat au constituit obiectivul principal al tezei.Studiile menționate la care se adaugă cercetarea compoziției corporeale în corelație cu nivelul androgenilor și SHBG la femeile cu hirsutism idiopatic mi-au deschis calea spre deschiderea celorlalte direcții de studiu menționate.

Ultimului capitol al tezei (SECTION III)prezintă principalele publicații care stau la temelia pregătirii mele profesionale și a determinării stadiului actual al cunoașterii în domeniile de interes.

SECTION I. PROFESSIONAL, SCIENTIFIC AND ACADEMIC ACHIEVEMENTS

Introduction

By definition, the term "career" is regarded as an evolution (ascending/advancing) of a person in a particular field of activity, which is usually desirable. Professional status is formally acquired through: diplomas, attestations, certificates, recognition, but comes as a consequence of actions that are carried out continuously and with dedication to the chosen career.

The stages I have spent in my teaching career in endocrinology had periods of continuous training and professional development. From the outset, the two components, didactic and research were permanently complemented by a physician profession that can be seen as a contribution to the community.

Teamworking and continued collaboration with all colleagues in the discipline were two things without which my professional evolution would not have been possible.Through everything I've done, I'm consider to be the product of the medical school of Iasi and especially of the East Endocrinology School.

The teaching profession requires continuous training and development of professors,in order to transmit the necessary knowledge to students. Theese will contribute to their personality development, training and propper competency in future profession.

Nowadays the general objectives of the university education are: increasing the quality of the educational process, raising the level of competence and performance of the graduates and the flexibility of the education. Thus, the role of the teaching staff is essential.

Without professionalism, continuous training and involvement in the contemporary scientific world it can not be achieved a higher quality of teaching and an improvement in the learning process.

Professional achievements

My professional career has started immediately after graduating from the General Medicine Faculty of "Grigore T. Popa"University of Medicine and Pharmacy, Iaşi, in 1984, when I was hired at the Vaslui Sanitary Department as a trainee physician. For three years, I have completed all six compulsory internships (internal medicine, surgery, obstetrics-gynecology, pediatrics, infectious diseases, emergency medicine) that I graduated through the specific exams.

After completing the internship, I was assigned as a general practitioner at the Bârlad Adult Hospital. For almost five years (1984-1989) I worked as a doctor in the 9th district of Bârlad with duties in preventive and curative medicine.

In 1989 I participated in the secondary school competition (the equivalent of the residency) by opting for the postgraduate endocrinology specialty at the Bârlad Adult Hospital. The first year of secondary education I conducted at the National Institute of Endocrinology "C. I. Parhon" Bucharest and the other two at Endocrinology Clinic of " St. Spiridon "Hospital Iasi.

As an endocrinologist I worked for a short period of time at the Bârlad Adult Hospital and, since 1992 I have been working as an endocrinologist at the Hospital for students in Iasi.

Since 1997 I became a primaryendocrinologist doctor.

Entire this experience gained from the clinical side of my professional development has helped me in my teaching career, giving me the opportunity to put into practice what I had learned in theory. This professional achievement has been another cornerstone in the developing and extended of my personal prowess, in understanding the suitableness of this knowledge.

Endocrinology as science and management of the patiens needs to colaborate with other medical specialties, especially surgery.

I have enriched my experience, knowledge and personal abilities by participating in postgraduate training courses in the fields of interest by obtaining professional attestations that allow me to practice the maneuvers and techniques that are so necessary and useful in my practical and current research work: clinical densitometry, endocrine-pediatric endocrinology, sexology, ultrasound competence and for the endocrine-metabolic.

Academic activity

I graduated the Faculty of General Medicine in 1984. In 1999, after I sustained my PhD thesis I obtained the title of Doctor of Medical Sciences. Then, I gained through contest the post of Assistant Professor at the Department of Endocrinology, Faculty of General Medicine, University of Medicine and Pharmacy "Gr. T. Popa"Iasi. In parallel, I worked as a primary endocrinologist at the Endocrinology Clinic, the St. Spiridon University Hospital in Iasi.

Since 2004, I worked as Lecturerand since 2014 as Associate Professor at the same Department, Faculty and University, which I have been working on at present.

Each of these steps of medical and didactic career consisted of periods of training and professional development, reinforcing my belief in an appropriate choice of teaching career. From the beginning, the two components, didactic activity and medical activity, were balanced in weight and complement each other, providing me with an adequate education framework with the students.

Since 1990 I was involved in the didactic process, initially by assisting within the practical classes with the students, and later as a PhD student. Besides the didactic activity, I participated, along with the whole team of the Endocrinology Discipline, in the development of teaching materials, which eventually led to the appearance of the book "Endocrinology. Diagnostic and Treatment Guide". This book was a guide not only for students, but also for resident physicians and specialists. The three editions (the last in 2008) emerged from the need to improve and update the knowledge in the field.

Following the same idea of improving the teaching method to students,I attended the post-graduate courseof pedagogy organized at the University of Philology, "Alexandru Ioan Cuza" from Iasi.

The entire experience gained in all these years made me realize the importance of getting students to be attracted to classes, raising their interest, especially by personal example and by emphasizing the practical importance of what they are studying. Organizing and conducting seminar activities within the laboratories we focused on the involvement of students in practical activities and centered on developing their capacity for expression and exposure in an academic way.

Over time, I've been endeavoring to improve my teaching method by using new, interactive methods to motivate and attract students to participate actively in classes and practical papers.

At the same time, a permanent concern regarded the review, renewal and harmonization of course content and internships with students in line with the latest trends in endocrinology as well as with other related disciplines.

Didactic position gained through contest:

- Associate professor – since 2014 until now, 2nd Medical Department, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.
- Lecturer – since 2004 until 2014, 2nd Medical Department, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.
- Assistant professor – since 1999 until 2004, 2nd Medical Department, Faculty of Medicine, ”Grigore T. Popa” University of Medicine and Pharmacy Iași.

To complete the psycho-pedagogical abilities and the specific methodical skills for superior education, I followed a post-university internship course for General Psychopedagogy organized by ”Al. I. Cuza” University, with graduation certificate seria F, no. 0022142.

Starting with the academic year 2004-2005 I worked as a lecturer. Thus, the didactic activity was diversified through the elaboration of materials presented during the classes with the students.

With the introduction of the Endocrinology discipline in the Faculty of Nursing, I developed the course support that was materialized in the book "Nursing Elements in Endocrinology" published in 2009.

The second discipline introduced in the norm "Methodology of Scientific Research" in the form of English language education, General Medicine 3rd year, required a new approach to the teaching act with the elaboration of specific course support. Besides the theoretical problems, the experience of over 10 years in clinical research has given me the opportunity to approach the theme of scientific research methodology and from the point of view of the practitioner.

We have also developed the course support for third year students in Dentistry, in the form of French education, by customizing the notions of endocrinology for this specialization.

The emergence of the e-learning platform marked a new stage in didactic activity not only through the new way of managing the activities and the results of the students but also by providing the course support which was updated and adapted for each specialization.

I participated actively in the post-graduate courses organized in the Endocrinology discipline where I held oral and practical courses (medical sexology, endocrine echocardiography, osteoporosis, growth disorder) and from the academic year 2013 to 2014 I held classes within the Master of Nutrition and Dietetics (Faculty of Medicine).

Since 1999 we have been conducting more than 30 licence theses both at the Faculty of General Medicine and at the Faculty of Nursing.

As a tutor of the year, I tried to create the necessary premises for open communication with students, I was receptive to their demands, and I gave them all the support when it was the case.

Scientific activities

My research activity began with admission to PhD in 1999. Idiopathic virilization syndrome, namely hirsutism, was the subject of the doctoral thesis. The involvement of binding protein (SHBG) in idiopathic hirsutism as well as studying the efficacy of oral contraceptives in combination with cyproterone acetate (in a sequential scheme) constituted the main objective of the thesis. Also (as a novelty for those years) I studied body composition in correlation with androgen levels and SHBG in women with idiopathic hirsutism. Under the guidance of Prof. Dr. Eusebie Zbranca, during the seven-year (1992-1999) PhD, I have been pursuing an in-depth knowledge of the field, developing clinical research skills, research reports and not the last row of developing attitudes on a higher level of scientific rigor, professional ethics, continuous learning and communication at different levels.

I have continued to pursue scientific research through participation in research teams, often multidisciplinary, with permanent focus on broad interest topics such as ovarian pathology, hormone-induced bone pathology, thyroid pathology.

The applicability of these professional competencies has also been reflected in my scientific research, where it has materialized through the publication of ISI articles with impact factor. The most relevant materials are related to the treatment protocols of thyroid gland pathology and rare endocrinologic sysndromes associations.

I chose this theme due to the relatively high incidence of affection in the Moldavian area, being 15.23 per 100,000 inhabitants in Iasi County between 2004 and 2007, but also due to the unsatisfactory efficiency of the early diagnosis of these deseases.

Participations in national and international conferences with original works as well as active participation in organizing the events of the Romanian Society of Endocrinology allowed me to have a good communication with my colleagues and a permanent updating from the country and abroad. This proved to be useful in time in the foundation of new didactic and scientificpartnerships.My capacity to organize and manage teaching is demonstrated by the published books, by the activity of coordinating diploma thesis and papers presented by students to scientific national and/or international congresses.

I wrote 26 books in Romanian, English and Franch.

*INTERNATIONAL VISIBILITY*is reflected and increased by:

- Web of Science H-index: 7;
- All Databases H- index: 7;
- total number of citations: 150;
- ISI papers 29;
- articles BDI 31;
- Using the ORCID platform that provides a persistent identity for humans.

Research projects

[1] Within the CNCSIS Type A Grant, "***Genetic Particularities in Modulation of Hormonal Mediators of Metabolic Stress. Pathogenic connection with cardiovascular reactivity***"(coordinator of the grant of Prof. Simona Fica, Bucharest),

as a member of UMF Iaşi team (coordinator Prof. Dr. Eusebie Zbranca) I participated in the study of the evaluation of seven adipocytokines: adiponectin, resistin, homocysteine, hsCRP, TNFα, IL-6 and leptin in PCOS. This study started from the premise that, analyzing the interrelationship between various hormones secreted by adipose tissue, as well as their relation to weight status, glucose and lipid metabolism and metabolic syndrome, we could find new data on the functionality of this tissue in women with PCOS.

[2] Another research topic I participated in as a team member was related to ***the influence of body weight and composition on bone metabolism*** (internal Grant of UMF Iasi - coordinated by Prof. Dumitru Brănişteanu). It was materialized by the publication in the Journal of Clinical Densitometry of the article "The influence of body weight, body compartments and related hormones on bone mass in pre- and postmenopausal women".

[3] Considering the contradictory results and the small number of selenium supplementation studies in thyroid autoimmune pathology, I participated in 2013 within the project titled "***Effect of selenium supplementation on antioxidant status, hormonal, autoimmune and ultrasonographic profile in euthyroid patients with chronic autoimmune thyroiditis***" to the UMF Iaşi internal grant competition; the project was rated with maximum points.

CHAPTER 1. NEW THEORIES ON ENDOCRINOLOGICAL DISORDERS

1.1. State of the Art

Endocrinology is intimately related to one key question: how does the brain manage to keep us alive and let our species survive? The answer to this is neuroendocrinology.

Harley Cushing postulated that*in this well concealed spot, almost to be covered with a thumbnail, lies the very main spring of primitive existence vegetative, emotional,reproductiveon which with more or less success, man has come to superimpose a cortex ofinhibitions* (Cushing, 1912).

These bidirectional interactions between neurons and endocrineglands do not only occur through hormones, but are partly executed by the autonomic system that is regulated by the hypothalamus and that innervates not only the endocrine glands, but all our organs.

The first who introduced the term ‘hormone’ was Ernest Henry Starling, at the begining of the 20th century. The story goes that he was talking to a colleague at a Cambridge dinner, and they were both struggling for a name for these secretions that could pass through another part of the body and stimulate it directly. A scholar of ancient Greek suggested the word "ormao", the Greek word for "excite" or "stir up" – and thus the word "hormone" entered the language (Henderson, 2005; Starling, 1912).

The hypothalamus acts as a central integrator for endocrine, autonomic, and higher brain functions (Kreier and Swaab, 2010).`

The story of the discovery of hormones abounds with bizarre experiments, remarkable characters, wrong turns, opportunism and quackery. However, there are elements of genius and amazing tales of survival. We can name the search for *the voice of an angel*, *the elixir of life*, finding of *the pink thyroid juice* and the story of *the ovarian hysteria*. The endocrine system may have been the last to really be discovered, because it is not an anatomical one (The Endocrinologist 2015).

The history of neuroendocrinology begins in 200 AD, with Galenus. He postulated that the brain excreted a residue from animal spirits (pituita), and continues into the last century, when researchers from different disciplines tried to understand how the brain regulates the vital functions of the body (Anand and Brobeck, 1951).

Some of our most recent "innovative" concepts had in fact already been thought up some 50–100 years earlier. Apparently, World War II and the migration and exile of many researchers interrupted the development of concepts in this field and made rediscovery necessary.

As clinicians and scientists or mere enthusiasts, we are all continually contributing to the progress of our subject, and thereby influencing its present and past.

Hormones's activities are exposed to genetic, epigenetic, immune and environmental interactions also. These play a highly important role in social behavior. The oxytocinergic system interacts with environmental cues and the deficits in this system have been linked to mental disorders associated with social impairments, such as autism spectrum disorder (ASD). this system is highly dependent on interindividual factors.

Neurohypophyseal hormones vasopressin (VP) and oxytocin (OT) might exert widespread influences on emotion and cognition in healthy subjects. They express some gender-related differences and interact with each other facilitating shifts between positive socially-oriented and defensive states. VP amplifies the reactivity to stressors showing also beneficial effects on attention, verbal learning as well as memory. Meanwhile OT reduces the amplitude of the stress response, improves emotion processing, and can play a negative effect on memory and verbal learning in healthy individuals.

Several data indicate the possible involvement of these neuropeptides in the pathophysiology of psychiatric conditions involving social interactions, such as autism, as well as in schizophrenia and depression (Iovino et al., 2018).

Documentation of yet-to-be-characterised pituitary disorders dates to ancient Egypt - a pharaoh depicted in 1365 BCE shows features of acromegaly. Aelius Galenus, in 150 CE, was the first who described the pituitary gland and suggested that it drains phlegm from the brain to the nasopharynx. Later on, various pituitary disorders were described, including amenorrhoea, acromegaly and diabetes insipidus (Brain, 1986).

In 1887, Minkowski first linked expansion of the pituitary gland to several clinical syndromes and open the way to acceptance that its anatomical expansion are what produce these syndromes, and the natural interest in operating on the pituitary (Minkowski, 1987). Hardy described microadenoma as "the pimento in an olive", but was met with much scepticism in 1968 when he presented the concept (Hardy, 1969).

Nowadays transsphenoidal surgical approach is leading the way for the treatment of pituitary adenomas.

In the field of endocrinology adrenal glands keep a special place. Bartholomaeus Eustachius first depicted the adrenal glands and named them ‘glandulae renibus incumbents’, because of their location close to the kidneys (Eustachi, 1783).

The first who performed a successful adrenalectomy was Knowsley Thornton in 1905. Precise preoperative assessment and diagnosis, had to await not just a complete understanding of adrenal biochemistry and reliable hormone assay but also the development of sophisticated localisation techniques, such as cross-sectional imaging with computed tomography (CT) and magnetic resonance imaging, supported by refinements such as selective adrenal venous sampling (Papadakis et al.,, 2015).

Excessive secretion of adrenal medulla results in the dramatic condition of phaeochromocytoma. One of the fascinating features of this tumour is its frequent (up to 25%) occurrence in familial form, as part of a genetically inherited disorder such as Multiple Endocrine Neoplasia Syndrome (MEN IIa, IIb) von Hippel-Lindau disease, neurofibromatosis 1 and succinate dehydrogenase (SDHD) gene mutations (Williams et al., 2003).

In China, around 1600 BCE was made use of burnt sponge and seaweed as a therapy for goitres. In about 1500 BCE Indian texts mention goitre as "galaganda", and describe its treatment. Later on, in 150 CE, Galen too suggested "spongia usta" (burnt sponge) for goitre (Niazi et al., 2011).

It was in the 20th century that the thyroid’s secretion of an iodinecontaining substance was examined. In 1914, Edward Kendall, Professor of Physiological Chemistry at the Mayo Clinic (Rochester, MN, USA), isolated crystalline thyroxine (T4). He found it had the same effects as the thyroid extract from which it came. More than a decade later, in London in 1926, Harrington defined the chemical formula of T4, and subsequently synthesised the hormone (Hennessey, 2015).

Maternal thyroid dysfunction during pregnancy may lead to persistent neurodevelopmental disorders in the offspring appearing in later life. This study aimed to review the available evidence concerning the relationship between maternal thyroid status during pregnancy and offspring behavioural and psychiatric disorders (Fetene et al., 2017).

During the recent years, neurodevelopmental disorders (NDDs) are more often related to neuroendocrinology (Keech et al., 2018).

During the recent years, applications of positional cloning in Endocrine Genetics with the identification of *RET,* menin, *PTEN* and *PRKAR1A* in the various forms of multiple endocrine tumor syndromes, and a number of other genes in developmental diseases affecting the pituitary, thyroid, parathyroid, pancreas, adrenal and gonadal glands, endocrinology has made a comeback to the forefront of "genomically"- influenced as well as post-genomic Endocrinology.

Some of the most traditional endocrine concepts could help us understand the complex directions recently unraveled in endocrine cancer genetics and its related fields.

The genomic concept suggests that "Endocrine" genes that control cellular signaling act as "conductors" since they regulate differentiation, growth and proliferation. Genes defy classic definitions of tumor suppressors and oncogenes and regulate gatekeepers, caretakers, and landscapers. These could help us to understand cellular regulation and pathophysiology and to design new treatments (Stratakis, 2005).

Endocrine disrupting compounds (EDCs) are exogenous chemicals that can alter endogenous hormone activity and homeostasis, thus potentially disrupting the action of sex and other natural hormones at all stages of human development. They play a fundamental role in brain development and sexual differentiation, exposure to EDCs in utero during critical stages of development can have lasting neurological and other physiological influences on the developing fetus and, ultimately, the child as well as adult.

The links between EDC exposures and aberrant neurodevelopment and behaviors are emphasing on EDC-induced transcriptional profiles.Exposure to these compounds can lead to permanent changes in gene expression and phenotype, which may in turn contribute to transgenerational inheritance of ASD (Moosa et al., 2018).

In the field of endocrinology there is an important demand for integration studies that combine multiple measures covering a broad range of social behaviors and link these to genetic epigenetic and environmental profiles.

The aim of this study is to take part in this effort to depict the intrinsic and extrinsic risk factors for endocrinological disorders.

This research direction has been realized by publishing the following articles:

1. Rusu C, **Preda C**, Sireteanu A, Vulpoi C.Risk factors in autism spectrum disorders: the role of genetic, epigenetic, immune and environmental interactions. *Environ Eng Manag J* 2015; 14(4): 901-917.
2. Denes J, Swords F, Rattenberry E, Stals K, Owens M, **Preda C**, Garcia IT et al. Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma: results from a large patient cohort. *J Clin Endocrinol Metab*2015; 100(3):531-541.
3. **Preda C**, Ungureanu MC, Vulpoi C.Endocrine disruptors in the environment and their impact on human health. *Environ Eng Manag J* 2012; 11(9): 1697-1706.
4. Arhire LI, Mihalache L, Pădureanu SS, Nita O, Gherasim A, Constantinescu D, **Preda C**. Changes in bone mineral parameters after sleeve gastrectomy: relationship with ghrelin and plasma adipokine levels. *Acta Endocrinologica (Buc)* 2018; 14(4): 498-504.

1.2. Endocrinological envolvement in the pathogeny of autism

1.2.1. Introduction

Particular development and activation of the immune system necessitate an entire network of signalling events that translate information from the outside environment to intracellular targets to elicit an appropriate response (Lettre and Rioux, 2008).

Imbalances in these signalling networks could occure and lead to the expansion and activation of autoreactive lymphocytes directed to self-antigens, overproduction of cytokines and secretion of autoantibodies leading to the destruction of normal tissue. These syndromes

may affect a particular anatomic structure or have systemic manifestation. The endocrine autoimmune disorders share many features (Frazer et al., 2007).

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopmental disorders defined by core deficits in social interaction and communication, restrictive interests and repetitive behaviors appearing before age 3 (American Psychiatric Association, 2013).

The spectrum encompasses autistic disorder, Asperger syndrome, pervasive developmental disorder not otherwise specified, childhood disintegrative disorder and Rett syndrome. Frequently autistic children associate comorbidities like intellectual disability, seizures, schizophrenia, sleep disorders or gastro- intestinal symptoms (e.g. diarrhea, constipation, bloating and gastro-esophageal reflux) (Rossignol and Frye, 2012).

The mechanism that leads to ASD is very complex, involving genetic, epigenetic, immune and environmental factors that could act in different proportions, at different developmental stages (prenatal, perinatal or postnatal) and on different pathways. The general prototype consists in an initial systemic dysfunction, such as immune dysregulation, inflammation, impaired detoxification or oxidative stress (Rossignol and Frye, 2012).

In this context, ASD may arise due to the harmful action of environmental factors. Autism is more frequent in males (4 male/ 1 female ratio) (Baron-Cohen et al., 2011) and different genetic, epigenetic, metabolic and social hypotheses tried to explain this finding. In USA, ASD prevalence has increased in time, from 1 in 3,000 individuals in 1966 (Lotter, 1966), to 1 in 150 in 2007 (Kuehn, 2007) and 1 in 88 in 2012, with specific prevalence in boys (1 in 54) comparative to girls (1 in 252) (CDC, 2012).

This recent increase pointed to environmental factors as a key issue for ASD determinism and stimulated research in the field. Surprisingly, research showed that ASD could be triggered in a genetically predisposed individual not only by classical external environmental factors (e.g. toxicants, pollutants, pesticides), but also by maternal imbalances or disorders (e.g. hormonal or inflammatory), as well as by disturbances of gut microbiota in the affected child (considered as internal environment).

Personal contribution – published paper:

Rusu C, **Preda C**, Sireteanu A, Vulpoi C.Risk factors in autism spectrum disorders: the role of genetic, epigenetic, immune and environmental interactions. *Environ Eng Manag J* 2015; 14(4): 901-917.

The aim of this review is to discuss actual theories concerning genetic, epigenetic, immunologic and environmental factors interplay in ASD determinism, as well as to point some of the new directions for ASD prevention and therapy.

1.2.2. Phenotypic approach to ASD studies

To overcome the difficulties of genomic studies (raised by the multitude of factors involved in ASD determinism), some studies in the literature tried to divide ASD cases

according to different clinical criteria and to relate each category with a specific genetic defect (Tadevosyan-Leyfer et al., 2003). These studies have identified genes involved in nervous system development and function, as well as genes related to endocrine and immune function (frequently disturbed in ASD) (Hu et al., 2009).

1.2.3. Genetic roots

Genetic ASD causes are represented by structural DNA defects that could be either sporadic (the parents of the affected child are normal) or could be inherited in the family.
Early on, ASD concordance in monozygotic twins was considered to be 90%, meaning that nongenetic factors (i.e. the environment) might have a little role in ASD determinism (Steffenburg et al., 1989).

However, recent studies have shown that ASD heritability (i.e. contribution of the genetic factors) is only 55% (Hallmayer et al., 2011), environmental influence being much more important than expected before.

DNA investigations have associated ASD with 2,193 genes, 2,806 single nucleotide polymorphisms (SNPs), 4,544 copy number variations (CNVs) and 158 linkage regions (Xu et al., 2012). Single gene disorders with high incidence of ASD, including Angelman, Fragile X, Rett, Smith- Lemli-Opitz and Timothy syndrome, as well as neurofibromatosis and tuberous sclerosis account for less than 20% of autistic patients (Benvenuto et al., 2009).

In these disorders genetic counseling and prenatal diagnosis easily allows the prevention of new cases, unlike the rest of the cases where the multitude of factors involved makes the management much more difficult. Copy number variation is the term used when a certain large DNA fragment is either missing (deletion) or present as extra copies (duplication); frequently duplications are associated with ASD, not deletions (deletions produce more severe consequences, being recorded in cases that associate ASD with intellectual disability and multiple congenital anomalies) (Girirajan et al., 2013).

Single nucleotide polymorphisms are very common DNA defects consisting in very small altered DNA sequences that can affect the function of a gene. More than 100 SNPs in genes involved in the detoxification of environmental pollutants have been involved in ASD, because they increase susceptibility to the adverse effects of environmental toxicants (Livingston et al., 2004).

Environmental agents may act as mutagens in at least two ways: either by contributing to oxidative stress (leading to DNA damage by free radicals) (Valko et al., 2005), or by inhibiting DNA repair systems (thereby leading to the accumulation of mutations) (Filipic and Hei, 2004). To maintain intracellular balance and protect against oxidative stress, cells produce glutathione. Mercury, cadmium and nickel are toxic because they reduce intracellular glutathione and bind to proteins used for DNA packing (Valko et al., 2005), leaving the DNA vulnerable to mutagenic effects of reactive oxygen species (Kinney et al., 2010).

The defects mentioned above are related to two key elements for ASD determinism:a) Synaptic development and functioning and intracellular Ca2+ signaling (Levitt and Campbell, 2009);
b) Epigenetic regulation of gene functioning.

1.2.4. Epigenetic mechanisms

Waddington introduced the term "epigenetics" to refer to causal mechanisms by which the genes of the genotype bring about phenotypic traits (Waddington, 1942). He has anticipated interactions between genes and between genes and the environment as an important foundation to understand development (Millan, 2013).

Thus, epigenetic mechanisms act on the genome to regulate gene expression, affecting the phenotype. This includes regulation by DNA methylation, histone modification, chromatin remodeling and microRNA expression, all processes being important for the development and function of the nervous system (Hu, 2013). The DNA encodes the information for all human traits. It is tightly packed with specific proteins called histones.

Chromatin represents the DNA and associated proteins within the nucleus. The tightness of wrapping (and thus the activity of the genetic material in the area) depends on the variant histone proteins used, as well as on the addition or removal of methyl groups to histone proteins (Posavec et al., 2013).

Mutations of the genes involved in this process have been found in ASD (Lasalle, 2013). Some environmental toxins act by reducing levels of DNA methylation (Baccarelli 2009). Fortunately, nutritional factors (e.g. folate and B vitamins) may counteract the harmful effect of chemical pollutants on DNA methylation levels. The typical pathway is the one carbon metabolism cycle that supplies methyl donors from the diet for methylation reactions to both DNA and histones (LaSalle, 2011).

Before implantation the embryo undergoes extensive DNA demethylation (methyl groups removed) followed by specific remethylation (methyl groups inserted) after implantation (Reik and Dean, 2001). Children with ASD and their mothers have prejudiced the capacity of methylation (James et al., 2010).

In the absence of periconceptional vitamin supplementation, maternal gene variants give less efficient one carbon metabolism and higher homocysteine levels. MTHFR is the key enzyme that regulates folate metabolism. MTHFR 677TT genotype decreases enzyme activity leading to inefficient folate metabolism, decreased blood folate, elevated plasma homocysteine and reduced methylation, mainly in individuals with low B vitamins levels (Hustad et al., 2007). Mutations in other genes involved in folate metabolism also increase maternal and fetal homocysteine with subsequent increase in ASD risk in the absence of maternal periconceptional vitamin intake (Boyles et al., 2006).

1.2.5. Oxidative stress and mitochondrial dysfunction

Mitochondria are complex, biochemically active and dynamic cellular components with a major role in energy production and management of reactive oxygen species (Shetty et al., 2012). They are providing energy for maintaining ion gradients, key elements in synaptic transmission (Toescu, 2000). The brain is extremely vulnerable to oxidative stress because of both high energy request and poor equippment with antioxidant enzyme defenses (Natelson, 2013), mitochondrial dysfunction increasing ASD risk.

Mitochondrial dysfunction leads to three main consequences: a) decreased energy production; b) increased production of reactive oxygen species (ROS) and oxidative damage; c) induction of apoptosis (cell death) (Rossignol and Frye, 2012).

All these changes have been recorded in autism, but may be also induced by pesticides (Franco et al., 2009). Most pesticides induce dysregulation of Ca2+-mediated signaling and production of mitochondrial ROS (Mariussen and Fonnum, 2006).

1.2.6. Immune dysfunction

Recent reviews of the immune dysregulation in autism have revealed different defects, the most important being altered cytokine profiles (Ashwood et al., 2003), neuroinflammation and auto-antibodies directed at nuclear brain proteins (Goines and Van de Water, 2010).

Cytokines are proteins that control the nature, duration and intensity of an immune response. They are produced by different immune cells, as well as by non-immune cells like neurons (that produce them and also respond to them) (Goines and Ashwood, 2013).

Cytokines are involved in normal neurodevelopment, including progenitor cell differentiation, cellular localization/migration within the nervous system and synaptic network formation (Deverman and Patterson, 2009). Levels of pro-inflammatory cytokines are elevated in ASD patients in a dose - response fashion (Ashwood et al., 2011), demonstrating an active neuro-inflammatory process (El-Ansary et al., 2013).

Many autistic individuals have food allergies (Jyonouchi, 2010) and allergic-like symptoms (Angelidou et al., 2011), but often with no positive test results, suggesting mast cell activation by non- allergic triggers (Theoharides et al., 2012). Mast cells are the "immune gate to the brain" (Theoharides, 1990).

They could be stimulated by allergic, environmental (mercury), infectious (viruses), mitochondrial, stress and toxic (propionic acid) triggers and release inflammatory and neurotoxic molecules that trigger focal brain allergies. They also increase blood brain barrier permeability, allowing circulating lymphocytes to enter the brain leading to focal encephalitis (Theoharides, 2013).

The "leaky gut" theory considers that increased gut permeability allows different substances to enter the blood stream and damage the central nervous system leading to ASD, (De Theije et al., 2011). Autoimmune diseases like type 1 diabetes mellitus, Hashimoto's thyroiditis or psoriasis were frequently recorded in families with autistic children, especially in the mother (Comi et al., 1999); maternal allergies, asthma, rheumatoid arthritis or celiac disease have also been related to ASD risk in children (Atladottir et al., 2009). Major consequences of the prenatal disruption of the immune development consist in atopy, allergy and autoimmunity in early childhood (Hertz-Picciotto et al., 2008).

Exposure to several types of pesticides may decrease immune competence and enhance autoimmunity (Corsini et al., 2008). Organo-phosphorus pesticides are particularly immunotoxic (Li, 2007), as well as pyrethroid pesticides (Gabbianelli et al., 2009). The chemical structure of the compound influences proinflammatory and immunosuppressive properties of the pesticide (Rooney et al., 2003).

1.2.7. Environmental causes

The term “environment” used here means any factor that is not part of the genome (i.e. genetic material) and may have a role in ASD determinism. It includes intrinsic factors (hormones, inflammatory mediators, microbiota that make up the microenvironment around the developing fetal or neonatal brain), as well as extrinsic factors (toxicants, environmental pollutants, medications, food additives, maternal disorders and even social influences that may have an impact on maternal, fetal or neonatal tissues).

Environmental agents that have been involved in ASD determinism may act before, during or after birth because even if the generation of new neurons is completed well before birth, their migration and differentiation, as well as the patterning of neural circuits continue after birth (Buss et al., 2006).

Advanced parental age is an important preconceptional factor related to ASD risk. Sperm from older men contain more DNA mutations (Kong et al., 2012), whereas older women are at higher risk of having ASD children due to disturbed epigenetic regulation. The season of conception was also correlated to ASD risk. Children conceived in winter months have an increased risk of autism (Zerbo et al., 2011).

This risk is correlated with maternal allergies before pregnancy (Croen et al., 2005), viral infections in the first months of pregnancy, nutritional factors (vitamin/mineral deficiency during winter time), with the precipitation rates (no sun exposure with subsequent lack of vitamin D) and with the use of pesticides in summertime (second and third trimester of pregnancy) (Lyall et al., 2013).

Results of the studies referring to toxicant environmental exposure should be interpreted with caution because genetic factors could influence the threshold for susceptibility to certain toxicants. Moreover, toxicant tissue levels may be very dynamic, depending on factors like caloric state, exercise, fever etc. For all these reasons, emerging testing methods incorporate tissue mobilization of toxicant using techniques such as caloric restriction (Gavrilescu et al., 2015; Rossignol et al., 2014).

A few recent meta-analyses identified significant ASD pregnancy-related risk factors: maternal disorders (diabetes, infections, bleeding during pregnancy and HTA), maternal medication (e.g. valproate), maternal exposure to organo- phosphate insecticides, first-born children (compared to the next ones) and mother born abroad (pregnancy close to migration moment; ASD risk probably related to maternal stress and pregnancy-induced low immunity for common infections).

Potential risk factors in the perinatal and neonatal period could be: abnormal fetal presentation, umbilical cord complications, fetal distress, birth trauma, multiple birth (twin pregnancy), maternal hemorrhage, summer birth, small for gestational age, meconium aspiration, neonatal anemia, ABO/Rh incompatibility and hyperbilirubinemia (Gentile et al., 2013). Risk factors are associated with hypoxia that leads to oxidative stress and neurotoxic effects (Gardener et al., 2009).

1.2.7.1. *Exogenous medically related factors*

Congenital rubella infection or an impaired immune response to rubella vaccination has been observed in some ASD children (Stubbs, 1976). Other viral infections (herpes simplex virus, cytomegalovirus, varicella zoster virus, mumps virus, influenza virus and

polyomaviruses) have been associated with ASD (Ciaranello and Ciaranello, 1995; Lintas et al., 2010). The most significant association was reported when the viral infection was recorded in the first trimester of pregnancy (Atladottir et al., 2010).

There are two possible mechanisms linking infections to ASD: either direct neurotoxic effect or neurotoxic effect mediated by the immune system. The direct neurotoxic theory appreciates that the pregnancy is an immune-suppressive condition and women could easily contract infections that damage the incompletely developed fetal brain (Sells et al., 1975).

For the immune mediated effects of viral infections there are two possible hypotheses: "molecular mimicry" (an immune cell recognizes a viral protein that resembles a human protein and triggers an immune response against both structures) and "bystander activation" (expansion of an immune response directed at tissues altered by inflammation induced by a viral infection) (Munz et al., 2009).

The viral infection could produce a transient increase of pro-inflammatory cytokines without viral persistence ("hit-and-go" mechanism) or could produce chronically elevated pro-inflammatory cytokines due to viral persistence (Libbey et al., 2005); cytokines may be produced directly in the brain or get access through the immature blood-brain barrier; these events could occur also in the maternal immune system, with subsequent attack of the fetus and abnormal neurological development (Libbey et al., 2005).

Vitamin D deficiency has increased in the last time due to life style modification (work inside, longer time spent inside watching television, use of solar filters and sun avoidance during pregnancy) (Cannell et al., 2008). Apart of the classic regulatory pathway of calcium metabolism, vitamin D is also involved in controlling the immune response by modulating immune cells (Baeke et al., 2010). It also promotes DNA synthesis and repair (Ellison et al., 2008).

Vitamin D level correlates with the latitude (Holick, 2005), the month of conception (Cannell et al., 2008), urban populations (Williams et al., 2006), air pollution (Windham et al., 2006), regions with high levels of precipitation and no sunny weather (Waldman et al., 2008), mother's metabolic status (obese individuals have a higher risk of vitamin D deficiency) and darker skin (Cannell et al., 2008), as well as increased access to cable television with longer time spent indoors (Waldman et al., 2008).

In females, estrogen increases neural vitamin D and protects against vitamin D deficiency, unlike testosterone that makes male brains susceptible to vitamin D deficiency, providing one reason why ASD is more frequent in males (Cannell et al., 2008).

Iron deficiency during the critical period of neurodevelopment could also trigger epigenetic mechanisms and increase ASD risk (Georgieff, 2008). Pre-pregnancy obesity ($\geq$ 90 kg) and excessive weight gain ($\geq$ 18 kg) during the pregnancy have been significantly associated to ASD (Krakowiak et al., 2012).

In women with non-insulindependent diabetes, increased fetal metabolism induces chronic intrauterine tissue hypoxia (Eidelman and Samueloff, 2002) and fetal iron deficiency (Georgieff, 2008), affecting neurodevelopment and neuronal connectivity (Georgieff, 2006). Moreover, increased cytokine levels disrupt normal fetal brain development by crossing the placental barrier (Krakowiak et al., 2012).

Mothers of ASD children frequently have auto-immune disorders, including rheumatoid arthritis, celiac disease and insulindependent diabetes (Atladottir et al., 2010), maternal autoimmune reaction having negative effects on fetal brain development (Careaga et al., 2010).

Valproic acid (frequently used as an anticonvulsivant) has been associated with a substantial increase in autism risk (Rasalam et al., 2005). It enhances DNA demethylation and interferes with the methylation processes necessary for normal brain development (Gadad et al., 2013). In humans, it seems that assisted reproductive technologies are not associated with an increased risk of autism, except ovulation-inducing drugs use (Hvidtjorn et al., 2011).

Pregnant women who receive paracetamol in the third trimester of pregnancy have an increased risk of HTA and subsequent ASD in the child (Rebordosa et al., 2010). Their ability to sulfate paracetamol is reduced, with subsequent activation of an immune response and pro-inflammatory cytokine signaling (Jetten et al., 2012).

There has been a lot of debate about the initial suggestion that MMR (measles – mumps – rubella) vaccine, through its thimerosal content, may contribute to autism (Wakefield et al., 1998). Thimerosal contains ethyl mercury and has been widely used as a preservative in many drug products and vaccines. The scientific consensus based on multiple epidemiologic studies rejected the relationship between thimerosal- containing vaccines and autism (Parker et al., 2004).

1.2.7.2. Maternal lifestyle factors

Short inter-pregnancy interval was associated with increased risk for ASD (Cheslack-Postava et al., 2011). A possible explanation of this finding could be maternal nutrient depletion, especially folate (van Eijsden et al., 2008). Essential nutrients (including folate) are preferentially distributed to the fetus, resulting in depleted stores in the mother, a state that remains for at least 12 months after the delivery (Milman et al., 2006).

Folic acid supplements have a protective role, especially in the presence of an inefficient folate metabolism (either in the mother or in the child) (Schmidt et al., 2011). No association has been found between average alcohol consumption and autism (Eliasen et al., 2010). However, high alcohol consumption facilitates folate deficiency and may increase ASD risk.

Maternal smoking has been associated with autism. A possible mechanism could be reduced blood flow to the fetal brain due to placental insufficiency produced by smoking and oxygen deprivation (Albuquerque et al., 2004).

Maternal smoking is also associated with stress and this triggers epigenetic ASD risk. Heavy metals from cigarette smoke (especially cadmium and lead) accumulate in maternal bones and are co-transferred with calcium to the fetus and infant during pregnancy and lactation (Sanders et al., 2012).

In the fetus they induce epigenetic alterations that predispose to autism. Maternal diet poor in leafy vegetables, meat and eggs could lead to folate deficiency with subsequent increase of ASD risk. For this reason in most of the countries worldwide peri-conceptional vitamins (including high doses of folic acid) are recommended and some countries decided to fortify bread with folic acid.

Some studies (McCanlies et al., 2012) have associated ASD in children with occupational workplace exposure of the parents (especially mother) during the three months preceding pregnancy through birth or weaning. Moreover, a home environment with polyvinyl chloride flooring, cable TV (with subsequent vitamin D deficiency due to less time spent outside), microwave, wireless technology and pesticides for pets, as well as home close to highways or fields with pesticides could also increase ASD risk.

1.2.7.3. Environmental chemicals

The effect of various toxicants in ASD determinism could be potentiated by the concomitant action of electromagnetic frequency and radiofrequency exposures (Juutilainen et al., 2006).

1.2.7.4. Endocrine-disrupting chemicals

Adequate levels of in utero thyroid hormones are critical for brain development. Maternal thyroid impairment has been associated with exposures to environmental chemicals (Caliman and Gavrilescu, 2009; Winneke, 2011).

Pesticides interfere with thyroid function by preventing iodine uptake and thyroid hormones synthesis (Colborn, 2004; Gavrilescu et al., 2015), with neurodevelopmental consequences in the child (Zimmermann, 2007). The human fetus does not start producing sufficient thyroid hormones until gestational week 18 (Burrow et al., 1994), meaning that adequate maternal thyroid hormones are critical to neurodevelopment in early fetal life (Pathak et al., 2011).

Polychlorinated biphenyls (PCB) were used as coolant fluids, plasticizers, adhezives, industrial oils, lubricants and pesticides, banned 40 years ago due to their toxicity, but they are still released into the environment due to their persistence or from building materials (Jamshidi et al., 2007).

Breast milk could be a source of exposure due to bioaccumulation of PCBs through the food chain (Hertz-Picciotto et al., 2008). In the prenatal brain, PCBs act on signaling pathways that regulate neuronal connectivity (Kodavanti, 2005). So, children with mutations in genes involved in these processes are more sensitive to PCBs and prone to ASD (Stamou et al., 2013).

Polybrominated diphenyl ethers (PBDE) are another class of endocrine-disrupting chemicals. They are used for flame retardants, foam in furniture, children's clothing and household materials. In spite of their banned production, PBDE levels in humans are high, as they bioaccumulate (Frederiksen et al., 2009).

Children react by robust inflammation to PBDE exposure during the critical developmental windows, with long term neurologic consequences and subsequent ASD (Goines and Ashwood, 2013). Both PCB and PBDE interact with specific receptors (Gu et al., 2012), disrupt endocrine systems (Lema et al., 2008) and interfere with calcium homeostasis (Langeveld et al., 2012), all of them leading to neurologic and immunologic consequences (Herbstman et al., 2010).

Bisphenol A (BPA) and phthalates have also been reported as endocrine disruptors. BPA is a plasticizer found in plastic drink containers, the lining of food cans and plastic food wrappings, dental resins and baby bottles, as well as thermal paper. BPA has estrogenic properties.

Phthalates are used in cosmetics, lotions, fragrances, vinyl flooring and a variety of plastics and have anti-androgenic effects. In-house flooring material made of polyvinyl chloride (a source of airborne phthalates), unlike wood flooring, has been associated with an increased risk of ASD (Larsson et al., 2009). Vinyl chloride is also a mutagenic agent, being metabolized to products that act directly on DNA, leading to chromosomal deletions (Chiang et al., 1997).

One possible mechanism by which endocrine- disrupting chemicals influence brain development and increase ASD risk is through disruption of thyroid homeostasis during pregnancy that affects nervous cells (Hertz-Picciotto et al., 2008). Alternative mechanisms are either by altering molecular signaling, including calcium (Shafer et al., 2005) or direct effects on neural development or the placental or blood-brain barriers. Endocrine- disrupting chemicals could also contribute to the male preponderence of ASD due to their effects on steroid hormones.

1.2.7.5. Pesticides

Pesticides are composed of a parent product, inert ingredients and agonists that enhance the functionality of the parent compound. All these compounds could be degraded to metabolites that distribute throughout the body, meaning that the mechanism by which pesticides determine ASD could be either direct (pesticide action) or indirect (metabolite action) (Shelton et al., 2012).

Pregnant women could be exposed to pesticides from very variable sources. They may be applying pesticides in or around their homes or to their pets, consuming food with residues of pesticides or pesticide metabolites or inhaling air from agricultural or urban spraying near their home or workplace (Gavrilescu, 2005; Shelton et al., 2012).

Exposure to organo-chlorine (OC) pesticides (e.g. hexachlorobenzene, DDT, dicofol and endosulfan – many of them banned – (Crinnion, 2009)) in early pregnancy has been associated with an ASD risk in children of women that lived within 500 m of fields treated with high doses of pesticides (Roberts et al., 2007).

Another study (Roberts et al., 2013) identified two critical periods of developmental vulnerability to ASD: one extended from one month before pregnancy till the end of the fifth month of pregnancy and the second one (postnatal) extending from 2 to 8 months of age. OCs interfere with calcium signaling, voltage sensitive sodium channels and GABA receptors, being neuro- and immuno-toxic (Heusinkveld and Westerink, 2012). The characteristic immune profile following OC exposure predisposes to allergic and asthmatic disorders, as well as autism (Gupta et al., 1998).

Organo-phosphorus pesticides (OP) (e.g. chlorpyrifos, diazinon) are the most commonly used pesticides in the world (Zaim and Jambulingam, 2007). ASD susceptibility is influenced by functional polymorphisms (SNPs) in paraoxonase PON-1, the enzyme involved in OP detoxification (D'Amelio et al., 2005).

OP interfere with synapse formation in mammalian brain by interfering with specific signaling pathways and Ca2+ signaling, conferring an increased ASD risk (Forster et al., 2010). OPs also induce persistent inflammatory states (Li, 2007). More recently, as OPs have been banned for residential use, pyrethroid sales have increased rapidly (Williams et al., 2008).

Pyrethroids (e.g. cyfluthrin) are a group of insecticides and repellants derived from natural compounds of Chrysantemum genus. They disrupt calcium signaling, interfere with voltage sensitive sodium channels and induce oxidative stress (Soderlund, 2012). Pyrethroids stimulate the expression of genes involved in cytokines production and signaling (Mense et al., 2006).

Imidacloprid is used in the agriculture, but it is also an active ingredient of flea and tick treatments for household pets (Cox, 2001). Its primary action is similar to that of OP (Pessah and Lein, 2008). Dermal absorbtion of imidacloprid can result from petting recently treated animals, but the dose that reaches and harms the fetus is unknown.

Depending on the chemical structure, pesticides could increase ASD risk through a variety of mechanisms, including: target voltage-gated sodium channels, acetyl-cholinesterase or GABA receptors, interference with the development of the serotonergic nervous system, changes in activity of monoamine oxidase, endocrine disruption, immune dysregulation, altered lipid metabolism and calcium signaling (Casida, 2009; Heusinkveld and Westerink, 2012; Malaviya et al., 1993; Pessah et al., 2010; Preda et al., 2012; Soderlund, 2012).

1.2.7.6. Air pollution and proximity to freeways

Different studies reported the following air pollutants as being associated with ASD risk: quinolone and styrene (Kalkbrenner et al., 2010), ozone (Becerra et al., 2013), nitrogen dioxide (Volk et al., 2013), pooled metals, mercury, lead, nickel, manganese, diesel particulate and methylene chloride (stronger association for boys compared to girls) (Roberts et al., 2013).

Collectively, significant association with ASD risk seems to be for traffic- related air pollution, big particulate matter and nitrogen dioxide, whereas small particulate matter and ozone do not show consistent association (Rossignol et al., 2014). Endocrine disruption may also be a pathway for air pollutants like diesel particulates, mercury and other metals, as they have been shown to influence levels of thyroid hormone (Takser et al., 2005).

1.2.7.7. Heavy metals

The most prominent heavy metals involved in ASD determinism are mercury, cadmium and nickel (Kinney et al., 2010). They act as mutagens in two ways: either by contributing to oxidative stress (DNA damage by free radicals) (Valko et al., 2005), or by inhibiting DNA repair systems (accumulation of mutations) (Filipic and Hei, 2004; Pavel et al., 2013).

Heavy metals are also associated with lower IQ, behavioral disturbances, endocrine disruptions (Winneke, 2011), immunotoxic properties leading to autoantibody production (Rowley and Monestier, 2005) and abnormal cytokine profiles. Some studies (Tian et al., 2011) suggest that individuals with ASD might have a particular immunologic susceptibility to heavy metals.

Glutathione S-transferases are the enzymes that catalyze the detoxification of heavy metals. Polymorphisms affecting their genes have been associated with an increased ASD risk (Rossignol et al., 2014).

Notably, SNPs in genes that impair toxicant elimination might not become functionally relevant in individuals with ASD until toxicant exposure levels reach a certain threshold and defense mechanisms are overwhelmed (Grandjean, 1995).

Mutagenicity of mercury has been proven (Agency for Toxic Substances and Disease Registry accessed 2009 – cited in (Kinney et al., 2010), the most dangerous being mercury acetate, that has a dose-dependent effect on the type of mutation (Silva- Pereira et al., 2005).

Apart of the genotoxic effect, mercury may bind groups like thiols, hydroxyls and carboxyls (Bridges and Zalups, 2010), increase dramatically intracellular calcium levels (Limke et al., 2003) and alter cytokine profile with subsequent development of autoimmunity (Kempuraj et al., 2010).

Ethyl mercury is a component of thimerosal, a widely used vaccine preservative. In vitro it has neurotoxic properties, may alter calcium signaling and cytokine production (Goth et al., 2006).

However, many independent epidemiological studies showed no association between thimerosal and ASD in humans (Price et al., 2010). Testosterone may increase toxicity of mercury (Muraoka and Itoh, 1980), whereas estrogen is protective, providing a possible explanation for increased ASD frequency in males (Oliveira et al., 2006).

Lead is neurotoxic and highly immuno-toxic (Mishra, 2009). At high levels, lead is immuno- suppressive, with increased production of regulatory cytokines and increased risk of infection (Valentino et al., 2007). At low levels, lead is immuno- stimulatory (Flohe et al., 2002). Pro-inflammatory status (Goebel et al., 2000) is frequently found in ASD (Li et al., 2009).

Lead toxicity may have unusual clinical presentation in some ASD individuals, with flu-like syndrome, weight loss, abdominal pain, diarrhea and vomiting (Newton et al., 2005), reason why periodic screening for lead exposure in children with ASD has been recommended (Filipek et al., 1999). Nickel produces reactive oxygen species (Galaris and Evangelou, 2002), inhibits DNA repair (Wozniak and Blasiak, 2004), but also potentiates the effect of other mutagens (Deng et al., 2006).

1.2.7.8. Electromagnetic frequency and radiofrequency exposures

Electromagnetic fields and radiofrequency radiations (EMF/RFR) are very diverse types of environmental radiations provided by different sources including X-rays used for diagnostic purposes, cell phones, wireless connections, microwaves etc. Electromagnetic fields enhance free radical activity, having a cumulative effect (De Iuliis et al., 2009). Free radicals destroy cells by damaging macromolecules (DNA, proteins and membrane components).

Moreover, it was discovered that in ASD individuals very low intensity EMF and RFR modulate glutathione, affecting mitochondrial metabolism (Choudhury et al., 2012). Their damaging effects may be reduced by supplementation with antioxidants and radical scavengers like vitamins E and C (Guney et al., 2007) and gingko biloba (Ilhan et al., 2004).

EMF/RFR also act on the physico-chemical characteristics of membranes (Beneduci et al., 2012), membrane potential (Linz et al., 1999) and calcium signaling (Nesin et al., 2012). EMF/RFR may also compromise barrier structures that separate blood flow from organs like brain (blood – brain barrier), gut, eye or placenta (Salford et al., 2012).

When these barriers become pathologically leaky, albumin, toxins, pro-inflammatory cytokines and infectious agents may cross the barriers, trigger immune responses and affect the developing fetus, finally producing ASD (Somosy et al., 1993). This mechanism has been

associated with non-thermal exposures comparable with normal cell phone radiation exposure (Nittby et al., 2008).

Some studies have documented the genotoxic effect of EMF/RFR (Sage and Carpenter, 2009). Many of the genetic defects predisposing to ASD are de novo mutations produced in sperm DNA by cell phone radiation (O'Roak et al., 2012). The proper mechanism of genotoxic action of EMF/RFR in ASD consists in oxidative stress and DNA damage by free radicals, challenge to DNA repair mechanisms or chromatin condensation (Herbert and Sage, 2013).

- ***Endogenous factors***
- *Microbiota*

The vast collection of microbes that live on or inside us (microbiota) and their collective genes (microbiome) have been recently proved as being involved in ASD due to the extensive use of genomic techniques (germ identification by DNA tests, not by germ culture) (Gonzalez et al., 2011).

The development of the human microbiome is a complex process, starting in pregnancy, when maternal bacteria are transported to the placenta via bloodstream, umbilical cord and amniotic fluid (Valles et al., 2012).

After birth, gut microbiota changes continuously, but in general gut microbial community is established in the first 3 years of life (Koenig et al., 2011). Gut bacteria have a set of digestive enzymes that are missing in the human host and complete the host set (Flint et al., 2008). Children with ASD frequently associate intestinal dysbiosis that determines abnormal digestion with additional growth substrates for bacteria that trigger dysbiosis (Williams et al., 2011).

Most of the microbiota germs are located in our gut and consists of approximately 1014 bacteria that balance the immune system, help digestion, produce vitamins and promote gastro-intestinal motility (Berg, 1996). The human microbiome represents the interface between our genes and our history of environmental exposures.

Early environmental exposures include physical contact with family members (that explains why family members share a core microbiome) and the diet (maternal milk). Some studies in the literature suggest that milk formulas could be involved in ASD determinism due to the reduced content of water and high molecular weight (Hahr, 2013).

The most important factors that influence gut microbiota are: mode of delivery (Dominguez-Bello et al., 2010), geographic origin (De Filippo et al., 2010), host genotype (Li et al., 2012), diet (Walker et al., 2011), antibiotics (Willing et al., 2011), probiotics (Rauch and Lynch, 2012), age (Tiihonen et al., 2010) and stress (Konturek et al., 2011).

Alterations of indigenous microbiota have been associated with many diseases, including obesity, metabolic syndrome, nonalcoholic steato-hepatitis, inflammatory bowel disease, irritable bowel syndrome, atherosclerosis, type I diabetes, autism, allergy, asthma and celiac disease (Backhed et al., 2012).

Gut microbiota could be involved in different ways in ASD pathogeny: alteration in sulfur metabolism, production of organic acids (propionic acid) and presence of bioactive peptides in urine (phenols produced by specific bacteria are transformed at liver level and released in urine) (Midtvedt, 2012).

Different microbiota unbalances have been identified in ASD, including increased levels of Clostridia (Parracho et al., 2005), Bacteroidetes (Finegold et al., 2010), Ruminococcus torques (Wang et al., 2013), Desulfovibrio (Finegold, 2011) and Sutterella spp. (Williams et al., 2012), as well as decreased levels of Firmicutes (Finegold et al., 2010) and Verrucomicrobia (Wang et al., 2011).

1.2.7.9. Mineral imbalances

Mineral imbalances associated with ASD are zinc and magnesium/calcium deficiency (frequent), iron, chromium, manganese, copper and cobalt deficiency (rare) and toxic metal burdens (aluminium, cadmium, lead, arsenic and mercury). An inverse relationship was found between zinc and lead, aluminium and cadmium concentrations, suggesting that these toxic metal burdens associate with infantile zinc deficiency.

Three types of metallomic profiles have been identified in ASD children: zinc and magnesium deficiency associated with burdens of cadmium and lead; burden of aluminium, mercury or arsenic; high sodium and potassium (Yasuda and Tsutsui, 2013). Most of the ASD cases are diagnosed clinically until 3 years of age and in these cases a mineral check is indicated for treatment/prevention purposes.

Zinc is a component of many enzymes and it is also involved in gene functioning. It plays important roles in nucleic acid/protein synthesis, cell division, as well as in tissue growth and repair, especially in pregnant women and infants.

In brain it plays an important role in synaptic transmission. Zinc deficiency observed in autistic children induces epigenetic mechanisms and by gene/environment interaction interferes with neuronal maturation during early development (Grabrucker, 2012).

Cadmium and arsenic also induce epigenetic alterations (Jakovcevski and Akbarian, 2012). Recently, it was shown that dietary restriction-induced zinc deficiency up-regulates intestinal zinc-importer, increasing the risk of high-uptake of toxic metals such as cadmium and lead (Goyer, 1997).

Similarly, deficiency in magnesium/calcium seems to enhance toxic effects of lead (Mahaffey et al., 1986). The most common lead exposure pathway in children is ingestion or inhalation of road dust, fossil fuel, asphalt and paints (lead chromate or lead carbonate) (Dixon et al., 2009), as well as maternal cigarette smoking (Razagui and Ghribi, 2005). Cadmium and lead from cigarette smoke accumulate in maternal bones and are co-transferred with calcium to the fetus and infant during pregnancy and lactation (Sanders et al., 2012).

1.2.7.10. Medication metabolism

Autistic children have a decreased ability to sulfate paracetamol (primary metabolic pathway for children) (Alberti et al., 1999). When paracetamol is metabolized through alternative routes it induces oxidative stress and immune dysregulation (Bauer and Kriebel, 2013).

1.2.8. Global mechanism

The most important and recent hypotheses concerning autism pathogeny are listed below:

- In individuals with vulnerable genetic background, advanced parental age and low birth-weight, the risk of ASD may be aggravated by environmental factors: infections provoking an immune activation response, maternal diabetes, obesity or poor nutrition,

fetal distress and birth trauma, faultyfetus preentation, hyperbilirubinemia and maternal bleeding. The environmental factors dysregulate epigenetic mechanisms resulting in an initial brain overgrowth and aberrant patterns of cerebral connectivity, clinically expressed as ASD (Gentile et al., 2013);

- Allergic, environmental, infectious, mitochondrial, stress or toxic triggers stimulate mast cells that release inflammatory and neurotoxic molecules (resulting in brain allergy) and increase blood-brain barrier permeability, leading to focal encephalitis (Theoharides, 2013);
- Impaired gastro-intestinal absorption of cysteine (main glutathione precursor) leads to local and systemic oxidative stress (because reactive oxygen species produced by metabolism cannot be neutralized anymore by glutathione), leading to disruption of normal epigenetic regulation of gene expression. If cysteine absorption is severely affected the individual will associate overt gastro-intestinal inflammation, whereas if cysteine absorption is mildly decreased, only immune and/or neurological development and function will be affected (Waly et al., 2012);
- Increased gut permeability facilitates the entrance of gluten, casein or lipo-polysaccharides into the blood stream and triggers peripheral inflammatory responses that lead to de novo production of cytokines in the brain with subsequent neuroinflammation (Critchfield et al., 2011);
- The individual lacks appropriate genetic machinery to excrete toxins and they accumulate, with toxic effects on the brain and the immune system. An environmental challenge during a critical window of development may have severe consequences, including abnormal neurodevelopment, altered immune phenotype and autism (Goines and Ashwood, 2013).

Probably the real mechanism is a mixture of all these theories, meaning that in a genetically predisposed individual different (and frequently combined) environmental factors trigger an immune reaction that leads to neuroinflammation with neurodevelopmental consequences (Fig. 1).

1.2.9. Management and therapy directions

As presented in detail above, autism cases are rarely produced by a single cause. In most of the instances the disorder is produced by many different factors that activate each other and finally lead to neurodevelopmental consequences.

For the moment there is no curative therapy for autism. However, because relatively often ASD is produced by a chain of reactions, simply by breaking one chain link we can stop the complex mechanism that leads to autism. Some of the most promising actions include:

- Vaccination of would-be mothers against viruses known to increase ASD risk (Millan, 2013);

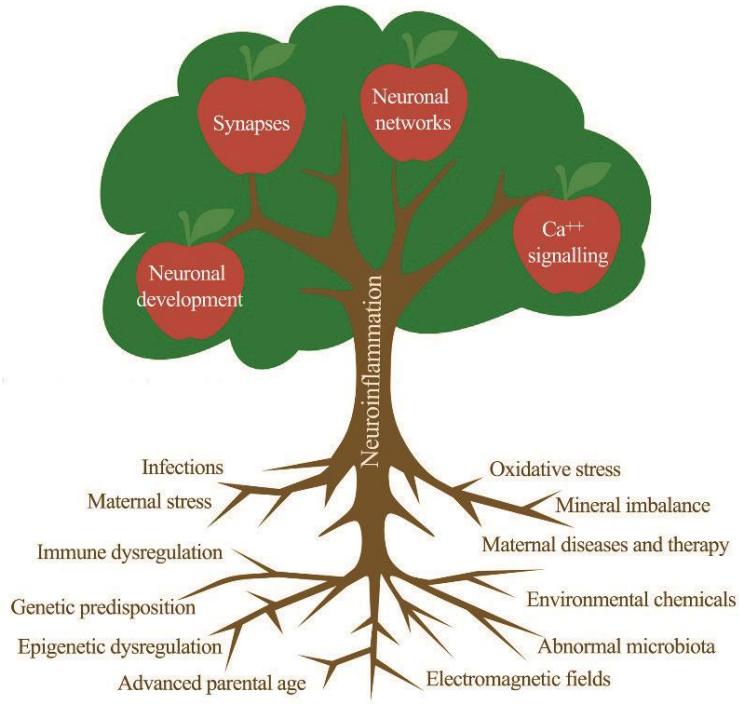


Fig. 1. Complex mechanism of autism spectrum disorders

- In mothers with less efficient folate metabolism (carrying at least one MTHFR 677T allele) folic acid administration should be increased and extended to three months before pregnancy (Schmidt et al., 2012);
- Strategies to create a beneficial shift in the microbiome include: antibiotics, probiotics (live microorganisms that are not part of the host microbiome, but confer a health benefit to the host) (Rauch and Lynch, 2012), prebiotics (nondigestible food components that are selectively fermented by beneficial members of the gut microbial community (Brownawell et al., 2012) and immune modulators (Backhed et al., 2012). Lactobacilli and Bifidobacteria from probiotics are also capable of transforming toxic mercury compounds into excretable metabolites (Brudnak, 2002);
- The nutritional approach supplements deficient nutrients (e.g. zinc) or vitamins (e.g. vitamin D) and detoxifies accumulated toxic metals (Yasuda and Tsutsui, 2013);
- Nutraceuticals are defined as "any substance that is food or a part of food and provides medical or health benefits, including the prevention and treatment of disease" (Alissa and Ferns, 2012). They are generally dietary supplements and promote healthy gut, lower body burdens of toxins, improve antioxidant capacity, enhance immune-modulatory systems and minimize stress and environmental contamination (Alanazi, 2013). Nutraceuticals could be multivitamins with high folate content for periconceptional supplementing (Schmidt et al., 2011), vitamin B12 and glutathione combined with low fructose and food additive/color organic diet (Patel and Curtis, 2007), essential fatty acids (especially omega-3, but results are controversial) (Vancassel et al., 2001),

tetrahydrobiopterin (Frye et al., 2010), casein-free (milk protein) and gluten-free (wheat protein) diets (Elder et al., 2006).

1.2.10. Final remarks

Autism is a very complex developmental disorder and genetic, epigenetic, immune and environmental factors should be considered in a comprehensive approach when investigating a case.

In most of the situations these factors act together in a sequence of events that leads to neuro- inflammation and abnormal brain development.

The knowledge of the factors involved provides ways for medical intervention and complication prevention. The recent increase in ASD prevalence underlines the growing importance of environmental factors in autism determinism.

Environmental factors involved in ASD do not refer only to classic extrinsic agents (like environmental pollutants, electromagnetic fields etc.), but also involve maternal disorders or lifestyle factors, as well as intrinsic factors (hormones, inflammatory mediators and gut bacteria), that may influence the developing fetal or neonatal brain.

Early diagnosis is a key issue because it allows a multimodal intervention plan, including education, medical intervention aiming to prevent complications and genetic counseling.

1.3. Neuroendocrinology on the edge of new associations of pathologies

1.3.1. Introduction

Rare endocrine syndromes and, especially different associations of them have an autoimmune and genetic background. They cause chronic inflammatory syndromes and result from the combination of a genetic susceptibility and environmental factors also. Collectively they are based on immune dysregulation through unknown mechanisms (Wiebolt et al., 2010).

The simultaneous occurrence of T1D is increased in monozygotic twins and ranges from 13 to 67Æ, 7%, compared to 0–12Æ, 4% in dizygotic twins and 0Æ, 5% in the general population. These rates already point to a complex genetic basis. By contrast, some rare cases of major endocrine autoimmune disease can be caused by a single mutation in one gene, for example in Autoimmune Polyglandular Syndrome type 1 (APS-1) (Huber et al., 2008).

On one hand, first evidences for a role of genetic factors in autoimmunity and vice versa originates from the observation that family members of a patient with an autoimmune disease have an increased risk to develop genetic disorders. On the other hand, twin studies suggest genetic influences on the aetiology of autoimmune disease. The reason of this is that the monozygotic (identical) twin pairs have high concordance rates (Dultz et al., 2009).

The prevalence of symptomatic pituitary adenomas (PAs) in the general population is 1:1063 to 1:1282 (Daly et al., 2006; Fernandez et al., 2010), whereas the prevalence of clinically diagnosed pheochromocytomas/paragangliomas (pheo/PGL) is 1:2500 to 1:6667 (Mazzaglia 2012; Eisenhofer et al., 2013).

Although both are relatively rare diseases, PAs and pheo/PGL can sometimes occur in the same patient or in the same family. Coexistence of the two diseases could be due to pure coincidence, but it is possible that in some cases the two conditions share a common pathogenic mechanism. Since the first description of a patient with acromegaly and pheochromocytoma in 1952 (Iversen 1952), 70 cases have been published with this rare disease combination.

The simultaneous occurrence of these two tumor types might be explained by the following:

1) a pheo/PGL-related gene mutation, which, in addition to the pheo/PGL, also causes PA, as suggested for the SDHX mutation being involved in PA formation (Beckers 2013; Xekouki and Stratakis 2012; Dwight et al., 2013);
2) a mutation in a familial PA gene that also causes pheo/PGL;
3) a digenic disease, ie, two gene abnormalities are present in the same patient or family causing the two diseases;
4) a single, possibly novel, gene causing both diseases;
5) ectopic hypothalamic hormone-secreting adrenal tumors causing pituitary enlargement mimicking PA; or
6) thedevelopment of a pituitary adenoma and a pheo/PGL in the same patient or same family due to pure coincidence.

Personal contribution – published paper:

Denes J, Swords F, Rattenberry E, Stals K, Owens M, **Preda C**, Garcia IT et al. Heterogeneous genetic background of the association of pheochromocytoma/paraganglioma and pituitary adenoma: results from a large patient cohort. *J Clin Endocrinol Metab*2015; 100(3):531-541.

The objective of the investigation was to study the possible coexistence of pituitary adenoma and pheo/PGL.

In the current study, are described 39 cases of sporadic or familial pheo/PGL and PA in which a germline genetic analysis, loss of heterozygosity (LOH), and pathological studies were performed. Eleven germline mutations in five different genes (five SDHB, one SDHC, one SDHD, two VHL, and two MEN1) and four germline variants of unknown significance in three different genes (two SDHA, one SDHB, and one SDHAF2) were identified in the studied genes in our patient cohort.

Tumor tissue analysis iden-tified LOH at the SDHB locus in three pituitary adenomas and LOH at the MEN1 locus in two pheochromocytomas. We have also identified a novel histological feature of SDHX-related PAs.

From the 9 patients with SDHB mutation, the one with sequence variant c.770dupT (p.Asn258GlufsTer17) was my patient. The association between: non-functional pituitary adenoma and bilateral phaeochromocytoma caught my attention. In the literature at this moment are only 21 patients (mine included) with pituitary adenoma and phaecromocytoma/paraganglioma with identified genetic mutation.

1.3.2. Materials and methods

1.3.2.1. Patients

The research team collected clinical data, genomic DNA, and tumor tissue, when available, from 39 patients with pheo/PGL and PA in a sporadic (n=19) or familial (n=20) setting. Probands from 23 aryl hydrocarbon receptor interacting protein (*AIP*) mutation negative familial isolated PA (FIPA) families (defined as two or more subjects with pituitary adenomas but no syndromic features of other diseases such as multiple endocrine neoplasia (MEN)-1 or Carney complex) served as controls.

Neurofibromatosis was ruled out based on clinical criteria according published guidelines (Ferner et al., 2007). The study was approved by the local ethics committee and all subjects gave written informed consent.

1.3.2.2. Genetic screening

Nucleic acid extraction

Genomic DNA was extracted from peripheral blood using a BACC2 DNA extraction kit (RPN-8502; GE Healthcare) according to the manufacturer's protocol. DNA extraction from formalin-fixed, paraffin embedded pituitary or pheo/PGL tissue was performed using a QIAamp DNA FFPE tissue kit (QIAGEN). Representative tumor tissue was marked by a pathologist to avoid areas showing suboptimal preservation and contamination with normal tissue.

Mutation testing

Sequence analysis of the *AIP* gene (NM_003977.2), MEN type 1 gene (*MEN1*; NM_130799.2), cyclindependent kinase inhibitor 1B gene (*CDKN1B*; coding region NM_004064.3, upstream open reading frame NM_004064.2) was performed using Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA), as previously described (Korbonits et al., 2012; Owens et al., 2009; Occhi et al., 2013).

Genes implicated in pheo/PGL [MYC associated factor X (*MAX*; NM_002382.3), rearranged during transfection tyrosine kinase receptor gene (*RET*; NM_020975.4), succinate dehydrogenase subunit A (*SDHA*; NM_004168.2), succinate dehydrogenase complex assembly factor 2 (*SDHAF2*; NM_017841.2), succinate dehydrogenase subunit B (*SDHB*; NM_003000.2), succinate dehydrogenase subunit C (*SDHC*; NM_003001.3), succinate dehydrogenase subunit D (*SDHD*; NM_003002.2), transmembrane protein 127 (*TMEM127*; NM_017849.3), and von Hippel-Lindau gene (*VHL*; NM_000551.3)].

All of these genes have been analyzed using a combination of next-generation sequencing, Sanger sequencing and MLPA, as previously described (Schouten et al., 2002; Rattenberry et al., 2013). In addition, fumarate hydratase (NM_000143) was studied in a subset of patients.

Tissue DNA analysis with PCR and sequencing was carried out according to standard protocols (Applied Biosys tems). The sequences were analyzed using Mutation Surveyor (version 4.0.6; Softgenetics). In silico analysis of variants was performed using the Polyphen2 (http//:genetics.bwh.harvard.edu) and ALAMUT 2.2.0 (http://www.interactive-biosoft ware.com/) softwares.

1.3.2.3. Loss of heterozygosity analysis

Microsatellites D1S170 and D1S3669 for the *SDHB* locus were identified on the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/) and

the University of California, Santa Cruz Genome Browser website (http:// genome.ucsc.edu/). Details of the microsatellites at the 11q13 locus (for *MEN1*) were previously described (Chahal et al., 2011).

Simple repeats were identified using the University of California, Santa Cruz website and designed accordingly for the specific region (Chahal et al., 2011). The NCBI36/hg18 assembly of the human genome was used for the localization of the markers.

Fragment analysis was carried out using standard protocols on an ABI 3730 (Applied Biosystems) and analyzed using GeneMarker (version 2.20; SoftGen- etics). All primer sequences are available on request.

1.3.2.4. Immunohistochemistry

Immunostaining for GHRH was performed using GHRH antibody 451–7 (Lyon, France), 1:2000 dilution, as previously de- scribed (Sassolas et al., 1983; Berger et al., 1984). Pheochromocytomas of patients with the *MEN1* mutation were stained for menin using a rabbit polyclonal antimenin antibody (Abcam; ab2605, dilution 1:500), as previously described (Harding et al., 2009).

Mouse pancreas showing islets and pheochromocytomas of patients without any known germline mutation were used as a positive control. SDHA and SDHB immunostaining was performed using a mouse monoclonal anti-SDHA antibody (2E3GC12FB2AE2, ab147159, dilution 1:200; Abcam) and a rabbit polyclonal anti-SDHB antibody (HPA002867, dilution 1:200; Sigma-Aldrich), as previously described (Gill et al., 2010).

Further immunostaining was performed using the antimitochondrial antibody 113-1 recognizing a 60- to 65-kDa nonglycosylated membrane protein (Merck Millipore; dilution 1:150) and an antibody directed against the endoplasmic reticulum lectin 1 (ERLEC1; dilution 1:100; Novus Biological). Immunoreactions were performed using the automated Leica Bond III system.

For antigen unmasking, EDTA at pH 8 was used for anti-113-1 and sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, at pH 6) for anti-ERLEC1. The primary antibody binding was visualized with the SuperSentitive immuno- histochemistry detection system from BioGenex. Sections were counterstained with Mayer's hemalum before being dehydrated and coverslipped.

1.3.2.5. Statistical analysis

The statistical analysis was performed using StatsDirect software (Addison-Wesley-Longman). Normal distribution of the data was tested by the Shapiro-Wilk test. The Student *t* test was used to compare numerical variables. The $\chi 2$ or Fisher's exact tests were used to compare categorical variables. The results are reported as mean ± SD. Values of $P < .05$ were considered statistically significant.

1.3.3. Results

1.3.3.1. Clinical data

The research team identified 39 patients with sporadic (n=19) or familial (n=20 from eight families) pheo/PGL and PA. The gender distribution did not differ significantly ($P=.6$) in our cohort (18 males, 21 females) compared with the control group (12 males, 11 females). The mean age at diagnosis was 43.7±18.2 years (mean±SD) for PA and 47.2±15.6 years for pheo/PGL.

There was no significant difference in age of onset of PAs compared with the control group (35 ± 15.4; *P*=.08). In the PA-pheo/PGL cohort, comparing patients with and without mutation, no difference was identified in the age at diagnosis of the PA [mutation positive group (n = 12) 43.4±18.9 y vs mutation negative group (n =16) 44.8 ±17.1 y, *P* = .8] or in the age of diagnosis of the pheo/PGL [mutation positive group (n=15) 46.7± 14.3 y vs mutation negative group (n=14) 48.4 ± 19.7 y, *P* = .8].

Table 1. Genes Tested in Pheo/PGL + Pituitary Adenoma Patient Cohort

Genes	Number of Patients With Sequence Variant	Sequence Variant	LOH in the Pituitary Adenoma	LOH in the Pheochromocytoma
SDHA	2(2 variants)[a]	c.969C>T(p.Gly323Gly)[b]	No LOH	Not tested
		c.91>CT (p.Arg31Ter)		
SDHB	9 (8 mutations and 1 variant)	c.298T>C(p.Ser100Pro)	3 LOH	Tested and identified in 1 case
		c.587G>A(p.Cys196Tyr)		
		SDHB del exons 6–8		
		c.423 +1G>A		
		c.770dupT(p.Asn258GlufsTer17)		
		Variant:c.80G>A (p.Arg27Gln)		
SDHC	2(2mutations)	c.380A>G (p.His127Arg)	NA	Not tested
SDHD	2(2mutations)	c.242C>T (p.Pro81Leu)	NA	Not tested
SDHAF2	1 (variant)	c.-52T>C	NA	Not tested
VHL	2[a]	c.340G>C (p.Gly114Arg)	No LOHc	Not tested
		c.589G>A (p.Asp197Asn)		
MEN1	2	c.1452delG (p.Thr557Ter)	Not tested	2LOH
		c.783 + 1G>A		
RET *TMEM127*	0			
*MAX*FH AIP	0			
CDKN1B	0			

Abbreviations: FH, fumarate hydratase; NA, not available.

[a] One patient had two variants, a *VHL* and an *SDHA* variant.

[b] Further details are cited in Supplemental Table 6.

[c] LOH is not obligatory in VHL-related tumors [Banks RE, Tirukonda P, Taylor C, et al. Genetic and epigenetic analysis of von Hippel-Lindau (VHL) gene alterations and relationship with clinical variables in sporadic renal cancer. Cancer Res. 2006;66:2000 –2011].

Nineteen patients had both pheo/PGL and PA, whereas a further 20 patients had pheo/PGL or PA in a setting detailed below. In two families (families 1 and 6), the pro-band had both PA and pheo/PGL, whereas other family members had either PA or pheo/PGL. In five families the pituitary and pheo/PGL tumors occurred in the same family but not in the same individual.

One patient with a *VHL* mutation and a family history of clear-cell renal tumor and multiple hemangioblastomas had a PA presenting at 15 years (no typical VHL manifestations at this stage) (Tudorancea et al., 2012). Two patients with *MEN1* mutations had a pheochromocytoma. One patient had acromegaly due to a GHRH-secreting pheochromocytoma (Mumby et al., 2014).

Most PAs were lactotroph adenomas (n=15), but somatotroph (n=6), clinically nonfunctioning (n=5, four of them showing positive FSH, LH or α-subunit immunostaining), and corticotroph (n=1) adenomas were also seen.

Twenty patients had macroadenomas and four patients had a microadenoma (for three patients PA size was not available). There was no significant difference (P=8) in the pituitary adenoma size compared with the control group. Therapeutic modalities for pituitary disease included surgery, medical therapy (cabergoline or bromocriptine and somatostatin analogues), or radiotherapy.

Twelve patients needed only one therapeutic intervention, and four patients needed two, three patients needed three, three patients needed four, and one patient needed five different therapeutic interventions (for three patients information on treatment modality was not available). One patient developed pituitary apoplexy.

Sixteen patients had pheochromocytomas and 14 patients had PGLs, of which 12 were head and neck PGLs and two were abdominal (retroperitoneal) PGLs.

1.3.3.2. Genetic screening

Germline alterations were identified in *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *VHL*, and *MEN1* genes in 19 patients with pheo/PGL and/or PAs. Fourteen of the 19 patients who harbored a genetic variant were index patients.

All patients harbored one gene mutation except one patient, who had a *VHL* mutation and an *SDHA* variant of unknown significance. Twenty patients (including 10 harboring both pheo/PGL and PA) had no identifiable mutations in any of the genes tested (**Table 1**). None of the patients in our cohort had *AIP* or *CDKN1B* mutations.

- *SDHX* **mutation**

The research team identified 11 kindreds (including 16 patients) with germline *SDHX* variants. Seven families had a pathogenic *SDH* mutation, whereas four had a variant of unknown significance.

All patients with *SDHX* mutations/variants had a pituitary macroad- enoma. In the pituitary adenomas, in which suitable sam- ple was available, we identified the loss of the wild-type allele in the adenoma sample compared with the germline DNA (**Figs. 2-4**).

In particular, patient 5 was interesting in whom the germline mutation was a large deletion affecting exons 6 – 8 of the *SDHB* gene, whereas in the tumor sample the whole gene was deleted with no detectable exons 6 – 8 and a reduced amount of the other exons. We identified two *SDHA* variants of unknown significance.

One of these (c.969C>T, p.Gly323Gly) was identified in a patient (patient 15) with a Wilms tumor (at the age of 1 y), retroperitoneal liposarcomas (32 and 40 y), a PGL in the retroperitoneum (50 y), a renal oncocytoma (50 y), and a nonfunctioning pituitary adenoma (NFPA; 53 y). His father had an NFPA operated at 44 years and again at 74 years. His mother (no known tumors) carried the c.969C<T variant.

The other *SDHA* variant was identified in a patient with a *VHL* mutation and PA (patient 21). We have also identified an *SDHB* variant (c.80G>A p.Arg27Gln, patient 17) of unknown significance. We have tested the proband's pheochromocytoma and showed LOH at the *SDHB* locus; however, the SDHB staining of the pheochromocytoma did not show loss of SDHB expression.

No pituitary tissue was available for testing in this family. An *SDHAF2* variant c.-52T>C was identified in a patient with somatotroph macroadenoma and head and neck PGL. The patient was not operated upon and therefore no tissue is available.

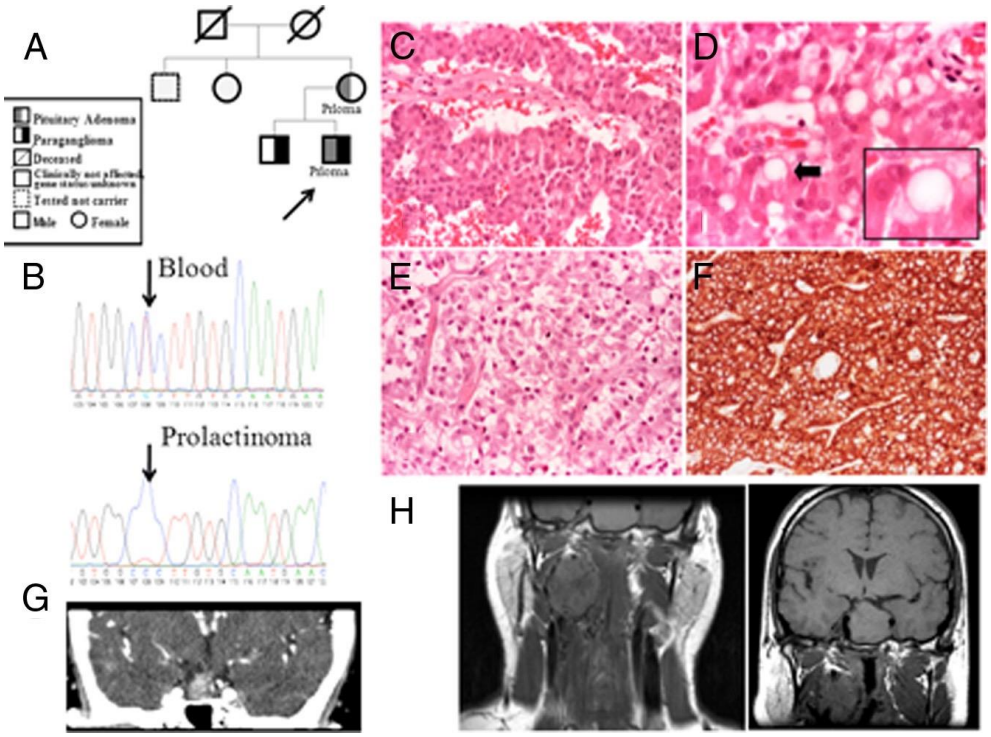


Fig. 2. Pedigree (A) and LOH (B) at the *SDHB* locus in the pituitary adenoma of patient 1 in family 1 is shown. C, H&E staining of the pituitary adenoma of the proband (patient 1 in family 1) shows predominant trabecular architecture (X20). D, Vacuoles at times filling the entire cytoplasm characterize this case (arrow) (H&E, X40). E, H&E staining (X20) of the pituitary adenoma of the proband's mother (patient 2 in family 1) also shows similar intracytoplasmic vacuoles. F, The immunoreaction with the anti-113-1 antibody (immunoperoxidase, X20) shows the mitochondria content. G, MRI imaging of proband's mother's pituitary adenoma. H, MRI imaging of the proband's pituitary adenoma and glomus vagale tumor. MRI, magnetic resonance imaging.

The research team identified two families with *SDH* mutations in which a family member with a PA did not carry the germline *SDHX* mutation: family 6 with two *SDHC* mutation- positive siblings had PA and/or PGL, whereas a first cousin had an NFPA but no

SDHC mutation; and family 7 in whom the parent and child both had *SDHD* mutation-positive PGL and another child had a microprolactinoma but no *SDHD* mutation. These cases are either phenocopies or could, theoretically, be explained by a digenic disease pattern in which the second disease- causing gene has not been identified.

- **VHL mutation**

An 18-year-old patient with a pathogenic *VHL* mutation [c.340G>C, a missense mutation affecting a surface aminoacid (Ong et al., 2007)], had an invasive GH- and prolactin (PRL)-positive PA.

***MEN1* mutation**

Two patients (patients 22 and 23) had a germline *MEN1* mutation and pheochromocytoma, whereas all the other tested genes were normal. Both pheochromocytomas showed LOH in the *MEN1* gene, supporting, although not proving, the pathogenic role of *MEN1* in these tumors (see **Fig. 5, A and B**). Although the association of pheo/ PGLs and an MEN1-like syndrome has been described in the literature in 13 cases, in only four of these have *MEN1* mutations been identified (Langer et al., 2002; Dackiw et al., 1999; Jamilloux et al., 2014), and none of them has been studied for LOH in the pheochromocytoma tissue.

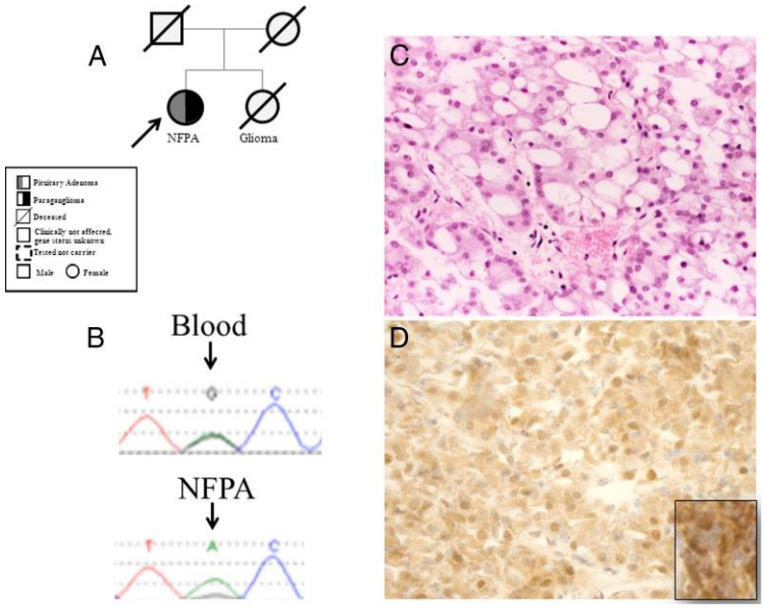


Fig. 3. Pedigree (A) and LOH (B) at the *SDHB* locus in the pituitary adenoma of patient 4; the microsatellite upstream of the mutation has also shown to be lost. C, H&E-stained section (X20) of this adenoma shows prominent vacuolar changes in most neoplastic cells; the cytoplasm otherwise appears weakly eosinophilic. D, *SDHB* staining suggesting lack of strong granular staining of the pituitary adenoma of the proband (immunoperoxidase, X20) (inset: positive SDHB staining as positive control in a paraganglioma).

1.3.3.3. Control patients

The research team studied 23 *MEN1*, *AIP*, and *CDKN1B* - negative FIPA family probands without features of Carney complex or a personal or family history of pheo/PGL. We analyzed their DNA for all the pheo/ PGL-related genes included in our panel to

investigate the role of these genes in FIPA families. No pheo/PGL-related gene mutations were found in these families.

1.3.3.4. Pathological features

The PAs of patients with *SDHX* mutations (patients 1 and 2 from family 1, patient 4, and patient 5) were characterized by intracytoplasmic vacuoles. The extent of vacuolization was not related to the histological type (prolactinoma or NFPA) of the tumor (**Figs. 2–4**). The number of vacuolated cells varied from about 50% to 80% of the neoplastic cell population. Vacuoles ranged from small and multiple (**Fig. 4C**) to large, occupying most of the cytoplasm and mimicking signet-ring cells (**Fig. 3C**).

None of the vacuoles indented the nucleus as commonly seen with accumulation of lipids. One case showed focal oncocytic changes identifiable on the hematoxylin and eosin (H&E) stained sections. The histochemical stain periodic acid-Schiff (PAS)/diastase-resistant periodic acid of Schiff did not reveal any glycogen accumulation. Vacuoles were not seen in the PA of the patient with the germline *VHL* mutation (without *SDH* mutation). The SDHB staining of PAs with the *SDHB* mutation showed either a loss of expression of SDHB or a faint expression (**Figs. 3D and 4E**).

Because *SDHX* mutations are known to alter mitochondrial function, immunostaining was performed for a mitochondrial membrane protein with the anti-113-1 antibody.

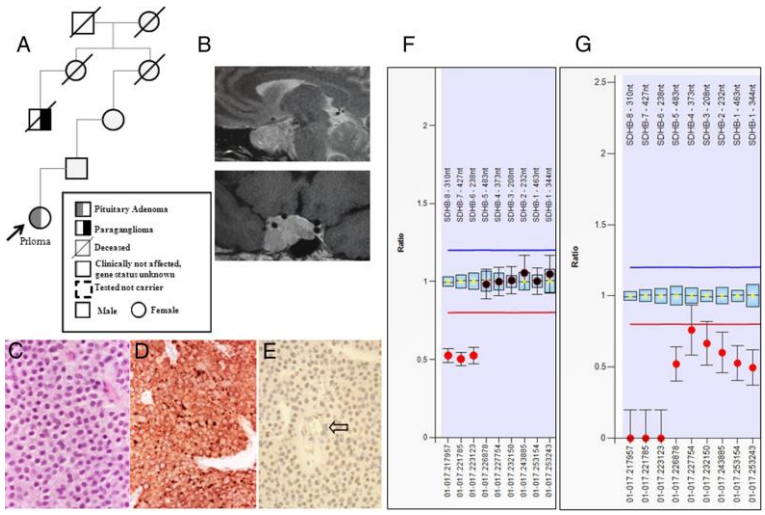


Fig. 4. Pedigree (A) and sagittal and coronal magnetic resonance images of the pituitary adenoma (B) are shown. C, H&E-stained section (X20) shows that the tumor of patient 5 contains multiple vacuoles. D, The immunoreaction with the anti-113-1 antibody (immunoperoxidase, X20) highlights the mitochondria content. E, SDHB immunostaining shows loss of expression in neoplastic cells, whereas endothelial cells (arrow) retain the expression (immunoperoxidase, X20). Loss of the *SDHB* gene in germline and pituitary tumor tissue in patient 5. F, Germline DNA shows a deletion affecting MLPA *SDHB* probes 6–8 in DNA derived from leukocytes. G, In pituitary adenoma tissue, a complete loss of genetic material at the *SDHB* probes 6–8 area and heterozygous loss of *SDHB* probes 1–5.

This staining documented variable accumulation of mitochondria in *SDHX* mutation-positive PA cells. Some adenomas in particular showed increased immunostaining compared

with the other cases (**Figs. 2F and 4D**) in keeping with the focal oncocytic changes observed in the H&E-stained sections.

Vacuoles did not appear to be rimmed by this protein, suggesting that vacuolization is not secondary to dilatation of mitochondria. To understand whether vacuoles were the result of swelling of the endoplasmic reticulum (ER), we immunostained our samples for the ER marker ERLEC1.None of the vacuoles was lined by this protein, indicating that they were not related to the ER.

Menin staining of the pheochromocytoma samples of the patients with *MEN1* mutations showed either no menin positive cells or weakly positive staining nuclei (**Fig.5**).

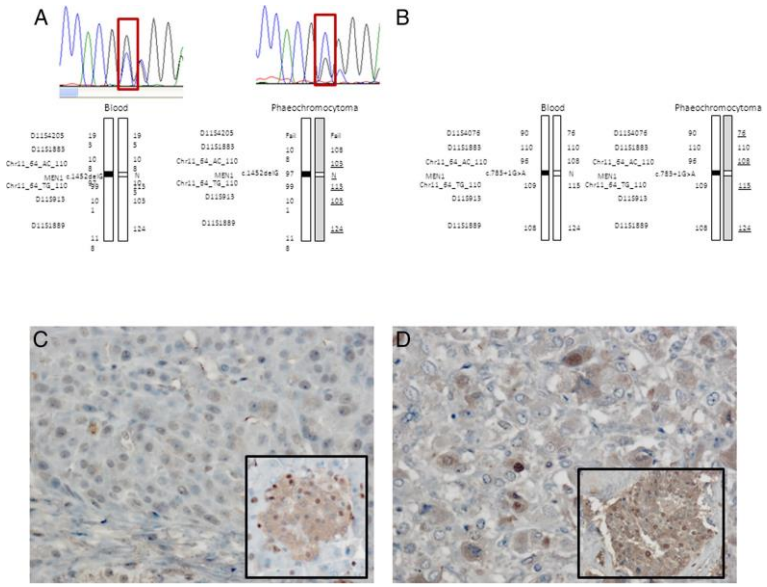


Fig. 5. A, LOH analysis at the *MEN1* locus of the pheochromocytoma of patient 22 and patient 23 (B). Underlined microsatellite results identify markers that show a reduction in peak height in the pheochromocytoma sample compared with blood, indicating LOH but suggesting that some nontumoral tissue was also retained in the operated samples. C, Pheochromocytoma of patient 22 shows a loss of menin staining (inset: positive menin staining in mouse Langerhans islet). D, The menin staining of the pheochromocytoma of patient 23 shows some weakly positive staining nuclei (inset: positive menin staining in a sporadic pheochromocytoma used as a positive control).

1.3.4. Discussion

Syndromic presentation of PA and pheo/PGL is rare, and it is not part of the classical multiple endocrine tumour syndromes. This study describes, we believe, the largest cohort of patients with PAs and pheo/PGLs. Systematic testing of this population for alterations of the known pituitary and pheo/PGL-related genes suggest that SDH mutations play a pathogenic role in the development of PAs in some of these patients.

Cases of other pheo/PGL genes associated with PA, *VHL* and *RET*, are exceptionally rare. On the other hand, the *MEN1* mutations can sometimes lead to pheo/PGLs, as suggested previously (Jamilloux et al., 2014), and here we present supporting LOH and immunostaining findings. An endocrine rather than genetic association occurs when pheochromocytomas secrete hypothalamic-releasing hormones (GHRH or CRH) mimicking the PA and pheo/PGL syndrome, described previously in eight cases.

Although in these cases only the adrenal gland harbors a tumor whereas the pituitary usually displays hyperplasia in response to the ectopic hormone secretion, this is a relevant clinical differential diagnostic scenario and should be kept in mind in patients with pituitary disease and pheo/PGLs. In approximately half of our cases, no germline abnormalities were seen, suggesting either the presence of other disease-causing genes or the coincidental occurrence of the pituitary and pheo/PGL tumors.

Because this is a multicentric study with a patient cohort from all over the world, with a heterogeneous genetic background, it is difficult to estimate whether the coincidence of these two tumors occurred randomly, or other, not-yet-specified genetic factors could be playing a role. Using the ranges of the available prevalence data for PAs and pheo/PGLs in the general population (Mazzaglia 2012; Eisenhofer et al., 2013), the coincidental chance for the two diseases occurring in the same patient ranges between 1 in 2.5 million and 1 in 8.5 million subjects. In our single center (Barts), we reviewed 828 patients with pituitary tumors and 150 with pheo/ PGL (Herincs et al., 2013; Srirangalingam et al., 2014).

Assuming a maximum population frequency of pheo/PGL of 1 in 2500, we predict that 0.33 cases in a population-based series of 828 pituitary adenoma patients would have a pheo/PGL, whereas the actual frequency in patients seen at our center was 2 in 828 (P = .048; Fisher's exact test on single proportions). Likewise, assuming the maximum population frequency of PA of 1 in 1000, we expect 0.06 cases in a population-based series of 150 pheo/PGL patients would have a PA, whereas the actual frequency is 2 in 150 (P = .01). Both of these data sets suggest an increased incidence.

Of the six suggested explanations for the coexistence of PA and pheo/PGL that we outlined in the introductory text, we could confirm the following options: 1) a pheo/ PGL-related gene causes PA, 2) a pituitary gene causes pheo/PGL, 5) ectopic hypothalamic hormone synthesis in a pheochromocytoma, and probably one or more families in our cohort match option, and 6) representing pure co- incidence. Regarding option 3, we have not found any patients with mutations in two genes, such as a classical pheo/PGL and a pituitary tumor gene. In addition, we found LOH at the SDH locus in pituitary adenomas and at the MEN1 locus in pheochromocytomas, suggesting, although not proving, that in these patients a single gene is responsible for both tumors.

Exome or whole-genome sequencing studies in the future might find novel genes causing both diseases (option 4). In our cohort 19 patients (48%) had a germline alteration, among them 17 (43%) with a genetic variant in the pheo/PGL genes. Large studies showed that about one-third of pheo/PGL patients (most familial cases and 10%–20% of the sporadic cases) carry a germline mutation in *RET*, *VHL*, *NF1*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, *SDHAF2*, *MAX*, or *TMEM127* genes (Almeida and Stratakis 2010; Gimenez-Roqueplo et al., 2012), suggesting that our cohort may have a slightly higher percentage of germline alterations.

More recently, three screening studies have been performed. One of them screened a group of patients (26 PGL patients and eight carriers) with a particular *SDHD* mutation due to a founder effect for the presence of a PA. One GH-secreting macroadenoma and three nonfunctioning microadenomas (suggested to be incidentalomas) were diagnosed in this patient cohort. No LOH was found at the *SDHD* locus in the GH-secreting PA (Dematti et al., 2013). In the second study, 309 PAs were screened for *SDH* mutations and a macroprolactinoma with two different somatic *SDHA* mutations with normal sequence in the

germline (Gill et al., 2014) was found. In the third study, screening has been performed in *SDHX*-mutated patients for nonpheo/PGL tumors. Two patients with *SDHD* mutations were found to have a PA, and in one of these cases, LOH at the *SDHD* locus was shown in the macroprolactinoma (Papathomas et al., 2014). Whether it is cost effective to measure prolactin in patients with pheo/PGLs needs to be studied further.

Summarizing present cases combined with the cases available in the literature (altogether 109 cases since 1952), the research team have tried to identify any particular features for each gene alteration for the tumor not classically associated with that gene. Twenty cases have a confirmed *SDHX* mutation with pituitary adenoma [(two *SDHA* (Gill et al., 2014), eight *SDHB* (Benn et al., 2006; Majumdar et al., 2010), two *SDHC* (Lopez-Jimenez et al., 2008), and eight *SDHD* (Varsavsky et al., 2013; Xekouki et al., 2012)]. *The patients with an SDH mutation had various PA types: nine macroprolactinomas, three somatotroph adenomas, and five NFPAs have been described. In three cases the PA subtypes could not be classified. All the PAs were macroadenomas, except for three nonfunctioning microadenomas (possibly incidentalomas). The patients needed one to four therapeutic interventions. Five patients needed a single therapeutic intervention, five patients needed two, one patient needed three, and two patients needed four therapeutic interven- tions*. Of the 109 patients, five patients had *RET* mutations (Brauer et al., 2004; Saito et al., 2010; Heinlen et al., 2011; Lugli et al., 2012); two cases with acromegaly, two cases with prolactinoma, and one NFPA (one macroadenoma and one microadenoma, and in three cases the adenoma size is not available). Four patients needed one therapeutic intervention (three surgeries and one medical treatment), whereas one patient needed medical therapy after transsphenoidal resection of the pituitary tumor. Two patients had a *VHL* mutation, one with a PRL and one with a GH- and PRL-secreting adenoma. Six patients had a confirmed *MEN1* mutation and pheo/PGL (Jamilloux et al., 2014): five patients with pheochromocytoma and one head and neck PGL.

It was identified a novel feature of the PAs of patients harboring SDHX variants. The adenoma tissues show extensive vacuolization of cytoplasm with features reminiscent of signet-ring cells or physalipherous cells (Klijanienko et al., 2011). *The origin of vacuoles remains unclear. Lipid and glycogen accumulation was suggested in the literature, but none of the vacuoles indented the nucleus as commonly seen in cells with accumulation of lipids and the histochemical stain PAS/diastase-resistant periodic acid of Schiff did not reveal any glycogen accumulation.* The vacuoles also do not resemble particle rich cytoplasmic structures, described in epithelial neoplasms (Necchi et al., 2011). Vacuolization of the nontumorous adenohypophyseal cells has been described in cases of fatal hypothermia in two separate studies (Ishikawa et al., 2004; Doberentz et al., 2011). Ishikawa suggested that the vacuoles are different from dilated cisternae of rough ER and from distended Golgi apparatus, which are the result of castration or gonadal dysfunction and raised the possibility that they are lipid droplets due to metabolic dysfunction initiated by the hypothermia (Ishikawa et al., 2004). Doberentz also noted cytoplasmic vacuolation of the anterior pituitary cells in the case of hypothermia, and they suggested that this could be due to gradually developing tissue hypoxia (Doberentz et al., 2011). Oncocytic PAs have recently been identified to contain somatic mutations affecting mitochondrial respiratory chain complex I,

but these tumors do not show the vacuolar changes we have identified in the *SDH*-related samples (Kurelac et al., 2013).

Inactivation of succinate dehydrogenase or VHL can lead to activation of the hypoxia inducible factor pathway and a pseudohypoxic state. Indeed, we have shown increased hypoxia inducible factor-1α in an *SDHD*-mutated case linked to pituitary adenoma (Xekouki et al., 2012). *It is not known whether the vacuoles seen in the SDH-related tumors are due to the pseudohypoxic state, but we did not observe this phenomenon in the VHL mutation-related PA.*

Immunostaining for a mitochondrial membrane pro- tein or for an ER marker did not prove that the vacuoles arise from these organelles. Electron microscopy was used to identify the nature of the vacuoles, but this was inconclusive due to the poor preservation of formalin-fixed tissue recovered from paraffin (data not shown). These vacuoles were not specifically described in the stud- ies of recently published *SDHX* mutations associated with PAs, but based on the available histological pictures, the presence of vacuoles cannot be ruled out (Xekouki et al., 2012). Vacuoles have been described in *SDHB* mutation-related renal carcinoma and were attributed to giant mitochondria (Housley et al., 2010), but the clear cytoplasm observed in these tumors can also represent glycogen or fat (Srigley et al., 2013). Large cytoplasmic vacuoles suggested to be mitochondria based on electron mi- croscopy have previously been described in PAs (Horoupian 1980), possibly due to ischemia. Acidophil stem cell adenomas can also contain paranuclear vacuoles resulting from giant mitochondria (Horvath et al., 1981).

The activity of certain mitochondrial enzymes involved in oxidative phosphorylation is decreased in cancer cells compared with normal tissue (Kroemer 2006). Taking into account that succinate dehydrogenase enzymes, being part of the mitochondrial complex II, play an important role in mitochondrial function, mutations that affect the activity of these enzymes might have a role in mitochondria dysfunction (Zhan and Desiderio 2010). *It is possible that the vacuoles represent a hallmark of PA in patients with the SDHX variant, but their nature remains to be further investigated. In addition, further study of the metabolic pathways in SDH-related endocrine tumors are awaited.*

This study has several shortcomings. First of all, the centers that are part of the study might attract more unusual genetic conditions, therefore representing a higher prevalence of these cases. In a significant portion of the patients, tumor samples were not available, often due to the lack of surgical intervention; therefore, no appropriate material was available for LOH or to study in further detail the unusual histological phenotype in the PAs.

1.3.5. Final remarks

In summary, germline mutations were identified in the studied genes in 11 of 27 kindreds with the combination of pheo/PGL and PAs. LOH at the SDHB locus in the PA samples and LOH at the MEN1 locus in the pheochro- mocytoma samples was demonstrated, suggesting, although not proving, the pathogenic role of these genes in these nonclassically disease-specific tissues. In addition, intracytoplasmic vacuoles in PAs of patients affected by SDH mutations were observed for the first time. Together with the single case reports available in the literature, this large cohort supports the hypothesis that in some families

SDH mutations may have a role in PA formation and MEN1 mutations may have a role in the development of pheochromocytoma. Whether screening for PAs in SDHX patients is warranted needs to be studied in the future, but this findings suggest that genetic testing for germline mutations in SDHX and MEN1 should be considered in patients with the constellation of pheo/PGLs and PAs.

1.4. Targeting endocrine system - what is it vulnerable to?

1.4.1. Introduction

A great number of natural or synthetic substances have been identified to disrupt thefunctioning of the endocrine system and to produce effects in hormone targets tissues and organs in both human and animals. These substances (chemicals) are referred to as endocrine disrupting chemicals (EDCs) (Henley and Korach, 2010; Mnif et al., 2011).

According to the WHO, a EDCs is an exogenous substance or mixture that alters function(s) of endocrine system and consequently causes adverse health effects in an intact organism, or its progeny or (sub)population (Danulescu et al., 2011; Bruni et al., 2002; Parent et al., 2011; Robu et al., 2007). The Environmental Protection Agency (EPA), define EDCs as an exogenous agent that interferes with synthesis, secretion, transport, metabolism, binding action, or elimination of natural blood-borne hormones that are responsible for homeostasis, reproduction, and developmental processes (Craig et al., 2011).

A subtype of EDCS are the neuroendocrine disruptors defined as *pollutants in the environment that are capable of acting as agonists/antagonists or modulators of the synthesis and/or metabolism of neuropeptides, neurotransmitters, or neurohormones which subsequently alter diverse physiological, behavioral, or hormonal processes to affect an animal's capacity to reproduce, develop and grow, or deal with stress and other challenges* (Van V oorhis et al., 1992).

Personal contribution – published paper:

Preda C, Ungureanu MC, Vulpoi C.Endocrine disruptors in the environment and their impact on human health. *Environ Eng Manag J* 2012; 11(9): 1697-1706.

The present review focuses on: endocrine disruptors mechanisms of action, examples of compounds that alter the endocrine system and the effect on human health.

1.4.2. Endocrine disruptors- general data

Endocrine Disruptors (EDCs) are compounds, of natural or synthetic provenience, which through environmental or inappropriate developmental exposures alters the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment (Diamanti- Kandarakis et al., 2009) (**Fig. 6**).

EDCs can influence the normal function of the endocrine system by affecting glands and hormones that regulate vital body functions: metabolic rate, sex development, insulin production and utilization, growth, stress response, gender behavior, reproduction.

EDCs may also disrupt the gene-controlled, normal signaling systems that determine the fetal development (Bernal and Jirle, 2010; Newbold, 2010) and act via nuclear receptors, through membrane receptors, neurotransmitter receptors, orphan receptors and enzymatic pathways involved in the synthesis of hormones (Craig et al., 2011).

The studies on endocrine disruption are not of recent data, since the phenomenon accompanied the industrial development, in particular industrial chemical synthesis (Solomon and Schettler, 2000). In the 1930s studies on laboratory animals demonstrated estrogenic effects of some industrial chemicals including bisphenol A, now widely used in plastics, resins and dental sealants (Solomon and Schettler, 2000; McCally, 2002).

The feminizing effect of the pesticide DDT (dichlorodiphenyltrichloroethane) in roosters was reported in the 1950s (Saalu and Osinubi, 2009). EDCs are environmental pollutants (pesticides, industrial byproducts and chemical used in manufacturing-particularly plastics) (Caliman and Gavrilescu, 2009; Robins et al., 2011) which are taken up through food, drinks or through the air and they are also absorbed transdermally (Wuttke et al., 2010).

The mechanism of action of EDCs is actively studied, but the consequences of endocrine disruption at the population level and the adaptations to cope with chronic EDCs exposure have been overlooked (Carere et al., 2006).

A recent report of Euopean Environment Agency reveals that, in the last 10 years, "many new computational predictors and both *in vitro* and *in vivo* assays for EDCs have been developed that greatly enhance the ability to study mechanisms of action and to screen large numbers of new and existing chemicals for hormone activity, so as to ensure their safety" (EEA, 2012).

Humans and animals are exposed to a complex range of chemical substances from the environment (**Fig. 7**). Chemicals in air, water soil and food, professional conditions and lifestyle factors, all contribute to a complex exposure situation in our daily life (Olujimi et al., 2010; Silins and Hogberg, 2011). The ability of exogenous substances to interfere with the endocrine system was known from the past: for centuries, farmers have observed reproductive problems in female sheep and cows grazing on pastures rich in certain clover species (containing estrogenic compounds such as coumestrol) (Marty et al., 2011).

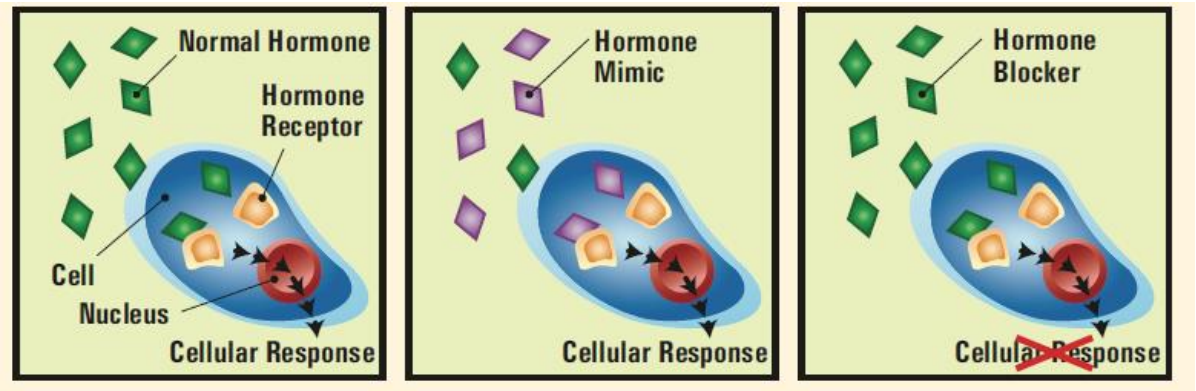
A decade ago, the focal point of both concern and action regarding EDCs was on hormone receptor agonists and antagonists, in particular oestrogen receptor (ER) agonists. The screening and assessment systems for ER- or androgen receptor (AR)-mediated hormone activity continue to be broadly used (EEA, 2012; Heinlein and Chang, 2002; Napoli et al., 2012).

The development and improvement of risk assessment procedures for EDCs exposure is an issue of many authorities world-wide (Hlihor et al., 2009; WHO, USA and the European Union). Several recent studies indicate that endocrine chemicals may interact in complex ways even in a way similar to that of many carcinogens (Caliman and Gavrilescu, 2009; EEA, 2012).

The existence of specific receptors in target cells allows the hormone- mimicking effect of endocrine disruptors. The 16th century Paracelsus observation's that "the dose

makes the poison" is no longer valid. Very low dose can enhance the production of receptors (receptor up- regulation) resulting in a stronger response, while higher doses can inhibit receptors (receptor down-regulation) resulting in a weaker response (Peterson Myers et al., 2009).

The timing of exposure to EDCs is important in determining its specific effect. Even low exposure during foetal or early life periods on EDCs has caused concern due to serious consequences later in life (Silins and Hogberg, 2011). Early in life exposure to endogenous sex hormones particularly in fetal life and infancy, organize the brain in a sexually dimorphic manner that becomes activated later in life. Exposure to exogenous substances such as EDCs is likely to have more profound detrimental consequences in developing organisms than in adults (Gore, 2010).



a b c

Fig. 6. Schematic representation of EDCs action when absorbed in the body: a) can decrease or increase normal hormone levels, b) mimic the body's natural hormones, c) alter the natural production of hormones (NIEHS, 2010; Swedenborg et al., 2009)

How the early life exposure can promote an adult onset disease is a serious question and is presumed to involved epigenetic mechanisms (Skinner et al., 2010). Synthetic EDCs such as: industrial lubricants, pesticides and plasticizers are frequently associated with alarming statistics regarding reproductive diseases, obesity and cancer (Patisaul and Jefferson, 2010).

1.4.3. Phytoestrogens

The increased effort to implement healthier eating lifestyles resulted in increased consumption of soy products which in turn has caused an important exposure to phytoestrogens (Newbold, 2010).

Phytoestrogens are plant compounds that are similar (as structure and/or function) to mammalian estrogens and their active metabolites, they have polyphenolic structures and can be classified in three major classes (Shanle and Xu, 2011).

One major classes is the flavonoids with subgroups as: flavanones (eriodictyol, hesperetin, homoeriodictyol, naringenin) found in citrus fruits and juices, flavones (apigenin, luteolin, tangeritin) found in parsley, celery, capsicum pepper, flavonols (fisetin, kaempferol, myricetin, pachypodol, quercetin, rhamnazin) found in kale, broccoli, onions, tomatoes,

lettuce, apples, grapes, red wine and catechins (proanthocyanides) found in chocolate, green tea, beans, apricots, cherries, berries.

Another major class is the isoflavonoids with subgroups as: isoflavones (biochanin A, clycitein, daidzein, formononetin, genistein) found in soy beans, isoflavans (equolmetabolite of daidzein), coumestans (coumestrol) found in clover, alfalfa, spinach. The lignans are also a major class of phytoestrogens and they are components of plant cell walls and found in many fiber-rich foods such as: grains, seeds, nuts and fruits (Senti, 1974; Thomas and Lutz, 2001; Patisaul and Jefferson, 2010).

Exposure to phytoestrogens occurs through dietary intake of food and beverages containing herbs, fruits and vegetables (mainly soy) (Shanle and Xu, 2011).

Soy is a popular food additive because is a vegetable protein high in fiber and unsaturated fats and free of lactose and cholesterol. Energy bars, sports drinks, cereals, granola bars and imitation dairy products are enriched with soy protein (Patisaul and Jefferson, 2010). About 25% of infant formulas are soy-based and urinary concentration of phytoestrogens-daidzein and genistein was 500 times higher in infants fed with soy-based formula compare with those fed with cow milk (Shanle and Xu, 2011).

Also textured soy protein is a meat substitute found in: hamburgers, sausages and hotdogs (Patisaul and Jefferson, 2010). Isoflavones are also used as an alternative for hormone replacement therapy in menopaused women in order to prevent osteoporosis or arteriosclerosis (Wuttke et al., 2010).

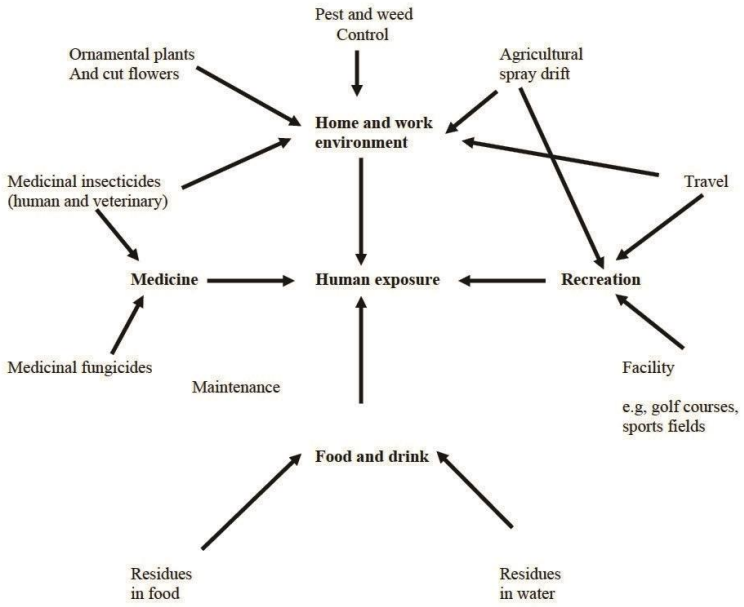


Fig. 7. Exposure routes of humans to EDCs (McKinlay et al., 2008; Olujimi et al., 2010)

The impact of soy or soy phytoestrogens consumption on human health, according with clinical and experimental studies is still unclear. The answer is complex and may depend on: health status, age, level of consumption (Patisaul and Jefferson, 2010). The phytoestrogens acts via estrogen receptors (ER). There are two subtypes: ERα and ERβ and after phytoestrogen binding an ER-dependent, gene transcription is activated. Once bound

isoflavones do not act as estrogen agonists but more like selective estrogen receptor modulators (SERM) (Patisaul and Jefferson, 2010).

Most phytoestrogens bind ERβ and this fact is significant in relation with the differentially distribution of ER throughout the body and the brain. Another pattern for the phytoestrogens action is the binding on membrane receptors with activation of the second messenger pathways (the first accepted transmembrane ER, GPR30, is capable of binding a wide range of EDCs including genistein) (Patisaul and Jefferson, 2010).

By stimulating sex hormone binding globulin (SHBG) synthesis in liver cells and competitively displacing either 17β-estradiol or testosterone from plasma SHBG, phytoestrogens can manipulate steroid synthesis and transport (Patisaul and Jefferson, 2010). Some phytoestrogens (e.g. coumestrol, genistein) interferes with enzymes needed for steroid biosynthesis.

Administration of coumestrol and equol to newborn mice enhances DNA methylation involving alterations in epigenetic processes (Skinner et al., 2010). Genistein and other phytoestrogens have been shown to cross the placenta and - in utero- exposure may interfere with the ovarian function later in life (Zama and Uzumcu, 2010). Genistein can affect adipose tissue fat deposition according to recent studies, the effects being dose-dependent and gender specific (Newbold, 2010).

It is unclear how phytoestrogens administration may impact with breast cancer risk, precocious or delayed puberty (Ozen and Darcan, 2011), brain and reproductive tract disregulation (androgen insufficiency with undermasculinisation of the male uro-genital tract) (Svechnikov et al., 2010), behavior disturbances, uterine fibroids development.

Consumers should be aware that soy contains endocrine disrupting compounds and make dietary choices accordingly (Patisaul and Jefferson, 2010). Pregnant women or those who want to become pregnant should use soy foods with caution (Patisaul and Jefferson, 2010). Also the effects of dietary isoflavones in newborns and climacteric/ postmenopausal women are of great concern and need to be further investigated (Wuttke et al., 2010).

1.4.4. Bisphenol A (BPA)

BPA was first described in 1891, initially developed as a pharmacological for estrogen replacement therapy (Robins et al., 2011). In the 1950's BPA was rediscovered and polymerized to make polycarbonate plastic and then until now it has been used in the plastic industry (Alonso-Magdalena et al., 2010).

BPA is the primary building block of polycarbonate plastic and component of epoxy resins being used in the manufacture of: food cans, polycarbonate baby bottles, beverage containers, dental sealants and composites (Newbold, 2010; Zoeller, 2010). BPA is also halogenated (brominated or chlorinated) to produce flame retardants (Newbold, 2010; Zoeller, 2010). BPA is one of the highest volume chemicals produced worldwide, human population being routinely exposed to this chemical through numerous sources and routes (Newbold, 2010). Daily exposure is possible by: carbonless print paper, lining inside cans, baby formula cans and plastic food containers (when heated) (Badawi et al., 2000), milk cartoons and other paper-board containers commonly used to package food and beverages (McAllister and al., 2010). BPA exposure has become an important health concern based on

it’s capability to enter the materials contained within them (Craig et al., 2011). Studies report that BPA is found in serum of pregnant women, in the amniotic fluid of their fetus, in placenta and in cord serum taken at birth (Zoeller, 2010).

Regarding the structure, BPA is similar to diethylstilbestrol, having two fenolic rings and bind to the nuclear estrogen receptor α and β. Taking into account the low affinity of BPA for estrogen receptor, it is likely that the estrogenic effects of BPA are due to non-genomic estrogen receptor signaling. BPA's molecular structure is similar to that of estradiol, one of the human body's three main estrogens, suggesting that BPA binds to estrogen receptors (**Fig. 8**) (Parker, 2012). In binding to the estrogen receptor, BPA can disrupt the body's hormonal system, with the most troubling consequences for fetuses, infants and young children (Parker, 2012; Shanle and Xu, 2011). MBP has a 100-fold to 1,000-fold stronger bond to the estrogen receptor than BPA; however, the structural basis for MBP’s high affinity for the estrogen receptor was not investigated further (http://www.foodproduct design.com/news/2012/10/study-says-metabolized- bpa-poses-bigger-threat.aspx).

Also BPA may interact with a variety of other cellular targets including: non-classical membrane- bound form of the estrogen receptor, nuclear receptor termed estrogen-related receptor gamma (Newbold, 2010). General hormone imbalance, as a result of BPA exposure, has been well documented in animal system (humans included) with disregulation of all sex hormones (Speranza, 2010). BPA can contribute in developing psychological dependence on drugs through the brain adverse effects (Speranza, 2010).

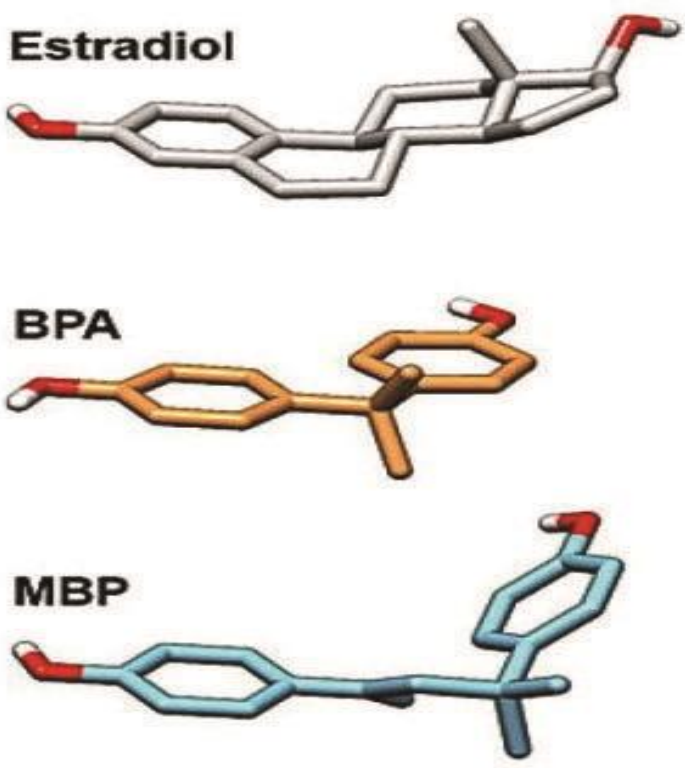


Fig. 8. Conformational structure of Estradiol, BPA and MBP with the relevant similarities (Parker, 2012)

Several experimental animal studies have shown that BPA decreases placental aromatase activity (Robins et al., 2011) and is associated with abortion (Zoeller, 2010) and a variety of abnormalities in the male and female reproductive and mammary gland tissues (Newbold, 2010).

The disruption of pancreatic β cell function and blood homeostasis (in mice) and the acceleration of adipocyte formation may connect BPA to the development of obesity (Newbold, 2010) and so considering as an "obesogen" (Hatch et al., 2010). In adult humans, epidemiological studies reflect BPA as an important risk factor for type 2 diabetes (Alonso-Magdalena et al., 2010). Also, BPA can interfere with thyroid receptor signaling both in vitro and in vivo (Zoeller, 2010). Perinatal exposure to low BPA doses caused: disrupted ovarian morphology, accelerated puberty and changes in body weight (Zama and Uzumcu, 2010). Disruption of the endocrine environment can alter metabolism and susceptibility to cardiovascular diseases may increase in humans (Speranza, 2010).

Much remains to be determined about the mechanism of action of BPA, haw BPA is metabolized, and whether animal models are relevant for modeling human exposure (Taylor et al., 2011).

1.4.5. Diethylstilbestrol (DES)

DES is a non-steroidal synthetic estrogen and during 1940-1971 was prescribed to pregnant women in an attempt to prevent miscarriages and premature births (Henley and Korach, 2010; Newbold, 2010; Craig et al., 2011). It was estimated that a range of 2 to 8 million pregnancies were exposed to DES (Newbold, 2010). Beginning with 1953 several studies showed that DES had no protective effects against miscarriages and premature births and the drug was banned on the use during pregnancy only in 1971 (Craig and al., 2011). The production or marketing of this this chemical is prohibited since 1997 (Ozen and Darcan, 2011). More than that, numerous studies showed multi-generational effects of DES on reproductive, cardiovascular, and immune system.

Today it is well known that perinatal DES exposure lead to a significant increase in neoplastic and benign lesions (Newbold, 2010) in both female and male offspring: anatomical malformations of the cervix, vagina and uterus, decreased fertility, vaginal clear cell adenocarcinoma, testicular hypoplasia, cryptorchidism and epididymal cysts (Henley and Korach, 2010).

DES has been a model for EDC action and has been studied due to its adverse impact on humans in utero (Shanle and Xu, 2011).

DES mimics the natural estrogen pattern, binding both α and β estrogen receptors; due to its high affinity for the receptors it is a potent transcriptional activator through genomic signaling (Shanle and Xu, 2011). More recent studies suggest another mechanism: non-genomic estrogen signaling (Nadal et al., 2000; Bredfeldt et al., 2010; Shanle and Xu, 2011). DES can be considered as an "obesogen" in relation with the development of obesity in perinatal DES treated individuals (Newbold, 2010). Also the effects on the sexual dimorphism of the brain in relation with DES use have been documented (Zama and Uzumcu, 2010). The DES paradigm was a clear example that prenatal exposure could lead to adult-onset disease (Newbold, 2010).

1.4.6. Phthalates

Phthalates are synthetic chemicals used to increase the flexibility of polyvinyl chloride plastics in beauty and infant products, medical devices and the enteric coating of some medication (Lovekamp- Swan and Davis, 2003; Craig et al., 2011; Robins et al., 2011), ink solvents, food packaging (Svechnikov et al., 2010). They are easily released in to the environment do to the fact that they are weakly bound to the plastic (Robins et al., 2011). The human exposure to these ubiquitous xenobiotics occurs by ingesting contaminated food and by applying make- up (Robins et al., 2011).

There are strong evidences that phthalates and their metabolites (diethylhexyl phthalate-DEHP and monoethylhexyl phthalate-MEHP) are potent reproductive teratogens in male and female animal models. Also in human granulosa-lutein cells, MEHP suppressed basal and stimulated estrogen secretion and decreased aromatase activity (Craig et al., 2011). Others than MEHP and DEHP, e.g. dioctylphthalate (DOP), diisononylphthalate (DiNP) and diisodecylphthalate (DiDP) posses endocrine- disrupting capacities, by interfering with the progesterone production of the ovary (Gregoraszczuk, 2002; Craig et al., 2011).

In male mice the phthalates exposure decreases the anogenital distance. In male, the development of testis is affected leading to abnormalities in spermatogenesis and hormonogenesis (**Fig. 9**) (Hu et al., 2009; Svechnikov et al., 2010; Robins et al., 2011).

Environmental effects have been assumed to contribute to the growing occurrence of testicular dysgenesis syndrome (TDS) in humans (i.e. cryptorchidism and hypospadias in newborn boys and testicular cancer and reduced sperm quality in adult males) (Hu et al., 2009). Also, prenatal exposure to phthalates affects Leydig cell function in the postnatal testis. Recent progress was performed in understanding of how Leydig cell factors contribute to phthalate-mediated TDS (Hu et al., 2009; Johnson et al., 2012).

In females phthalates have been associated with: uterine abnormalities, anovulation, and foetal development in pregnant women (Silins and Hogberg, 2011), implantation or placentation (Robins et al., 2011).

Several studies have correlated phthalates levels with abnormal pubertal development (Colon et al., 2000; Qiao et al., 2007; Robins et al., 2011) and with occurrence of obesity (Hatch et al., 2010).

However, additional studies are needed for more definitive conclusions regarding the consequences of phthalates exposure on human health.

1.4.7.Pesticides

In order to increase agricultural productivity, numerous pesticides have been discovered and used since 1939, without guidelines or restriction (Mnif et al., 2011). They are widely used not only for agricultural purpose but also for municipal, home and medical use. Despite the benefits (control of agricultural pests and plant disease vectors) pesticide may persist in soils and aquatic water sediments, move up trophic chains and affect top predators (Mnif et al., 2011).

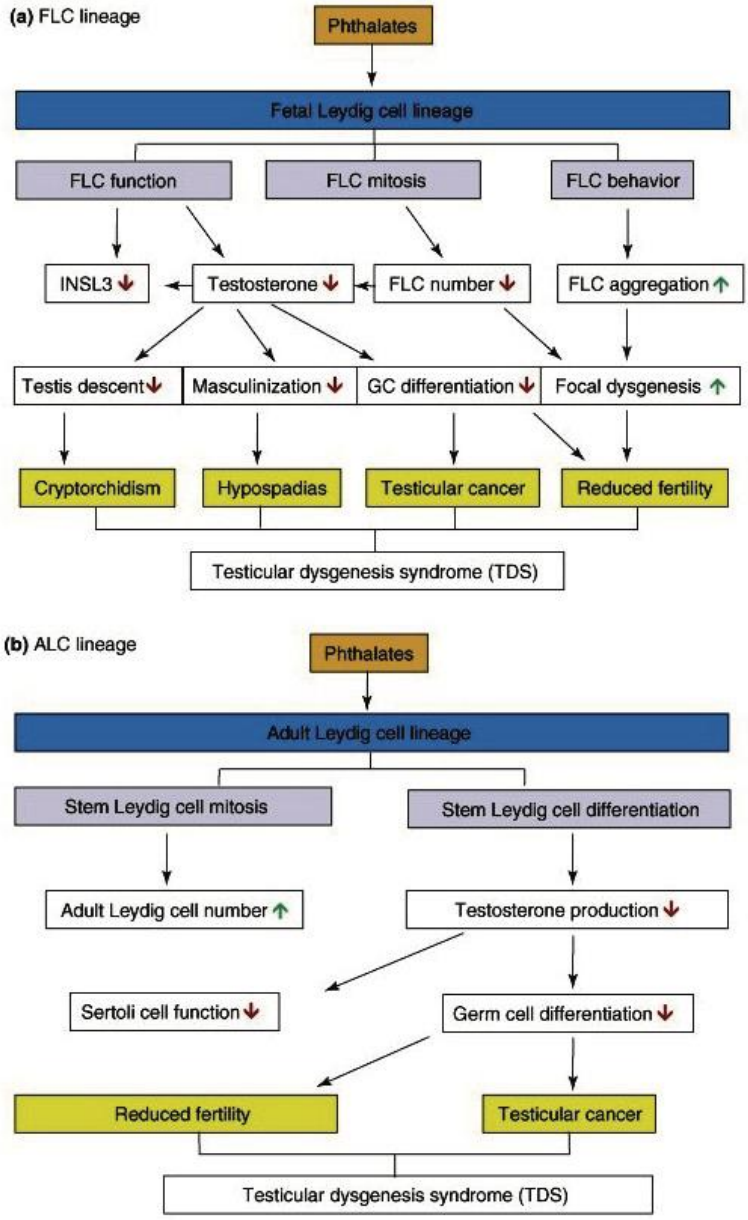


Fig. 9. Effects of phthalates on (a) fetal Leydig cell (FLC) lineage: (b) adult Leydig cell (ALC) lineage after in utero exposures, causing testicular dysgenesis syndrome (FLC, fetal Leydig cell; INSL3, insulin-like growth factor 3; GC, germ cell; ↓ = decrease or inhibition; ↑ = increase or stimulation) (taken from Hu et al., 2009)

It has been suggested that disease such as cancer, allergies, neurological disorders and reproductive disorders may be connected to pesticide exposure (Mnif et al., 2011).

About 105 substances can be listed as pesticides divided in three major groups: insecticides (46%), herbicides (21%) and fungicides (31%) with different chemical structures: organochlorides, organophosphates, carbamates (Čeh and Majdič, 2010; Mnif et al., 2011).

Human epidemiological studies have shown that there is an important correlation between occupational or nutritional exposure to pesticide and different human pathologies mainly in female fertility (Fuortes et al., 1997; Bretveld et al., 2006; Zama and Uzumcu, 2010).

Pesticide may act as endocrine disruptors via multiples mechanisms: agonist receptor (estrogen receptor, androgen receptor, estrogen related receptor, pregnane X receptor, arylhydrocarbon receptor), antagonist receptor and interfering with the synthesis, transport, metabolism and excretion of hormones (Andersen and Cook, 2002; Čeh and Majdič, 2010; Mnif et al., 2011).

The disruptive effect of pesticides has consequences on: reproductive and sexual development, gametogenesis and early development of the fetus, intellectual function and central nervous system function (**Table 2**) (Caliman and Gavrilescu, 2009; Zama and Uzumcu, 2010; Mnif et al., 2011).

Table 2. Pesticides disrupting the hormone and reproductive system (Andersen and Cook, 2002; Čeh and Majdič, 2010; Mnif et al., 2011)

Effects	*Pesticides*
Estrogenic activity	Amitraz Lindane Parathion-methyl Permethrin Triadimefon s-Triazines
Anti-androgenic activity	Atrazine Lindane Linuron Procynidon Vinclozolin Pyrethroids
Disrupt steroid metabolism	Atrazine Carbofuran Conazole Lindane
Disturb thyroid function	Amitrole Dithiocarbamates Ioxynil Metribuzin Certain pyrethroids Trifluralin
Influence on gonadothrophic hormones	Amitraz Atrazine Certain organophosphates Some dithiocarbamates
Influence on spermatogenesis	Cooper fungicides Certain pyrethroids Some dithiocarbamates Glyphosate Some organophosphates
Reproductive toxicity	2,4 – D Some dithiocarbamates Some organophosphates

Pesticides such as: thiram, molinate, metam sodium, chlordimeform, amitraz, triazole, dichloroacetic acid, atrazine, propazine, simazine and linuron may impair neuroendocrine

functions through their effect on the central nervous system and the hypothalamic-hypophyseal-gonadal axis in animal models (Ozen and Darcan, 2011). The pesticide prochloraz suppresses both estrogen and androgen synthesis through enzymatic inhibition also in animals (Ozen and Darcan, 2011).

Dichlorodiphenyltrichloroethane (DDT) an organochlorine pesticide, used in the 1940's as a broad-spectrum insecticide, was banned in the 1970's due to the estrogenic effect and interference in pubertal development (Tiemann, 2008; Craig et al., 2011; Ozen and Darcan, 2011; Shanle and Xu, 2011).

Methoxychlor, an organochlorine pesticide introduced as an alternative to DDT, was also banned in the United States due to its endocrine disrupting proprieties (Shanle and Xu, 2011). It was detected in human adipose tissue, it was shown to impair reproductive behavior and functions in male rat but there are no studies on the effect of this pesticide on precocious puberty in humans (Ozen and Darcan, 2011; Shanle and Xu, 2011). Epigenetic analyses using bisulfite-sequencing PCR and methylation- specific PCR showed that methoxychlor caused hyper-metilation in ER β promoter sequences and had no effect in ER α promoter (Zama and Uzumcu, 2010).

Not only the direct contact with the pesticide is dangerous but also the residential proximity to agricultural activity may lead to: low birth weight, fetal death or childhood cancer (Reynolds et al., 2002; Mnif et al., 2011).

In a Danish study more frequent genital abnormalities in boys and earlier puberty in girls were observed in children of greenhouse owners even so, no pesticide analysis was done in this study (Ozen and Darcan, 2011).

The risk of breast-estrogen dependent cancer and prostate cancer is raised by several epidemiological studies but the relation with pesticide use is not yet formally demonstrated (Mnif et al., 2011).

The combined actions of pesticides are very important in the risk assessment process because mixtures of these substances may cause higher toxic effects that those from a single compound (Mnif et al., 2011).

1.4.8. Dioxins

Dioxins are a class of chemicals (polychlorinated dibenzo-p-dioxins) which are formed as by-products of incomplete combustion of chlorinated waste and in contact of plastics with hot surfaces (Svechnikov et al., 2010; Ozen and Darcan, 2011).

Exposure to dioxins (such as 2,3,7,8- tetrachlorodibenzeno-p-dioxin: TCDD) is possible using products such as: plastic plates and glasses, cleaning substances or paper whitened by chlorine in contact with hot surfaces (Ozen and Darcan, 2011). Also they may be transferred to humans from animal meat or milk. Other sources and the evolution of total environmental releases of dioxins from all quantifiable sources between 1987 and 2000 are shown in **Fig. 10** (USEPA, 2006).

Dioxins bind to aryl hydrocarbon receptor (AhR) and regulate the transcription of target genes (Svechnikov et al., 2010). AhR regulates expression of metabolic enzymes and have many similarities with ER signaling (Shanle and Xu, 2011).

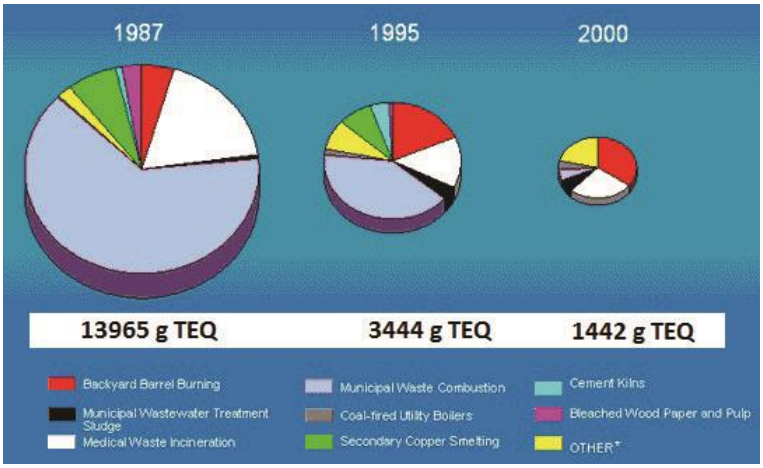


Fig. 10. Dioxin sources contribution (total environmental releases of dioxins from all quantifiable sources decreased by 90% between 1987 and 2000) (taken from USEPA, 2006)

The disruptive actions of dioxins may lead to: reduced expression of sex steroids and LH receptors, inactivation of steroid hormone synthesis or altered steroidogenesis (Badawi et al., 2000; Ohsako et al., 2001; Fukuzawa et al., 2004; Baba et al., 2005; Mutoh et al., 2006; Svechnikov et al., 2010).

1.4.9. Cigarette smoke compounds - endocrine disruptors

Constituents in cigarette smoke as: benzo (a) pirene and cadmium can operate as endocrine disruptors by different mechanisms resulting in either estrogenic or anti-estrogenic effects (Dechanet et al., 2011). Benzo (a) pirene has estrogen-like properties, but in human tissue this estrogenic effect must be demonstrated (Dechanet et al., 2011).

Cadmium also interferes in steroidogenesis and may act as an estrogen-like factor (by binding to ER). Given the fact that chemical structure of cadmium is close to that of calcium (they all have 2+ oxidation states and are similar in size when they are ionized) this may facilitates interaction with intra- cellular calcium signaling (Dechanet et al., 2011). Also the FSH and LH intra-cellular signaling mechanism can be altered by cadmium.

An abnormal endocrine profile as: higher level of FSH, higher level of testosterone and lower estrogen level during ovarian stimulation in vitro fertilization, was mentioned in several studies (Van Voorhis et al., 1992; Cooper et al., 1995; Barbieri et al., 2005) there for exposure to cigarette smoke affect every step of the reproductive process according to time, dose, type and duration of exposure (Dechanet et al., 2011).

1.4.10. Final remarks

Exposure to endocrine disrupting substances is a common circumstance in nature. Even the disrupting mechanism of action is actively studied, the consequences at the population level and the adaptation to chronic exposure have been disregarded. The impacts of endocrine disruptors on endocrine pathway are complex and often occur through multiple direct and indirect mechanisms making it hard to anticipate the endpoints of their toxicity in

animals and humans. Moreover, exposure to EDCs may lead to different effects in different tissues at different life stages. The role of endocrine disruptors as epigenotoxic agents raise the issue of epigenome altering that may influence the health of actual and future population. The influence on reproduction, development and growth, metabolic rate, gender behavior convert EDCs into real health hazards.

The chronic exposure to low levels to an increasing number of chemicals (with possible endocrine-disruptig properties) must determine the regulatory agencies to embrace modern endocrine principles into their risk assessments methodology.

Further studies are necessary to understand the deleterious effects of endocrine disruptor compounds on the endocrine system.

1.5. The effects of bariatric surgery on ghrelin hormone

1.5.1. Introduction

Obesity has become a global epidemic with over 650 million individuals affected by this disease worldwide (Friedrich, 2017). Obesity is strongly associated with hypertension, type 2 diabetes or insulin resistance, dyslipidemia, coronary heart disease, nonalcoholic fatty liver disease (ranging from simple steatosis to steatohepatitis, hepatic cirrhosis and end-stage liver disease), hepatocellular carcinoma and multiple other types of cancer, including colonic and gynecological cancers. This is why the treatment of obesity has become one of the major concerns of health systems in the affected areas, including primarily economically developed countries. To date, bariatric surgery is the only treatment that has shown long-term usefulness.

Of the hormones that are involved in the etiology of obesity, we mention: Norepinephrine – increases energy consumption; Serotonin – decreases food intake; Neuropeptide Y – anabolic promoter – increases food intake and promotes energy storage; CRH – decreases intake of food; Leptin, which is a satiety factor, lowers the expression of neuropeptide Y in the hypothalamus, the main physiological role being the sign of starvation to the brain, because it rapidly falls to the food restriction, the circulating level correlating with the body's fat reserves (Agrawal et al., 2016).

It also leads to cardiometabolic complications and increased risk of cancer, that shortens life expectancy and decreases quality of life. The social and material impact is devastating. Unfortunately, most drug treatments are not effective. Aggressive diets, physical exercise, and cognitive behavioral approaches can result in about 5-15% loss of initial weight, rarely maintained over time and unaccompanied by the remission of complications (Yanovski et al., 2014; Jensen et al., 2014).

Bariatric surgery has been officially approved for nearly three decades (Ann Intern Med 1991) and has strengthened its position over time through a variety of high performance, accessible (laparoscopic) and safe techniques. The number of surgeries has grown impressively (Kang and Le, 2017), both the restrictive and malabsorptive (with or without restrictive component) ones, with comparable results. Studies have confirmed that bariatric surgery is superior to conventional medical treatments, both with regard to stable weight loss

and especially to the regression of complications, reason why it gained the name of metabolic surgery (Sjostrom et al., 2007).

The multitude of evidence of dramatic weight reduction along with a reduction of obesity-related complications and comorbidities including type 2 diabetes mellitus, hypertension, dyslipidemia, nonalcoholic fatty liver disease and obstructive sleep apnea resulted in the recommendation of metabolic surgery in the guidelines for morbid or complicated obesity (Sheka et al., 2018), sometimes as the only efficient treatment.

However, there is evidence that bariatric procedures have nutritional and metabolic abnormalities still incompletely understood as adverse effects. Bone metabolism is often adversely affected, although the degree of this unwanted complication is not sufficiently estimated and the physiopathological mechanisms incompletely elucidated (Chang et al., 2014).

Studies show that after bariatric surgery there is an increase in bone-turnover markers, and a continuous decrease in bone mass and its quality (Stein and Silverberg, 2014).

Sudden weight loss results in skeletal mechanical unloading with consequences on bone mass, strength and size. Mechanical loading results in bone formation by osteocytes (Frost 1987), that act as a 'mechanostat'.

The easiest explanation would be the nutritional deficiency, the decrease in key osteogenic components such as calcium, phosphorus and vitamin D, but it turned out that these changes are just the "tip of an iceberg" of bone remodeling.

Ghrelin is a peptide that releases growth hormone, being synthesized primarily in the stomach. In humans, ghrelin is an appetite-stimulating hormone and at the same time has anabolic effect, decreasing energy expenditure (Mihalache et al., 2016). Thus, it stores energy, acting on the bone by increasing osteoblast proliferation and differentiation through its effects on growth hormone (Maccarinelli et al., 2005) and via mitogen-activated proteinkinase (MAPK) pathway (Delhanty et al., 2006). Ghrelin has an anabolic effect on bone tissue and positively influences trabecular bone density (Napoli et al., 2011). However, literature data on the action of ghrelin on osteoclasts are contradictory, and even less data exist regarding the involvement of ghrelin in bone metabolism after bariatric surgery.

Ghrelin acting through GHS-R theoretically causes weight gain based on the increase in height of individuals and/or lean tissue, similar to GH administration. Basically, the available data clearly demonstrate that central or peripheral administration of ghrelin increases the body fat mass, adipogenesis and lipogenesis (by increasing PPARγ level), with concomitant reduction of lipolysis and use of lipids as energy substrates (Mihalache, 2016).

Peripheral daily administration of acyl-ghrelin for two weeks caused a significant increase in fat mass as measured by dual energy X-ray absorptiometry (Li, 2016). On the other hand, the blockade of the ghrelin receptor abolished the effect of acyl-ghrelin on adiposity (Davies et al., 2009). In vitro experiments demonstrated that ghrelin increases white adipose tissue volume by either stimulating adipogenesis or inhibiting lipolysis and lipid efflux from adipocytes.

Choi reported that ghrelin stimulates adipogenesis via activation of ghrelin receptor subtype 1a in vitro culture of rat preadipocytes (Choi et al., 2003). In contrast, Ott demonstrated no direct effect of ghrelin on adipogenesis by using a well-characterized brown

adipocyte model, even though ghrelin directly suppressed expression of adiponectin, an adipokine involved in the pathogenesis of insulin resistance and obesity (Ott et al., 2002).

Using a stable cell line overexpressing ghrelin, Zhang demonstrated that ghrelin inhibits adipogenesis in 3T3-L1 preadipocytes (Zhang et al., 2004). Ghrelin exposure stimulates proliferation in 3T3-L1 cells and prevents the progression of adipocyte differentiation. Ghrelin may inhibit adipogenesis by a mechanism involving down-regulation of PPAR-g activity (Bhattacharya et al., 2014).

Leptin is an adipokine released from white adipose tissue in proportion to the size of fat depots, with the role of informing central nervous system about the body energy deposits. Leptin level decreases significantly after bariatric surgery in proportion to the loss of adipose tissue. This decrease affects bone metabolism by influencing both bone formation (upregulation of osteoblast function and bone formation) and bone resorption (increase in osteoclast production and activity by promoting bone resorption) (Pizzorno, 2016; Folli et al., 2012). Decreased leptin after bariatric surgery inversely correlates with increased bone formation and resorption markers, tipping the balance towards bone loss.

Adiponectin, expressed exclusively by adipocytes, has low circulating levels in obesity and increases after bariatric surgery. Adiponectin receptors have been identified on both osteoblasts and osteoclasts. At osteoblast level, these receptors will promote their differentiation, while suppressing the formation and activity of osteoclasts (Pizzorno 2016; Folli et al., 2012). It is the most important adipokine that negatively correlates with BMD no matter of gender or post menopausal status (Biver et al., 2011).

Through multifactorial mechanisms, some incompletely known, obesity itself and its surgical treatment make the bone vulnerable and increase the risk of fracture. Although, especially after 2012, data from studies began to be systematically analyzed (Gagnon and Schafer, 2018), no consensus has been reached regarding the risk groups, their preoperative assessment, and the type of intervention recommended.

Personal contribution – published paper:

Arhire LI, Mihalache L, Pădureanu SS, Nita O, Gherasim A, Constantinescu D, **Preda C**. Changes in bone mineral parameters after sleeve gastrectomy: relationship with ghrelin and plasma adipokine levels. *Acta Endocrinologica (Buc)* 2018; 14(4): 498-504.

The aim of this study was to evaluate the evolution of bone mass parameters in the first year after laparoscopic sleeve gastrectomy in relation to anthropometric and body composition parameters and specific hormones of obesity (ghrelin, adiponectin, leptin).

1.5.2. Materials and methods

1.5.2.1.Study population

We included in this study patients with obesity that were consecutively evaluated and underwent metabolic surgery over a course of 18 months at the Center for Obesity and Bariatric Surgery of "Sf. Spiridon" Emergency Hospital Iași.

In our center, patients are only considered for surgery if they meet the current criteria for metabolic surgery according to international guidelines and undergo the complex multidisciplinary assessment (Fried et al., 2014). After surgery, patients are required to return for nutritional and medical follow-up at 1, 3, 6, 12 months after surgery, then yearly, but, as in all bariatric centers, some patients are lost to follow-up (Harper et al., 2007). We included in this study all consecutive patients who gave informed consent, even if they did not present to the postoperative follow-ups; also, we included all patients who underwent bariatric surgery during the selected timeframe, which implied that not all patients had the 6 months and 12 months follow-up during the course of the study.

1.5.2.2. Studied variables

We measured all studied variables preoperative and at 6 and 12 months postoperative. Anthropometric parameters (weight, height, and waist circumference - WC) were assessed according to the recommendations of the World Health Organization and allowed for the calculation of body mass index (BMI) (WHO 1995). We also calculated excess weight (EW) using the difference between real weight of patients and ideal weight as defined by the Devine formula (Devine 1974). The postoperative evolution of weight was described as percentage of excess weight loss (%EWL), i.e. (weight (kg) lost over a period of time/ EW) x100.

Bone mineral density (BMD) and body composition (BC) were measured using dual-energy x-ray absorptiometry - DEXA (Hologic Delphi A; Hologic Inc., USA). Measurements were made according to the standard protocol and with daily calibration by two experienced technicians certified by the International Society for Clinical Densitometry (ISCD). BMD and whole body and regional body composition including fat mass and lean body mass were measured after an overnight fast, with the participant in the supine position and wearing a hospital gown. Body composition values were analyzed using software version 11.2 (Hologic) that allowed calculation of: bone mineral content (BMC-g), fat mass (g and %), lean mass (g), BMD (g/cm2) - bone mineral content (g)/bone area (cm2). Bone mineral density before and after bariatric surgery is usually assessed through imagistic methods and correlated with anthropometric parameters, metabolic, nutritional or hormonal parameters (25-hydroxyvitamin D, plasma PTH, ghrelin, leptin and adiponectin concentrations).

We determined the plasma values of specific hormonal parameters (acylated-ghrelin, adiponectin and leptin) by ELISA, using the equipment of the Genetics and Immunology Laboratory of "Sf. Spiridon" Hospital: ELISA reader, ELISA ASYS washer, SIGMA centrifuge and Zanussi fridge. Plasmatic acylated ghrelin was quantified using commercially available ELISA kits (BioVendor Laboratory, United States) based on a double-antibody sandwich technique.

1.5.2.3. Statistical analysis

Data was analyzed using Microsoft Office Excel and SPSS version 17.0. Numerical data were expressed as means and standard deviation (SD), minimum and maximum. Significant differences between numerical data were found using t student test and paired-samples t student (in this case descriptive statistics took into account only those pairs). We determined statistically significant correlations using Pearson's correlations, and we used a p value <0.05 to define statistical significance for all calculations.

1.5.2.4. Ethical issues

The ethics committee of the "Grigore T. Popa" University of Medicine and Pharmacy approved the current study and all patients gave their informed consent prior to participation in the study.

1.5.3. Results

We included 75 patients in the study (82.67% women), with and average age of 42.11±11.45 years (43.31±12.5 years in men, 41.85±11.3 years in women, p>0.05). Fifty-two patients were also evaluated at 6 months after surgery, and 26 patients were evaluated at 12 months after surgery as well. The average BMI before surgery was 45.15±6.78 kg/m2, with patients presenting EW of 54.96±18.08 kg.

Table 3. Clinical anthropometric and body composition parameters of study population prior to surgery

Parameter	Category	Mean±SD	Minimum	Maximum	p
Weight (kg) preop.	Men Women Total Men	147.577±20.56 118.347±17.74 123.413±21.26 45.9007±4.65	110.0 90.0 90.0 38.06	180.0 165.0 180.0 54.34	<0.001
BMI (kg/m2) preop.	Women Total Men	45.0009±7.17 45.1568±6.78 139.462±10.48	35.06 35.06 122.0	66.51 66.51 156.0	0.667
WC (cm) preop.	Women Total	123.550±14.4 126.384±15.02	101.0 101.0	170.0 170.0	<0.001
EW (kg) preopr.	Men Women Total Men	67.389±16.89 52.355±17.34 54.961±18.08 1.23±0.23	37.8 26.0 26.0 0.858	97.2 91.0 97.2 1.6	0.006
BMD (g/cm2) preop.	Women Total Men	1.16±0.08 1.18±0.12 3.22±0.4	0.994 0.858 2.34	1.36 1.6 3.64	0.108
BMC (kg) preop.	Women Total Men	2.44±0.2 2.57±0.4 61.01±14.23	1.92 1.92 35.30	3.17 3.64 78.04	<0.001
Fat (kg) preop.	Women Total Men	57.43±11.99 58.04±12.33 40.63±4.88	38.79 35.30 32.20	87.87 87.87 46.6	0.433
% fat preop.	Women Total Men	46.96±3.83 45.88±4.64 84.14±7.8	40 32.20 71.9	56.9 56.9 97.63	<0.001
Lean (kg) preop.	Women Total	61.58±7.62 65.41±11.4	44.41 44.41	78.36 97.63	<0.001

BMI=body mass index; WC=waist circumference; EW=excess weight; BMD=bone mineral density; BMC=bone mineral content.

There were no statistical differences between men and women in terms of BMI and BMD measured by DEXA, prior to surgery, but women presented a significantly higher percentage of body fat compared to men and also a significantly smaller WC. These data are presented in **Table 3**.

Table 4. The evolution of clinical anthropometric and body composition parameters at 6 and 12 months after surgery

Parameter	Preoperative value	Value at 6 months postoperative	P*	Value at 12 months postoperative	P**	P***
Weight (kg)	124.135±20.85	91.48±16.84	<0.001	89.442±18.75	<0.001	<0.001
BMI (kg/m2)	45.43±7.14	33.06±5.69	<0.001	32.05±6.29	<0.001	<0.001
WC (cm)	127.36±15.26	101.69±14.38	<0.001	98.78±15.52	<0.001	0.028
%EWL		63.15±16.91		69.13±22.64		
% fat	45.72±5.4	36.79±6.34	<0.001	30.85±5.41	<0.001	<0.001
BMD (g/cm2)	1.169±.12	1.177±.07	0.781	1.21±.12	0.686	0.184
BMC (kg)	2.61±.425	2.55±.438	0.035	2.68±.541	0.007	0.075
Lean (kg)	65.58±12.42	55.91±11.22	<0.001	58.51±13.55	<0.001	0.259

BMI=body mass index; WC=waist circumference; EWL=excess weight loss; BMD=bone mineral density; BMC=bone mineral content.
*6 months postop. *vs*. preop.
**12 months postop. *vs*. preop
***12 months postop *vs*. 6 months preop.

We noticed a significant improvement in anthropometric parameters after surgery (weight, BMI, WC), which leads to 63.15±16.91 %EWL at 6 months after surgery and 69.13±22.64 %EWL at 12 months after surgery. We also noticed a significant reduction in lean body mass in the first 6 months after surgery, but not afterwards (6 to 12 months after surgery).

We observed no reduction in BMD after surgery and also a significant improvement in BMC at 12 months after surgery compared to preoperative (**Table 4**).

Our results showed that the value of adiponectin presented a significant increase after surgery (both at 6 and 12 months) and leptin showed a significant decrease at 6 months and 12 months postoperative compared to preoperative, but not at 12 months compared to 6 months postoperative. We observed a decrease in ghrelin level postoperative compared to preoperative, but this did not have a statistically significant value (**Table 5**). We found a significant inverse correlation between BMD and ghrelin preoperative (r=-0.448, p=0.009), but not with BMC, and the significance of this correlation did not persist postoperative; also,

there were no significant correlations between adiponectin or leptin and BMC or BMD either preoperative or postoperative.

Table 5. The evolution of ghrelin, adiponectin and leptin after bariatric surgery

Parameter	Preoperative value	Value at 6 months postoperative	P*	Value at 12 months postoperative	P**	P***
Ghrelin (pg/mL)	54.31±23.5	52.21±22.8	0.417	48.30±18.2	0.275	0.244
Adiponectin (ug/mL)	11.41±3.44	15.05±4.69	<0.001	15.24±6.34	0.014	0.021
Leptin (ng/mL)	42.54±12.14	14.87±6.81	<0.001	15.7±8.29	0.003	0.552

*6 months postop. *vs*. preop.
**12 months postop. *vs*. preop
***12 months postop *vs*. 6 months preop.

1.5.4. Discussions

Human studies regarding the role of ghrelin on bone mineral density are limited and conducted on different study populations, leading to contradictory and inconsistent results. In one study on 137 older men, plasma ghrelin level correlated positively with BMD (Gonnelli et al., 2008). Similar results were reported by Amini et al. which showed a significant positive correlation between plasma ghrelin level and BMD in women, suggesting that ghrelin would have a positive effect on BMD in women, independently of BMI, physical activity, age, smoking status or alcohol intake (Peyvand et al., 2013). Another study showed that both plasma ghrelin and BMD were significantly reduced at 11 months after sleeve gastrectomy (Coates et al., 2004). Different results were obtained in other studies on different populations (Korean middle-aged men, twins and older men and women), where there was no correlation between plasma ghrelin level and BMD (Oh et al., 2005; Makovey et al., 2007; Weiss et al., 2006). In contrast to these results, we found a negative correlation between plasma ghrelin and BMD preoperative, which was not maintained after surgery.

A metaanalysis published in 2011 showed that adiponectin correlated inversely, whereas leptin correlated positively with BMD especially in post-menopausal women. Increased levels of leptin are predictive of decreased risk of fracture, whereas an increased level of adiponectin is predictive for an increased risk of vertebrae fracture only in men. No significant association between ghrelin and BMD was demonstrated. Hence, adiponectin remains in this study the most relevant adipokine for BMD (Biver et al., 2011). Mpalaris evaluated BMD together with assessing plasma concentration of leptin, adiponectin and ghrelin in 110 healthy post-menopausal women and showed an inverse correlation between adiponectin and BMD, independently of body weight; leptin was positively associated with BMD, but depending on weight, and ghrelin had no significant correlation with BMD

(Mpalaris et al., 2016). Other authors found a positive correlation between adiponectin and BMD in postmenopausal women (Stojanovic et al., 2018). We found no significant correlations between adiponectin or leptin and BMD in our study population.

In bariatric surgery, laparoscopic sleeve gastrectomy (LSG) has gained much influence in recent years over Roux-en-Y gastric by-pass (RYGB): results on weight and metabolic parameters are encouraging, and numerous studies report significant and sustainable weight loss, with fewer adverse effects, among which we note reduced alteration in bone metabolism (Gehrer et al., 2010).

There is a relatively small number of studies reporting the possible relation between bone parameters (BMD, BMC) and the evolution of plasma ghrelin and adipokines after bariatric surgery, especially after LSG. Some studies demonstrated that the postoperative evolution of BMD was comparable between LSG and RYGB, the type of procedure having no influence on the bone mass (Vilarrasa et al., 2013; Kim and Brethauer 2015).

Some studies reported a reduction in BMD after surgery, but most studies referred in fact to the evolution after RYGB and other malabsorptive procedures, few authors studying particularly the effect of LSG on BMD and BMC (Kim and Brethauer 2015). Carrasco et al. evaluated alterations in BMD after bariatric surgery (both procedures – LSG and RYGB) in relation to plasma ghrelin and adiponectin. Percentage of EWL was 79.1±3.8 at one year after RYGB and 74.9 ± 4.1 at one year after LSG (no significant differences between these values). They showed a significant reduction in BMD only after RYGB and identified the significant reduction in ghrelin as the main factor which correlated with loss in BMD (Carrasco et al., 2014). Another study demonstrated that the effect of the two types of procedures on BMD was comparable at one year after the intervention, but menopausal women had a higher risk for low bone mass, even if osteoporosis was less frequent (Gehrer et al., 2010). However, Maghrabi et al. reported less reduction of BMD at 24 months after surgery for LSG compared to RYGB (Maghrabi et al., 2015). Some studies reported an increase in BMC postoperative, a small insignificant reduction in BMD, simultaneously with the significant reduction in adipose tissue and lean body mass, suggesting that bone loss after bariatric surgery was related to the degree of weight loss and modification in body composition (Adamczyk et al., 2015). Other authors reported a progressive increase in BMD in the first two years after LSG, but the modification in BMD was not associated with weight loss (Ruiz-Tovar et al., 2013).

There were also some reports that the evolution of BMD after LSG depended on gender, with a significant reduction of BMD seen only in women (Wang et al., 2018). Our study brings valuable results, as it refers only to patients who underwent LSG and we showed that BMD and BMC were not decreased at 6 and 12 months after surgery, on the contrary, BMC presented a significant increase one year after surgery.

1.5.5. Final remarks

Numerous studies attempt to elucidate the causes of post-bariatric bone alteration, the relationship between them, and the possible protective factors. To date, comparing the results of these studies, due to the heterogeneity of the methods and study populations, brings a weak power of evidence.

CHAPTER 2. GROWTH HORMONE PATHOLOGY BETWEEN EXCESS AND DEFICIENCY

2.1. State of the Art

Giants have been a subject of fascination throughout history. Whereas descriptions of giants have existed in the lay literature for millennia, the first attempt at a medical description was published by Johannes Wier in 1567(Nemec, 1974). However, it was Pierre Marie, in 1886, who established the term “acromegaly” for the first time and established a distinct clinical diagnosis with clear clinical descriptions in 2 patients with the characteristic presentation (Marie, 1886).

An interesting initiation into the world of giants is to examine the possibility that the Egyptian Pharaoh Akhenaten had acromegaly. The monuments of Akhenaten, who ruled about 1358 B.C., have been studied in great detail ever since his acromegalic facies and eunuchoid appearance led to the theory that Akhenaten suffered from a growth hormone secreting pituitary adenoma and hypogonadism. This idea, however, is refuted by John Wass who comments that Akhenaten, being the father-in-law of Tutankhamun, had a daughter and was therefore fertile (Alfred and Sandison, 1963).

Multiple autopsy findings revealed a consistent correlation between acromegaly and pituitary enlargement. In 1909, Harvey Cushing postulated a “hormone of growth” as the underlying pathophysiological trigger involved in pituitary hypersecretion in patients with acromegaly. This theory was supported by his observations of clinical remission in patients with acromegaly in whom he had performed hypophysectomy. In this paper, the author present some of the early accounts of acromegaly and gigantism, and describe its historical evolution as a medical and surgical entity (Marie, 1886; Herder 2009-2016).

The giants described in the ancient scriptures were said to be of tremendous stature and mighty in war. One of these legendary creatures was king Og of Bashan was said to be so tall that he could roast a fish by holding it at arms length towards the sun. A history of acromegaly without these wonderfully vivid stories of gigantism would be incomplete although eventually we will have to spoil the fun and call for the measurements to be scienti~cally validated (Enderle, 1998).

The killing of Goliath by David possibly represents the first account of the physical disadvantage caused by a large pituitary tumour. Any interpretation regarding Goliath’s downfall being the result of chiasmal compression by a suprasellar pituitary tumour could only be conceived this century since it is only during this time that pituitary disease has been recognised (Sheaves, 1999).

Further more, the philosophies attributed to the ancient greeks and romans dominated medical practice in Europe for many centuries and partly explains why the function of the pituitary was so profoundly misinterpreted for so long. Little progress in medicine was made during the 1000 or so years of the Byzantine empire but significant advances in science appeared at the time of the Renaissance.

The father of this new style of scientific medicine was Claude Bernard who proposed that ‘internal secretions’ affected the body after entering the bloodstream from glandular structures. About him was said that "he is not merely a physiologist, he is the physiology" (Henderson, 1928).

Subsequently it was discovered that pituitary hyperfunction caused by a pituitary tumour was indeed the cause of acromegaly. The cause of acromegaly could be further determined after the discovery of growth hormone (GH) and insulin-like growth factor I (IGF-I) and after demonstrating an association with GH hypersecretion and elevated circulating IGF-I. From the beginning of the 20th century, acromegaly could be treated by pituitary surgery and/or radiotherapy. After 1970, medical therapies were introduced that could control acromegaly. First, dopamine agonists were introduced, followed by somatostatin analogues and GH receptor blockers (Pearce, 2000).

In 1887 it had been noted that a pituitary tumor was present in most patients with acromegaly. Untill the beginning of the 20^{th} century relationship between growth disorders and the pituitary was contested. Since 1908 pituitary surgery became established treatment in growth hormone (GH) hypersecretion (Lindholm, 2006).

In 1922 it was demonstrated that injection of pituitary extract to animals caused excessive growth and soon after the opposite: removal of the pituitary caused growth retardation. A huge number of studies on the effects of GH were subsequently reported as were trials with GH treatment (Evans and Long, 1922). They were impeded by failure to recognize the impact of species specificity of GH (Smith, 1927) and only ffter this issue was clarified in 1957, treatment with human growth hormone proved effective (Sumner, 1969, Franklin, 2009).

In 1985 it was realized that Creutzfeldt-Jakob's disease might be transmitted through human growth hormone. At this time recombinant GH had become available. In 1971 the structure of human GH was established. In the same period both GH releasing and inhibiting hormones were identified and an analogue of somatostatin had evolved into the first effective pharmacological treatment for acromegaly (PES, 1985).

In the last three decades, short and long term research projects and clinical trials have provided relevant information on the efficacy and safety of GH replacement therapy in adults with GH deficiency (AGHD). The knowledge acquired has been compiled into guidelines that offer clinicians an evidence-based, practical approach for the management of AGHD (Boguszewski, 2017). However, their are still open questions in some key areas in which recommendations are supported only by moderate or weak evidence (Aguiar-Oliveira ad Bartke, 2019).

The high diversity of actions of GH can be explained only by the fact that the hormone plays many different roles by activat- ing a high number of proteins involved in cell signaling and displaying different mechanisms of action. The possibil- ity exists that, rather than a hormone, GH is a prohormone that depending on the tissue may be proteolytically cleaved giving origin to different and shorter GH derivatives with tissue-specific properties. In addition, GH may activate the proliferation of tissue-specific stem cells that then would act in tissue repair after an injury. In this sense, GH is safe if administered in the appropriate doses and frequency. A clas- sical GH-dependent adverse effect such as hyperglycemia is not important if the hormone is administered before physical exercise (Devesa et al., 2016) .

The development of long-acting GH preparations has created new therapeutic possibilities by decreasing injection frequency, improving adherence and thereby potentially maximizing clinical outcomes.

This research direction has been realized by publishing the following articles:

1. Solomon E, Brănișteanu D, Dumbravă A, Solomon RG, Kiss L, Glod M, **Preda C**. Executive functioning and quality of life in acromegaly. *Psychol Res Behav Manag*2019; 12: 39-44.
2. Pascanu I, Pop R, Barbu CG, Dumitrescu CP, Gherlan I, Mărginean O, **Preda C,** Procopiuc C, Vulpoi C, Hermanussen M. Development of synthetic growth charts for romanian population.*Acta Endocrinologica (Buc)* 2016; 12(3): 309-3018.
3. **Preda C**, Ungureanu MC, Leustean L, Cristea C, Vulpoi C. Ethical issues related to the use of human growth hormone in idiopathic short stature. *Rev Rom Bioet* 2013; 11(4): 31-37.

2.2. Growth hormone hypersecretion and executive functioning impairment

2.2.1. Introduction

Acromegaly is a rare chronic illness caused in >95% of the cases by excessive secretion of growth hormone (GH) due to pituitary adenoma; it is associated with increased mortality and morbidity (Melmed 2009; Martín-Rodríguez et al., 2013; Niculescu et al., 2017). The impact of GH and IGF-1 on regulating brain functions and their neurotrophic role has been recently documented (Sievers et al., 2009). IGF-1 has an important role in cognition. It improves cerebral circulation, increases neuronal activity, and inhibits neuronal apoptosis (Åberg et al., 2006; Hallberg and Nyberg 2012). The prefrontal cortex, hippocampus, and limbic structures have many GH and IGF-1 receptors (Lai et al., 1993; Pereira 2015). This may explain the decrease in executive function – the prefrontal cortex is responsible for this complex neuropsychological construct. “Dysexecutive syndrome” is usually associated with difficulties in setting goals, having future-oriented behavior, as well as planning, organizing, and flexible thinking during problem solving (Suchy 2009; Salthouse 2011; Bowie and Harvey 2006; Heflin et al., 2011).

In healthy condition, GH and IGF-1 have protective effects on the cognitive (eg, memory) and mood brain functioning (Nyberg 2002). Moreover, clinical studies have demonstrated that both memory and general cognitive functions are improved when a patient with pituitary deficiency is successfully treated with recombinant GH (Deijen et al., 2011; Al-Delaimy et al., 2009; Burman et al., 1996).

On the other hand, supraphysiological levels of GH and IGF-1 are correlated with cognitive dysfunction, but no pathological mechanistic associations have yet been discovered. One of the hypotheses is that excessive levels of brain IGF-1 might cause insulin resistance of the cerebrum leading to hyperphosphorylation and amyloid accumulation. These phenomena result in synaptic loss (Crespo and Webb 2014).

There are many brain regions with a higher density of GH and IGF-1 receptors, including the hippocampus and the prefrontal cortex. These areas seem to be particularly vulnerable in acromegaly (Lai et al., 1993; Pereira 2015).

Personal contribution – published paper:

Solomon E, Brănișteanu D, Dumbravă A, Solomon RG, Kiss L, Glod M, **Preda C**. Executive functioning and quality of life inacromegaly. *Psychol Res Behav Manag*2019; 12: 39-44.

Given the fact that these brain regions are essential for specific neurocognitive functioning, we hypothesize that patients diagnosed with acromegaly may suffer from impaired executive function. The purpose of the study was to evaluate the executive function and quality of life (QoL) in acromegaly patients compared to healthy controls. Our goal was to indicate that acromegaly is associated with impaired executive functioning and this decreases QoL in acromegaly sufferers.

2.2.2. Materials and methods

2.2.2.1. Study design

This was a cross-sectional case–control study that assessed the executive functioning and the QoL of patients with acromegaly vs healthy subjects.

2.2.2.2. Subjects

Both the acromegaly and the control groups included 19 subjects, 14 women and five men. Volunteers were matched for age, sex, and education. The participants from the acromegaly group were recruited between January 2015 and February 2016 from the Endocrinology Department of Saint Spiridon Academic Hospital of Iasi. To eliminate confounding variables, we excluded those with current diagnosis or history of any of the following: 1) stroke; 2) Parkinson's disease; 3) traumatic brain injury; 4) major psychiatric disorders (including schizophrenia, dementia, and generalized anxiety disorders); 5) smoking, drug or alcohol abuse; 6) diabetes mellitus; or 7) uncontrolled systemic arterial hypertension.
The acromegaly group consisted of patients seeking inpatient endocrinology treatment. In total, 27 acromegaly patients were initially invited to participate in the study. Four refused, one patient was excluded due to a stroke, two did not complete all the tests, and one patient was illiterate.

The control group was represented by healthy volunteers – mostly relatives of the patients. The inclusion criteria were the absence of current systemic pathology such as systemic arterial hypertension, diabetes mellitus, or other metabolic or endocrine disturbances, as well as present or previous drug abuse.

All the patients gave informed written consent to participate in the study.

The diagnosis of active acromegaly was based on clinical features, biological parameters (GH profile during oral glucose-tolerance test [OGTT] over 2 hours and IGF-1), and pituitary neuroimaging. Biochemically controlled acromegaly was based on the following two criteria: 1) GH levels <1 μg/L during the OGTT and 2) IGF-1 levels within 2 SD of age and gender-adjusted norms. The global function of the anterior pituitary was also

assessed (prolactin, adrenocorticotropic hormone [ACTH], thyroid-stimulating hormone [TSH], follicle-stimulating hormone [FSH], luteinizing hormone [LH] along with cortisol, thyroxine, and estradiol/testosterone).

2.2.2.3. Assessment instruments

QoL in acromegaly was assessed using the Acromegaly Quality of Life Questionnaire (AcroQoL). It contained 22 questions with five possible responses scored on a Likert-type scale (from 1 to 5) that evaluates physical and psychological domains. The psychological domain is subdivided into two subscales regarding the appearance and personal relationships. For each, a lower score is associated with a worse QoL.

We applied the following instruments to evaluate the executive functioning:

- Trail making test (TMT) part A estimates attention, motor speed, and visual research skills. Part B assesses higher cognitive functions such as mental flexibility, ability to execute and modify an action plan, and to maintain and apply two criteria simultaneously.
- The Stroop test is a common neuropsychological instrument to measure frontal lobe function by evaluating the inhibition of a pre-potent automatic response.
- The Stroop test consists of three different conditions that are based on the same number of stimuli.
- Simple and constrained phonemic fluency can assess high cognitive function including the executive domain specific to the frontal brain function. This task involves many complex operations such as finding the right words, initiating verbal responses, inhibiting responses that do not meet the criteria, correcting wrong outputs, and shifting attention to a new search.

2.2.2.4. Ethical considerations

All subjects signed an informed consent form. The study was approved by the ethical committee of "Grigore T. Popa" University of Medicine and Pharmacy from Iași, Romania. The study was conducted according to the Declaration of Helsinki.

2.2.2.5. Statistical methods

Statistical analysis used SPSS version 19. Sample distribution was evaluated with the Kolmogorov–Smirnov test. We used parametric tests (Student's *t*-test) for continuous variables, independent, unequal variance assumed, and Pearson's method for correlations to compare the possible relationship between depression and QoL. Statistical significance level was set at a 95% CI ($P<0.05$).

2.2.3. Results

2.2.3.1. Subjects characteristics

The mean age of acromegaly patients was 53.68±11.84 and 55±12.28 years for the control group ($P=0.739$). The characteristics of acromegaly subjects along with the functional state of the pituitary are shown in **Table 6**. **Table 7** illustrates the demographic data of acromegaly and control group.

We used the Hamilton Depression Scale to assess the level of depression because depressed people may achieve lower scores on some cognitive measures due to psychomotor slowness. We further analyzed the correlation between the Hamilton Depression Scale scores

and the AcroQoL global score because some questions are similar in both question- naires. There was a significant negative relationship between the depression score obtained with Hamilton scale (r=–0.45; P=0.005) and the global AcroQoL score. Subjects with a higher depression have a lower QoL as seen by a lower score on the AcroQoL.

2.2.3.2.AcroQol

The average scores in the acromegaly group are significantly lower (M=60.80, SD=13.37) than the control group (M=75.97, SD=13.68, P=0.001).

We investigated the three dimensions in the AcroQoL. There are significant differences between the acromegaly group and the control group in terms of the physical effects (P=0.001) and appearance (P<0.001) but not for personal relationships (P=0.421).

The average score for physical dimension in the acromegaly group was 20.89 (SD=6.72) vs 28.52 (SD=6.2) for the controls; for appearance it was 20.47 (SD=5.91) vs 27.94 (SD=5.03) and for personal relationships 25.63 (SD=4.36) vs 27.10 (SD=6.58) for the acromegaly group and the controls, respectively.

Table 6. Characteristics of the patients with controlled and uncontrolled acromegaly

	Controlled (n=8), M±SD	**Uncontrolled (n=11), M±SD**
age (years)	56.875±8.084	50.417±12.345
gender (F/M)	6/2	8/3
Disease duration	12.375±13.738	8.583±8.489
GH (ng/dl)	0.585±0.2853	3.023±3.207
IGF-1 (ng/dl)	199.875±91.279	305.750±199.379
Fasting blood glucose (mg/dl)	113.264±25.602	103.667±21.833
TSH (μiU/ml)	0.832±0.777	0.530±0.334
fT4 (pg/ml)	1.036±0.188	1.029±0.124
ACTH (pg/ml)	29.313±11.912	29.346±13.835
cortisol (μg/dl)	8.683±4.389	22.116±41.120
Prolactin (ng/ml)	3.780±2.161	4.455±5.762
Microadenoma, n (%)	3 (37.5)	0 (0)
Macroadenoma, n (%)	5 (62.5)	11 (100)

Abbreviations: ACTH, adrenocorticotropic hormone; F, female; FT4, free thyroxine; GH, growth hormone; IGF-1, insulin-like growth factor-1; M, male; TSH, thyroid- stimulating hormone.

Table 7. Demographic data of acromegaly and control groups

	Patients	**Controls**	***P*-value**
Total N	19	19	
Female (%)	73.68	73.68	
Male (%)	26.32	26.32	
age (years)	53.68±11.84	55±12.28	0.739
educational level (years)	11.315±2.38	12.73±3.052	0.118
hamilton scale score	11.842±5.32	10.421±4.63	0.068

2.2.3.3.TMT

The average time to solve the TMT part A was 58.94 seconds (SD=39.74) in the acromegaly group; the control group needed 45.5 seconds (SD=17.24) to solve the same part of the test (*P*=0.195). The average time for solving TMT part B for subjects in the acromegaly group was 161.42 (SD=110.78) vs 74.77 (SD=35.26) for the controls (*P*=0.003).

We evaluated another parameter to analyze the differences between the two groups (acromegaly and control groups): the time difference needed to finish the two samples (time for part B minus time for part A). The average time B – A in the acromegaly group was 104 seconds (SD=87.28), which was significantly elevated (*P*=0.002) vs the control group (M=29.27 seconds; SD=25.93), suggesting that part B is more difficult to solve vs part A.

2.2.3.4. Stroop test

The Stroop test evaluated the time needed to read an incongruent list via ID (score difference) and IR (ratio score). The *t*-test for independent samples showed no significant differences between the acromegaly group and the control group. The average score difference was 79.44 (SD=34.6) for the acromegaly group, and for the control group it was 71.42 (SD=24.51), *P*=0.419. The acromegaly group had a mean ratio score of 0.308 (SD=0.086), and the control group had 0.310 (SD=0.063). The differences are not significant (*P*=0.949).

2.2.3.5. Simple and constrained phonemic fluency

The acromegaly group provided fewer words than the control group for letters "A" (*P*=0.01) and "I" (*P*=0.002) and no significant difference for letter "P" (*P*=0.116). No significant differences were found for the constrained phonemic fluency. The acromegaly group achieved a mean of 4.73 (SD=3.12) and the control group 5.88 (SD=2.84), *P*=0.250.
All the neuropsychological test results are shown in **Table 8**.

2.2.4. Discussion

The QoL of acromegaly patients is an important topic. Patient's expectations, living standards, and social integration are important concepts in evaluating the QoL. This study showed that acromegaly is associated with a decreased QoL. The acromegaly group had global AcroQoL scores lower than the control group, suggesting a lower QoL. This study reports a global score of 60.8 (SD=13.37), which is similar to studies in Germany (62.1 [SD=18.2]), Belgium (67.1 [51.1–78.4]), and in France (59.8 [SD=17.9]) (Psaras et al., 2011; T'sjoen et al., 2007; Matta et al., 2008).

Although acromegaly has a negative impact on the body side and appearance, patients feel that their social life is not affected. This is important because social relations play a crucial role in psychological well-being. Subjects with acromegaly showed more visible physical effect including hyper- tension, polyarthralgia, osteoporosis, and kyphosis. Similar results were reported by Webb, but Szcześniak reported worse social relationships than the general population (Szcześniak et al., 2017; Webb et al., 2006; Webb and Badia 2007).

Table 8. Neuropsychological test results for acromegaly group and healthy controls

	Items	**All patients**		
		Acromegaly group (SD)	**Healthy controls (SD)**	***P*-value**
AcroQol	Physical side	20.89 (6.72)	28.52 (6.2)	0.001
	Appearance	20.47 (5.91)	27.94 (5.03)	0.000
	Interpersonal relations	25.63 (4.36)	27.1 (6.58)	0.634
	Total score	60.8 (13.37)	75.97 (13.68)	0.001
TMT	Part A (s)	58.94 (39.74)	45.5 (17.24)	0.195
	Part B (s)	161.42 (110.78)	74.77 (35.26)	0.003
	B-A (s)	104	29.27	0.002
Stroop test	ID	79.44 (34.6)	71.42 (24.51)	0.419
Simple phonemic fluency	A	8.47 (4.62)	13.05 (5.50)	0.01
	I	6.21 (3.66)	11.10 (5.44)	0.002
	P	12.84 (5.06)	15.36 (4.59)	0.116
Constrained phonemic fluency		4.73 (3.12)	5.88 (2.84)	0.250

Notes: iD (the score difference) calculates the amount of time needed for naming the color from the incongruent list and the time needed recognizing the color from the congruent list; simple phonemic fluency was quantified by counting the number of words that patients were able to give for each of the three letters that we have provided: P, A, I; constrained phonemic fluency was evaluated by the number of words the subjects could provide in 1 minute beginning with the letter "m" and having only four letters. **Abbreviations:** AcroQoL, Acromegaly Quality of Life Questionnaire; TMT, trail making test.

This study illustrates a significant relationship between dysexecutive syndrome and QoL. Unlike other acromegaly comorbid conditions such as cardiovascular disease, diabetes mellitus, osteoporosis, and sleep apnea, dysex- ecutive syndrome is an "invisible" and silent factor with a negative impact on QoL. Cognitive dysfunctions – particularly impairment of executive function – are subtle changes. Minimizing dysexecutive syndrome is an important step in improving QoL: cognitive-behavioral therapy including the "Think Healthy" technique had encouraging results(Kunzler et al., 2018).

Executive function is one of the highest neurocognitive functions. This neuropsychological construct involves complex cognitive skills such as an ability to plan and organize with efficient working memory and flexibility. It is the capacity to reject inappropriate stimuli and to integrate past experiences into present actions.

Executive function is a new and exciting concept that has been recently investigated outside neuropsychology. Impaired executive function has been discovered in brain tumor, stroke in frontal lobes, Parkinson's disease, fibromyalgia syndrome, and type 1 diabetes mellitus (Muñoz Ladrón de Guevara et al., 2018; Perez et al., 2017).

This study showed that acromegaly patients have a decreased executive function. The study explored different aspects of the executive function such as working memory and semantic inhibition. The acromegaly patients had lower scores than the controls in all tests with a statistically sig- nificant difference in the TMT part B and simple phonemic fluency.

The cognitive impairment is subtler. This might be because of the delay in diagnosing acromegaly. It takes 5–10 years on average. The patient is usually not aware of these changes and blames "ugly aging" associated with a deficit of memory, attention, as well as goal setting and execution.

Moreover, physicians tend to focus only on GH normalization and the treatment of the life-threatening consequences of the disease. Metabolic abnormalities such as diabetes, abnormal lipid profile, hypertension, heart failure or sleep apnea are severe complications that motivate the patient to seek adequate treatment, but cognitive dysfunctions can also decrease QoL.

Neurocognitive impairment in acromegaly has been evaluated in multiple studies with contradictory results. Sievers reported that patients with acromegaly have a decrease in cognitive functioning as follows: 33% with reduced attention, 24.1% with reduced memory, and 16.7% with reduced executive function; 67.3% could not reach the cutoff level in at least one test (Sievers et al., 2012). Leon-Carrion proved that short-term and long-term memory are most severely impaired, and the prefrontal and middle temporal cortices have decreased activity (Leon-Carrion et al., 2010).

Neuropsychological tests revealed that lower memory scores are associated with untreated disease duration with few deficits in executive functions (Sievers et al., 2009). Tiemensma reported no cognitive dysfunction in patients with long-term cured acromegaly (Tiemensma et al., 2010).

Most studies that have analyzed cognitive function report that patients diagnosed with acromegaly achieve lower scores than control groups on memory and executive functioning (Martín-Rodríguez et al., 2013; Leon-Carrion et al., 2010; Brummelman et al., 2012; Yedinak and Fleseriu 2014; Tanriverdi et al., 2009). Tanriverdi used cognitive electroencephalographic (EEG) – evoked potentials to show that there is a reduction of P300 amplitudes in brain areas in acromegaly patients (Tanriverdi et al., 2009). These areas are important for cognitive functioning and include the inferior parietal lobe, frontal lobe, hippocampus, and medial temporal lobe (Brummelman et al., 2012). An MRI study compared 44 patients with acromegaly to healthy controls and found that acromegaly led to alterations of the macroscopic brain tissue architecture that appear during the first 10 years of the disease; patients had increased gray matter volume (+3.7%) and white matter volume (+5.1%), which might be due to excessive GH and IGF-1 on neuronal or glial cells (Sievers et al., 2009). Low resolution brain electromagnetic tomography (LORETA) was used to estimate the electrical brain activity in 16 untreated acromegaly patients vs 16 matched controls – there was decreased activity in the right inferior frontal lobe alpha, the right dorsolateral prefrontal cortex β-2, and the right parahippocampal cortex β-3 waves (Leon-Carrion et al., 2010). A combination of EEG and LORETA in acromegaly patients showed lower α and β cortical activity in the left temporal cortex vs healthy controls (Martín-Rodríguez et al., 2013).

2.2.5. Conclusion

This study suggests that executive functioning is affected in acromegaly. These results indicate that clinicians should consider cognitive tests to evaluate executive functioning in

addition to standard screening for comorbidities. We highlight the benefit of an interdisciplinary psychological intervention to acromegaly disease that can improve the patients' QoL.

Further studies with a larger number of subjects and a larger battery of tests are needed to investigate the cognitive functions in acromegaly. Another aspect that might possibly be evaluated is a correlation between the degree of cognitive dysfunction with the disease-specific hormone levels (GH, IGF-1, etc) and imaging parameters (LORETA).

2.3. Clinical evaluation of growth hormone

2.3.1. Introduction

The clinical evaluation of height and weight is based on comparisons with specific growth references. Yet, disagreement however exists upon which chart is the right chart to use (Radcliffe et al., 2007). In many countries, national references for height, weight and body mass index (BMI) are available. Also for Romania several regional tudies have been performed (Chirita et al., 2012; Pascanu et al., 2014), but none of these studies can be considered a national representative sample. As the only available growth chart for the Romanian population has been published more than four decades ago (Popa et al., 1974; Cordeanu 2009), current recommendations from the protocol of growth hormone treatment in short stature in Romania suggest using the Swiss growth charts developed in 1989 by Prader for clinical purposes (Prader et al., 1989).

In recent years globally applicable international growth standards and references have been suggested for countries that lack national growth references (WHO, 2010). The idea of such standards goes back to recommendations of a Working Group on infant growth established by the World Health Organization (WHO), and may be justified for infants and very young children who tend to grow similarly under modern affluent conditions. Yet, it appears questionable whether WHO growth standards and references should be used for clinical purposes in Romanian children, particularly as the Romanian population at present undergoes major economic transition that may have major influences also on infant and child growth. It was, thus, considered appropriate to newly develop a national growth chart for Romanian children. Constructing national growth charts is demanding work and most notably, expensive work. It was therefore decided to apply the method of generating synthetic references for the Romanian population.

Personal contribution – published paper:

Pascanu I, Pop R, Barbu CG, Dumitrescu CP, Gherlan I, Mărginean O, **Preda C,** Procopiuc C, Vulpoi C, Hermanussen M. Development of synthetic growth charts for romanian population.*Acta Endocrinologica (Buc)* 2016; 12(3): 309-3018.

Synthetic growth references are based on Principal Component Analysis and the Likelihood principle. Based on a global reference combination of longitudinal and cross-sectional modern and historic growth studies with data on height and weight, mean values and a limited number of Principal Components, that characterize the variability of growth in

this global sample, are known (Hermanussen et al., 2016). This information is then used to derive estimates of height, weight, BMI and respective centiles from available auxological data of the Romanian population.

2.3.2. Material and methods

Methods used for generating synthetic growth charts are described in detail elsewhere (Hermanussen et al., 2016).

In short, the methodology comprises 2 steps:

- Step 1:
- Principal Component Analysis was applied to characterize the global variance of the mean values for height, weight and BMI in 196 female and 197 male longitudinal and cross-sectional growth studies from 53 countries published since 1831 (all studies contained information on height, data on height and weight were available in 87 female and 89 male studies).
- Five principal components were able to explain 98.4 % of the between-study variance in mean height, 99.2 % of this variance in mean weight, and 93 % (females) and 94 % (males) of this variance in mean BMI. These components define a growth model that describes the basic human growth pattern from birth to maturity. The component of this growth model can now be used for generating "synthetic" references for height, weight, and BMI of any population of interest that lacks complete annual data of these parameters.
- Step 2:
- The Maximum Likelihood Principle is then applied and standard techniques for non-linear optimization are used. This step generates a "synthetic" growth curve that is the most likely curve for the population of interest given the following assumptions:
  a) the curve belongs to a population similar to the ones for which the Principal Component Analysis was performed,
  b) all differences between the curve and the observed mean heights are errors of these means distributed according to a known standard error of the mean.
- By applying a Bayesian rational, synthetic growth curve find that curve that best compromises between the data available from the population of interest and the global patterns obtained from the Principal Component Analysis.

We used local Romanian data from 9 studies with information on height and weight obtained between 1999 and 2016 (**Table 9**). Not all of these studies, however, appeared fully plausible. Data from the Childhood Obesity Surveillance Initiative appeared unsuitable because of some uncertainty in respect to the definition of age (INSP 2013). A "rural" study was excluded because the probands were obtained from general practitioners' offices without clear selection criteria, and also the numbers appeared limited. We also excluded data from Cluj given the fact that information was available only for height and the fact that it included only subjects from urban areas.

Also given the proximity of Targu Mures and Cluj and the fact that the Mures data was more recent, we considered including the former. Six studies (**Table 9**) remained, and

were selected for generating the National Synthetic Growth References for Romanian Children. The 6 studies selected added to 8407 measurements of children 3-18 years of age. This represents approximately 0.22% of the Romanian population below 18 years according to the latest census.

The selected studies included subjects measured in schools/kindergartens. Age is reported in years. A 6 years old subject is defined like any subject with the age between 5.5 to 6.49 years. Height and weight were measured by trained medical personnel at a precision of 0.1 cm and 0.1 kg. For every parameter the mean of three measurements was used. All children were measured at normal temperature, in light clothes, without footwear.

Table 9. Description of the studies analyzed

No Location	Number of subjects	Socio-economic status	Ethnicity	Age	Measurement location	Period	Published
Iasi	556	Not available (NA)	NA	7-13	Schools	2016	No
Timisoara	2130	NA	NA	3-18	Schools, kindergartens	2011-2012	NO
Mures	1923	NA	NA	6-14	Schools	2013-2014	No
Bucuresti 1	1108	NA	NA	6-23	Schools	2011	Yes (11)
Childhood Obesity Surveillance Initiative	4348	NA	NA	8-9	Schools	2013	Yes (10)
Rural	134	NA	NA	8-15	General practice office (GPO)	2011	Abstract only (12)
Bucuresti 2	1163	NA	NA	3-18	Schools	2015	No
Cluj	7953	NA	NA	0-18	GPO, Schools, kindergartens	1999 2012-2013	Yes (13)
Petroșani	1527	NA	NA	3-16	Schools, kindergartens	2016	No

The studies provided information from all main regions of the country, recorded in the last five years: Timisoara, Tirgu-Mures, Bucharest (2 cohorts), Petroșani and Iasi. The age range varied between the studies, but overall, covered the full age range from 3 to 18 years. For weight charts, we used the same data sets, excluding one of the Bucharest studies, considered redundant. Height data is showed as means and standard deviations. Weight data was expressed as medians (3 data sets) or means and standard deviations. Based on the number of subjects in each growth study we a priori considered a measurement error of 0.7

(girls) cm and 0.5 (boys) cm for height; 0.7 (girls) kg and 0.5 (boys) kg for weight; and 0.3 kg/m2 for BMI.

Original centiles for height and weight were not recorded as this information is provided by the synthetic approach. For generating the synthetic charts, 6 age groups were selected: boys: 4,7,11,13,15, and 18 years; girls: 4,7,9,11,13, and 16 years. These age groups were chosen as they provided information on prepubertal, pubertal and final height. Centiles for weight and BMI were calculated using LMS method (Cole 1990).

MedCalc v. 12.5 was used for statistical analysis with a level of significance α=0.05. Data were tested for normal distribution using the Kolmogorov- Smirnov method. For data comparison, non-parametric tests were used (Wilcoxon).

Table 10. Synthetic means and SD for height – boys

Age (mo.)	Mean (cm)	SD (cm)	**Age (mo.)**	Mean (cm)	SD (cm)	**Age (mo.)**	Mean (cm)	SD (cm)	**Age (mo.)**	Mean (cm)	SD (cm)	**Age (mo.)**	Mean (cm)	SD (cm)	**Age (mo.)**	Mean (cm)	SD (cm)	**Age (mo.)**	Mean (cm)	SD (cm)
0	50.6	2.0	**33**	94.9	4.0	**66**	114.4	4.9	**99**	130.9	5.7	**132**	145.3	6.6	**165**	163.6	8.2	**198**	175.6	6.8
1	54.2	2.2	**34**	95.6	4.0	**67**	114.9	4.9	**100**	131.3	5.7	**133**	145.8	6.6	**166**	164.2	8.3	**199**	175.7	6.8
2	57.7	2.3	**35**	96.4	4.1	**68**	115.5	5.0	**101**	131.8	5.7	**134**	146.3	6.7	**167**	164.8	8.3	**200**	175.8	6.8
3	61.3	2.4	**36**	97.1	4.1	**69**	116.0	5.0	**102**	132.3	5.8	**135**	146.8	6.7	**168**	165.5	8.3	**201**	176.0	6.7
4	63.6	2.5	**37**	97.7	4.1	**70**	116.6	5.0	**103**	132.7	5.8	**136**	147.3	6.8	**169**	165.9	8.3	**202**	176.1	6.7
5	65.9	2.5	**38**	98.3	4.2	**71**	117.1	5.1	**104**	133.2	5.8	**137**	147.8	6.8	**170**	166.4	8.2	**203**	176.2	6.7
6	68.1	2.5	**39**	98.9	4.2	**72**	117.7	5.1	**105**	133.6	5.8	**138**	148.3	6.9	**171**	166.9	8.2	**204**	176.4	6.6
7	69.6	2.5	**40**	99.5	4.2	**73**	118.2	5.1	**106**	134.1	5.9	**139**	148.8	6.9	**172**	167.4	8.2	**205**	176.4	6.6
8	71.2	2.6	**41**	100.1	4.3	**74**	118.7	5.1	**107**	134.6	5.9	**140**	149.3	7.0	**173**	167.9	8.1	**206**	176.5	6.6
9	72.7	2.6	**42**	100.7	4.3	**75**	119.2	5.2	**108**	135.0	5.9	**141**	149.8	7.0	**174**	168.4	8.1	**207**	176.6	6.6
10	73.9	2.7	**43**	101.3	4.3	**76**	119.7	5.2	**109**	135.4	5.9	**142**	150.3	7.1	**175**	168.9	8.0	**208**	176.6	6.6
11	75.2	2.8	**44**	101.9	4.4	**77**	120.2	5.2	**110**	135.9	5.9	**143**	150.8	7.1	**176**	169.4	8.0	**209**	176.7	6.6
12	76.5	2.8	**45**	102.5	4.4	**78**	120.7	5.2	**111**	136.3	6.0	**144**	151.2	7.2	**177**	169.9	8.0	**210**	176.7	6.6
13	77.5	2.9	**46**	103.1	4.4	**79**	121.2	5.2	**112**	136.7	6.0	**145**	151.8	7.2	**178**	170.3	7.9	**211**	176.8	6.6
14	78.6	3.0	**47**	103.7	4.5	**80**	121.7	5.3	**113**	137.2	6.0	**146**	152.4	7.3	**179**	170.8	7.9	**212**	176.9	6.5
15	79.6	3.0	**48**	104.3	4.5	**81**	122.2	5.3	**114**	137.6	6.0	**147**	153.0	7.4	**180**	171.3	7.9	**213**	176.9	6.5
16	80.7	3.1	**49**	104.9	4.5	**82**	122.7	5.3	**115**	138.0	6.1	**148**	153.5	7.4	**181**	171.6	7.8	**214**	177.0	6.5
17	81.7	3.2	**50**	105.5	4.5	**83**	123.2	5.3	**116**	138.5	6.1	**149**	154.1	7.5	**182**	171.9	7.7	**215**	177.1	6.5
18	82.8	3.2	**51**	106.0	4.6	**84**	123.7	5.4	**117**	138.9	6.1	**150**	154.7	7.6	**183**	172.2	7.7	**216**	177.1	6.5
19	83.7	3.3	**52**	106.6	4.6	**85**	124.2	5.4	**118**	139.3	6.1	**151**	155.3	7.7	**184**	172.5	7.6			
20	84.6	3.4	**53**	107.2	4.6	**86**	124.7	5.4	**119**	139.8	6.2	**152**	155.8	7.7	**185**	172.8	7.5			
21	85.5	3.4	**54**	107.7	4.6	**87**	125.2	5.4	**120**	140.2	6.2	**153**	156.4	7.8	**186**	173.0	7.5			
22	86.4	3.5	**55**	108.3	4.6	**88**	125.7	5.5	**121**	140.6	6.2	**154**	157.0	7.9	**187**	173.3	7.4			
23	87.3	3.5	**56**	108.9	4.7	**89**	126.1	5.5	**122**	141.1	6.2	**155**	157.6	8.0	**188**	173.6	7.3			
24	88.2	3.6	**57**	109.4	4.7	**90**	126.6	5.5	**123**	141.5	6.3	**156**	158.1	8.0	**189**	173.9	7.3			
25	88.9	3.6	**58**	110.0	4.7	**91**	127.1	5.5	**124**	141.9	6.3	**157**	158.7	8.0	**190**	174.2	7.2			
26	89.7	3.7	**59**	110.5	4.7	**92**	127.6	5.5	**125**	142.4	6.3	**158**	159.4	8.1	**191**	174.5	7.1			
27	90.4	3.7	**60**	111.1	4.7	**93**	128.1	5.6	**126**	142.8	6.4	**159**	160.0	8.1	**192**	174.8	7.1			
28	91.2	3.8	**61**	111.7	4.8	**94**	128.5	5.6	**127**	143.2	6.4	**160**	160.6	8.1	**193**	174.9	7.0			
29	91.9	3.8	**62**	112.2	4.8	**95**	129.0	5.6	**128**	143.6	6.4	**161**	161.2	8.1	**194**	175.0	7.0			
30	92.6	3.9	**63**	112.7	4.8	**96**	129.5	5.6	**129**	144.1	6.5	**162**	161.8	8.2	**195**	175.2	7.0			
31	93.4	3.9	**64**	113.3	4.9	**97**	130.0	5.7	**130**	144.5	6.5	**163**	162.4	8.2	**196**	175.3	6.9			
32	94.1	3.9	**65**	113.8	4.9	**98**	130.4	5.7	**131**	144.9	6.5	**164**	163.0	8.2	**197**	175.4	6.9			

Legend: mo. – months; SD – standard deviation; cm - centimeters.

All the studies taken into consideration were approved by the local ethics committee and written consent was obtained from the parents/legal representatives, which contain information about the possibility of secondary data analysis. Given the fact that for generating the synthetic growth curves we use secondary research, having no contact with

and no possibility to identify any of the subjects, a new ethics approval was considered redundant.

2.3.3. Results

2.3.3.1. Height

Tables 10 and 11 show the computed synthetic means and SD scores for height for both sexes. **Fig. 11** depicts the synthetic growth curves derived for both boys and girls.

When comparing the synthetic references with the existing references currently used in Romania (Swiss, WHO and CDC), there are statistically significant differences between either 2 standards compared ($p<0.0001$).

Romanian boys are in average taller than the Swiss with 0.51±0.84cm ($p=0.038$), 1.71±0.42cm than the US ($p<0.0001$) and 1.77±0.57cm ($p<0.0001$) than the international WHO reference. For girls, the mean differences are 0.35±0.65cm for the Swiss, 1.03±0.91cm for the US and 0.81±0.59cm than the WHO (**Fig. 14a**).

The maximum difference between the Romanian and the CDC tables for boys is 2.3cm at the age of 12.4 years and the minimum is 0.93cm at the age of 17.6 years. Using the same 2 references for girls, the maximum difference is 2.6cm at the age of 4.6 years and the minimum 0.009cm at the age of 12.1 years.

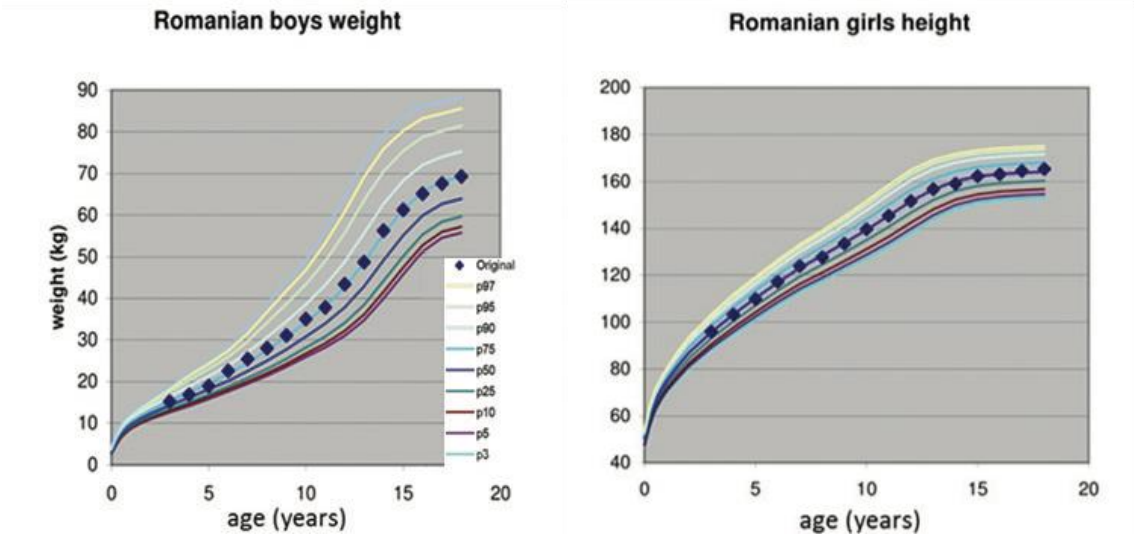


Fig.12. Synthetic weight centiles, Legend - p3 – 3rd percentile, p5 – 5th percentile, p10 – 10th percentile, p25 – 25th percentile, p50 – 50th percentile, p75 – 75th percentile, p90 – 90th percentile, p95 – 95th percentile, p97 – 97th percentile.

The maximum difference between the Prader and Romanian growth standards for boys is attained at 14 years (2.5cm), while the minimum is 0.0008cm at 9.9 years. For girls, the corresponding values are 1.39cm maximum (12 years) and respectively 0.003cm minimum (14 years).

The same comparison using the Romanian and WHO standards leads to a maximum difference for boys 2.45cm at 9 years and a minimum of 0.39cm at 2 years of age. For girls

the maximum difference is found at 7 years (2.21cm) and the minimum at 12.5years (0.049cm).

Tables 4-5 show the common centiles for weight analysis and **Fig.12** depicts the corresponding curves. While weight centiles are useful, the recent recommendations are for

Table 12. Weight references (kg) for boys

Age (years)	p3	p5	p10	p25	p50	p75	p90	p95	p97
0	2.62	2.72	2.89	3.16	3.47	3.78	4.07	4.25	4.36
0.25	4.85	5.02	5.30	5.76	6.28	6.82	7.32	7.63	7.82
0.5	6.62	6.80	7.10	7.60	8.16	8.74	9.29	9.62	9.83
0.75	7.71	7.91	8.23	8.78	9.41	10.06	10.66	11.04	11.28
1	8.73	8.93	9.25	9.80	10.42	11.07	11.68	12.06	12.30
1.5	10.03	10.24	10.58	11.18	11.89	12.64	13.37	13.84	14.14
2	11.01	11.24	11.63	12.30	13.10	13.95	14.79	15.33	15.68
3	12.64	12.93	13.42	14.28	15.31	16.44	17.56	18.29	18.76
4	14.18	14.52	15.10	16.15	17.44	18.90	20.40	21.41	22.08
5	15.89	16.27	16.93	18.12	19.60	21.30	23.08	24.29	25.09
6	17.65	18.09	18.83	20.18	21.88	23.85	25.94	27.38	28.35
7	19.49	20.00	20.88	22.48	24.55	27.00	29.67	31.55	32.84
8	21.41	22.01	23.05	24.99	27.53	30.65	34.18	36.75	38.55
9	23.57	24.26	25.46	27.72	30.72	34.44	38.70	41.85	44.07
10	25.91	26.73	28.15	30.79	34.27	38.51	43.29	46.73	49.13
11	28.25	29.19	30.83	33.93	38.04	43.14	48.98	53.25	56.26
12	31.04	32.16	34.10	37.79	42.68	48.76	55.71	60.79	64.36
13	34.75	36.10	38.46	42.87	48.66	55.70	63.54	69.12	72.97
14	39.98	41.54	44.21	49.13	55.38	62.70	70.50	75.86	79.45
15	45.75	47.37	50.12	55.09	61.24	68.23	75.45	80.28	83.46
16	51.24	52.75	55.29	59.89	65.57	72.04	78.73	83.23	86.20
17	54.56	55.98	58.38	62.69	68.01	74.02	80.22	84.37	87.12
18	55.76	57.18	59.60	63.93	69.25	75.27	81.45	85.58	88.31

Legend: Kg – kilograms; p3 – 3rd percentile; p5 – 5th percentile; p10 – 10th percentile; p25 – 25th percentile; p50 – 50th percentile; p75 – 75th percentile; p90– 90th percentile; p95 – 95th percentile; p97 – 97th percentile.

the employment of BMI for weight disturbances definitions, especially after the age of 10 years. In **Tables 14 and 15** the common used BMI centiles can be found and **Fig.13** shows the corresponding charts.

For girls the same average differences are 0.41±0.32kg/sqm for the WHO reference and 0.63±0.38kg/sqm for the IOTF standard (**Fig. 14b**).

A comparison between the new Romanian synthetic BMI references and the common used references world-wide (WHO and IOTF) shows there are statistically significant differences between these references ($p<0.0001$).

The maximum differences for boys are 1.17 kg/sqm at age 11.1 years between the Romanian and WHO references and 1.48 kg/sqm (14 years) for the IOTF comparison. The minimum corresponding differences are 0.04 kg/sqm at 5 months (WHO) and 0.001 at 2.5years (IOTF).For girls these maximum differences are of 0.91 kg/sqm at 2 months (WHO), respectively 1.14 kg/ sqm at 12 years (IOTF). The smallest difference was 0.007 kg/sqm at 16.6 years for the WHO and 0.003 kg/ sqm at 3.4 years for the IOTF.

On average the new Romanian synthetic references show that boys have a 0.68±0.31 kg/sqm higher BMI than the WHO and 0.9±0.53 kg/sqm than the IOTF reference population.

Table 13. Weight references (kg) for girls

Age (years)	p3	p5	p10	p25	p50	p75	p90	p95	p97
0	2.48	2.57	2.72	2.97	3.24	3.51	3.76	3.91	4.00
0.25	4.44	4.60	4.87	5.31	5.81	6.32	6.78	7.07	7.25
0.5	5.94	6.12	6.42	6.92	7.49	8.08	8.63	8.97	9.19
0.75	6.90	7.09	7.41	7.95	8.56	9.21	9.82	10.21	10.45
1	7.74	7.93	8.25	8.80	9.44	10.12	10.76	11.17	11.43
1.5	8.90	9.12	9.49	10.12	10.86	11.63	12.38	12.85	13.15
2	9.90	10.15	10.57	11.28	12.12	13.00	13.84	14.37	14.71
3	11.70	11.99	12.48	13.34	14.37	15.50	16.62	17.35	17.82
4	13.36	13.70	14.28	15.31	16.58	18.01	19.48	20.46	21.11
5	14.90	15.31	16.00	17.26	18.85	20.71	22.69	24.05	24.97
6	16.86	17.31	18.08	19.48	21.27	23.38	25.65	27.24	28.31
7	18.51	19.05	19.99	21.72	23.97	26.68	29.66	31.77	33.23
8	20.43	21.07	22.19	24.30	27.06	30.44	34.24	36.99	38.91
9	22.55	23.31	24.64	27.14	30.42	34.43	38.94	42.19	44.45
10	24.70	25.62	27.23	30.25	34.23	39.13	44.64	48.62	51.39
11	27.31	28.42	30.34	33.97	38.77	44.66	51.26	56.01	59.31
12	30.09	31.44	33.76	38.06	43.56	50.05	56.98	61.75	64.96
13	41.70	42.89	44.92	48.58	53.15	58.39	63.87	67.58	70.05
14	38.99	40.27	42.44	46.39	51.35	57.09	63.12	67.22	69.96
15	41.70	42.89	44.92	48.58	53.15	58.39	63.87	67.58	70.05
16	43.58	44.71	46.64	50.13	54.52	59.60	64.97	68.65	71.11
17	44.35	45.48	47.41	50.92	55.37	60.56	66.11	69.94	72.52
18	44.61	45.75	47.70	51.27	55.78	61.05	66.70	70.60	73.23

Legend: Kg – kilograms; p3 – 3rd percentile; p5 – 5th percentile; p10 – 10th percentile; p25 – 25th percentile; p50 – 50th percentile; p75 – 75th percentile; p90– 90th percentile; p95 – 95th percentile, p97 – 97th percentile.

2.3.4. Discussion

From 1997 to 2003, the WHO performed a Multicenter Growth Study collecting data from six countries (United States, Brazil, Ghana, India, Norway, and Oman) on the growth of children from affluent families. The key assumption was that "environmental differences rather than genetic endowments are the principal determinants of disparities in physical growth" (Garza C and Onis 2004). These children were exclusively or predominantly breastfed for at least 4-6 months, complementary foods were introduced by 6 months of age, and breastfeeding was continued to at least 12 months of age, and they showed similar growth patterns. Since it was considered not feasible to perform similar longitudinal growth studies in older children, WHO developed a growth reference based on the 1977 USA growth study as an international "normative", though in fact descriptive, chart from 5 to 19 years of age (de Onis et al., 2007).

Table 14. BMI reference (kg/m2) for boys

Age (years)	p3	p5	p10	p25	p50	p75	p90	p95	p97
0	11.35	11.56	11.91	12.51	13.19	13.92	14.62	15.06	15.34
0.25	14.55	14.81	15.23	15.96	16.80	17.70	18.55	19.10	19.44
0.5	15.10	15.37	15.81	16.58	17.48	18.43	19.35	19.94	20.32
0.75	15.06	15.35	15.82	16.64	17.61	18.65	19.66	20.32	20.74
1	14.91	15.19	15.66	16.47	17.44	18.48	19.51	20.18	20.61
1.5	14.45	14.71	15.16	15.95	16.89	17.92	18.95	19.62	20.06
2	14.13	14.38	14.80	15.53	16.41	17.38	18.35	18.99	19.40
3	14.05	14.27	14.64	15.28	16.06	16.92	17.79	18.36	18.74
4	13.94	14.15	14.48	15.08	15.81	16.61	17.43	17.97	18.32
5	13.76	13.96	14.30	14.90	15.65	16.48	17.35	17.92	18.31
6	13.72	13.93	14.29	14.93	15.73	16.65	17.62	18.28	18.73
7	13.74	13.97	14.36	15.08	15.99	17.06	18.23	19.06	19.62
8	13.97	14.21	14.63	15.39	16.38	17.56	18.88	19.82	20.48
9	14.34	14.59	15.02	15.83	16.88	18.17	19.62	20.68	21.43
10	14.72	15.00	15.46	16.34	17.48	18.90	20.52	21.73	22.60
11	15.17	15.46	15.95	16.88	18.11	19.64	21.41	22.74	23.70
12	15.51	15.82	16.35	17.35	18.67	20.31	22.23	23.66	24.70
13	16.02	16.34	16.90	17.94	19.33	21.07	23.10	24.63	25.74
14	16.47	16.81	17.41	18.52	19.99	21.83	23.97	25.58	26.74
15	17.06	17.41	18.01	19.14	20.60	22.40	24.45	25.95	27.02
16	17.61	17.96	18.57	19.71	21.20	23.01	25.07	26.58	27.64
17	17.99	18.35	18.98	20.14	21.66	23.52	25.62	27.16	28.24
18	18.15	18.53	19.18	20.38	21.95	23.86	26.01	27.57	28.67

Legend: BMI – body mass index; kg/m2 – kilograms/square meters; p3 – 3rd percentile; p5 – 5th percentile; p10 – 10th percentile; p25 – 25th percentile; p50 - 50th percentile; p75 – 75th percentile; p90 – 90th percentile; p95 – 95th percentile; p97 – 97th percentile.

Previous clinical experience in Romanian children strongly questions that WHO standards/ references are truly suitable for the Romanian population. Particularly in view of current secular trends in height and the marked economic progress in Romania, it appeared desirable to actualize previous National Romanian growth charts.

Recent meta-analyses of historic data (NCD 2016) summarized currently known empirical data on a century of trends in global adult human height from 1914 to 2014 and reports variation between and within populations of up to 20 cm. This proves the need for up to date growth curves.

The aim of this study was to derive synthetic national growth references for the Romanian population. This approach has been proven costeffective and in statistical agreement with the classical studies for constructing growth charts and has been used in countries where recent growth charts are not available (Georgia, Kazakhstan) (Hermanussen M et al., 2010) or for specific ethnic sub-populations (Redlefsen et al., 2007).

There are previous regional studies aiming to provide new growth references (Chirita et al., 2012), but there is no recent one with a national representative sample. Limitations of the studies include: all data are from school measurements, which are subject to sampling error. Medical history was not analyzed, therefore children with conditions which might impair normal growth and development were not excluded.

Table 15. BMI reference (kg/m2) for girls

Age (years)	p3	p5	p10	p25	p50	p75	p90	p95	p97
0	11.26	11.45	11.77	12.31	12.94	13.60	14.23	14.62	14.87
0.25	13.62	13.87	14.28	14.99	15.81	16.69	17.53	18.06	18.40
0.5	14.44	14.70	15.12	15.85	16.70	17.62	18.51	19.08	19.44
0.75	14.61	14.87	15.30	16.07	16.97	17.96	18.93	19.56	19.97
1	14.56	14.82	15.25	16.00	16.90	17.89	18.86	19.50	19.91
1.5	14.20	14.44	14.85	15.58	16.47	17.46	18.48	19.15	19.60
2	13.92	14.15	14.53	15.22	16.07	17.04	18.05	18.73	19.18
3	13.67	13.88	14.25	14.91	15.73	16.67	17.65	18.33	18.78
4	13.59	13.79	14.14	14.77	15.57	16.49	17.49	18.18	18.65
5	13.39	13.61	13.97	14.64	15.49	16.50	17.61	18.40	18.94
6	13.46	13.67	14.04	14.72	15.59	16.64	17.80	18.64	19.23
7	13.46	13.70	14.11	14.87	15.85	17.06	18.42	19.42	20.12
8	13.69	13.95	14.39	15.21	16.28	17.57	19.02	20.08	20.82
9	13.95	14.23	14.70	15.58	16.73	18.12	19.68	20.82	21.62
10	14.30	14.60	15.12	16.08	17.34	18.88	20.61	21.88	22.77
11	14.72	15.04	15.60	16.65	18.01	19.67	21.55	22.92	23.88
12	15.04	15.41	16.05	17.24	18.81	20.76	22.99	24.64	25.81
13	15.60	15.99	16.65	17.90	19.54	21.58	23.92	25.66	26.89
14	16.32	16.69	17.34	18.55	20.12	22.03	24.19	25.75	26.85
15	16.94	17.30	17.92	19.06	20.52	22.28	24.20	25.58	26.52
16	17.31	17.67	18.29	19.41	20.83	22.50	24.30	25.56	26.41
17	17.51	17.86	18.47	19.57	20.96	22.60	24.35	25.56	26.39
18	17.57	17.92	18.53	19.63	21.00	22.60	24.29	25.45	26.24

Legend: BMI – body mass index; kg/m2 – kilograms/square meters; p3 – 3rd percentile; p5 – 5th percentile; p10 – 10th percentile; p25 – 25th percentile; p50 - 50th percentile; p75 – 75th percentile; p90 – 90th percentile; p95 – 95th percentile; p97 – 97th percentile.

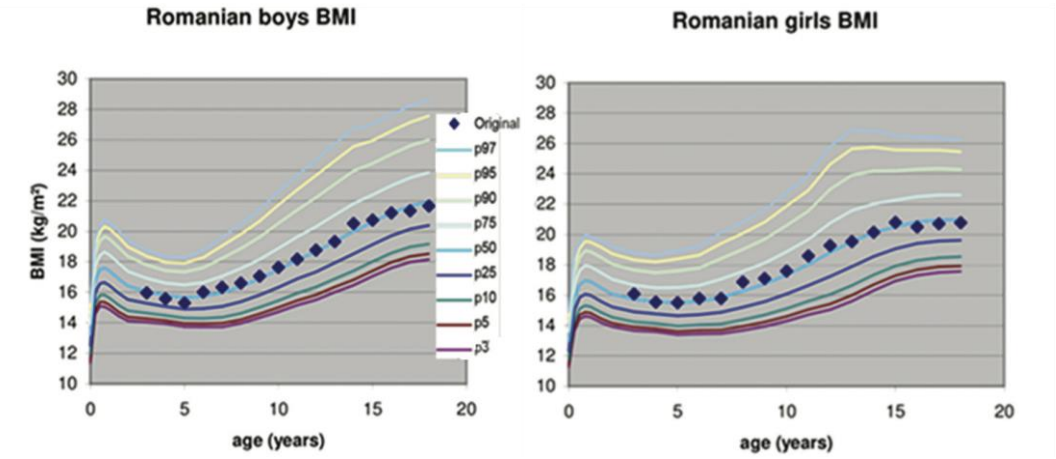


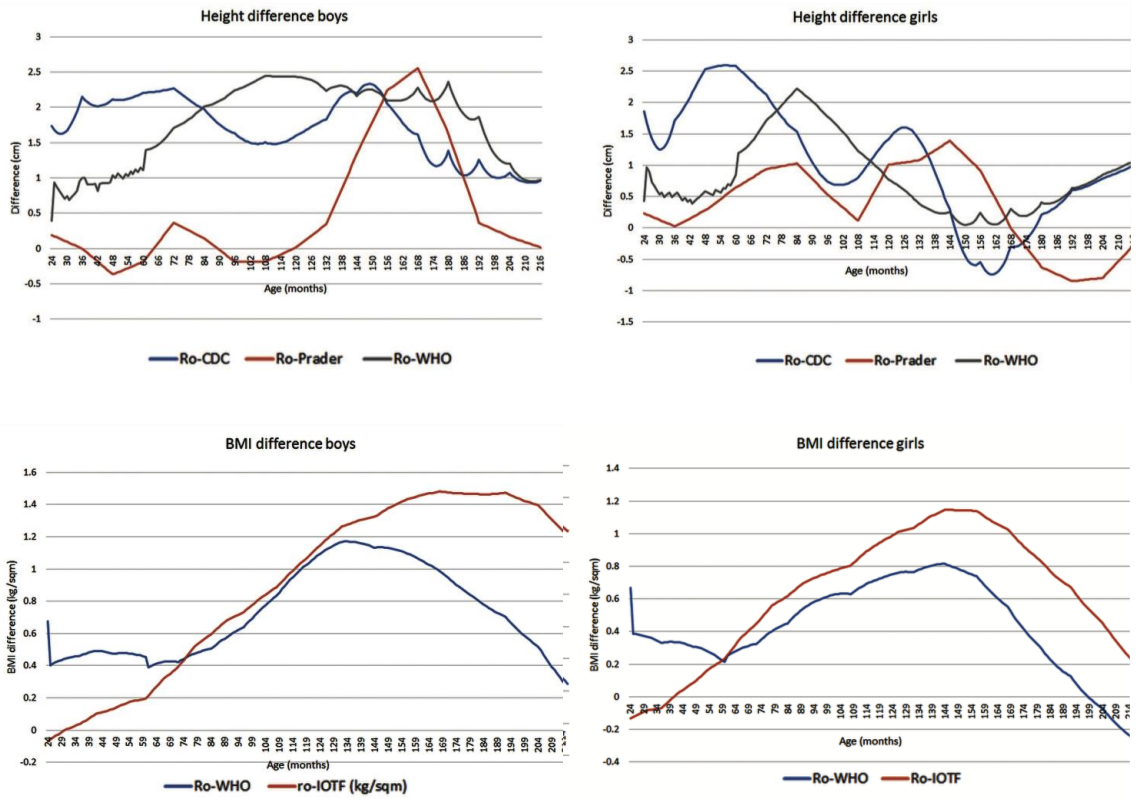
Fig. 13. Synthetic BMI centiles Legend - p3 – 3rd percentile, p5 – 5th percentile, p10 – 10th percentile, p25 – 25th percentile, p50 – 50th percentile, p75 – 75th percentile, p90 – 90th percentile, p95 – 95th percentile, p97 – 97th percentile.

The majority of the studiesconsisted of children from urban areas.It is worth mentioning that in one sub-study (data from Targu Mures) children in urban areas have

higher odds of being short, but not overweight comparing to their rural counter parts (Pascanu et al., 2016). Further studies regarding the existing ethnic and maybe regional differences are needed. According to the latest census, 3% of the Romanian population is of Rromanes ethnicity (INS 2011), almost the same percentage as the Turkish population in Germany (Statisches Bundesamt 2014). In Germany (Redlefsen et al., 2007) and also in the Netherlands (Fredriks et al., 2003; Schönbeck et al., 2015), special growth charts are available for ethnic minorities.

2.3.5. Conclusions

We suggest synthetic growth references based upon recent growth data from 6 different Romanian regions as new National Growth Charts for Romanian children.



a b

Fig. 14. Height (a) and BMI (b) differences between the synthetic and the common used references. Legend - Ro - Romanian synthetic reference, CDC - US growth reference, WHO - World Health Organization's growth reference, Prader - Swiss growth reference, IOTF - International Obesity Task Force reference.

2.4. Human growth hormone treatment in idiopathic short stature – pros and cons

2.4.1. Introduction

The use of the growth hormone therapy in children with idiopathic short stature is a subject of great ethical debate. Prior to the advent of biosynthetic GH in 1985, only the children with classic GH deficiency were eligible for treatment. The natural growth hormone derived from the pituitary of cadavers has been used since 1958 until 1985, when several cases of Creutzfeld-Jacob's disease were reported for indiv iduals that ten to fifteen years before had rece ived cadaver-derived GH . Based on the assumption that the infectious vector causing the disease was transferred along with the cadaver-derived GH, this product was removed fro m the market. The biosynthetic process of GH involves a chemical synthesis of the DNA fragment encoding the first 24 amino acids and complementary DNA copies of messenger RNA prepared from human pituitary cells. The entire DNA sequence is introduced into a bacterium, Escherichia coli, which enables the synthesisofGH.

In October 1985, the Food and Drug Administration (FDA) approved the new drug named Protropin for use in treating ch ildren with growth hormone deficiency or chronic renal failure. In December 1996, the recombinant growth hormone was approved for use in treating girls with Turner's syndrome. In July 2003, FDA approved biosynthetic GH for the treatment of children with short stature of unknown etiology (idiopathic short stature-ISS), sometimes known as " short, but otherwise normal" (Cuttler 2005).

A virtually unlimited resource of human pituitary – derived GH has led to an increasing number of approved indications. In Turner's syndrome numerous studies demonstrate that GH can accelerate growth and lead to a greater height than predicted (an average gain of 9 cm over the predicted height without treatment) (Carel et al., 2005). Cases of GH-treated children with chronic renal insufficiency on dialysis or transplant revealed significantly greater growth rates in the first and second year of treatment. The treatment with GH for more than 2 years before the onset of puberty in children born small for gestational age (SGA) sustains an accelerated growth rate and the normalization of height in contrast to untreated SGA control subjects (Allen 2006). Children with Prader- Willi's syndrome show a higher growth-rate in response to GH therapy similar to other severly GH deficient children (Eiholzer 2005; Loke 2005). The treatment with GH will allow some of these children to achieve a height closer to lower adult normal range.

Personal contribution – published paper:

Preda C, Ungureanu MC, Leustean L, Cristea C, Vulpoi C. Ethical issues related to the use of human growth hormone in idiopathic short stature. *Rev Rom Bioet* 2013; 11(4): 31-37.

2.4.2. GH treatment and idiopathic short stature

Being short is part of the natural diversity of the human race. Such terms such as "short normal stature", "normal variant short stature" and "familial short stature" have been used for a while (Wygold 2002). Idiopathic short stature, on the other hand, is a relatively

recent term and refers to children who are very short compared to their peers, for unknown or hereditary reasons (Lifshitz 1998).

There is no universal consensus regarding the definition of idiopathic short stature that remains as a heterogeneous group and a diagnosis of exclusion. The definition should be adapted depending on race, geographical area and even historical period. There is also an absence of generally accepted criteria for diagnosing: inadequate secretion, partial GH deficiency or dysregulation ofGH secretion.

By definition, the GH secretion is assumed to be normal in sense of a normal GH peak after a standard provocation test, but subtle disorders of spontaneous GH secretion may be involved in some children labelled as idiopathic short stature (Martin 2013). The fact that biochemical tests for GH deficiency are unreliable led, inevitably, to this grey area of "insufficiency" of GH.

In the past decade, there have been many studies reporting the use of GH therapy in idiopathic short stature. There is a general consensus that the GH treatment leads to an increased growth rate in the first years of treatment, but the evidence, however, rarely extends beyond an apparent gain in final height (Martin 2013; Buchlis et al., 1998; Lee et al., 2006; Ross et al., 2004; Wit et al., 2005).

Many of the controversies surrounding the treatment with growth hormone are related to the absence of well-defined objectives for therapy, the lack of consensus on definition of short stature, costs, medicalisation of short normal children and the capability of informed consent in children.

The ethical issues associated with the attempt to enhance the stature of a short, but otherwise healthy child, have been renewed (Voss 2006). Is idiopathic short stature a social prejudice, and more than that, may it lead to a psychological disability? First of all, the term " idiopathic " is unfortunate since it implies a pathology (Allen 2006). In any normal population, there will be short normal children who will be at the lower segment of the Gaussian distribution. Treating short, normal children with growth hormone might open a Pandora box (Lifshitz 1998). The treatment of one group of children may create illness in another previously healthy group (Allen and Fost 2004; Sandberg and Colsman 2005). It is very important to know if the added height with growth hormone is a real gain for the psychological benefit, or just a placebo effect (Lifshitz 1998).

There are also important economic issues regarding the growth hormone therapy: there are many children who lack acces to the most basic health care and may seem ethically inappropriate (violation of justice and equity principle) to spend colossal monetary resources to provide GH therapy to anyone who is not classically GH deficient or resistant (Leschek et al., 2004). But is this opinion acceptable for the parents (for whom every centimeter counts) or for those children who reaching adulthood and having judgment is to late to be treated with GH ?

The biosynthetic growth hormone is one of most expensive treatment regimensavailable.Theannualcostfor one child weighing 30 kg is approximately $ 15 000 to 20 000. The cost per inch (2.5 cm) of the adult height growth is estimated to be $ 35 000 to 52 000 (Allen 2006). Treatment costs of adolescents using higher "pubertal" doses to maximize adult height can exceed $ 50 000 per year. With the idiopathic short stature indication, it has been estimated that more than 400 000 children aged 4 to 15 years are now

eligible for GH therapy. Not all children with idiopathic short stature will be treated with the growth hormone but the cost will still be considerable (Lee et al., 2009). There is a debate about whether the growth hormone for idiopathic short stature is a medical treatment or an enhancement therapy. The growth hormone therapy for idiopathic short stature represents one of the major challenges for the health care system.

Autonomy and responsibility in children are two concepts very difficult to comprehend. Young children do not understand the risks and benefits of therapy and cannot give valid informed consent or assent (Ciuca 2009). The average age of 10.3 years was chosen by health care providers for ma king such medical decision. But many children with idiopathic short stature are under 10 years of age.

Parents and legal guardians are those who make the decision for their children. It is normal to be concerned that your "short" child is disadvantaged and might be discriminated in school. On the other hand, demands for the treatment may also be motivated, less by concerns for the children, than by aspiration of their parents. The parents' attempt to modify their child's appearance may signal tacit disapproval and make them feel unacceptable as they are (Voss 2000). Are there children who would prefer to remain short or adults to accept retroactively the non-use of a cure from various reasons ?

According to the literature, short people have emotional and behavioral problems: social isolation, low self-esteem, less marital success, low job satisfaction (Sandberg and Colsman 2005). Some authors have suggested that short children are conditioned to behave in a socially immature manner and that the stereotypical anxious, introverted short child could well be the result of "experiences associated with the small stature". Parental overprotection has in fact been shown to be a strong predictor of victimization by peers in school (Voss 2006). Even if short children may report higher rates of bullying or victimization some authors consider that they do not experience significant psychosocial problems attributable to their short stature (Kemp et al., 2005) and that there is no statistically significant relationship between height and friendship, popularity or reputation among peers, and therefore social behaviour, friendship and acceptance among peers had a minimal impact on extreme stature (Ross et al., 2004; Sandberg et al., 2004). The Wessex Growth Study (WGS), a prospective cohort study, had a unique recruitment technique of unselected population of short, but healthy children, and has shown no evidence of maladaptation or psychological dysfunction, before, during and after puberty (Voss 2006). Because psychological problems are not common in short children, it is not surprising that attentively designed studies failed to demonstrate a relationship between adult height of GH treated individuals and quality of life (Ross et al., 2004; Sandberg et al., 2004). This lack of evidence for predictable psychological benefit in children with idiopathic short stature who have been treated with GH does not mean that children with short stature should not be treated with GH (Ross et al., 2004; Sandberg et al., 2004). It is acceptable that in children with Turner's syndrome or chronic renal insufficiency, the stress of dealing with other diseases could exacerbate an adverse psychological effect caused by their short statur. Health care providers need to carefully assess the parents' perception of short stature but is questionable whether they could ensure families that children with this condition will not experience social, emotional or behavioural issues compared with their taller peers (Kemp et al., 2005)

On the other hand, although the modern society wants to avoid discrimination, the media promotes aestethic models which disadvantage short or even midle-height persons.Most sports relate the performance with being tall and some professional standards exclude the short stature (police, army). Even in politics indiv iduals would rather give their vote to taller people expressing power and trust, with notable exceptions of course (but not in the left central area of the gaussian distribution).

There are concerns regarding the safety of GH therapy and the incidence of the side effects in children with idiopathic short stature. To date, GH treatment has been relatively safe, with no significant side effects reported (Germak 1996; Guyda 1999).

The experience of the past 20 years has shown that GH therapy in children with idiopathic short stature is generally safe, but continued surveillance is necessary because pharmacological doses used to treat non-GH deficient children continue to rise in response to dose-related benefits in growth out-come. The long-term risks of prolonged treatment with higher doses of growth hormone remain unknown, since there is still no evidence about the potential side effects after long periods of time from treatment. Further studies are important to assess the safety of long-term growth hormone therapy in children with idiopathic short stature (Finkelstein 2002).

Being tall is indisputably viewed as a benefit in our culture, and is associated with multiple advantages, including higher income, academic achievement, self-esteem and social status. The concepts of normality and abnormality are very difficult to define, as they subsume many sociocultural variables (Leschek et al., 2004).

The stigma of the short stature can be addressed in various ways: parents, teachers, nurses can be important by interacting with the short child (Miller et al., 2006). Parents can encourage them to participate in sports and other social activities. Role modelling, promoting positive self-image can improve the child's self confidence.

2.4.3. Final remarks

Children with idiopathic short stature and their families must be encouraged to avoid pereceiving height as a disability and to achieve performance on their own terms. Parents of children with id iopathic short stature may need to undergo counselling about the limitations of growth hormone therapy.

The medical decision to use growth hormone for idiopathic short stature is controversial and individual. Before starting treatment, physicians should explain to the parents the advantages and disadvantages of daily treatment with growth hormone emphasized on: potential side effects, limited expectations in final height, adequate monitoring, cost and the (unknown long-term) risks.

GH therapy would be ethically acceptable in the following cases: children with classic growth hormone deficiency, ch ildren with chronic renal failure who are a wait ing kidney transplantation, girls with Turner's syndrome, children whose extreme short stature keeps them from participating in basic activities of their daily living, and who have a condition for which the efficacy of growth hormone therapy has been demonstrated.

Idiopathic short stature is one of many conditions for which the elective treatment may, or may not be damaging to patients. Therefore, more studies are necessary to determine the risks and benefits of this type of treatment.

We believe that GH-treatment in idiopathic short stature is disputable: if analysing the benefits, potential risks (theoretically possible but virtually unattested), autonomy (children with no or limited discernment), justice and equity, the decision involving all four classical commandments of bioethics. The final decision should depend on the value scale of each family although the economic factor remains limited.

CHAPTER 3. THE ALCHEMY OF THE THYROID GLAND

3.1. State of the Art

A healthy thyroid is a critical component of one's overall health, and many people are struggling with thyroid disorders such as hypothyroidism, specifically Hashimoto's autoimmune thyroiditis. In this autoimmune condition, the immune system attacks the thyroid gland, with the resulting inflammation leading to an underactive thyroid gland or hypothyroidism. Hashimoto's disease is the most common form of hypothyroidism and was the first condition ever to be classified as an autoimmune disease.

Taking into account the thyroid health, we must focus on a multitude of environmental factors that may affect thyroid function, including gluten, gut health, stress, excess iodine, and vitamin D deficiency. The dietary changes are always the first step in treating Hashimoto's, and replacement thyroid hormone therapy is often necessary for a successful outcome.

Baumann, in 1895, characterized iodine as an essential element of thyroid tissue by and its efficacy to prevent goiter was demonstrated by Marine in Northern USA in 1916-1920 (Baumann and Fromm, 1895).

Goiter and cretinism with severe endemic manifestations had been almost entirely eliminated from continental Western Europe and Northern America before the 1930's;however large populations elsewhere and even some places in Western Europe (Sicily) were still affected up to the 2000's (Halpern et al., 1991).

Iodine deficiency has complex public health consequences which are not limited to endemic goiter and cretinism. Its related systemic disorders include also increased neonatal death rate and decreased intellectual development. Although these consequences are not included in the current estimation of the Global Burden Disease related to iodine deficiency (Hetzel, 1983).

In isolated places severe iodine deficiency still heats as a public health problem, mostly in hard-to-reach and/or politically neglected populations. To take under control we emphasize the importance of maintaining international cooperation efforts, in order to monitor iodine status where iodine deficiency is now adequately controlled, and identify at risk population where it is not.

The goal should be now global eradication of severe iodine deficiency. Commercial distribution of iodized salt remains the most appropriate strategy. A randomized clinical trial in New Guinea clearly showed in the 1970's that correcting severe iodine deficiency early in pregnancy prevents endemic neurological cretinism. This supports the essential role of thyroid hormones of maternal origin on the normal fetal development, during the first trimester of pregnancy (i.e. when fetal thyroid is still not functional). A randomized clinical trial in Congo (RD) in the 1970's also showed that correcting severe iodine deficiency during pregnancy prevents myxoedematous cretinism, particularly prevalent in affected Congolese areas (Kassebaum et al., 2016).

The EURopean micronutrient RECommendations Aligned (EURRECA) Network of Excellence is working towards developing aligned micronutrient recommendations across Europe (Ashwell et al., 2008).

An adequacy selenium status may influence iodine metabolism because of selenium's role in the iodothyronine deiodinase (IDI) enzymes, which convert the pro-hormone thyroxine (T-4) to the more potent active form tri-iodothyronine (T-3) in various tissues. Additionally, selenium plays a role of antioxidant in glutathione peroxidase, which may protect the thyroid gland from oxidative damage due to excess H2O2 produced during thyroid hormone synthesis.

Thus selenium deficiency may exacerbate some effects of iodine deficiency and may have a role in the aetiology of iodine deficiency disorders (Arthur and Beckett, 1999).

Studying the detrimental effects of marginal selenium intakes on thyroid status, Derumeaux et al found an inverse correlation between selenium status and thyroid volume in females in France, and also a protective effect of selenium against goitre and thyroid tissue damage (Derumeaux et al., 2003).

Low selenium level may compromise even further our declining iodine status, resulting in an inhibition of the conversion of T-4 to T-3, as recent studies suggested (). This paper reports on three further studies investigating (a) the relationship between seleniumstatus and thyroid status in a New Zealand population and (b) the effect of selenium supplementation on thyroid hormones (thyroid stimulating hormone (TSH) and the ratio of T-3/T-4) (Vanderpas and, Moreno-Reyes, 2017).

Although the importance of selenium for bone metabolism is unknown, some clinical conditions such as Kashin-Beck osteoarthropathy have been associated with selenium deficiency. Although selenium deficiency induces growth retardation in rats, it has not been established whether this growth inhibition is associated with changes in bone metabolism.

Maintenance of “selenostasis” via optimal intake not only aids preservation of general health but also contributes substantially to the prevention of thyroid disease (Duntas, 2010).

Low serum selenium concentrations have been associated with a diagnosis of differentiated thyroid cancer in small studies in selenium deficient areas. Also low 25-hydroxyvitamin D concentrations have been associated with several malignancies.

Recent studies confirm the association between serum TSH and advanced thyroid cancer and a potential association between selenium concentrations and higher thyroid cancer stage but no such association for 25-hydroxyvitamin D concentrations. Larger prospective studies will be required to confirm this association and its causative in nature. It seems likely

that selenium concentrations would influence thyroid cancer development via an independent mechanism from that of TSH (Jonklaas et al., 2013).

Following the same idea, it is known that the factors associated with postthyroidectomy hypocalcaemia were defined to be "gender, preoperative diagnosis, parathyroid gland injury, nodule size and *vitamin D deficiency*". It is a multifactorial issue and it might be proper to define etiopathogenic pathway (Ozogul, 2014).

This research direction has been materialized by publishing the follwing articles:

1. **Preda C**, Vasiliu I, Bredetean O, Ciobanu DG, Ungureanu MC, Leustean EL, Grigorovici A, Oprisa C, Vulpoi C. Selenium in the environment: essential or toxic to human health? *Environ Eng Manag J* 2015; 15(4): 913-921.
2. **Preda C,** Ungureanu MC, Leuștean L, Cristea C, Mogoș V, Vulpoi C, Gavrilescu M. Human health related to iodine environmental occurrence and its deficiency in water and food. *Environ Eng Manag J* 2013; 12(5): 1045-1049.
3. Vulpoi C, Ungureanu MC, Azoicăi DA, Vulpoi-Naghel I, Anton MC, **Preda C.** Screening for iodine deficiency – more than a medical approach. *Eur Sci J* 2014; 3:175-179.
4. **Preda C**, Vasiliu I, Mihalache L, Armașu I, Șerban IL, Șerban DN, Stoica B, Ciobanu DG, Bredețean O, Strungaru SA, Nicoară M, Plavan G, Vulpoi C. Selenium- essential antioxidant element. The example of autoimune thyroiditis. Rev Chim Bucharest 2017; 68(7):1617-1621.
5. Grigorovici A, Costache M, Velicescu C, Savin G, Ciobanu D, **Preda C**. Disecația radicală a gâtului în cancerul tiroidian avansat. *Chirurgia* 2010; 105 (5): 669-672.
6. Grigorovici A**,** Varcus F, Mogoș S, Călin A, Hînganu D, Hînganu MV, **Preda C.** Hypocalcemia after thyroidectomy for advanced local malignancies. *Rev Chim Bucharest* 2019; 70(3):1053-1057.
7. Mihalache L, Arhire LI, Gherasim A, Graur M, **Preda C**. A rare case of severe type 4 polyglandular autoimmune syndrome in a young adult. *Acta Endocrinologica (Buc)* 2016; 12(1): 104-110.

3.2. SELENIUM AND IODINE - AKNOWLEDGMENT AND PERSPECTIVES

3.2.1. Introduction

Micronutrients, mostly iodine and selenium, are required for thyroid hormone synthesis and function. Iodine is an essential component of thyroid hormones and its deficiency is considered as the most common cause of preventable brain damage in the world. About 800 million people are affected by iodine deficiency disorders that include goiter, hypothyroidism, mental retardation, and a wide spectrum of other growth and developmental abnormalities.

Iodine supplementation, under form of iodized salt and iodized vegetable oil, produced dramatic improvements in many areas, even though iodine deficiency is still a problem not only for developing countries. In fact, certain subpopulations like vegetarians may not reach an adequate iodine intake even in countries considered iodine-sufficient. A

reduction in dietary iodine content could also be related to increased adherence to dietary recommendations to reduce salt intake for preventing hypertension.

Furthermore, iodine intakes are declining in many countries where, after endemic goiter eradication, the lack of monitoring of iodine nutrition can lead to a reappearance of goiter and other iodine deficiency disorders.

Three different selenium-dependent iodothyronine deiodinases (types I, II, and III) can both activate and inactivate thyroid hormones, making selenium an essential micronutrient for normal development, growth, and metabolism. Furthermore, selenium is found as selenocysteine in the catalytic center of enzymes protecting the thyroid from free radicals damage. In this way, selenium deficiency can exacerbate the effects of iodine deficiency and the same is true for vitamin A or iron deficiency. Substances introduced with food, such as thiocyanate and isoflavones or certain herbal preparations, can interfere with micronutrients and influence thyroid function.

There is an important relationship between environment, health and diseases. Elements from: soil, water and air are major factors involves in important mechanisms vital for the proper functioning of the human body.

It is the case of iodine which is mandatory for the synthesis of thyroid hormones. As an important constitutive of thyroid hormones, iodine is involved in all their actions: growth, development and metabolisms. It is required throughout the life-cycle and is obtained through the diet (Brantsaeter et all., 2013).

Deficient iodine intake is well known to be associated with reduced thyroid hormone production. The result of this deficit will be the goiter (thyroid enlargement) and a series of other disturbances, so called iodine deficiency disorders (IDD) (Zygmunt et all., 2012).

The excess of iodine can also have adverse effects depending on thyroid function, extent and duration of iodine excess (Brent, 2010). So, an optimal (regular and adequate) iodine amount reduces susceptibility to thyroid diseases which represents major public health problems.

The iodine deficiency is an old but still existing issue not only from the medical point of view but also from the environmental one. Soils from mountain regions (Himalayas, Alps) are iodine deficient. This problem is aggravated by deforestation and soil erosion.

The food from iodine deficient regions will never provide the right amount of iodine to the population living there. Iodine deficiency results mainly from geological rather than social and economic conditions. Besides nutritional iodine deficiency, a variety of other environmental, socio-economics and cultural factors are involved in aggravation of iodine deficiency (Kapil, 2007).

Iodine insufficiency is not only a problem in developing countries but also a major public health problem in many countries from Europe and Western world (Brantsaeter et all., 2013). In many parts of developed world the iodine deficiency has reemerged despite various programs to reduce its incidence (Blumenthal et all., 2012).

Therefore, in the last few decades, there has been remarkable progress in the global effort to eliminate iodine deficiency which is the world's greatest single cause of preventable brain damage (Brantsaeter et al., 2013).

Selenium (Se_{79}^{34}) was first isolated in 1817 by Jacob Berzelius but its importance in

human health and ecosystem was recognized in 1957. The name is derived from Selene, the Greek goddess of the moon and is a nonmetal of the same family as sulfur and oxygen (Mehdi et al., 2013). Se is present in the environment (water, soil and air) in very low concentrations (< 1μg/g). The natural environment has a profound influence on the Se content of soil, crops and human tissues. Se is indispensable for normal plant growth and functioning of animal organisms (Bem, 1981). Se from soil and water enters the food chain through the root ways of plants and aquatic organisms (Saranac et al., 2011). Se is an essential element for human health. Food as source of Se in human nutrition can contain inorganic (selenite, selenite) or organic (Se-amino acids, Se-methylated and Se-proteins) forms of Se. The organic forms are more available than the inorganic ones (Ježek et al., 2012). The body pool of Se is: 30% in the liver, 15% in the kidney, 30% in the muscle and 10% in the plasma. Most of Se in tissues and fluids (blood) is found in proteins (seleno-proteins, selenotrisulphides and other acid-labile Se compounds) (FOA/WHO, 2001).

While moderate Se deficiency has no or subtle clinical symptoms, severe Se deficiency leads to: Keshan disease, characterized by failure of myocardium function (cardiomyopathy) and Kashin- Beck disease, characterized by osteoartropathy (damage of cartilages causing deformations of bone structures) (Ježek et al., 2012). Low Se supply is linked to the incidence of prostate cancer; also growth retardation, impaired bone metabolism and osteopenia were found in Se-deficient male rats. Se deficiency was associated with cardiovascular diseases, thrombosis and atherosclerosis (Kohrle et al., 2005). The toxic effect appears when chronically over limit daily intake of Se is present (Ježek et al., 2012). In seleniferous areas it is possible that people consuming locally grown food may manifest signs of Se toxicity (selenosis).

The environmental conditions and agricultural practices have a great influence on Se content in different foods. The Se load in vegetables, wild- grown mushrooms fruits, meat, fish and water depends on factors such as: soil composition, agricultural practices, plant species, and pollution. Therefore, the average adult Se intake can vary by geographic area and others multiple variables. China, India, Middle–East and some European countries are extremely low in soil Se resulting in Se deficiency in the local population (Dharmasena, 2014). In contrast, soil from driest regions tends to concentrate high quantities of Se. Also, alkaline soils release more Se than acid ones (Joy et al., 2015).

The safe level of total Se intake for an 70 kg adult who subsists on a normal diet (reference dose- RfD) has been set at 350 μg/day corresponding to 5 μg Se/kg body weight/day (Dharmasena, 2014). The role of Se is very important in living organisms. The understanding the Se soil-plant-animal axis is mandatory in covering the requirement of the organisms in this element (Mehdi et al., 2013).

There are many issues related to environmental Se and human health, in terms of the link between soil-plants-human body and Se, need for Se supplementation in general population in order to prevent severe deficiency but also to avoid Se excess or toxicity. The lack of suitable frameworks in general population represents an issue for the assessment of health/economic impact of Se deficiency.

Personal contribution – published papers:

Preda C, Vasiliu I, Bredetean O, Ciobanu DG, Ungureanu MC, Leustean EL, Grigorovici A, Oprisa C, Vulpoi C. Selenium in the environment: essential or toxic to human health? *Environ Eng Manag J* 2015; 15(4): 913-921.

Preda C, Ungureanu MC, Leuștean L, Cristea C, Mogoș V, Vulpoi C, Gavrilescu M. Human health related to iodine environmental occurrence and its deficiency in water and food. *Environ Eng Manag J* 2013; 12(5): 1045-1049.

Aim of this section is to highlight the role of micronutrients in thyroid function and diseases their role. Only by research trials o large populational groups, Se and iodine deficiencies could be managed corectly.

3.2.2. Iodine and Se in the environment

3.2.2.1. Iodine: general data

Iodine is a non-metallic microelement from the halogen family and exists in nature as a monovalent anion. The isotope I127 is the stable, naturally occurring iodine (Risher and Keith, 2009).

Like the other halogens, free iodine occurs mainly as a diatomicmolecule I_2, which is toxic for eyes and lungs. Iodine in air can combine with water particles and precipitate into water or soils, where it combines with organic matter and remains in the same place for a long period of time. A number of analytical methods can be used to determine iodine level in air, water, soils and foods: ion chromatography, colorimetry, arsenic-cerium catalytic spectrometry (Risher and Keith, 2009).

3.2.2.2. Iodine cycle

Iodine is quite scarce in the environment, and even sea water contains a very low concentration of iodide well below that of fluoride, chloride and bromide (Rokita). The concentration in the earth's crust is 0,5 mg/kg and in the atmosphere the iodine concentration is 10-20 ng/m² (Risher and Keith, 2009).

Iodine concentration in water and soil reflects the environmental iodine distribution and is an indirect index of environmental pollution and also is directly associated with the content of food iodine which affects living quality (Lu et all., 2005).

Environmental transport and distribution of iodine are performed by a complex of physical, chemical and biological mechanisms so called global iodine cycle (**Fig. 15**).

During iodine global cycle, iodine is moved from soil and ocean sediment to water and the atmosphere (Risher and Keith, 2009).This cycling through terrestrial ecosystem is poorly understood, due to its complex environmental chemistry and low natural abundance (Landis et all., 2012).

The primary source of iodine on land is volatilization from the ocean surface (marine algae emit organic iodine vapour) (Risher and Keith, 2009). Brown algae of the Laminariales (kelps) remained a major source of iodine. They represent an important pump in the global biochemical cycle of iodine. In coastal regions Laminariales are a major contributor to the iodine flux from the ocean to the atmosphere through the production of volatile halocarbons (Kupper et all., 2008). Same orders of brown algae have developed a capability to accumulate

and to use iodine in physiological adaptation to stress. Marine halogenated natural products have biological functions as defense compounds and signaling molecules for the producing organism (La Barre et all., 2010).

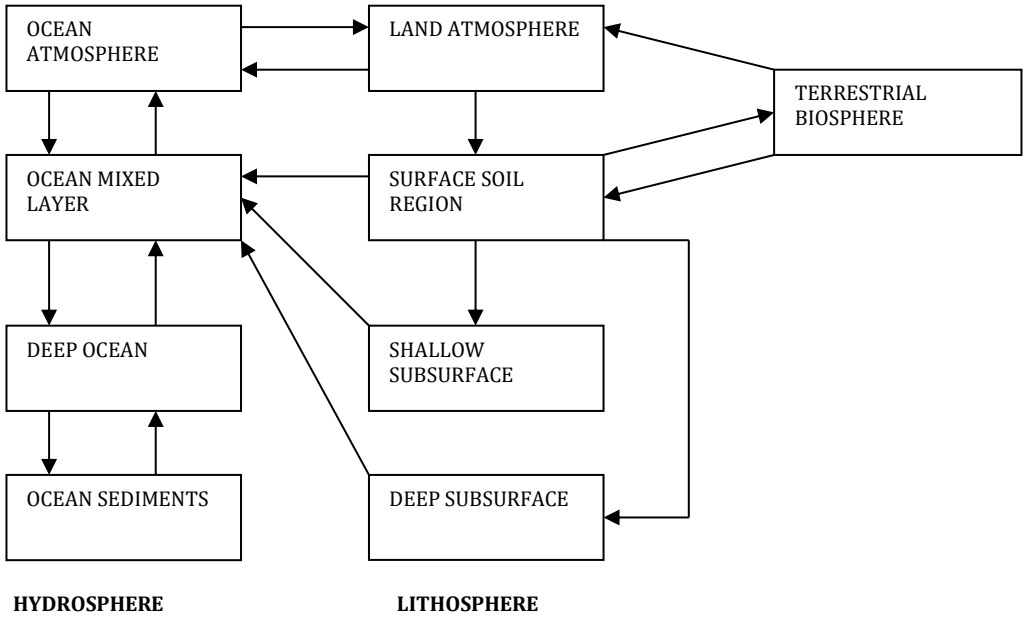


Fig. 15. Three groups of compartments with steady-state iodine content interconnected in order to show transfer rates among the ocean, land and terrestrial biosphere (after Risher and Keith, 2009)

Combustions of fossil fuels also transfer the iodine to the atmosphere. Decay of the vegetation, volcanic activity, weathering of rock and human activities all contribute to the deposition of iodine in soil (Risher and Keith, 2009).

3.2.2.3. Iodine sources

The iodine **soil** status represents the relation between the supply of iodine and the soil's capacity to retain it. The coastal zones have the highest amount of iodine but the lowest capacity to retain it. There are many factors involved in the complex mechanism of iodine fixation in soil: acidity conditions, the chemical form of the iodine, the soil's organic content and texture (Johnson et all., 2003) (**Table 16**).

Table 16. Textural classification of soils according with iodine content (Johnson et all., 2003).

SOIL	PEAT	CLAY	SILT	SAND
I (μg/g)	7	4,3	3	2,2

Organic-rich soils are not good providers of iodine in the food chain due to poor bioavailability (iodine is strongly fixed and not bioavailable).

Iodide is the most mobile form in the soil comparing with iodate; acidic soil conditions are suited for iodide and alkaline oxidizing conditions are suited for iodate form (less soluble) (Johnson et all., 2003).

The iodine in surface **waters** is the best index of the environmental iodine status. The general level of iodine reported in rivers and lakes ranges from 1-10 μg/l (Johnson et all., 2003). The concentration of iodine differs depending on the water origin (**Table 17**).

Table 17. Iodine content in sea, rain and river water (Risher and Keith, 2009)

	I concentration
SEAWATER	45-60 μg/l
RAINWATER OVER OCEANS	1-15 μg/l
RAINWATER OVER LAND	0,1-15 μg/l
RIVER WATER	0,1-18 μg/l

The **food** chain make iodine available to humans. The sources of dietary iodine are: food and food additives (kelp, seaweed, iodinated salt, red coloring, iodine additives to bread/flour, therapeutics (Burek and Talor, 2009).

Seafood is the most important source of iodine in the diet (the content of iodine in: marine seafood 160-3200 μg/kg, shellfish 0.798-1.6 mg/kg, kelp and seaweeds 1-2 mg/kg) (Risher and Keith, 2009).

The grazing animals and their products are also important suppliers of iodine, furthermore, during food production the level of iodine rise in milk, eggs and meat (use of fish flour as chicken food, iodates used as oxidants and a sanitizing agent in bread manufacturing, iodophors used as antiseptics in the dairy industry) (Johnson et all., 2003). In fact, the major source of iodine in highly developed countries are: dairy products (27-47 μg of iodine/kg), eggs (93 μg of iodine/kg), grain and cereal products (47 μg of iodine/kg) (Risher and Keith, 2009).

The iodine content in plants depends on the intake-retention ratio and a correct manipulation of this mechanism could improve iodine biofortification of crops leading to the prevention of iodine deficiency diseases (Landin et all., 2012). The iodine is not concentrated in the seeds but in leaves and so, the proportion of leaves make the difference regarding the iodine content (legumes > vegetables > fruits) (Johnson et all., 2003).

The absorption of dietary iodine is reduced by: nitrates, fluorides, calcium, magnesium, smoking, thiocyanates. Iodine, in large quantities (from radiological contrast media, antiarrhythmic drug amiodarone, dental and skin disinfectants) may interfere with the normal distribution of iodine in human body.

3.2.2.2. Physiochemical characteristics of Se

Se is a nonmetal mineral, a trace element and an essential micronutrient (Winther et al., 2014). Six Se isotopes coexist in nature (mass number: 74, 76, 77, 78, 80 and 82). At ordinary temperature Se is a solid substance. Se occurs naturally in inorganic forms: selenite (SeO_3^{-2}), selenide (Se^{-2}), selenate (SeO_4^{-2}) and selenium element and organic forms: selenomethionine and selenocysteine. The inorganic, anionic forms are highly soluble, mobile, bioavailable and potentially toxic. The organic forms come from decomposition of plants that accumulate Se (Mehdi et al., 2013).

3.2.2.2.1. Selenium in soil

The basic source of dietary Se for humans and animals is soil. Soils are the major

source of Se for plants, soil Se existing in various forms: elemental Se, selenates, selenides and organic Se compounds. Different geographic locations have different Se content. Se availability for plants is decreased by low pH and high concentrations of sulfur and phosphorus (Hall et al., 2013). Se tends to be concentrated in the soil of the driest regions in the world. The toxic effect on animals occurs in these regions.

Alkaline soils release more Se than acid ones (Mehdi et al., 2013). Soil with Se content lower than 0.3 mg/kg-1 is insufficient and higher than 3 mg/kg-1 is toxic. The average Se content varies with the soil type, climate and area (Ježek et al., 2012).

The chemical form of Se in the soil is determined by soil pH and redox potential: Se binds to iron oxide clay minerals and organic material. Forest soils efficiently retain Se and then incorporate it into low-molecular-weight fractions of humic substance (FAO/WHO, 2001).

Soil type may influence the selenium content of food crops. Use of soil-specific composition data can improve estimates of dietary mineral supplies. In some countries such as Malawi total Se concentrations in maize grain from calcareous soils was greater than in grain from non-calcareous soils (Joy et al., 2015).

3.2.2.2.2. Selenium in water

Aqueous Se can exist in three oxidation states: selenide, selenite and selenate in natural waters. The dominant hydrolysis complexes for selenide, selenite and selenate are: HSe^-, $HSeO_3^-$ and SeO_4^{2-}, respectively, in the pH range of 3 to 9. Porcella (1991) quoted by Parkman and Hultberg (2002) described the biological pathways by which Se is transported in freshwater systems (**Fig.16**).

The major entries into the food web are: incorporation of Se by phytoplankton and microheterotrophs and uptake from sediments by benthic organisms. Both these two pathways may transform Se to new forms or pass it to higher trophic levels by being eaten (Parkman and Hultberg, 2002).

Se compounds toxicity to aquatic organisms is a very important issue. Se-methionine is extremely toxic to Daphnis at concentrations of 4 to 8g/l-1 while lethal concentrations for midge larvae were about 1000 times higher. Se at the same concentrations in mixtures of sea water and seleniferous leachate from coal fly-ash was less toxic than the pure sodium selenite.

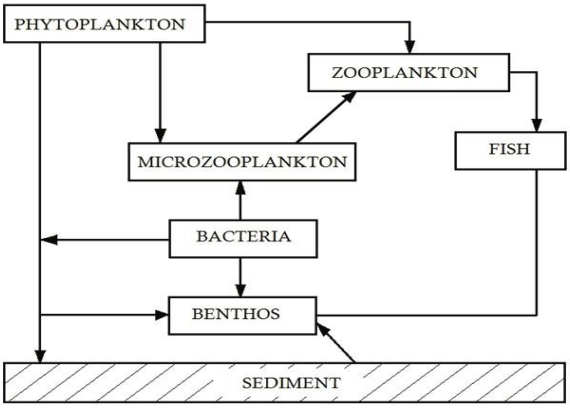


Fig. 16. Potential C and Se pathways in a model freshwater ecosystem (Porcella et al., 1991 cited by Parkman and Hultberg, 2002)

There are different models to determine the transport in water and sediments (Pintilie et al., 2007) and also the uptake kinetics, toxic effects on growth, reproduction and mortality of various chemical forms of Se to multiple aquatic organisms. The fish may be considered as sensitive as lower organisms for certain Se-exposure.

The most important aspect of Se in aquatic organisms is not the direct toxicity to the organisms themselves, but the position in the food chain and the dietary source of the Se they provide to organisms that feed on them (Parkman and Hultberg, 2002).

3.2.2.2.3. Selenium in plants

The role of Se in the life cycle of plants which absorb organic selenium compounds accumulated in the soils of semiarid areas is very important (Revanasiddappa and Kumar, 2001). Se concentration in plants is in direct relation with its surrounding soil content.There are seleniferous plants, Se accumulating plants and others plants with an average content of Se (Mehdietal., 2013).Uptake of Se by plants is influenced not only by the Se content in soil, but also by the Se form, soil reaction, soil redox potential, mineral structure of soil, mineral fertilizers, atmosphere and rain precipitation (**Fig.17**). According to the amount of Se accumulated, plants can be grouped in three categories:

1) Selenium non-accumulators containing up to 25 mg Se/kg^{-1} of dry matter (cereals, potatoes, grass,
2) Secondary selenium accumulators absorb from 25 up to 100 mg Se/kg^{-1} (various species as Aster, Astragalus, Atriplex).
3) Selenium accumulators can contain 100-10000 mg Se/kg^{-1} of dry matter (various species as Stanleya, Haplopappus) (Ježek et al., 2012).

Transformation and assimilation of Se in plants is in relation with sulfur metabolism. Most plants take up selenite because of its similarity to sulfate and metabolize it via the sulfur assimilation pathway (Mehdawi et al., 2011).

The toxic effect of Se in plants is attributed to interactions with sulfur metabolism. Replacement of sulfur cysteine and methionine amino acids with selenium amino acids can disturb the biochemical reactions and enzymatic functions within the cells (Ježek et al., 2012).

3.2.3.Se, Iodine and thyroid

3.2.3.1. Iodine general data

The healthy human body contains 15-20 mg of iodine, of which about 70-80% is present in the thyroid gland (Kapil, 2007). Iodine is essential for the synthesis of thyroid hormones which are extremely important due to their action on virtually every cell in the body. Growth and development are major process depending on thyroid hormones which implies a normal iodine intake.

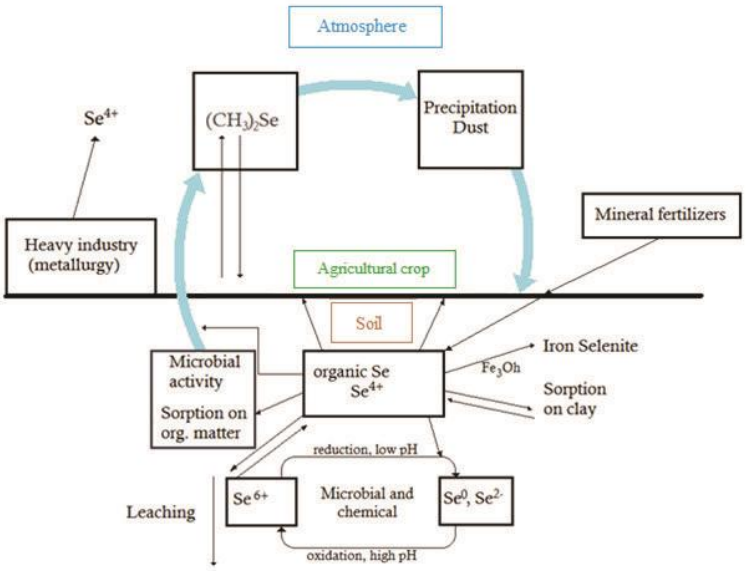


Fig. 17. Selenium cycle in an agroecosystem (Gissel-Nielsen, 1998, cited by Ježek et al., 2012)

Thyroid function and developmental abnormalities arise if the physiological requirements of iodine are not met. Public health consequences of severe iodine deficiency include: goiter, mental retardation, infant mortality and decreased fertility. Iodine deficiency is considered also as a variable involved in thyroid cancer etiology (Lukas et al., 2012).

About a third of the world's population live in iodine deficient areas. The iodine deficiency is exacerbated by the concomitant decrement in other nutrients such as: selenium, iron, zinc and vitamin A (Nyenwe and Dagogo-Jack, 2009).

3.2.3.1.1. Iodine and synthesis of thyroid hormones

Iodine from food, water or supplements is absorbed as molecular iodine by the stomach and duodenum; inorganic compounds of iodine are also rapidly absorbed when inhaled in vapour or aerosol form through the lungs. In the gastrointestinal tract, iodine, in form of water-soluble salts and food iodine are 100% absorbed compared to inorganig iodine and iodate which require reduction to iodide in the gut (Risher and Keith, 2009). The active transport of iodine from the blood into the thyroid is regulated by: Thyroid Stimulating Hormone (TSH) and by the concentration of iodine in the blood (Yarrington and Pearce, 2011). The thyroid gland has evolved not only to trap iodine avidly from dietary sources but also to store this element in organic form in order to maintain the secretion of thyroid hormones during periods of iodine deficiency.

In the thyroid gland iodine is oxidized to form "active" iodine which iodinate the tyrosine molecules and by coupling iodinated tyrosine residues form thyroid hormones: thyroxine (T4) and triiodothyronine (T3) (Yarrington and Pearce, 2011) (**Fig.18**).

The thyroid hormones acts through specific receptors in target organs leading to specific effects such as: increased basal metabolic rate, stimulation of normal growth and most of all normal levels of thyroid hormone are essential to the development of the fetal and neonatal brain.

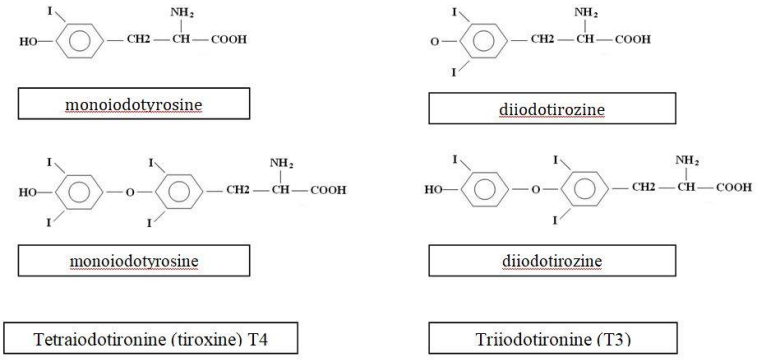


Fig. 18. Structure of thyroid hormones and precursors

3.2.3.1.2. Iodine deficiency disorders (IDD)

Iodine deficiency is defined by the WHO as a population median urinary iodine concentration (UIC) that falls below 100 μg/L: 50-99 μg/L is mild, 20-49 μg/L is moderate and < 20 μg/L is severe (Skeaff et al., 2012).

The most severe IDD are encountered when iodine deficiency affects embryonic and fetal development. Many of the underlying anatomical lesions occur during the early stages of intra-uterine development and the damage is irreversible by birth. So, the effects of severe iodine deficiency depends upon the period of life exposed to this deficit (**Table 18**).

Mild iodine deficiency may influence developmental impairment in children. There are limited studies reporting association between prenatal iodine status or suboptimal maternal iodine intake and cognitive function of infants and children up to 18 months (Brantsaeter et al., 2013; Gunnarsdottir and Dhal, 2012). The psychomotor and mental performances of a population are adversely affected when fetuses and neonates are exposed to moderate or mild levels of iodine deficiency.

Table 18. Functional and developmental disorders associated with severe iodine deficiency.

PERIOD	DISORDERS
FETAL	Abortions Stillbirths Congenital anomalies Endemic cretinism
CHILD	Goiter Impaired mental function Retarded physical development Increased susceptibility to nuclear radiation
ADULT	Goiter Impaired mental function Hypothyroidism Increased susceptibility to nuclear radiation

In pregnant women with normal thyroid function, mild iodine deficiency is present in 66% during the first trimester. In the third trimester the prevalence of iodine deficiency is present in ¾ of cases (Bruker-Davis et al., 2012).

While the neurocognitive development is dramaticaly damaged in severe form of iodine deficiency the consequences of mild forms of iodine deficiency is still less documented.

The population most assessed to reveal iodine deficiency is school-age children because it is very efficient and practical to survey such group (Zygmunt et al., 2012).

In order to evaluate the iodine status several criteria are used: assessment of thyroid volume by ultrasound (when more than 5% of school children population present enlargement of the thyroid it is called endemic goiter), measurement of urinary iodine concentration (UIC), measurement of neonatal TSH (recommended only to detect severe iodine deficiency), measurement of plasma tyroglobulin (Zbranca et al., 2008; Vandevijvere et al., 2012).

The best parameters to evaluate the iodine deficiency are: goiter incidence and iodine urinary concentration (Vulpoi et al., 2002). Urinary iodine concentration (UIC) is a good marker for recent dietary intake of iodine. It is now the index of choice for evaluating the degree of iodine deficiency and for monitoring its correction (Zygmunt et al., 2012). Using all this parameters is possible to classify the degree of iodine deficit (**Table 19**).

3.2.3.1.3. Prevention of IDD

More than 2 billion people are at risk to develop iodine deficiency (Campbell et al., 2012). According to World Health Organisation (WHO), between 1993-2003, a number of 54 countries suffered from iodine deficiency and 3 billion peoples had insufficient iodine intakes, infants, pregnant and lactating women being among those most affected (Mirmiran et al., 2012).

The recommendations of WHO-United Nation Children's Found (UNICEF)-International Council for the Control of Iodine Deficiency Disorders (ICCIDD) for adequate iodine intake are: 150 μg/ day for adults, 220-290 μg/ day for pregnant and lactating women (Campbell et al., 2012).

Table 19. Classification of the iodine deficiency according to: goiter, TSH and IUC (Zbranca et al., 2008).

PARAMETER	NORMAL	MILD	MODERATE	SEVERE
Schoolchildren and adults				
goiter incidence	< 5	5- 19,9	20- 29,9	≥ 30
UIC (μg/dl)	> 10	5- 9,9	2- 4,9	< 2
Newborns				
incidence of TSH> 5mUI/l (%)	< 3	3- 19,9	20-39,9	≥ 40
UIC (μg/dl)	> 10	3,5-9,9	1,5-3,4	< 1,5

The global strategy to prevent IDD is universal salt iodization, which is safe, cost-effective and sustainable. Even the risk of iodine excess is low, regular monitoring of urinary iodine is recommended also, more research on the impact of the excess iodine intake is necessary (Wu et al., 2012).

WHO and UNICEF recommendation for controlling iodine deficiency disorders is universal salt iodization (USI), which consist of adding iodine (as iodate or as iodide) to all salt for human consumption. This procedure is highly effective and low-cost. The current WHO-UNICEF-ICCIDD guideline recommends salt iodization in the range of 20-40 mg of iodine per kg of salt (Campbell et al., 2012).

The two most commonly used vehicles for iodine supplementation are: salt iodization and iodized oil injections. There are operational difficulties in the implementation of iodized oil injection program (availability of trained staff to administer injections and population approach to this procedure) and also for the iodized salt program (lack of infrastructure, resistance of the population to change the type of salt they are consuming) (Pandav, 2012).

Since 1999 the number of products to which iodised salt could be added was increased due to the change of dietary habits to include: breakfast cereals, breakfast biscuits, crisp bread and pickling brine in the production of meat products (Vandevijvere, 2012).

Biofortification of vegetables with iodine provides a mild but significative increase in urinary iodine concentration and together with the habitual use of iodized salt may contribute to the improvement of iodine nutritional status of populations (Tonacchera et al., 2013).

The fortification of bread significantly increased urinary iodine concentration but not to a level compatible with iodine sufficiency, suggesting that supplements are still required despite the iodized salt initiative in bread and cereal production (Clifton et al., 2013).

Iodine enrichment in yest- creating a technology of iodine-enriched yeast production is of strong necessity and could be applied in both human and animal nutrition (Dolinska et al., 2012).

To prevent the iodine deficiency in pregnant women was necessary in some countries to offer iodine, iron and folic acid tablets and for neonates and children under 5 years iodine and iron supplements (Tonmukayakul et al., 2012).

For the developing countries the main methods used to combat iodine deficiency are: iodization of salt (requires special packaging and national commercial networks), intramuscular injection of iodinated oil (costly and requires medical intervention), oral intake of iodinated oil (costly, requires distribution infrastructure and long-term compliance) (Fish et al., 1993).

The benefits of correcting iodine deficiency (decreased prevalence of goiter and other thyroid disturbances) are far more important than the possible thyroid disorders induced by the iodine supplementations (hyperthyroidism or hypothyroidism in elderly) (Lombardi et al., 2013).

Unfortunately not all countries have legislation for mandatory salt iodization and iodized salt quality and thereby population iodine status is not controlled appropriately in many countries (Mirmiran et al., 2012).

Despite the universal salt iodization, mild iodine deficiency has re-emerged in some country from Europe, Australia due to changes in food pattern (Skeaff et al., 2012).

Besides medical intervention (enhancement of iodine in food and drink or by oral or parenteral iodine administration) the environmental measures are necessary: preventing the removal of iodine by flooding, improving the ability of the soil to fix iodine, changing crops development, reducing iodine volatilization from the soil-plant system (Johnson et al., 2013).

Also there are several environmental contaminants (perchlorate, thyocianate and

nitrate) which in pharmacological doses can affect iodine uptake and so the thyroid function (Pistea et al., 2013; Yarrington and Pearce, 2011; Zewdie et al., 2010).

The consumers, food producers, media representatives, public health professionals and healthcare workers must be educated by all interventions and measures possible in order to better understand the necessity of a healthy diet (Vandevijvere, 2012).

3.2.3.2.1. Selenium metabolism

Se enters the food chain through plants and the amount of Se in food is directly correlated with the level of Se in soil (Li et al., 2014). In animals the absorption of Se takes place in the duodenum and caecum. Inorganic forms are absorbed by simple diffusion or by active transport through a sodium pump.

Organic forms (selenomethionine, selenocysteine) are absorbed in the small intestine by an active mechanism. The hepatic Se concentration reflects the level of intestinal absorption. Se is transported by blood in form of selenoprotein P. Urine is the dominant route of excretion of Se in animals (Mehdi et al., 2013). From food 70-95% of organic forms are absorbed, than metabolized as proteins. The inorganic forms are absorbed and deposited in tissues in small extent (Ježek et al., 2012).

In the human body Se has different concentrations in different organs: 30% in the liver, 15% in the kidney, 30% in the muscle and 10% in the plasma. Most of the Se in tissues is found in proteins (seleno-proteins, selenotrisulphides and other acide- labile Se compounds) (FOA/WHO).

3.2.3.2.2. Selenium and nutrition

Different types of food, such as biological materials and dairy products are important sources of Se in human diet (Shaltout et al., 2011). Serum Se levels are influenced by the dietary Se concentrations, which in turn are dependent on the soil Se content, form and distribution of Se in foods (Se from plant source foods is more bioavailable than animal source foods) (Lander et al., 2008).

The recommended Se daily intake in adulthood varies greatly according to different authors, countries and organizations (**Table 20**). Schrauzer and White (Dharmasena, 2014) estimated that daily Se intake per person is in the range of 90- 168 μg. In Asia, Africa and many European countries the daily Se intake is under the recommended dose.

Se deficiency has negative consequences mostly in seniors, pregnant and lactating women, growing and developing children (Ježek et al., 2012), thus for different life stages there are different dietary allowances (**Table 21**). In order to provide a safe total Se intake the reference dose (RfD) from all nutritional sources for a 70 kg adult has been set at 350 μg/day, corresponding to 5 μg Se/kg body weight/day (Dharmasena, 2014).

There are different ways to increase the dietary Se intake: increased consumption of foods rich in Se (meet, fish or grains), nutritional educational programs, water Se supplementation, pharmacological substances, Se fortification of food and bio fortification (Lopez-Bellido and Bellido, 2013).

It is necessary to adopt agricultural-based programs to enhance the mineral composition of food in regions with risk of Se deficiency by enriching fertilizers with Se (Yan et al., 2010; Chilimba et al., 2011). In 1984, in order to improve Se content in food, Finland implemented the Se-enriched fertilizers (agronomic biofortification) which have continued to date. All types of cereal grains and all grain fractions are enriched with Se when

Se forms are added to fertilizers (Hurst et al., 2013).

Se supplementation of fertilizers has affected all domestic agricultural products. The mean Se intake of the population was calculated on the basis of food consumption statistics, urinary Se excretion data and average serum concentration of both urban and rural people. Enrichment of fertilizers with sodium selenite in areas with low Se seems to be an efficient way to increase Se concentration in food (Varo et al., 1988).

Table 20. Recommended daily intake in adults (men/women) for different countries and organizations, based on the minimum quantity necessary to optimize the GPX activity (WHO consider recommendable minimum intake to attend 2/3 from optimum activity of GPX) DRI (Dietary Reference Intake), RNI (Reference Nutrient Intake), PRI (Population Reference Intake), NRE (Normative Requirement Estimate) (Lopez-Bellido Garrido and Lopez Bellido, 2013, with permission)

RDA (Recommended Dietary Allowance) μg Se per day							
	DRI/RDI USA	*RNI/RDAUK*	*PRI/RDA EU*	*NRE WHO*	*RDA USA*	*RNI/RDA WHO*	*RDI/RDAAUS*
Country/ Organization	USA 1989 (Thomson, 2004)	UK 1991	EU 1993 (Thomson, 2004)	WHO 1996	USA 2000	WHO 2004	AUSTRALIA & NE 2005 (Hawkeford and Zhao, 2007)
Men	70	75	55	40	55	34	70
Women	55	60	55	30	55	26	60

Table 21. Maximum safe daily intake of Se for particular age categories (Ježek et al., 2012)

Life stage	*Age*	*Dose (μg Se.day^{-1})*
Infants	0-6 months	45
	7-12 months	60
Children	1-3 years	90
	4-8 years	150
	9-13 years	280
Adults	>14 years	400

3.2.3.2.3. Role of selenium in the human body

Se is an essential micronutrient important for many aspects of human health (Verma et al., 2011) which plays a major part in optimal endocrine response, immunomodulation and inflammatory process (Dharmasena, 2014).

Se is incorporated into cysteine forming the 21st amino acid used during protein synthesis. Selenoproteins have vital functions in the body: essential antioxidant enzymes that fight cancer, regulators of thyroid function, structural proteins in sperm required for fertility, reduce virulence associated with certain viral infections (Weeks and Hanna, 2012).

The effect of Se in humans is concentration- dependent, ranging from an antioxidant activity in the nanomolar-micromolar range to a potentially prooxidant activity at concentrations higher than required for selenoprotein synthesis (Negro, 2008). There are two distinct families of Se-containing enzymes: glutathione peroxidases and thioredoxin reductase involved in controlling tissue concentrations of oxygen-containing metabolites and

iodothyronine deiodinases which are essential in the conversion of thyroid hormone thyroxin to its active form- triiodothyronine (FAO/WHO, 2001).

The selenoenzimes are capable of modifying cell function by acting as antioxidants and the redox status; also they influence cell growth, apoptosis, and modify the action of cell signaling systems and transcription factors. Se is incorporated co- translationally into the selenoproteins as a selenocysteine residue that is fully ionized at physiological pH and acts as a very efficient catalyst. Most of known selenoproteins are expressed in the thyroid gland: glutathione peroxidases (GPXs), thyoredoxin reductase (TRs) and iodothyronine deiodinase (type D1, D2 and D3). Selenoprotein P, W, selenophosphate synthetase and many others exist in different organs and have multiple actions, many of them unknown (Beckett and Arthur, 2005) (**Table 22**).

The testis contains high concentrations of Se and experiments on selenoprotein P-knockout mice indicate that Se is essential in testicular function. GPX4 provides the link between Se, sperm and male fertility (Beckett and Arthur, 2005). Se has insulin-mimetic properties: an insulin-like effect of Se in cultured rat adipocytes include stimulating glucose transport, phosphodiesterase activity and ribosomal phosphorylation (Beckett and Arthur, 2005).

The beneficial effect of Se on autoimmune mechanism is a complex one in which the inhibitory effect on HLA-DR molecule expression and anti-oxidative capacity are involved (Balázs and Kaczur, 2012). The thyroid contains more Se per gram of tissue than any other organ (Effraimidis and Wiersinga, 2014).

Adequate Se intake assures thyroid hormone synthesis and metabolism and also protects the gland from damage from excessive iodine exposure. In regions with both deficit in Se and iodine it is mandatory to normalize Se intake before iodine supplementation to prevent endemic goiter (Saranac et al., 2011).

3.2.3.2.4. Selenium deficiency

Se deficiency can affect human health in different ways. The severe endemic defficiency is associated with: Keshan disease (congestive cardiomiopathy), Kaschin-Beck disease (chronic, endemic osteochondropathy) and the mild one with limited expression of various Se-dependent enzymes (Lopez-Bellido Garrido and Lopez Bellido, 2013). Causes of deficiency are low dietary intake or poor intestinal absorption.

The non-endemic form is more common in individuals maintained on parenteral or enteral feeding for long periods of time. In infants Se deficiency may be present when formulas with low Se content or without added Se are used. Clinical manifestations are very uncommon and nonspecific: myalgia, muscular weakness, congestive heart failure.

In order to develop such conditions the daily Se intake must be under 10 μg per day. The endemic forms Keshan disease and Kaschin-Beck disease present variable distribution depending on geochemical factors. Acid soils high in organic matter and iron oxide content may be responsible for fixing Se in forms which are poorly absorbed by staple crops (FAO/WHO, 2001).

Table 22. Mammalian selenoproteins and their function (Beckett and Arthur, 2005)

Selenoproteins	*Proposed Function*
Gluthatione peroxidase (GPXs)	
GPX1	Antioxidant in cell cytosol; Se store?
GPX2	Antioxidant in gastrointestinal tract
GPX3	Antioxidant in extracellular space and plasma
GPX4	Membrane antioxidant; structural protein in sperm; apoptosis?
GPX5	Unknown
GPX6	GPX1 homologue?
Thyoredoxin reductase (TRs)	Multiple roles including dithiol-disulphide oxoreductase[SEP]Detoxifies peroxides, reduces thioredoxin (control of cell growth); maintains redox state of transcription factors
TR1	Mainly cytosolic, ubiquitous
TR2	Expressed by testes
TR3	Mitochondrial, ubiquitous
Iodothyronine deiodinases	
Type D1 and D2	Converts thyroxine (T4) to bioactive 3,5,3$^{'}$-tri-iodothyronine (T3)
Type D1 and D3	Converts thyroxine (T4) to bioinactive 3$^{'}$,3$^{'}$,5$^{'}$reverse T3
Selenoprotein P	Selenium-transport protein. Antioxidant on endotelium
Selenoprotein W	Antioxidant in cardiac and scheletal muscle?
Selenophosphate synthetase (SPS2)	Synthesis of selenophosphate for selenoprotein synthesis
15 kDa Selenoprotein (Sep 15)	Protects against cancer?
H, I, K, M, N, O, R, S, T, V	Role largely unknown

The clinical features of Keshan disease are acute or chronic episode of a heart disease characterized by cardiogenic shock, enlarged heart, congestive heart failure, cardiac arrhythmias and ECG changes. There are 4 types of clinical manifestations: acute, chronic, subacute and insidious. During autopsy, moderate enlargement with dilation of all heart chambers was found in most cases. Histopathologically, multifocal necrosis and fibrous replacement of myocardium are scattered throughout the heart muscle (Chen, 2012).

Kashin-Beck disease is an endemic osteochondropathy. The disease starts in childhood and affects the growth of joint cartilage, the joints become deformed and painful, and the worst forms result in dwarfism. The joints most frequently involved are: finger, wrist, ankles, knees and elbows. Geographically Kashin-Beck disease has a typical endemic distribution in Eastern Siberia of Russia, China and North Korea. Three major environmental hypotheses including endemic selenium deficiency, cereal contamination by mycotoxin-producing fungi and high humic acid levels in drinking water have been proposed (Farooq et al., 2012).

3.2.3.2.5. Selenium toxicity

Se is a complex element due to its properties of being both essential and toxic, leaving a narrow range within which intake is healthy. Organic and inorganic forms of Se have similar toxic effects. Selenosis is the most common disease as is due to over limit intake of Se from food.

The clinical sigs are: abnormalities of nervous system, fragility and loss of hair and nails, nausea, mottled teeth, hives, diarrhea (Ježek et al., 2012, Nazemi et al., 2012). In humans, at higher levels, Se becomes toxic and nonspecific replacement of cysteine by selenocystein in protein disrupts protein function causing toxicity and death (El Mehdawi et al., 2011).

The upper limit in relation with the toxic effect varies with the geographic region and population characteristic. In some US regions with naturally high Se content in soil, a daily intake of 724 μg per day has no toxic effects. In China selenosis occurs when the daily Se intake is over 910 μg per day. Moreover, when daily Se doses of 1600-3200 μg were used in order to treat cancer only mild symptoms of toxicity were present (Ježek et al., 2012). Exposure to air pollution can lead to Se excess causing brain damage in young people and Se increase in frontal lobe with age in exposed subjects (Calderón-Garcidueñas et al., 2013).

The signs and symptoms of human overexposure to Se are not well defined. Common clinical features are: icteric skin, gastrointestinal disturbances, hair loss and nail dystrophy when food supply exceeds 900 μg/day. There are no sensitive biochemical markers for Se intoxication. In their absence it is suggested that the tolerable upper intake level for Se should be 400 μg/day for adults (FOA/WHO, 2001). The recommended daily intake, tolerable upper nutrient intake level, insufficient and toxic intake for Se are shown in **Fig.19**.

3.2.3.2.6. Selenium supplementation in thyroid disorders

The role of Se supplementation is still debated even though there are in vitro and in vivo data on its positive effect in reducing cancer-associated mortality, severity of autoimmune disease, oxidative damage and improving mental health, reproductive performances, evolution of AIDS (Weeks and Hanna, 2012). Autoimmune thyroid diseases arise due to complex interactions between environmental and genetic factors. Low birth weight, iodine and selenium excess or deficiency, reproductive span, parity, stress, seasonal variation, radiation, smoking, allergy, viral and bacterial infections have an important role in the development of thyroid an autoimmune disorders (Prummel et al., 2004).

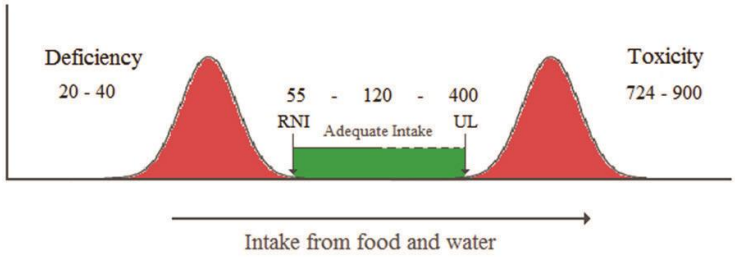


Fig. 19. Distribution and requirements to prevent Se deficiency and toxicity (the values are in μg Se per day; RNI= recommended nutrient intake, UL= upper limit)

The thyroid is the endocrine gland with the highest Se content because it expresses

specific selenoproteins. Se status appears to have an important impact on thyroid metabolism (selenoproteins have a major role in the synthesis and action of thyroid hormones) and thereby seems to be involved in thyroid pathology (Balázs and Rácz, 2013; Bhuyan et al., 2012).

In a recent crosssectional, prospective European study (Krassas et al., 2014) a linear correlation of Se levels and Se protein P was found in patients with thyroid disorders, indicating a less than optimal Se status. Patients with Graves' disease and Hashimoto thyroiditis had significantly lower Se levels compared with patients with non-autoimmune disease. The lower levels of Se in patients with autoimmune thyroid disorders suggest that Se supplementation may be useful.

The effect of Se supplementation in autoimmune thyroid disorders is controversial however the European Food Safety Authority (EFSA, 2009) advise for a normal thyroid function a daily dose of 200 μg/day with a maximum recommended dose of 350 μg/ day

Combination of myo-inositol and Se improves the subclinical hypothyroidism in autoimmune thyroiditis (Nordio and Pajalich, 2013). Se (selenomethionine 200μg per day) and l-thyroxin therapy is effective in decreasing anti-thyroid peroxidase antibodies in patients with autoimmune thyroiditis (Duntas et al., 2003). Se 200μg per day for 9 months is effective in increasing GPx activity and decreasing thyroid autoantibody levels (Turker et al., 2006). In children with autoimmune thyroiditis, 100- 200 μg sodium selenite per day does not decrease thyroid autoantibody levels (Bonfig et al., 2010).

Se supplementation could alleviate inflammatory thyroid lesions by inhibiting IL-2 expression and others cytokines (Tan et al., 2013). Treatment with 200μg selenium-enriched yeast for 12 months in autoimmune thyroiditis was used to assess the quality of life (Winther et al., 2014).

Although clinical applications still need to be defined, in pregnant women with Hashimoto thyroiditis Se supplementation significantly decreases the rate of postpartum thyroiditis and hypothyroidism (Drutel et al., 2013).

Also, the beneficial effect of Se on mild Graves's inflammatory orbitopathy is being studied (Dharmasena, 2014). A Cochrane systematic review (van Zuuren et al., 2013 cited by Effraimidis and Wiersinga, 2014) concluded that present data do not allow confident decision making about the use of selenium supplementation for Hashimoto's thyroiditis. Further studies are needed to support the beneficial effect of Se in thyroid disorders.

3.2.3.7. The "protective" effect of Se against methyl- mercury (MeHg) exposure

For many years Se was considered as a „natural" Hg antagonist that conteracts symptoms of toxicity related to high Hg exposure.The interactions between Se and Hg are important for the understanding of the environmental behavior and toxicological effects of these two elements. This is the subject of interdisciplinary research which involves: geology, medicine and other disciplines.

The antagonistic effect of Se on the toxicity of Hg in mammals and aquatic organisms is very complex and still in debate in terms of mechanism that explain the protective effect of Se compounds on mercuric mercury (Hg^{2+}) and methylmercury (CH_3Hg^{+}) toxicity.

Animal studies have indicated that the toxic effects of MeHg increase with decreasing Se intake. In brain, the toxic effect is directly correlated to the Hg-to-Se molar ratio and is

dramatically increased when this ratio is greater than 1:1 (Brockman, 2011 cited by Zhang, 2014).

Also, others authors suggest that the protective effect of selenite on the toxicity of Hg2+ in mammals is determined by in vivo formation of mercuric selenide (HgSe), a stable and biologically inert complex (Zhan, 2014). The most recent proposed mechanism explains the toxicity of Hg by the restricted synthesis and activity of selenoenzymes (Ralston and Raymond, 2010).

In humans maternal exposure to MeHg during pregnancy is directly correlated with later fetal neurodevelopment. Based on this conventional assumption, epidemiological studies were performed in order to assess the adverse effects of maternal exposure to MeHg on fetal development, but the results of these studies are contradictory (Zhan, 2014).

In fact, it is possible that the toxicity of MeHg is not correlated with maternal exposure to MeHg but with a relative deficiency in Se (Khan and Wang, 2009 cited by Zhang, 2014). The observed toxicity of Hg is at least partially attributable to Se deficiency caused by Se-Hg complexation (Khan and Wang, 2009; Zhang, 2014).

3.2.3.2.8. Pitfalls in the assessment of Se in the environment and humans

The determination of Se is of considerable interest because of its contrasting biological effects: toxic element as well as a trace element for animals and humans. Sampling of biological material for Se determination is not difficult (care must be taken only that sample to be representative), for water sampling only filtration and concentration are necessary due to the low Se content. Air sampling is much more difficult due to the volatility of its compounds.

Sample decompositions procedures for Se determination are various: instrumental nuclear activation analysis (INAA), atomic absorption spectrometry (AAS), elecrothermal atomic absorption spectrometry (ET AAS), hydride generation atomic absorption spectrometry (HG AAS), fluorimetry, X-ray fluorescence analysis (XFA), and gas-liquid chromatography (GCL) (Bem, 1981) but a high care is needed for selection of a proper analytical procedure for determination of Se in foods and biological material because of biased results produced (Falandysz, 2013).

Chromogenic reagents used for Se determination by various spectrophotometric methods are: dithiozone, chromotropic acid, J-acid, Variamine Blu (Revanasiddappa and Kumar, 2001).

Se content in human hair is a useful indicator for human Se intake and status (Li et al., 2014) and so are measurements in plasma, serum or in such tissues as kidney and liver. An indirect method to asses Se is the measurement of GPx activity in erythrocytes.

3.2.4. Final remarks

The iodine deficiency is one of the must important public health problem not only in developing countries but also for the Western Europe and Australia. In spite of many efforts that began more than 30 years ago, the issue of iodine deficiency reemerge.

The medical measures will not solve the entire problem, a multidisciplinary approach is necessary: educational programs for healthcare providers and population, social and

economical measures and environmental strategies in order to prevent the removal of iodine from the soil and the volatilization of iodine from the soil-plant system.

Se is essential to humans playing an important role as antioxidant, regulator of thyroid function and many others organs as component of structural proteins. Se can be considered a required dietary nutrient but the right amount for different populations it is still in debate. This important nutrient for human health can become a real menace when a certain dosage is exceeded. If severe diseases are directly related to Se insufficiency, Se excess can lead to severe illnesses.

The importance of environmental elements: pollution, precipitations, fertilizers, soil type on Se supply, absorption and excretion makes more complicated the issue on optimal intake. There are many problems to solve: normal Se concentration in human body, optimal intake and therapeutic effect of pharmacological doses of this micronutrient in various diseases.

Researchers in various scientific fields have to collaborate in order to develop new methods and technologies able to provide solutions and answers to these complex problems.

3.3. Demographic approach of iodine deficiency

3.3.1. Introduction

The huge amount of knowledge, in all science fields, led to division in numerous subspecialties. These (limited) domains permitted a detailed examination of specific problems. However, this approach has two main limitations. First of all, a single, isolated discipline may distinguish the details but cannot offer a global image of the investigated problems. On the other hand, not every problem has its one appropriate discipline, and their complexity makes necessary a complex approach, from several subspecialties, sometimes even from specialties of different fields of knowledge (Lingnan, 2011).

Interdisciplinarity means more than putting together different disciplines which keep their one point of view, as in pluridisciplinarity, it presumes a true connexion of different methodologies, an integration of particular aspects of knowledge (Zaman&Goschin, 2010).

Medicine is one of the best models of the necessity of interdisciplinarity and even transdiciplinarity, especially in research and politic of health fields. National health programs are an example of application of epidemiological, clinical, social, and economic data in order to solve or at least diminish a general problem of health. We present our self experience of screening for an endemic problem in the eastern part of Romania, Moldavia.

*Similar features apply to iodine deficiency which demands an interdisciplinary approach. It is a global public health issue because iodine plays a major role in the thyroid hormone synthesis and is essential for normal neurological development. This review summarizes the publications on iodine status. Most of the related studies on indine in in the WHO Eastern Mediterranean Region (EMR) countries are coming from the main national and international databases. Here datas were systematically searched in order to prevent the iodine deficiency disorders (IDDs).*IDDs countries is currently under control without significant side effects in the WHO EMR (Mohammadi et al., 2018).

Overall, despite enormous efforts to control IDDs, still IDD remains a serious public health problem in some countries of the region, requiring urgent control and prevention measures (Jiang et al., 2019).

On the last decade of the 20th century iodine deficiency was not yet solved in Europe. From the last WHO, UNICEF, and ICCIDD settlements a region is considered iodine deficient if more than 5% of the population have goiter (or thyroid volume more than 97 percentile) and median urinary iodine (good marker for iodine intake) is less than 10 g/dL. At that time, reevaluation of iodine status had shown that, in spite of salt iodination, iodine deficiency (IDD) was controlled in only 5 European countries, persisting, from minor to severe, in the rest of the continent. Starting from these data, a project named ThyroMobil evaluated iodine deficiency in 12 countries, including Romania (Delange, 1997).

However, none of the 3 investigated Romanian zones was in Moldavia. We decided to evaluate the iodine supply in some Moldavian regions, starting with the main city (Iasi), previously considered with minor to moderate IDD, and to compare the results with data from other Romanian and European regions

According to WHO definition, goiter prevalence and urinary iodine are the best parameter to evaluate IDD. Goiter prevalence is nowadays appreciated by ultrasonography, most accurate than palpation, being a quantitative and not qualitative method. Urinary iodine offers an image of recent dietary iodine intake. It is considered the choice index for evaluating and correcting iodine deficiency (Preda, 2014).

Personal contribution – published paper:

Vulpoi C, Ungureanu MC, Azoicăi DA, Vulpoi-Naghel I, Anton MC, **Preda C.** Screening for iodine deficiency – more than a medical approach. *Eur Sci J* 2014; 3:175-179.

As well as ThyroMobil study, our study also was performed in schoolchildren. Schoolchildren are considered one of the best target groups, both because their thyroid vulnerable to iodine deficiency, offer a better image of the iodine status, and the selection and survey of a children group is more practical (Zygmunt, 2012). The initial team was composed by of few enthusiastic endocrinologists.

3.3.2. Material and Methods

Establishing the **study design**, some unexpected problems arrived:

1) first, we needed to choose a representative sample. Iasi is a city of 300,000 stable inhabitants, with 13.5% children between 5 and 18 years old, with an equal sex distribution. However, the problem was not the number of children, but their family environment, since alimentary habits were important for our research. We asked the help of an epidemiologist who selected schools with a representative variety of children.
2) after selecting the schools, we needed the informed consent of children tutors (family and school), so we had to discuss with the teacher and to explain our goals. On the

initiative of a young team of schoolteachers, there were established meetings with the parents, where we did explain the procedures and the goals of our studies.

There were no invasive methods and practically all the parents permitted their children to enter in the study.

We have examined 914 children (466 boys and 448 girls) with a mean of 9.76±4.04 years of age. The including criteria were residency in the Iasi city and the absence of any known thyroid disease or capable to influence nutrition status and/or thyroid function. The study protocol included general examination, with evaluation of height, weight (by a nurse), and puberty stage (by a physician), thyroid ultrasonography, and urinary sample (taken by a nurse and analyzed by a biochemist). In order to avoid inter-observer differences, all thyroid ultrasonography were performed by the same physician.

3.3.3. Results

Some important issues have emerged, which deserve to be mentioned since they were different of the expected results and some of them have been bases for other studies:

- urinary iodine was realized at 10% children, by randomization (minimum 2 children for every age and sex). Mean value was 9.93g/dL, at the inferior limit of the normal (WHO recommendations), pleading for minor IDD.
- mean thyroid volume (**Table 23**) was close to the values from regions with sufficient iodine supply (with the exception of teen-agers, with a slightly higher volume). Superior limit of the thyroid volume (97 percentile) was smaller than that proposed by the ThyroMobil study. We considered our limit more appropriate for the evaluation of IDD, since using the ThyroMobil results; goiter prevalence had an unrealistic value of 0.1%, uncorrelated to the other data. With our one limits, goiter prevalence was 4.5%, under the 5% limit for IDD. Our skeptical view concerning the accuracy of ThyroMobil limits for all European countries was confirmed by other researcher (Liesenkötter, 1997).

Table 23. Mean thyroid volume in children

Author		Mean thyroid volume (mL) / Age (years)									
		6	7	8	9	10	11	12	13	14	15
Delange, 1997 Europe	Girls	4.9	6.3	6.7	8	9.3	9.8	11.7	13.8	14.9	15.6
	Boys	5.4	5.7	6.1	6.8	7.8	9	10.4	12	13.9	16
Liesenkötter, 1997 Germany	Girls	3.7	4.5	4.5	4.5	4.9	7	8.6	9.5	10	9.5
	Boys	3.2	3.7	3.9	3.8	4.4	6.8	6.9	6.9	8.2	7.2
Vulpoi, 1997 Romania	Girls	3.1	3.4	4.5	5.4	6.3	8.3	8.3	9.8	11	10.7
	Boys	4	3.2	3.7	4.9	6.5	7.1	6.4	7.9	12.3	12

- corroborating the two parameters we could affirm that Iasi region is one of borderline

iodine deficiency, showing a significant improvement comparing to anterior data, mainly due to salt iodination.

- since the clinical examination included anthropometric parameters, we have noticed the secular trend of height enhancement (**Table 24**). This was a good argument to evaluate a larger cohort, which confirmed the first results (Vulpoi, 2005). Due to inherent differences between geographical regions, global growth charts may not be appropriate for all zones (Bonthuis, 2002).

Table 24. Height evolution

Age (years)	Height (cm)			
	Boys		Girls	
	1979*	1997	1979*	1997
3	96.5	96.7	95.3	97.1
4	101.6	106.3	100.9	105
5	108.9	113.7	108.4	113
6	115.5	120.9	114.5	120
7	119.9	126.8	119.2	124.8
8	126.4	128.3	126.7	129.2
9	132.4	134.8	131.6	134.5
10	136.4	142.3	136.4	146.5
11	141.3	149.7	142.5	153.9
12	147.7	150.7	149.3	154.1
13	152.6	159.7	154	155.9
14	160.3	166.4	157.4	161.5
15	165.1	169.4	158.4	161.1
16	171.3	174.2	159.1	162.9

*-national growth chart

3.3.4. Discussions

National growth charts should be used, but in Romania they date from decades. A study with a representative sample for at least the region of Moldavia is programmed this year.

On the other hand, there is another factor which affects iodine metabolism, even in perfect enviromental conditions.

Iodine uptake is mediated by thyroid follicular cells from the blood plasma which leads to the synthesis of thyroid hormones. The ingested iodine is bound to serum proteins, especially to albumins. Meanwhile, the rest of the iodine which remains unlinked and free in bloodstream, is removed from the body through urine.

Iodine uptake is the result of active transport mechanism mediated by the sodium iodide symporter (NIS) protein, which is found in the basolateral membrane of thyroid follicular cells. It will cause an iodide concentration inside follicular cells of thyroid tissue 20 to 50 times higher than in the plasma (Triggiani et al., 2009).

These data convinced us to extend our study and we have evaluated in the same manner iodine status in 3 other regions of Moldavia. An improvement was remarked there too, from medium/severe to mild/medium deficiency. The most significant difference in linear

growth, comparing to older growth chart, was found at the age of 11, probably due to earlier puberty onset. Children linear growth is evaluated using growth charts.

This complex transport mechanism across the cell membrane is driven by the electrochemical gradient of sodium (the intracellular concentration of sodium is approximately 12 mM and extracellular concentration 140 mM). Once inside the follicular cells, the iodide diffuses to the apical membrane, where it is metabolically oxidized through the action of thyroid peroxidase to iodinium (I+) which in turn iodinates tyrosine residues of the thyroglobulin proteins in the follicle colloid. Thus, NIS is essential for the synthesis of thyroid hormones (T3 and T4) (Wang et al., 2018).

NIS is the plasma membrane glycoprotein that mediates active iodide transport in the thyroid and other tissues, such as the salivary, gastric mucosa, rectal mucosa, bronchial mucosa, placenta and mammary glands. NIS mediates the uptake and accumulation of iodine and its activity is crucial for the development of the central nervous system and disease prevention (Dai et al., 1996).

It was discovered in 1996 and from then on researches have further shown that NIS functionality and iodine transport is dependent on the activity of the sodium potassium activated adenosine 5-triphosphatase pump (Na+, K+-ATPase) (Sandell and Kolthoff, 1934).

The molecular mechanisms by which F inhibits NIS expression and functionality which in turn contributes to impaired iodide absorption, diminished iodide concentrating ability and iodine deficiency disorders (Filer et al., 2017).

NIS expression and activity seem inhibited by thyroglobulin (Tg), tumour necrosis factor alpha (TNF-), transforming growth factor beta 1 (TGF-1), interleukin 6 (IL-6) and Interleukin 1 beta (IL-1), interferon- (IFN-), insulin like growth factor 1 (IGF-1) and phosphoinositide 3-kinase (PI3K) and how fluoride upregulates expression and activity of these biomarkers (Riesco-Eizaguirre et al., 2009).

Prolactin and megalin have crucial roles in regulation of NIS expression and iodine homeostasis and the effect of fluoride in down regulating prolactin and megalin expression. Among many other issues, there is a potential conflict between public health policies such as water fluoridation and its contribution to iodine deficiency, neurodevelopmental and pathological disorders. Further studies are warranted to examine these associations.

We performed a study which we considered first easy to realize, with a purely endocrinological team. The subject was clear, the methods easy to perform. However, even in this simple problem more than one provider had to interact. More than that, not all of them were health professional. This enlarged team permitted not only the goal achievement, but also opened new gates for other (interdisciplinary) studies.

In the late 90‘s interdisciplinarity was not clear defined, in Romania as in other places (Nair, 2008). It is now, when isolated work is increasingly less conceivable. Consultation, examination solicitation, prescription is no more sufficient in the view of this paradigm shift (de Lorenzi, 2009; Chettiparamb, 2007).

3.3.5. Conclusion

Although unique by the main determinants and the clinical approach, endocrinology is a frontier discipline, interfering with many other specialties. In the field of science, this means that any discipline will bring its own point of view and try to harmonize it with the

others, realizing hybridization, a zone prone to innovations and capable to identify errors of the constitutive disciplines. In the clinical field, the interdisciplinarity comes natural, since every disease is a sum of different interacting factors.

3.4. Selenium-essential antioxidant element - the example of autoimune thyroiditis

3.4.1. Introduction

Se is an essential element for human health being involved in redox processes, intracellular signaling, autoimmunity, apoptosis and cell proliferation (Mehdi et al., 2012). This is possible due to the activity of at least 30 selenoproteins variants encoded by 25 human genes (Winther et al., 2016).

Several selenoproteins are located in the thyroid gland: thioredoxin reductases (TRs), three deiodinase isoforms (D1, D2 and D3) and glutathione peroxidase (GPXs). TR1 has an antioxidant effect. D1 and 2 convert thyroxine (T4) in tironine (T3). GPX1 and 4 protect thyreocites from damage caused by oxygen free radicals (Pirola et al., 2016).

Selenium exhibits anti- inflammatory and antioxidative actions and was shown to have an important role in lymphocytic chronic thyroiditis (Duntas 2015). Se affects the autoimmune system by controlling the production of ROS (Reactive Oxygen Species) (Chakrabarti et al., 2016) and also may indirectly inhibit TNF activation and cytokine release.

The inhibition of the expression of HLA-DR molecules is possible to be another explanation of the protective effect of Se in thyroid autoimmune process. All this data suggests the possible relationship between the Se deficiency and autoimmune thyroiditis and the Se supplementation has been considered in this regard (Krassas et al., 2014).

Nowadays, there are no ways to stop the autoimmune process in chronic thyroiditis and the treatment with levothyroxine is necessary only in the stage of hypothyroidism. The results of clinical studies regarding the effect of Se in thyroid autoimmunity are discordant but it is true that the dose used varies widely as well as the duration of treatment (Pekar et al., 2015; Drutel et al., 2013).

Since the normal plasma Se concentrations varies between 75 and 150 μg/L or 0.8 ± 0.36 μmol/L, related to the dietary intake (under 400 μg/day due to toxic effects), (Drutel et al., 2013) it is very difficult to establish the right amount of Se or the duration of therapeutical intervention necessary to induce clinical effects. Anyway, most clinical trials showed that 100-200 ìg/day of selenomethionine or sodium selenite for 3 to 12 months was related to the decrease of TPOAb titers (De Farias 2015).

Personal contribution – published paper:

Preda C, Vasiliu I, Mihalache L, Armașu I, Șerban IL, Șerban DN, Stoica B, Ciobanu DG, Bredețean O, Strungaru SA, Nicoară M, Plavan G, Vulpoi C. Selenium- essential antioxidant element. The example of autoimune thyroiditis. Rev Chim Bucharest 2017; 68(7):1617-1621.

Taking into account that in several regions of Romania the soil content in Se is very low and given current knowledge that selenium therapy might reduce aggression and prevent autoimmune thyroid dysfunction (remaining the only actual therapeutic alternative), its effectiveness needs to be supported by more evidences. Based on these data, we wanted to evaluate the efficacy of Se supplementation in patients with autoimmune thyroiditis and normal thyroid function in terms of serum levels of TPOAb and glutathione peroxidase activity.

3.4.2. Material and methods

This randomized-controlled trial was conducted between January 2014-January 2016 in the Department of Endocrinology of the Hospital St. Spiridon, University of Medicine and Pharmacy Grigore T. Popa, Iasi. Patients were eligible if they had euthyroid autoimmune thyroiditis.

The diagnosis was assessed by the presence of detectable TPOAb levels and normal TSH. One hundred women with euthyroid autoimmune thyroiditis and normal iodine intake were randomized into 2 groups (Group I, n=50, treated with selenomethionine 100ig/day, Group II, n=50, control).

Serum concentrations of TPOAb, TSH and Se were performed in all patients at baseline and after 3 months. Serum levels of GPx1 were determined only for the treated patients at baseline and after 3 months. Inclusion criteria: adult female patients, with normal thyroid function (TSH = 0.4-4 μIU/mL) and autoimmune thyroiditis (TPOAb> 35 UI/mL), able to sign informed consent.

Variables studied: age, titers of TPOAb, TSH, GPx1 and plasma Se levels. The study protocol was approved by the Ethics Committee of our institution (University of Medicine and Pharmacy, Iasi) and the written informed consent was obtained from all participants.

Serum levels of TSH (reference values 0.4-4μUI/ml), TPOAb (0-35 UI/mL) were measured by chemiluminescence (Human TSH, TPOAb CLIA Kit). Se measurements were performed by atomic absorption spectrometry using the GF with platform HR-CS-AAS contra 600 Analytic Jena.

The method we used involves several steps: fresh blood samples were preserved by freezing at – 25^0 C; they were defrosted and homogenized before metal digestion; metal digestion for Se analysis also had several steps. A mixture of 1ml total blood with 3 mL of nitric acid 65% and 1ml hydrogen peroxide 30% was allowed 15 minutes to react. To realize the digestion process we kept the samples for 5 min at 145oC, 10 min at 190o and 10 min at 100^0C.

After the digestion process the samples were transferred into 25 mL decontaminated Duran volumetric flasks. The flasks containing the samples were filled with ultrapure water up to a volume of 25 mL, shacked few times and then the content was transferred in 15 ml bottles for the final metal analysis. Se concentrations were expressed in μg/L. GPx1 activity was determined using a commercially available kit (RANSEL control based on Paglia and Valentine method – ELISA Kit for Glutathione Peroxidase 1 from Cloud-clone Corp®). The values of GPx1 were expressed in mU/dL.

Statistical analysis was performed using SPSS software version 18 (SPSS Inc., Chicago, IL, USA). The results are presented as mean ± standard deviation (SD).

3.4.3. Results

Of the 123 recruited patients, 100 women fulfilled the inclusion criteria and were enrolled in the study. 50 women (mean age 46.24±12.50) were randomized to receive selenomethionine and 50 women (mean age 50.50 ±13.48) were randomized to receive placebo. **Table 25** summarizes the results: TSH, TPOAb, GPx1 and Se concentrations at baseline and after 3 months.

There were no significant differences in mean serum levels of TSH at baseline between the two groups. After 3 months, TSH presented a significant increase in group I (2.09 vs 2.49 UI/ mL; p=0.001) and in group II (1.91 vs 2.38 UI/mL; p=0.008), but in respect with normal ranges.

Mean serum TPOAb levels at baseline was significantly higher in treated group vs placebo (362.99 vs 284.79 UI/mL; p=0.045).This distribution was a consequence of the randomization. Individual values of TPOAb in patients treated with Se after 3 months, were in indirect correlation with initial values, with an important decrease of TPOAb in 20% of cases (r= -0.196; R2= 0.038; p=0.125) (**Fig. 20**).

The dynamics of average values in the TPOAb showed a decrease by 15.2%, statistically significant in patients treated with Se (p=0.002), which did not occur in untreated patients (p=0.850).

Mean serum levels of GPx1 in group I at baseline and after 3 months were not significant different (0. 64±0.37 vs 0.64±0.38 mU/dL, p=0.979). At baseline mean Se level was similar in the two groups and after 3 months significantly increased in group I (560.14 vs 270.50; p=0.001) but also in placebo group (316.13 vs 236.49; p=0.014) even if to a lesser extent.

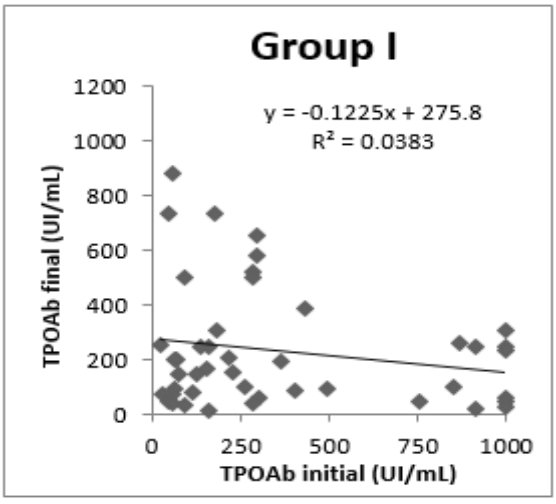


Fig. 20. Evolution of individual values of TPOAb in Se-treated group between baseline and end of study.

Considering normal values of Se between 75-150µg/L we have divided both groups in three categories: < 75, 75-150 and >150 µg/L. In group I, patients with Se levels < 75 µg/L, at baseline, presented the highest levels of TPOAb and those with Se > 150µg/L, on the contrary, the lowest concentrations of antibodies (p=0.023). The same tendency was present in the placebo group.

After 3 months, in group I, the concentration of TPAb decreased with the increase of Se levels, this fact was not noticed group II. Also, the concentrations of GPx1 showed the same pattern. All these data are summarized in **Table 26**.

At baseline, in the group I the individual values of Se and GPx were in significant direct correlation, moderate in intensity (r= +0.401; R2= 0.1606; p=0.006) (**Fig. 21**). After 3 months of treatment, the individual values of Se and TSH were in a weak indirect correlation, statistically insignificant (r= -0.232; R2= 0.0537; p=0.161) in the group I and independent in the group II (r= +0.024; R2= 0.006; p=0.866).

Table 25. Baseline and 3 monts biological profile (tsh, atpoab, se and gpx1) of the patients with chronic autoimmune thyroiditis randomised to selenomethionine or placebo

	GROUP I (n=50)	GROUP II (n=50)	p value
Selenomethionine supplementation (µg/day)	100	0	-
TSH (µUI/ml)			
- at baseline	2.08±1.00	1.91±1.06	0.392
- at 3 months	2.49±1.27	2.38±1.30	0.677
TPOAb (UI/ml)			
- at baseline	362.99±348.24	284.79±235.06	**0.045**
- at 3 months	307.87±306.1	289.96±287.78	0.781
Se (µg/L)			
- at baseline	257.69±240.51	236.49±211.63	0.652
- at 3 months	560.14±363.09	316.13±160.27	**0.001**
GPx1 (mU/dl)			
- at baseline	0.64±0.37	-	-
- at 3 months	0.64±0.38	-	-

In the treated group, the individual values of Se and TPOAb at baseline showed an indirect correlation with a moderate but significant intensity (r=- 0.308; R2= 0.0006; p=0.037) which decreased in intensity and was statistically insignificant at the end of the study (r= +0.267; R2= 0.0715; p=0.105) (**Fig.22**). In the placebo group Se and TPOAb were independent values at baseline (r= -0.086; R2= 0. 0074; p=0.553) and at 3 months (r= +0.014; R2= 0.0002; p=0.924) (**Fig. 22**).

Table 26. Variations in tpoab according to the serum concentration in Se

Marker	Group 1				Group 2			
	Se<75	**75-150**	**Se>150**	**p**	**Se<75**	**75-150**	**Se>150**	**P**
Baseline								
TPOAb	**439±88**	**428±116**	**264±63**	0.023	**396±157**	**292±78**	**242±61**	**0.577**
3 Months								
TPOAb	*	**164±68**	**201±42**	**0.049**	**306±180**	**317±90**	**271±57**	**0.501**

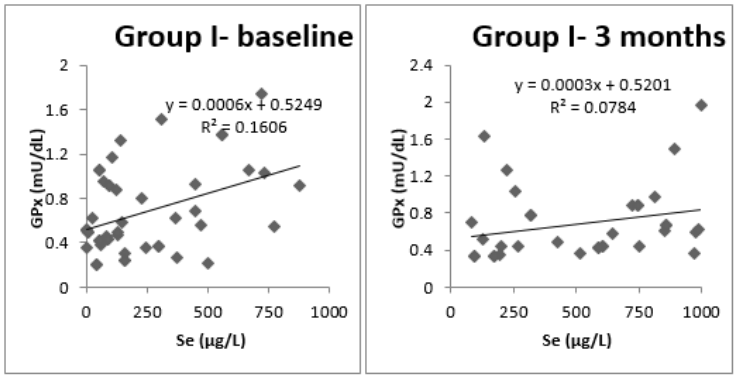


Fig. 21. Correlation between GPx1 and Se in group I (direct, significant at baseline and non-significant after 3 months of treatment)

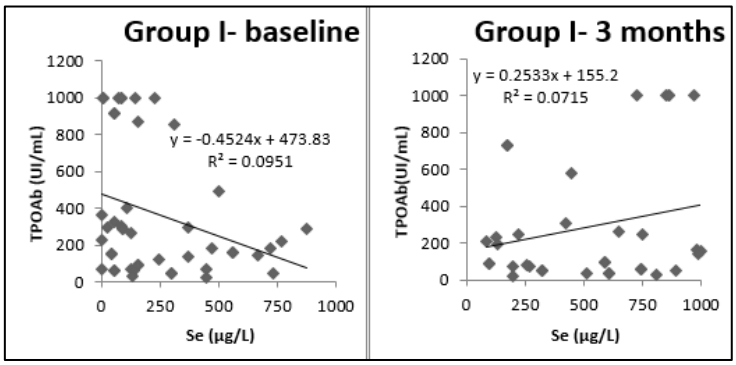


Fig. 22. Correlation between TPOAb and Se in group I (direct, significant at baseline and non-significant after 3 months of treatment)

3.3.4. Discussions

Our results showed a mild decrease of TPOAb and a weak negative correlation between Se and antithyroid antibodies suggesting that Se supplementation may improve the course of thyroid autoimmunity. There is no consensus regarding the dose of Selenium useful in autoimmune thyroiditis – the dose that would be effective by reducing the intensity of the immune response without being toxic.

The majority of previous studies demonstrate the effectiveness of a 200 µg Se per day (Turker et al., 2006). *Some immune functions were enhanced by a 50 µg Se, but the activity of the glutathione peroxidase, which is involved in other immune processes such as thyroiditis was not. The optimization of the immune function seems to appear at 100µg/day. Taking into account the potential adverse effects of the excessive supplementation of selenium such as insulin resistance (Wang et al., 2014), we chose the 100 µg over 200 µg Se/day. The medication was well tolerated; none of the patients reported side effects or intolerance phenomena.*

- *The effect of Selenium supplementation on TPOAb*

After 3 months of Se treatment, a significant decrease, by 15.2%, was observed in the TPOAb titer, which was not noticed in the untreated group. If the antibody titer is very high

at the initiation of treatment, Se supplementation was proved more efficient since there was a significant decrease in the ATPO level (Esposito et al., 2017).

The same effect was observed in our study. As a consequence the indirect correlation between Se and TPOAb present at baseline was no longer valid at the end probably due to an amelioration of the autoimmune process. An important issue is the dose and duration of treatment. Three months of 100 µg/day Se may not be enough to normalize the level of TPOAb. Esposito showed that 166 µg/day of Selenomethionine for six months has a limited impact on TPOAb. It seems that 200µg/day for 6-9 months have a greater impact on autoimmune process (Esposito et al., 2017).

- *The effect of Selenium supplementation on TSH*

There are different opinion regarding the impact on Se treatment on TSH values: Pirola report the restoration of euthyroidism in patients with sub-clinical hypothyroidism after 4 months of 83 Selenomethionine µg/ day (Pirola et al., 2016). The decrease of TSH was also mentionated by Winther, in a population of 361 subjects with autoimmune thyroiditis under Se for a period of 5 years (Winther et al., 2015).

In our study, even if low values of TSH had a tendency to correlate with selenium level, this correlation was not statistically significant concordant with most of the literature studies (De Farias 2015). *However, we noticed a growing tendency of TSH, which was statistically significant, both in the treated and the untreated women, while the TSH remained within the normal limits. Increased TSH in treated patients has been found in other studies and was attributed to T3 decreament* due to the decrease in the activity of peripheral deiodinases (Duntas 2010).

- *The effect of Selenium supplementation on GPx1*

Previous studies have shown that the administration of sodium selenite induced a dose proportional increase in the GPx1 activity (Wajner et al., 2015). A minimum intake of 65µg/day was needed to optimize and 95ìg/day to maximize the GPx1 activity. It is considered that the daily selenium intake must provide 2/3 of the maximum GPx activity (Kabata-Pendias et al., 2007).

` A comparaison between studies is difficult because GPx1 normal values vary depending on the detection method as well as on the population investigated. *Our results showed that mean serum levels of GPx1 at baseline and after 3 months were not significantly different in patients with euthyroid autoimmune thyroiditis after Se supplementation*.

A tendency towards parallelism was noticed at baseline between GPx and TSH values (lower TSH values corresponded to lower GPx values), but this correlation was not significant. *At the end of the study, the tendency of the correlation reversed with lower TSH values and higher GPx values, which indicated an improvement of the function; however, this tendency was not significant.*

The GPx1 and TPOAb individual values were statistically independent both at the beginning and at the end of the study. This lack of correlation may be explained by the dose which we used (100µg/day), since the dose necessary to fill in the deficit storage in GPx1 is greater than the supplementation dose.

3.3.5. Conclusions

Our study demonstrated the existence of an indirect correlation between ATPO and the value of selenium. Selenium supplementation induces a decrease in antibody titer, more significant if the initial values were high. Selenium supplementation did not result in functional changes (TSH). GPx was directly and significantly correlated with the value of selenium, both at baseline and after 3 months, suggesting an improvement in oxidative stress.

3.5. Endocrinological roots of the management protocols in thyroid malignancies

3.5.1. Introduction

Thyroid cancer is a rare malignant tumour, but is the most common cancer of the endocrine system. It affects women more on a age of 25-65 years (Bailey, 2001). The most common type of thyroid cancer is represented by papillary thyroid carcinoma, approximately 85% of all thyroid cancer (Pearce et al., 2004).

Most of the papillary thyroid cancer shows good prognosis but some of them has aggressive behavior such as local invasion, lymph nodes extension or distant metastasis (Gulben et al., 2008). There are various factors which have been known to be associated with prognosis of thyroid cancer: age, gender, tumour size, extrathyroid extension and distant metastasis (Passler et al., 2004; Joo et al., 2015).

The most effective treatment for thyroid cancer is surgery which involves various techniques. Advances in our understanding of thyroid lesions, especially those entities with an indeterminate behavior, has led clinicians to question the most appropriate surgical management of such thyroid nodules (Rossi et al., 2019).

Recent studies have shown that the non-invasive encapsulated follicular variant of papillary thyroid carcinomas (NI-EFVPC) exhibits poor histopathologic diagnostic reproducibility and have been over-treated as conventional thyroid cancer. The new terminology of "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) was accordingly introduced to replace NI-EFVPC in 2015 by an international thyroid working group. NIFTPs are biologically similar to follicular adenomas lacking lymph node metastases and/or recurrence. The revision is motivatedby the rare finding, in some studies, of lymph nodes with metastatic NIFTP (Alves et al., 2018).

The introduction of NIFTP has resulted in significant impact on the clinical management of thyroid nodules. Recent revisions in the morphological criteria for NIFTP emphasize the need to adhere to very stringent histomorphologic criteria when making a diagnosis of NIFTP. The adoption of NIFTP terminology instead of NI-EFVPC is associated with conservative lobectomy without radioactive iodine treatment in the majority of cases (Trimboli et al., 2019).

Radical neck dissection has an important role in the surgical management of cervical carcinoma. It is used especially to control cervical lymph node metastasis (Razack et al., 1981; Razack et al., 1979).

Nodal metastasis occurs in more than 70% of patients with medullary thyroid carcinoma (MTC) with a palpable primary tumour; total thyroidectomy and bilateral neck dissection is recommended as minimal surgery (Ahn and Chung, 2019).

The concept of ”lymph node removal” in thyroid cancer was introduced for the first time by Kocher in 1880 and the dissection technique was described later (1906) by George Crile (Crile, 1906).

The 1960s and 1970s were marked by a significant change in the treatment of malignant thyroid disorders. This can be exemplified by conservative surgery (with tissue preservation and implicit function) that developed new surgical techniques. In 1953, Pietrantoni, a sustainer of elective bilateral dissection of the neck, nevertheless recommended the maintenance of accessory spinal nerves and at least an internal jugular vein (Muller et al., 2002; Edis, 1977). Functional neck dissection have less morbidity than radical neck dissection, but the same oncological outcome (Ferlito et al., 2004; Bocca, 1975; Welch and McHenry, 2013).

Another studies reported that even with extensive neck dissection, a biological cure is rarely achieved in patients who have more than 10 positive nodes or more than 3 compartments involved (Tissel et al., 1986; Machens et al., 2000; Weber et al., 2001; Ahn and Chung, 2019; Welch and McHenry, 2013).

Papillary thyroid carcinoma, Hurthle cell, and medullary thyroid carcinoma usually metastasize to cervical lymph nodes. Studies reported that almost 35% of patients with papillary thyroid carcinoma and 75% of patients with medullary thyroid carcinoma will present clinically evident cervical lymph nodes metastases (Schlumberger, 1998; Kloos et al., 2009). Patients with thyroid cancer and macroscopic lymph node metastases will undergo surgery. It will be performed a lateral compartment neck dissection (Welch and McHenry, 2013).

American Thyroid Association (ATA) together with ATA Thyroid Nodules and Differentiated Thyroid Cancer Guidelines Task Force have reviewedand made recommendations related to the suggested new classification of encapsulated follicular variant papillary thyroid carcinoma (eFVPTC) without capsular or vascular invasion to noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). The manuscript proposing the new classification and related literature were assessed. It is recommended that the histopathologic nomenclature for eFVPTC without invasion be reclassified as a NIFTP, given the excellent prognosis of this neoplastic variant. This is a weak recommendation based on moderate-quality evidence. It is also noted that prospective studies are needed to validate the observed patient outcomes (and test performance in predicting thyroid cancer outcomes), as well as implications on patients' psychosocial health and economics (Haugen et al., 2017).

Studies registered an increased mortality rate in patients over 55 years of age, with differentiated thyroid cancer and regional lymph node metastases (Lundgren et al., 2006; Welch and McHenry, 2013).

There have been a lot of debates over the influence of prophylactic or therapeutic cervical lymphadenectomy on prognosis, such as recurrence or survival rate for the thyroid cancer patients with or without limph nodes metastasis (Attie, 1988; DeGroot et al., 1990; Joo et al., 2015). Many studies reported higher frequency of recurrence in patients found to

have limphatic nodes metastasis at the time of initial surgery with no significant change in overall survival, while some report decreased disease free survival (Attie, 1988; DeGroot et al., 1990; Joo et al., 2015). While lymph node metastasis may not influence overall survival, it presents a significant risk of regional recurrence which diminishes quality of life during periods of recovery after initial treatment (Joo et al., 2015).

In the absence of alternative effective treatments, surgery combined with postoperative radioiodine treatment continues to be the first choice for thyroid cancer patients with bilateral neck metastasis. Researchers believe that a more complete resection results in greater success (Guo et al., 2018).

Monoclonal antibodies targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4), programed cell death 1 (PD-1), or its ligand (PD-L1) have become the mainstay for advanced malignancies. The incidence of endocrine adverse events provoked by these immune checkpoint inhibitors (ICI) is based on data from randomized controlled trials, which have their drawbacks. Patients treated with Ipilimumab experienced hypophysitis in 5.6% (95% CI, 3.9-8.1), which was higher than nivolumab (0.5%; 95% CI, 0.2-1.2) and pembrolizumab (1.1%; 95% CI, 0.5-2.6) (de Filette et al., 2019).

Meanwhile, PD-1/PD-L1 inhibitors had a higher incidence of thyroid dysfunction - particularly hypothyroidism (nivolumab, 8.0%; 95% CI, 6.4-9.8; pembrolizumab, 8.5%; 95% CI, 7.5-9.7; PD-L1, 5.5%; 95% CI, 4.4-6.8; ipilimumab, 3.8%; 95% CI, 2.6-5.5) (Ibrahimpasic et al., 2019).

Combination therapy was associated with a high incidence of hypothyroidism (10.2-16.4%), hyperthyroidism (9.4-10.4%), hypophysitis (8.8-10.5%), and primary adrenal insufficiency (5.2-7.6%). Diabetes mellitus and primary adrenal insufficiency were less frequent findings on monotherapy. There seems to be a high incidence of endocrine adverse events provoked by single agent checkpoint blockade, further reinforced by combined treatment (Abdel-Rahman et al., 2016).

Personal contribution – published paper:

Grigorovici A, Costache M, Velicescu C, Savin G, Ciobanu D, **Preda C**. Disecația radicală a gâtului în cancerul tiroidian avansat. *Chirurgia* 2010; 105 (5): 669-672.

Total thyroidectomy with or without limited lymph node dissection, combined with specific oncologic treatment remains the ideal solution in thyroid cancer with long-term average results. In this study we want to highlight the better prognosis of patients with advanced thyroid cancer that underwent radical neck dissection.

Our aim is to assess the most important medium and long term advantages and disadvantages of the surgical recomanded treatment in order to reach the oncological security and the best recurrence rate.

3.5.2. Materials and methods

We retrospectively reviewed the medical records of patients admitted to the III-rd Surgical Unit of ”St. Spiridon” Emergency Hospital Iași, between January 2000 to September

2009. The evaluation of the results was done with data from the subsequent surgical follow-up. All patients were investigated and diagnosed during prior admissions at the Endocrinology Clinic or the Otorhinolaryngology Clinic.

The study group comprised 140 women and 49 males (W: M ratio = 2.85). Distribution by age group reveals a maximum incidence of thyroid cancer in the sixth decade of life, with an average of 51.48 years (extreme 19-81 years) (**Fig. 23**). We used the staging of TNM set by AJCC (Bychkov, 2019). (**Tables 27 and 28**) Anaplastic carcinomas are considered at stage T4. Regional lymph nodes are those in the median and lateral cervical compartment and superior mediastinum (**Tables 27 and 28**).

In the Third Surgical Unit were performed during this period 189 interventions for malignant thyroid disorders, of which 59 total thyroidectomy with radical/radically modified neck dissection and 34 with selective dissection in central or lateral neck compartment.

The surgical technique consisted of *total thyroidectomy accompanied by radical neck dissection*, which included the lymph nodes removal of the lateral or median neck compartment, the resection of sternocleidomastoid muscle, subhioid muscles, recurrent nerve when it was embedded in the tumour, resection of the clavicle (for approaching the upper mediastinum) and the internal jugular vein, possibly carotid adventitious, using 48-hour thyroid aspiration drainage. In the absence of complications the patients left the hospital on the third postoperative day. *All patients received thyroid stimulating hormone suppressive hormonal therapy and radioactive iodine therapy after surgery.*

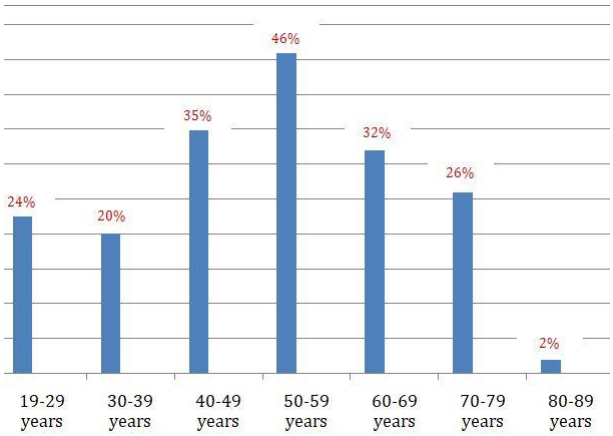


Fig. 23. Distribution by age groups of patients with thyroid neoplasm

Long-term *follow up* was done at 6, 12, and 24 months in Third Surgical Unit and Endocrinologic Unit, where hormone replacement therapy and radioiodine therapy (selected cases) were initiated. If routine tests indicated recurrence, enhanced CT was performed to confirm if additional surgery was needed.

Table 27. TNM definitions (Bychkov, 2019)

Primary tumor (pT) for papillary, follicular, poorly differentiated, Hürthle cell and anaplastic thyroid carcinomas:	Regional lymph node (pN):	Distant metastasis (M):
TX: Primary tumor cannot be assessed	**NX:** Regional lymph nodes cannot be assessed	**M0:** No distant metastasis
T0: No evidence of primary tumor	**N0:** No evidence of regional lymph node metastasis	**M1:** Distant metastasis
T1: Tumor ≤ 2 cm in greatest dimension limited to the thyroid	**N0a*:** One or more cytologic or histologically confirmed benign lymph nodes	
T1a: Tumor ≤ 1 cm in greatest dimension limited to the thyroid	**N0b*:** No radiologic or clinical evidence of locoregional lymph node metastasis	
T1b: Tumor > 1 cm but ≤ 2 cm in greatest dimension limited to the thyroid	**N1*:** Metastasis to regional nodes	
T2: Tumor > 2 cm but ≤ 4 cm in greatest dimension limited to the thyroid	**N1a*:** Metastasis to level VI or VII (pretracheal, paratracheal, prelaryngeal / Delphian or upper mediastinal) lymph nodes; this can be unilateral or bilateral disease	
T3: Tumor > 4 cm limited to the thyroid or gross extrathyroidal extension invading only strap muscles	**N1b*:** Metastasis to unilateral, bilateral or contralateral lateral neck lymph nodes (levels I, II, III, IV or V) or retropharyngeal lymph nodes	
T4: Includes gross extrathyroidal extension into major neck structures		
T4a: Tumour invades subcutaneous soft tissues, larynx, trachea, esophagus or recurrent laryngeal nerve		
T4b: Tumour invades prevertebral fascia or carotid artery or mediastinal vessels		
Primary tumor (pT) for medullary thyroid carcinomas:		
TX - T3: *Definitions are similar to the above*		
T4: Advanced disease		
T4a: Moderately advanced disease; tumor of any size with extension into subcutaneous soft tissue, larynx, trachea, esophagus or recurrent laryngeal nerve		
T4b: tumor of any size with extension toward the spine or blood vessels, invading the prevertebral fascia or encasing the carotid artery or mediastinal vessels		

3.5.3. Results

Between January 2000 and September 2009, 189 thyroid cancer patients initiated surgical treatment at the Third Surgical Unit. Of these, 65 underwent total thyroidectomy, 34

underwent total thyroidectomy with selective neck dissection, 59 total thyroidectomy with radical or radically modified neck dissection, 15 underwent procedure in order to complete a total thyroidectomy (due to the discovery of occult thyroid cancer in patients with lobectomy for benign tumour), 12 reinterventions to complete thyroidectomy with radical or radically modified neck dissection (of which 8 for lymph node recurrence in less than 2 years), 2 tracheostomies and 2 exploratory cervicotomies.

Of the 189 patients treated for malignant thyroid disease, 138 were papillary thyroid carcinomas, 26 follicular carcinomas, 12 medullary carcinomas, 8 anaplastic carcinomas, 3 metastases from another cancer localization, and 2 non-Hodgkin's malignant lymphomas (**Fig. 24**).

Table 28. AJCC prognostic stage grouping (Bychkov, 2019)

Differentiated thyroid cancer			
Age at diagnosis < 55 years			
Stage I:	any T	any N	M0
Stage II:	any T	any N	M1
Age at diagnosis ≥ 55 years			
Stage I:	T1	N0 / NX	M0
	T2	N0 / NX	M0
Stage II:	T1	N1	M0
	T2	N1	M0
	T3a / T3b	any N	M0
Stage III:	T4a	any N	M0
Stage IVA:	T4b	any N	M0
Stage IVB:	any T	any N	M1
Medullary thyroid cancer:			
Stage I:	T1	N0	M0
Stage II:	T2	N0	M0
	T3	N0	M0
Stage III:	T1 - 3	N1a	M0
Stage IVA:	T4a	any N	M0
	T1 - 3	N1b	M0
Stage IVB:	T4b	any N	M0
Stage IVC:	any T	any N	M1
Anaplastic thyroid cancer:			
Stage IVA:	T1 - T3a	N0 / NX	M0
Stage IVB:	T1 - T3a	N1	M0
	T3b	any N	M0
	T4	any N	M0
Stage IVC:	any T	any N	M1

From the study group, 68 patients were diagnosed in the Endocrinology Clinic with malignant puncture confirmed subsequently by paraffin examination, 106 patients with suspected puncture, which subsequently, through paraffin examination, proved to be thyroid

neoplasm and 15 were diagnosed postoperatively in the histopathologically exam to paraffin (patients who had thyroid lobectomy and who subsequently underwent thyroidectomy).

Patients with suspicious puncture that were found to be benign for paraffin exams were excluded from the study. There has been a steady increase in the incidence of thyroid carcinoma through a real increase in incidence and probably early diagnosis of this condition.

For papillary thyroid neoplasms the staging is different for those under 55 years of age (stage I any T, any N, M0 and stage II - any T, any N, M1) and over 55 years old (stage I - T1N0M0, stage II - T2N0M0, Stage III T1-3, N1M0, T3N0M0, Stage IV T4N0-1M0, Any T, Any N, M1). For medullary thyroid tumours, the classification is the same as for papillary neoplasms over 55 years, and the anaplasia is considered Stage IV (**Table 28**).

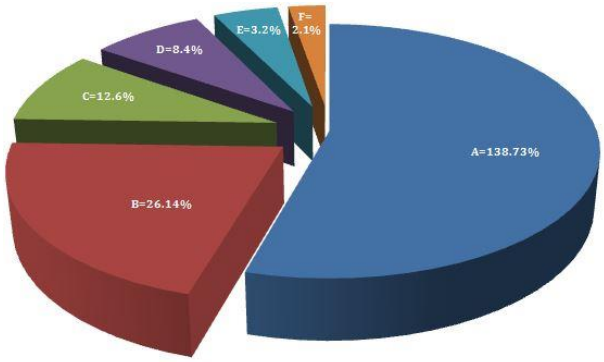


Fig. 24. Histologic types of cancer; A=papillar cancer; B=anaplastic cancer; C=follicular cancer; D=thyroid metastases; E=medullar cancer; F=LMNH

91 patients from the study group that underwent surgery were in the following stages:

- 68 patients in stage I (regardless of histological form);
- 72 patients in stage II (regardless of histological form);
- 26 patients in stage III (regardless of histological form);
- 23 patients in stage IV (regardless of histological form) –**Fig.25**;

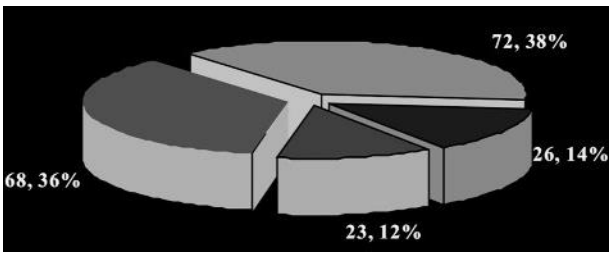


Fig. 25. Patients number depending on tumour stage

In the study group, the following surgical interventions were performed: 65 total thyroidectomies plus 34 total thyroidectomies with selective neck dissections (52.38%) for Stage I or II patients, except for medullary thyroid cancer; 59 total thyroidectomies accompanied by radical or radically modified neck dissection (31.21%), for Stage III or IV patients or those with non-medullary thyroid neoplasm at any stage; 2 palliative interventions (tracheostomies) and 2 exploratory cervicotomies in advanced thyroid tumour, surgically outdated.

In the 59 patients with radical or radically dissected dissection, the following surgical procedures were performed:

- 29 radical dissections (with internal jugular vein resection, sternocleidomastoidian, spinal nerve accessor and limph nodes removal - levels I-V);
- 30 radically modified dissections (with the preservation of at least one non-lymphatic structure compared to those with radical dissection or the preservation of another anatomical element).

It should be noted that of the 59 patients, 3 had bilateral dissection and two had radically modified dissection in the first stage and secondly (6 or 10 months after the first intervention) radical dissection on the opposite side.

One of the patients who required a modified radical dissection of the neck with the approach of the upper mediastinum showed the peculiarity that thyroid tumour had mechanical compression on the superior cave vein. This led to the development of superior cavo-cave collateral circulation at the level of anterolateral thoracic regions.

Regarding the immediate postoperative complications, of the 189 surgical patients, there were 6 cases of bleeding (3.17%) (in which the surgery haemostasis was done), 7 (3.70%) cases of unilaterally recurrent nerve damage (patients in stage IV with recurrent nerve invasion), the remaining 176 (93.12%) being without immediate complications.

Follow up showed that of the 189 patients, 6 months after surgery, 15 patients (7.93%) had permanent hypoparathyroidism. Also at 24 months there were 8 (4.23%) postoperative recurrences that needed surgery (3 cases at <1 year and 5 cases at> 1 year), all 8 patients being in the group of the 12 thyroid that underwent radical neck dissection after total thyroidectomy in the first stage.

Thus, relapse for radical neck dissection is 13.55% (5.08% before 1 year and the rest 8.47% after one year). In the 8 patients with relapse, unilateral dissection was performed. In two patients it was performed the controlateral dissection after controlateral lymph node invasion at 6 and 10 months postoperatively.

3.5.4. Discussions

As we mentioned above, radical neck dissection technique was described by George Crile in 1906. This procedure has often been used in the staging of metastatic cancers of the head and neck. The procedure included removal of cervical lymph nodes and sacrificing internal jugular vein, major auricular nerves as well as sternocleidomastoid, digastric and stilohioid muscles. Subsequently, the technique was modified, allowing for the preservation of important cervical anatomical structures (Crile, 1987). Today, the term radical dissection of the neck refers to the functional dissection - Bocca - of the neck (Bocca, 1980).

In turn, the radically modified dissection may be of three types: type I with the maintenance of the spinal accessory nerve, type II with the maintenance of internal jugular vein and / or the spinal accessory nerve and type III with internal jugular vein preservation and / or the accessory spinal nerve and the sternocleidomastoid muscle.

The results evaluation in thyroid cancer surgery is done by assessing the occurrence of tumour recurrence which is the most important prognostic factor.

All of these findings suggest that a more conservative approach may be a preferred management strategy over immediate completion surgery, despite a slightly higher risk of structural recurrence. Regarding the follow-up of post-thyroidectomy patients, it is reasonable that an initial risk stratification system based on clinicohistological findings be used to guide the short-term follow-up prior to evaluating the response to initial therapy and that the dynamic risk stratification system based on the response to initial therapy be used to guide long-term follow-up (Park and Yoon, 2019).

There is a virtual consensus among surgeons that patients with clinically palpable cervical metastases should undergo neck dissection.

In the 189 patients included in this study we used the Spiro classification from 1994 for statistically recording of the datas provided by the Third Surgery Unit:

1. radical neck dissection (includes dissection of 4 or 5 ganglion levels):
   - conventional radical dissection;
   - radically modified dissection;
   - extensive radical dissection;
   - radically extensive modified dissection.
2. selective neck dissection (3levels of lymph node dissection):
   - suprahioid neck dissection;
   - jugular dissection (levels II-IV);
   - dissection of other (any) 3 nodal levels.
3. limited neck dissection (dissection of less than 2 levels of lymph nodes):
   - paratracheal lymph node dissection;
   - dissection of mediastinal lymph nodes;
   - dissection of other (any) 1 or 2 lymph nodes levels.

The lymph nodes considered in the radical neck dissection are as follows: level I submental and submandibular lymph nodes, level II superior jugular lymph node (anterior, posterior, superior, inferior), level III middle jugular lymph nodes (anterior, posterior, superior, inferior), level IV inferior jugular lymph nodes (anterior, posterior, superior, inferior), level V lymph nodes group of the posterior cervical triangle, level VI pretracheal, paratracheal and precricoid lymph nodes, and level VII superior mediastinal lymph nodes.

Neck dissection plays an essential role in the management of head and neck cancer. The dilemma of approaching the radical neck dissection is that the most differentiated types of thyroid cancer commonly give clinically significant cervical metastases. This phenomenon occurs especially in young patients with papillary thyroid carcinoma (Qu et al., 2019).

One of the indications of modified radical neck dissection in well differentiated thyroid cancer is to establish the topography of cervical lymph adenopathy either by palpation or radiology.

Confirmation of metastatic disease can be achieved by a fine needle preoperative aspiration performed under echographic guidance. Biopsy of the "sentinel" lymph node of the jugular-carotid chain using mapping with methylene blue stains can be a feasible and valuable method for estimating lymphatic status in the neck compartment (Dzodic, 2006).

Prophylactic intervention is recommended for cases of papillary carcinoma demonstrating two or more of the following four features: male gender, age 55 years or older, maximum tumour diameter greater than 3 cm, and extrathyroid massive extension (Ito, 2007).

Recent studies reported the frequent occurrence of metastases in the anterior cervical median (64.1%) and lateral (44.5%) regions (Wada, 2003).

Instead of radical neck dissection, in the past, many surgeons have supported local removals of "picking berry" designed to eliminate coarse lymphadenopathy (Raina, 1983; Nicolosi, 1993).

However, these procedures are associated with a higher rate of local recurrence requiring corrective surgery associated with a higher complication rate (Musacchio, 2003), close to that of radical dissections. The same is true for limited modified neck dissection, where the superior extension of the surgery is limited to the spinal accessor nerve (Pingpank, 2002; Gemsenjäger, 2003; Kupferman, 2004).

In early thyroid cancer (Stage I or II), is sufficient total thyroidectomy with the removal of lymph nodes from the median or lateral neck compartment (selective neck dissection), but this is not recommended in medullary thyroid cancer, when radical neck dissection is indicated regardless of stage (Moley and DeBenedetti, 1999).

In advanced thyroid cancer (stage III or IV) as well as in medullary thyroid cancer total thyroidectomy accompanied by unilateral or bilateral neck dissection is the recommended procedure to improve the recurrence rate (Moley and DeBenedetti, 1999).

The controversy consists in preserving or removing internal jugular veins together with adjacent lymph nodes. Recent studies performed by ultrasonographic evaluation of internal jugular veins allowed the hemodynamic states to be recorded in different patients positions. Their results show that internal jugular vein is not the primary pathway of cerebral venous drainage in sitting position and calm breathing (Gallo, 2013;Chen, 2019).

Generally, young patients with papillary thyroid cancer have a better prognosis than older patients despite their more frequent ganglion metastases. Some studies suggest that radically modified neck dissection (bilateral) can prevent reintervention for lymph nodes recurrence but it does not seem to influence prognosis in young patients (Voutilainen et al., 2001). Both internal jugular veins can be resected, but at least 6 weeks apart.

In contrast, the survival of elderly patients appears to be low in those with recurrent lymph nodes. Thus, elderly patients with risk factors for controlateral lymph nodes recurrence are the ideal candidates for radically modified bilateral dissection (Ohshima et al., 2000).

Although used, it seems that selective neck dissection leads to increased recurrence, making it an inefficient technique for advanced thyroid cancer (especially non-papillary cancer). However, there are other studies demonstrating that this technique is safe enough to prevent recurrence.

The incidence of chronic neuropathic pain after neck dissections is approximately 40%. Due to its location, the superficial cervical plexus occupies an anatomic position that exposes it to the risk of structural changes (fibrosis, withdrawal phenomena associated with radiotherapy or neuroma formation) posttiroidectomy. Standard drug therapy in these patients includes pharmacological treatments due to neuropathic pain (gabapentinoids, tricyclic antidepressants). Modern techniques of pain therapy with invasive ultrasound allow

for the successful treatment of these postoperative complications by pulse radiofrequency ablation of nerve structures (Valls, 2019).

To minimize surgical morbidity and neck scarring, minimally invasive thyroididectomy and endoscopic robotic thyroidectomy have developed in the last 20 years. They use the cervical, axillary, anterior thoracic, mammar or postauricular approach. Of these, the transaxillary gas-free approach, the bilateral axillary-mammary approach, the post-auricular approach of facial lifting, and the transoral vestibular approach are currently in use. The main advantage of endoscopic thyroididectomy is the cosmetic, while the rate of complications is similar to that of conventionally operated patients and requires a team of experienced surgeons. Operating time is significantly higher in this case (Berber, 2016; Tae, 2019).

Modern radiological techniques allow a precise identification of the lymph nodes involved in the paraneoplastic process, the nodal compartments of the neck and even their mapping. This allows for dissection to be oriented to lymph nodes compartment (or selective) instead of the extended one, but requires close cooperation between an experienced radiologist and the operative team. There are evidences that proove this more conservative strategy allows local control of the disease, while morbidity is minimized. A better cosmetic result is also obtained (Fama, 2015; Sakofaras, 2019).

3.5.5. Conclusions

Thus, the radical neck dissection in advanced thyroid cancer, although a complex surgical technique, with possible incidents and postoperative complications, is the primary therapeutic solution in advanced thyroid cancers with long-term good results in a specialized surgery center. More other retrospective and prospectives studies are needed to succeed in establishing a management protocol for each type of thyroid malignancy.

3.6. Advances in postthyroidectomy hypocalcemia

3.6.1. Introduction

Postoperative hypoparathyroidism is most commonly caused by the extirpation, injury or devascularisation of parathyroid glands. It can be considered a medical and surgical emergency (Kazaure and Sosa, 2018).

Thyroid cancer is a rare entity among human malign tumours, covering less than 1% of all cancers, but is the most common endocrine malignancy. The incidence of thyroid cancer in the US is about 7.7 per 100,000 inhabitants/year. Of the histological types, the highest incidence is papillary, 5.7 per 100,000 inhabitants/year, followed by follicular and then medullary form.

The incidence of thyroid cancer has increased by about 5% annually over the past 10 years and the mortality rate has increased from 0.8% per year from 2002 to 2011 (Cooper et al., 2009; Nguycn et al., 2015).

Women are 3-4 times more often affected than men. The effect of Graves disease on the postoperative complications in patients undergoing total thyroidectomy is unclear. This difference may reflect not only real incidence, but also difficult access to medical services and early detection (Kwon et al., 2019).

The staging of thyroid cancer is based on TNM (Tumor, Nodes, Metastases), with a few peculiarities of other cancers. Thus, both the histological diagnosis and the age of the patient are taken into account in the evaluation of the prognosis and the establishment of the therapeutic course. Clinical staging is performed by inspecting and palpating the thyroid gland and regional lymph nodes.

Laryngoscopy is also indispensable for highlighting the mobility of vocal cords. Paraclinical examinations include radioactive isotope, ultrasonography, computer tomography and MRI scanning. Diagnosis of thyroid cancer should be confirmed by puncture-needle biopsy or open biopsy. Staging should be completed by a biopsy on suspected lymph node (Edge and Compton, 2010).

Personal contribution – published paper:

Grigorovici A**,** Varcus F, Mogoș S, Călin A, Hînganu D, Hînganu MV, **Preda C.** Hypocalcemia after thyroidectomy for advanced local malignancies. *Rev Chim Bucharest* 2019; 70(3):1053-1057.

The aim of this study is to establish correlations between the advanced thyroid cancer staging, the choice of individual treatment, the results obtained, the frequency and intensity of transient and/or permanent post-operative hypocalcemia.

The results of the study are compared with the data from the literature as well as with the previous experience of the 3rd Surgery Clinic of St. Spiridon Iasi Hospital, in order to highlight the causes of these complications. This makes possible to develop surgical, oncological and endocrine management protocols for patients diagnosed with advanced thyroid cancer in order to avoid these relatively frequent and important complications.

3.6.2. Materials and methods

This paper is a retrospective study of 213 cases of thyroid cancer, operated in the 4th Surgery Clinic of the "St. Spiridon" Emergency Clinical Hospital in Iasi between January 2012 and December 2018. In particular, 116 cases diagnosed in advanced stages (III and IV) were analyzed in the Endocrinology Clinic of the St. Spiridon Clinical Hospital.

The data were taken from the table provided by the statistical service of the St. Spiridon Clinical Hospital, as well as from the surgery protocols and histopathological exams. The inclusion criteria in the batch were the primary or secondary diagnosis of malignant thyroid cancer and the over 18 years of age in both genders.

During this period 194 surgical interventions for thyroid cancers were performed. Of these, 145 were performed in female and 49 in male patients, achieving an M: W ratio of 1: 2.95.

3.6.3. Results

Most patients were investigated and diagnosed under a prior admission to the Endocrinology Clinic. The evaluation consisted of the general and local clinical examinations, serum levels of thyroid hormones, parathormone and calcium. The ENT clinical examination and thyroid ultrasonography were also performed.

Indirect laryngoscopy was performed in order to identify possible paralysis of vocal cords, eventual compression or invasion of thyroid nodules. In most cases, thin needle biopsy puncture and computer tomography were performed. This highlights the anatomical extension of the tumor and the possible invasion of adjacent structures, thus guiding the extent of surgery.

Percentage of female patients accounted for 74.74% of all cases, while male patients accounted for 25.25% (**Fig. 26**) .

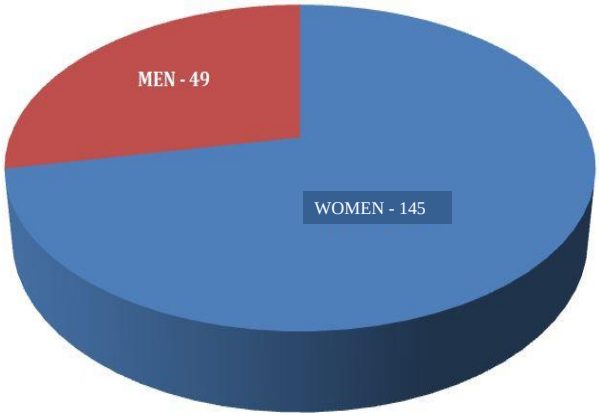


Fig. 26. Distribution by gender within the study group

Distribution by age group shows the proportion of the disorder in patients over 50 years of age (**Fig. 27**).

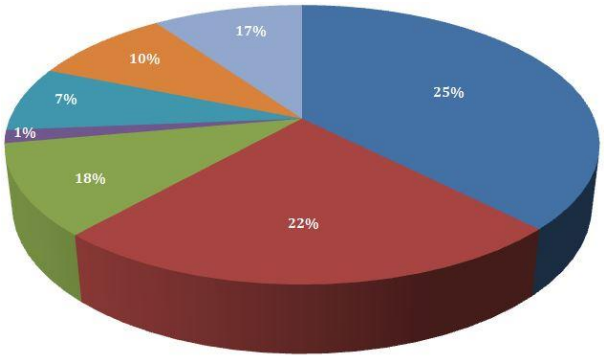


Fig. 27. Percentual distribution of patients by age: 1% between 18-20, 7% between 21-30, 10% between 31-40, 17% between 41-50, 18% 71 and on, 22% between 51-60 and 25% between 61-70 years old

Thus, out of a total of 194 cases, patients aged 61-70 years are the highest-age group, with 49 cases and 25.52%, followed by the 51-60 age group, accounting for 22% of the total cases - 43 patients.

The fourth decade of life includes 33 patients, 31-40 year-old patients are 18 cases, while 18- to 30-year-old patients are 16 cases.

Patients over the age of 71 mark a decrease in incidence after the peak of 61-70 years, including 35 cases - 18.2% of the total. The minimum age in the study group is 19 years and the maximum of 84 and a medium age of 55.3 years (**Fig. 28**).

To analyze the distribution of thyroid cancers subtypes among the investigated patients, we took the data from the histopathological bulletins attached to their admission sheets. Of the 194 cases considered, the highest histological weight is papillary thyroid cancer, with 79.89% of the total cases. This is followed by medullary thyroid cancer with 27 cases, anaplastic thyroid cancer - 17 cases and follicular thyroid cancer - 7 cases.

Three other cases were non-thyroidal cancers: a case of neuroendocrine tumor, a thyroid locomotor plasmocytoma and a renal neoplasm metastasis case.

Analyzing the relationship between the histological subtype and the age for advanced cancers, it can be observed how the proportion of papillary thyroid cancer decreases from 88.46% for the age group of 45-50 years, to 84.61% for the age group 51-60 years at 54.05% for 61-70 years and only 66.66% for patients over 70 years of age.

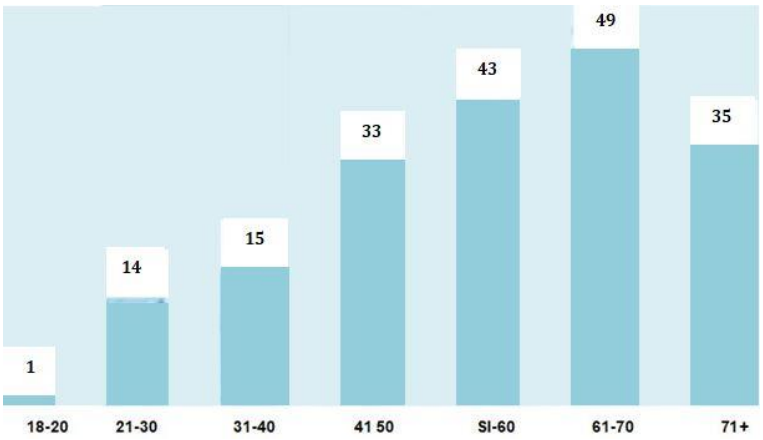


Fig. 28. Patients distribution by age

Anaplastic thyroid cancer increases from 0 cases for the 45-50 age group, 2 cases (7.69%) for 51-60 years, 7 cases for 61-70 years (18.9%) and 7 cases (25.92%) for patients aged over 70 years. Follicular thyroid cancer occurs only in the age group of 61-70 years, in a number of 5 cases.

Medullar thyroid cancer is distributed relatively constant between the four age groups: 3 cases (11.5%) for the age group 45-50 years, 2 cases (7.69%) corresponding to the 5th decade of life, 5 cases (13.51%) for 61-70 years and 2 cases (7.4%) for patients over 70 years of age (**Fig. 29**).

3.6.4. Discussions

The rate of intraoperative complications related to recurrent laryngeal nerve injury, intraoperative haemorrhage requiring haemostasis and the necessity to achieve a definitive tracheostomy is 13%, 17% and 3.44%, respectively.

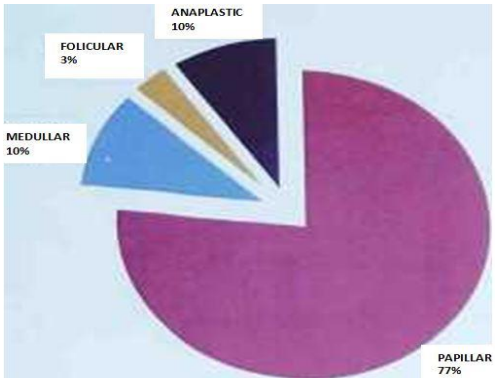


Fig. 29. Thyroid cancer type percentage

Regarding the rate of postoperative complications, these are present in 26.72% of patients, while the complications strictly related to the operative act are 17.24%. By excluding short-term complications (transient hypocalcaemia and postoperative haemorrhage), it appears that a number of 16 complications were the direct consequence of the operative act. A total of 12 patients (10.34%) experienced these complications (Table 29).

Transient hypocalcaemia (16 cases, 29% of all complications), defined as 6-month remission hypocalcaemia, was associated with complications related to the operative act whereas permanent hypocalcaemia required supplemental calcium medication throughout life (4 cases, 7.27% of total complications). This is the consequence of parathyroid gland damage during total thyroidectomy intervention.

The most commonly associated intervention with permanent hypocalcaemia is total thyroidectomy (2 cases, 50% of cases of permanent hypocalcaemia), interventions associated with one case being total thyroidectomy with selective neck dissection and totalization of thyroididectomy with radical dissection of the neck. Transient hypocalcaemia was most commonly associated with simple total thyroidectomy (3 out of a total of 16 cases, 18.75%).

Two cases were recorded following total thyroidectomy accompanied by tumor excision, unilateral thyroidectomy with radical neck dissection, total thyroidectomies with radically modified dissection of the neck and enlarged total thyroidectomies.

One case was recorded as a consequence of total thyroidectomy with selective dissection, extended thyroidectomy with radically neck dissection and tracheostoma, and total thyroidectomy with radical dissection and definitive tracheostomy.

The predominance of female gender encountered in international and national literature is verified in terms of statistics of the 4th Surgery Clinic in 2012-2018.

Table 29. Postoperative complications depending on type of the intervention, in the case of advanced thyroid cancers; TT= total thyroidectomy, TL=total lobistectomy, RD=radical dissection of the neck, SD=selective cervical dissection, MRD=modified cervical radical dissection, ETT=extended total thyroidectomy, PTT=permanent tracheostomy, TE= tumor excision, TOT= thyroidectomy totalization

Complications by intervention	TOTAL	TT+ SD	TL+ PTT	ETT+ RD+ PTT	TT	ETT+ MRD	TT+ TE	TE	WS	TT+ MRD	ETT+ RD	TT+ RD+ PTT	TT+ PTT	TOT+ RD	TT+ RD	ETT
Oesophageal/ hypopharyngeal/ tracheal fistula	3		1	1	1											
Permanent hypocalcaemia	4	1			2									1		
Unilateral vocal cord paresis	1					1										
Paraseptal emphysema Phrenic nerve paralysis	1									1						
Bilateral vocal cord paresis	1											1				
Unilateral recurrent nerve paralysis	1				1											
Pneumomediastinum Subcutaneous emphysema	1												1			
Transient hypocalcaemia	16	1		1	3	1	2			2	1	1			2	2
Postoperative bleeding/ Wound infection	3			1							1				1	

There are important international studies that highlight the differences in the rate of complications between different centers and operating teams, underlyening the importance of evaluating post-operative complications in the same center over a period of time. This is explained by the direct correlation between the risk of complications and the expertise and experience of the operating team (Fitzgerald, 2013; Nixon et al., 2013; Brandt et al., 2013).

Another study of our research center, between 2000-2002 revealed a complication rate of 6.87%. The studied group comprised all stages, including stages I and II, while the present study examines the complications of Stages III and IV . Also, the percentage of patients that required post-operative reintervention was reported to be 3.17%, while only 2 cases (1.72%) were documented in the present study.

Permanent hypoparathyroidism was reported in 7.93% of patients, superior to the 3.44% found in this group. *Considering these aspects, it appears that the experience of the operative team has contributed to the decrease of the rate of postoperative complications.*

In a multicenter prospective study focused on the relationship between the rate of postoperative complications (recurrent lesion and hypoparathyroidism) and the experience of the operating team, an association has been found between the experience of the operating team and the surgical performance in thyroid cancer surgery, in the sense that the complications were the most common in the case of surgeons at the beginning or, on the contrary, at the end of their careers.

Graves disease significantly increased the risk of transient lesion of the recurrent lacrimal nerve and transient hypoparathyroidism and could be a predictive factor for recurrent postoperative nerve damage and hypoparathyroidism after total thyroidectomy. From this point of view, better results were obtained because of the reducing of their complications rate (Duclos et al., 2012; Guo et al., 2013; Kisakol et al., 2003).

Regarding the correlation between surgical intervention and the frequency of postoperative complications, it can be seen that total thyroidectomy with the radical neck dissection and tracheostomy has involved most complications strictly related to the operative act. The patient who underwent this treatment had postoperative hypopharyngeal fistula, transient hypocalcaemia and bleeding, all these requiring reintervention.

These complications also correlate with the stage and type of cancer, the patient presenting an IV C grade, thyroid anaplastic carcinoma with a diameter of approximately 15 cm, with right internal jugular vein thrombosis and a right subclavian vein thrombosis and direct invasion of the subhyoid muscles, cervical vessels and recurrent bilateral laryngeal nerve (Sitges-serra et al., 2010; Thomusch et al., 2003; Weetman et al., 1990).

Another type of intervention with multiple postoperative complications was extensive total thyroidectomy with radical neck dissection, in which the patient presented both complications directly related to the operative act and other complications: respiratory insufficiency and acute renal insufficiency. The patient had a fourth-degree papillary cancer with multiple comorbidities.

Routine administration of calcium vitamin D for two weeks was performed in all patients with total thyroidectomy, preventing symptomatic hypocalcaemia. The dose used was 3g per day 3 times a day in the first week and 1.5g three times a day in the second week. If symptoms of hypocalcaemia persist, it is recommended to supplement with intravenous calcium gluconate, one ampoule every 12 hours, for 7-10 days under the control of serum calcium.

Hypoparathyroidism is considered permanent if calcium supplementation is still needed, more than 6 months after surgery. For patients with hoarseness, an indirect laryngoscopy was programmed at 1, 3 and 6 months postoperatively, respectively, until the vocal cords function. After 6 months recurrent paralysis is considered permanent (Shore and Waghorn, 2011; Wilhelm et al., 2016; Cui et al., 2019).

Depending on the postoperative progression, patients are usually discharged from the hospital in 2-3 days postoperatively after removing the drainage tube, this being done when the drained liquid does not exceed 20 ml for two consecutive days. Patients with various postoperative complications had a longer hospitalization period, depending on the complication and the solution to solve it (Christou et al., 2013; Cozzolino et al., 2004; Bergenfelz et al., 2008).

Follow-up after initial surgery is performed in the Endocrinology Clinic, whrere are done further investigations: a local clinical examination, dosing of serum TSH levels, leukoglobulin or calcitonin, depending on the type of cancer, and cervical ultrasound at 6-12 months, depending on the patient's risk for recurrence. The ultrasound should include the thyroid lodge and the central and lateral lymph nodes. If a positive result (ultrasound ganglia larger than 5/8/5 - 8 cm diameter) is highlighted, it is done a biopsy with a fine needle for cytology with tireglobulin dosing.

3.6.5. Conclusions

An important post-operative complication of total thyroidectomy is given by hypocalcemia. This one can determine severe symptoms and also increases hospitalization time. The primary cause is secondary hypoparathyroidism following damage to, or

devascularisation of, one or more parathyroid glands during surgery. The present study can be continued with further researches in order to improve and develop surgical, oncological and endocrine management protocols for patients diagnosed with advanced thyroid cancerand to to avoid these relatively frequent and important complications.

3.7. Perspectives in polyglandular autoimmune syndromes (pas)

3.7.1. Introduction

Polyglandular autoimmune syndromes (PAS) represent the association of different autoimmune diseases, frequently endocrine disorders, where the diagnosis is established in the presence of at least 2 endocrine autoimmune diseases.While some of these disorders are quite frequent (e.g. hypoparathyroidism, thyroiditis, diabetes, candidiasis), others are rare (e.g. myasthenia gravis, Addison's disease); while some disorders are symptomatic and allow for a rapid diagnosis, others are asymptomatic or often diagnosed after a long evolution with very few symptoms.

Some authors consider that the diagnosis of polyglandular autoimmune syndrome represents an inappropriate medical term because the condition that it refers to often implies the association of non-endocrine autoimmune diseases (Eisenbarth et al., 2004). However, it is extremely important to recognize and diagnose these syndromes, the risk of association of several autoimmune diseases inthesamepatientandlast,butnotleast,thefactthat the relatives of these patients have an increased risk for any of the autoimmune disorders present and therefore require early diagnosis and treatment (Eisenbarth et al., 2003).

Significant clinical observations about the association of several endocrine disorders or about the multiple glandular insufficiencies in the same patient go as far back as the early 1900s (1904 in Germany, by Paul Ehrlich, 1908 in France by Claude and Gourot) (Carpenter et al., 1964).

In 1980, a group of authors led by Neufeld proposed a classification of PAS into 4 distinctive types: type 1 defines the association between Addison's disease, hypoparathyroidism and cutaneo-mucosal chronic candidiasis; type 2 represents the association between adrenal insufficiency and autoimmune thyroiditis; type 3 is represented by autoimmune thyroiditis associated with any other autoimmune disease except for adrenal insufficiency; and type 4 is represented by the association between other endocrine disorders or autoimmune diseases (Neufeld et al., 1980).

More recently, Kahaly considered both epidemiological and genetic criteria and proposed a two-fold classification of these syndromes, namely type I (PAS I, very rare, juvenile forms) and type II (PAS II, more frequent forms, discovered in adults, with or without adrenal insufficiency) (Kahaly 2009). Irrespective of the classification employed, one may observe that thyroid autoimmunity is quasi-constant in patients with type 2, 3 or 4 PAS (the Neufeld system) or type II (the Kahaly system).

Since thyroid autoimmunity often coexists with other significant autoimmune disorders and since hypo- or hyperthyroidism may have a negative impact upon the evolution of most associated disorders, it is imperative that patients should be properly evaluated and

monitored. Autoimmune thyroiditis is generally considered the specific manifestation of organ autoimmunity and is usually associated with other autoimmune or endocrine disorders (Wémeau et al., 2013). While polyserositis is frequently encountered in hypothyroidism, anasarca is a rare medical condition.

Personal contribution – published paper:

Mihalache L, Arhire LI, Gherasim A, Graur M, **Preda C**. A rare case of severe type 4 polyglandular autoimmune syndrome in a young adult. *Acta Endocrinologica (Buc)* 2016; 12(1): 104-110.

We present a case of type 4 PAS (hypothyroidism, type 1 diabetes mellitus, celiac disease), where the presence of pleural, pericardial and peritoneal effusions and important peripheral oedema raised major problems of differential diagnosis, in the context of severe hyperglycaemia and serious malabsorption syndrome.

3.7.2. Material and methods

We illustrate these by the case of a patient who, after an extensive period of bed rest, a 20-year old patient was brought to the emergency room with serious slowness and generalized oedema. The patient was informed about the required diagnostic and treatment procedures and signed the informed consent form. The initial tests excluded the following causes of the symptoms: renal (absent proteinuria, normal urea and creatinine), hepatic (normal liver enzymes and billirubin; liver steatosis in ultrasonography) and cardiac (no signs of cardiac failure).

Given his generalized oedema and weakness, the patient was suspected of severe malnutrition or hypothyroidism; moreover, the high blood glucose value of 790 mg/ dl in a person with no previous diagnosis of diabetes eventually motivated the patient's admission to the Clinical Centre for Diabetes, Nutrition and Metabolic Diseases.

The patient's admission note included the following basic information: conscious but depressed patient, underweight (weight = 44 kg, height = 162 cm, BMI = 16.7kg/m2), with slow deterioration of general state in the previous 4-5 years, aggravated in the previous 6 months (leading to bed rest), lack of appetite, slowness in movements and cold intolerance.

3.7.3. Results

Clinical observations included: pale yellowish, dry and exfoliated skin, important oedema of hands and face (**Fig. 29**), lower limbs, scrotum (confirmed by ultrasonography, **Fig.30**), bilateral rough vesicular breath sounds, diminished in the lower posterior chest walls; abnormally quiet heart sounds, BP=100/60 mmHg, distension of the abdomen, diffusely tender on palpation.

The abdominal *ultrasonography* revealed the presence of small quantities of liquid in the inter-hepatic-renal region and in the iliac fossae; the ECG revealed the presence of low voltage waves which are characteristic of pericarditis. The *chest X-rays* confirmed the diagnosis of pleural effusion and pericarditis (**Fig. 31**).

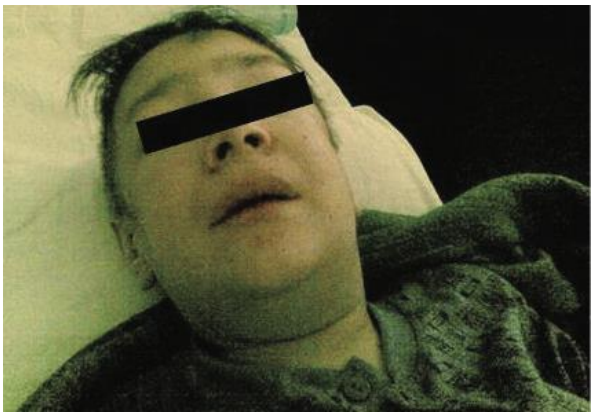


Fig. 29. Facial oedema in the patient with anasarca.

The initial treatment recommendations included i.v. fluid and insulin therapy for a progressive diminishing of blood glucose values (glycated Hb initial value was 13.8%).

Blood test values confirmed the diagnosis of myxedema (*TSH=100 μUI/mL, fT4 = 0.835 pmol/L*) and the patient began immediate hormonal substitution therapy with levothyroxine 100 μg/day for 14 days and then 150 μg/day (the patient's weight and celiac disease were taken into consideration).

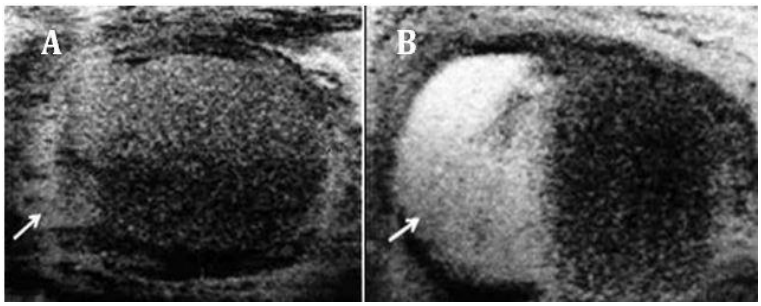


Fig. 30. Ultrasonography of the right testicle in A, showing 10 ml volume and normal structure; B=right scrotum ultrasonography showing scrotal oedema

Also, a *short-term corticotherapy* (100 mg hydrocortisone hemisuccinate, 4 days) was recommended in order to avoid a functional adrenal crisis, because thyroid hormones are known to accelerate cortisol metabolism. The patient also presented normochrome normocitary anaemia, the initial values indicating Hb=9 g/dL and Ht=25.5%.

Following the patient's rehydration, the blood values indicated Hb=5.7 g/dL and Ht=15.6%, which required the administration of iso-group iso-Rh blood. Anemia tends to have a mixed etiology especially in patients with severe malnutrition and due to hemodilution of hypothyroidism.

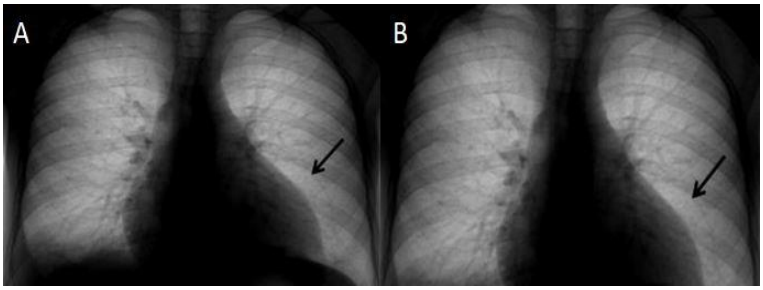


Fig. 31. Chest X-rays showing pleural (A) and pericardial (B) effusions.

Complex diagnostic problems and therapy decisions required that the patient be admitted to hospital for a rather long period of time, namely for 6 weeks. Here is a summary of the medical problems that we had to address:

- understanding the aetiology of hypothyroidism, the exclusion of other endocrine disorders, and the selection of an adequate endocrine treatment
- the patient was tested for anti-TPO antibodies (64 UI/mL, normal values < 20 UI/mL) and anti-TG antibodies (17UI/mL, normal values < 20 UI/mL).

These findings were corroborated with the ones elicited from the patient's full thyroid examination (including the thyroid ultrasonography, **Fig.32**) and they ultimately confirmed the diagnosis of autoimmune thyroiditis. Other forms of primary hypothyroidism were excluded due to clinical examination, medical history and thyroid ultrasound.

The patient was also tested for the *FSH, LH and testosterone levels*, which, in accordance with the 3-4 Tanner pubertal stage, revealed values in normal ranges. The delay in pubertal evolution was considered in the context of long lasting untreated hypothyroidism.

While both ACTH and cortisol values were within normal ranges, the PRL value was raised due to hypothyroidism.

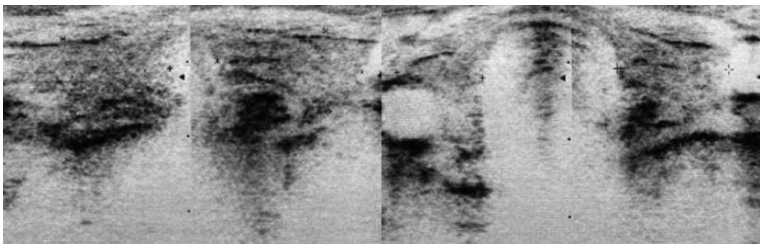


Fig. 32. Thyroid ultrasonography shows thyroid volume of 15 ml, hypoechogenic, non-homogeneous echostructure

The *CT scan of the brain* (to exclude a possible pituitary gland hyperplasia secondary to severe long-lasting hypothyroidism) revealed no abnormalities. The *X-ray of the wrist* revealed the presence of fertile growing cartilage and a delayed bone age (bone age=12 years, **Fig. 33**), due to the long-lasting and severe form of hypothyroidism.

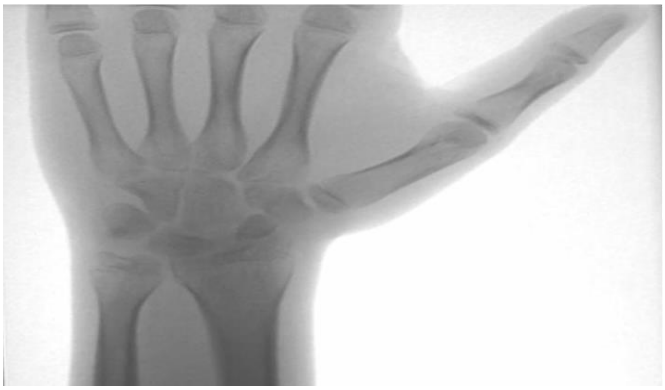


Fig. 33. X-ray of the wrist revealed the presence of fertile growing cartilage and a delayed bone age of 12 year

The diagnosis confirmedthe type 1 diabetes mellitus and allowed the selection of an adequate treatment. Given his attested autoimmune thyroid disorder, the patient was tested to determine the level of anti- GAD antibodies (24.3 UI/mL, normal values 0-5 UI/ mL) and the level of C peptide (0.28 ng/mL, normal values 0.9-7.1 ng/mL). The detected levels confirmed the diagnosis.

The patient went into partial remission during admission, which required negative titration of insulin doses, from a basal-bolus regime, to a regime with one injection of a long-acting insulin analogue. It was also necessary to laboriously educate the young man and his mother regarding insulin administration, the importance and technique for self-monitoring of blood glucose, the preventive and curative treatment of hypoglycaemia and the adequate diet.

However, we need to address the suspicion of a celiac disease. During his admission time, the patient did not respond well to treatment; in fact his overall treatment response was considerably delayed and did not match all our medical expectations (e.g. slow reduction of oedema and polyserositis, despite the correct administration of hormonal substitution treatment; persistent hypoalbuminemia which was considered the reason for the persistence of oedema; anemia, overall weakness, and weight loss within the context of malnutrition).

As a consequence, the patient was referred to a parental regime to correct his protein-caloric deficit (repeated infusions with desodated human albumin, dextrose solutions, iv vitamins and minerals); he was then referred to the enteral administration of vitamin and mineral supplements, and the per os correction of his protein deficit.

The confirmed presence of two autoimmune diseases raised our suspicions for celiac disease. As a consequence, endoscopy was performed and intestinal mucosa biopsy collected. The morphological aspects detected were typical of the Marsh II celiac disease; the specific antibodies present also confirmed the diagnosis (IgG antigliadin antibodies 29 mg/L, normal values <18 mg/L, IgA tissue antitransglutaminase antibodies 24 U/mL, normal values <10 U/mL, IgG tissue antitransglutaminase antibodies 37 U/mL, normal values <10 U/mL).

Following these results, we initiated a specific nutritional therapy (i.e. a hyperproteic, normoglucidic and normolipidic diet; the avoidance of foods with a high glycemic index, the selection of foods with a high content of proteins and a high biological value; gluten-free products).

We present in **Table 30** the evolution of the patient's parameters, which were initially altered. **Table 31** presents other parameters, which were not modified during admission, but were necessary for monitoring and diagnosis.

Table 30. The evolution of the patient's parameters after 6 weeks of hospitalization

Parameters	Normal values	Hospital admission values	Hospital discharge values
Glycemia	70-110 mg/dL	790 mg/dL	126 mg/dL
Hb	13-17.5 g/dL	5.7 g/dL	11.8 g/dL
Ht	40.1-51%	16.4%	33.8%
Total protein	63-83 g/l	59 g/l	68 g/l
Serum albumin	3.5-5.2 mg/dL	2.8 mg/dL	3.9 mg/dL
Serum iron	50-158 μg/dL	40 μg/dL	68 μg/dL
Ferritin	30-350 ng/dL	29.8 ng/dL	34 ng/dL
Total calcium	8.4-10.2 mg/dL	7.46 mg/dL	9 mg/dL
Magnesium	1.6-2.6 mg/dL	2.59 mg/dL	2.57 mg/dL
TSH	0.4-6 μUI/mL	100 μUI/mL	65 μUI/mL
fT4	12-22 pmol/L	0.835 pmol/L	16.82 pmol/L

All medical facts should be considered with direct reference to the patient's social background. Thus, in accordance with his declaration, the patient came from a very large and poor family, with a low level of education and with no health insurance. This explains the long evolution of the patient's PAS, especially hypothyroidism, without any medical interventions and no diagnosis.

During his hospitalization, the patient was referred to a constant thyroid substitution therapy, in progressively increasing doses (100 μg/day for the first two weeks and 150 μg/day after) under clinical, ECG and TSH monitoring, reaching the cruise dose of 150 μg levothyroxine/day.

3.7.4. Discussion

The patient was very late diagnosed with autoimmune thyroiditis and who was long suffering from myxedema and anasarca. It turned out that the patient also suffered from two other associated autoimmune diseases: type 1 diabetes mellitus and celiac disease.

The clinical characteristics of each of these diseases were masked by their association (the dehydration secondary to hyperglycemia was masked by the oedematous syndrome from hyperthyroidism, the reduced metabolic rate in hypothyroidism masked the hypercatabolic signs characteristic to the development of type 1 diabetes mellitus, and the coexistence of celiac disease and malabsorption masked the polyuria and polydipsia secondary to hyperglycemia).

The poor prognosis of the patient derived not only from the association of these autoimmune diseasesfound in an advanced state of evolution, but also from some severe co-existent malnutrition. The patient's social background also impeded any previous medical evaluations and early diagnosis.

Table 31. Other blood test values

Parameters	Normal values	Levels on hospital admission
Urea	15-45 mg/dL	48 mg/dL
Creatinine	0.7-1.3 mg/dL	0.51 mg/dL
ALAT	5-38 U/L	36 U/L
ASAT	5-41 U/L	28 U/L
Total bilirubin	0.2-1.2 mg/dL	0.43 mg/dL
Uric acid	3.5-7.2 mg/dL	2.50 mg/dL
Cholesterol	120-200 mg/dL	178 mg/dL
Triglyceride	35-150 mg/dL	107 mg/dL
Anti-Tiroglobuline antibodies	<20 UI/mL	17 UI/mL
Anti-TPO antibodies	<20 UI/mL	64 UI/mL
C peptide	0.9-7.1 ng/mL	0.28 ng/mL
Anti-GAD antibodies	0-5 UI/mL	24.3 UI/mL
FSH	1.5-17.4 mUI/mL	15.63 mUI/mL
LH	4.0-8.6 mUI/mL	3.53 mUI/mL
Testosterone	2.49-8.36 ng/mL	3.96 ng/mL
Cortisol	5-25 μg/dL	26.5 μg/dL
Prolactin	86-324 μUI/mL	767.1 μUI/mL
Anti-gliadin IgG antibodies	<18 mg/L	29 mg/L
Anti-transglutaminase IgA antibodies	<10 U/mL	24 U/mL
Anti-transglutaminase IgG antibodies	<10 U/mL	37 U/mL

Eventually, the medical team acknowledged a positive evolution of most of his clinical, biological and imagistic parameters (i.e. slow improvement of the patient's general state, slow oedema attenuation, progressive reduction of TSH with normal fT4 after 6 weeks of treatment, reduction of pericardial effusion and disappearance of the pleural and peritoneal effusions).

There was also a slow positivenutritionalevolution,withsomeimprovement in the protein, vitamin and mineral parameters. The patient and the family requested hospital discharge approval after 6 weeks of hospitalization.

Various cases of autoimmune thyroiditis with hypothyroidism are often reported in the medical literature, yet their associations with type 1 diabetes and celiac disease are rarely found and discussed. Benedini recently reported on a case of a 74 year old woman diagnosed with a type 3 PAS in association with type 1 diabetes mellitus, autoimmune thyroid disease diagnosed in hypothyroidism, and pernicious anemia (Benedini et al., 2015). In this case, the woman was diagnosed with diabetic ketoacidosis upon her emergency admission to hospital; subsequently, the patient was discovered to suffer from other autoimmune conditions, which initially masked all her symptoms and delayed overall diagnosis, hence, the screening for this type of patients is justified (Riley et al., 1981).

Recent data suggests that the development of diabetes usually precedes the diagnosis of hypothyroidism (Mouradian and Abourizk 1983). Many research studies offer strong medical evidence to support the association between type 1 diabetes mellitus and thyroid autoimmunity and hence recommend the introduction of routine screening tests for this category of patients(Reghina et al., 2012; Al-Khawari et al., 2015).

There is another study involving type 1 diabetes patients that reveals another interesting aspect, namely that the prevalence of antithyroid antibodies and parietal gastric cell antibodies increases with age and diabetes duration. At the same time, the presence of anti-GAD antibodies is regarded in this study as a marker for the development of other autoimmune diseases in teenagers (Karavanaki et al., 2009).

The special nature of our investigated case also resides in the fact that, on hospital admission, our 20-year old patient presented symptoms of anasarca, unlike the presence of only polyserositis, which is often seen in severe hypothyroidism (Lindsay 1997; Gottehrer et al., 1990).

A similar case (Gotyo et al., 2010) was that of a 50-year old female patient who requested medical assistance for weight gain, important oedema and large quantity ascites, and in whom the ultrasonography showed pericardites. In fact, all these modifications resulted from severe hypothyroidism.

In young patients the association between pleural and pericardial effusion was present in the context of Graves-Basedow disease (also an autoimmune thyroid condition) and type 1 diabetes mellitus (Algun et al., 2001).

Another specific feature of this case was the absence of the adrenal deficiency which is usually associated with autoimmune thyroiditis, diabetes mellitus and celiac disease in type 2 PAS. In our case the adrenal axis was unimpaired as evidence by the clinical examination and hormonal tests.

Celiac disease is another autoimmune condition of multifactorial aetiology and which is highly dependent upon the genetic background. Furthermore, it is often associated with other autoimmune diseases. The clinical manifestations are varied enough, including the classical malabsorption syndrome, but there are also asymptomatic cases (Fasano 2006).

The association between thyroiditis and the celiac disease raises serious problems about the therapy with levothyroxine because the levels of TSH normalize after a gluten-free diet, requiring lower doses of levothyroxine, due to the important alteration of the intestinal barrier in celiac disease.

The association between celiac disease and type 1 diabetes mellitus or autoimmune thyroiditis is frequently mentioned in the literature (Belei et al., 2015; Jinga et al., 2014), and there is enough evidence for the existence of some genetic susceptibility especially related to the HLA histocompatibility genes (Denham and Hill 2013).

However, to our knowledge, there are not many reported cases of PAS in adults that include celiac disease. This rare association has been reported in children (Lammer et al., 2008), yet predominantly in the context of genetic diseases (e.g. Down's syndrome). One study involving children with type 1 diabetes mellitus revealed the association with celiac disease at a rate of 7.8% and with anti- thyroid antibodies at a higher rate, 29% for anti-TPO antibodies and 23% for anti-Tg antibodies (Ergur et al., 2010).

The authors opined that patients with type 1 diabetes should be screened annually for antibodies specific to celiac disease and autoimmune thyroiditis, regardless of the presence of any specific symptoms.

A relatively similar study involving a much larger group of type 1 diabetes patients suggests the need for patients' annual screening for thyroid autoimmunity (especially in women) and celiac disease (especially in younger patients) (Messaaoui et al., 2012).

After a particular and unfortunate sequence of events, all medical facts were related to the patient's social background. The nescience leaded to long evolution of the patient's PAS, especially hypothyroidism, without any medical interventions and no diagnosis. We do believe that the main condition for PAS is regarding this aspect, together with patient's own comorbidities.

Furthermore, these researchers have also reported the presence of certain HLA haplotypes, which would confer increased susceptibility to each autoimmune disease. All these facts and data support the idea that celiac disease is the second most frequent autoimmune disease in patients with type 1 diabetes mellitus, after autoimmune thyroiditis (Volta et al., 2011) and it is asymptomatic in over 50 % of the cases (Holmes 2001).

3.7.5. Conclusion

The association of type 1 diabetes mellitus with autoimmune thyroid disorder and with celiac disease may result in type 4 PAS. The case reported in this paper was difficult to approach because it required the differential diagnosis of a severe oedematous syndrome with polyserositis in a seriously ill patient, with multiple organ problems.

In our daily medical practice, this pathological association raises important diagnosis problems, mostly because the symptomatology of each of these diseases can be hidden by the others.

As a consequence, therapy decisions are difficult to take and put into practice. Moreover, the malabsorption syndrome usually associated with celiac disease often hinders our most adequate treatment solutions.

SECTION II - FUTURE PROJECTS IN THE ACADEMIC, PROFESSIONAL AND RESEARCH FIELD

In this part of the manuscript I shall refer to the future plans for both academic and research career. The academic career future relays on the results of the clinical studies, in order to disseminate their findings.

In the near future, the project "Effect of selenium supplementation on the antioxidant status, hormonal, autoimmune and ultrasonographic profile in euthyroid subjects with chronic autoimmune thyroiditis" is under way. The main objective is to create an experimental model for assessing the effect of micronutrients in autoimmune thyroiditis, in our case with selenium specificity but with the possibility of extrapolation to other categories of micronutrients. The results are expected to help clarify issues related to the need for selenium supplementation and its therapeutic effect in thyroid pathology.

The data on selenium concentration in the body will provide indirect information on the selenium intake in the environment. Due to the variables studied, an estimate of the salt iodine effects that has been implemented in Romania since 2003 can also be made.

I propose to participate with my colleagues in drafting the Endocrinology Manual for students in Romanian and French.

I propose the creation of an endocrinology atlas in which iconography is the main asset in increasing the quality of practical skills of students (diagnosis based on clinical examination and corroboration with imaging explorations such as ultrasound, scintigraphy, computed tomography, nuclear magnetic resonance for the main endocrine disorders).

As a long-term goal I propose to participate in competitions for CNCSIS-funded projects, to develop collaboration relations and to exchange experience with national and foreign educational and research units.

A priority will also be active participation in projects and actions initiated by faculty and university for research funds.

There have been significant breakthroughs in the research in the field of endocrinology throughout the recent years. This has led to the *discovery of new treatments* for endocrine conditions and new findings could bring about dramatic changes to the way endocrine conditions are currently managed, especially considering all the innovative treatments on the horizon.

One of my research perspectives is focused on the treatments for endocrine diseases used at the present time regarding the management of symptoms in affected patients. This helps to improve the quality of life of patients significantly, but relies on continuous treatment that the patient will need to take for the rest of their life to maintain the effect. From my opinion, there is a significant need for discovery about how the function of the endocrine system can be altered for medical purposes.

In the case of hypothyroidism, the mainstream treatment to be recommended is hormone replacement therapy to reduce symptoms associated with low levels of thyroid hormones. A better understanding of the function of the thyroid gland enables the possibility of restoring its function and would allow ongoing treatments to be ceased.

For all of these I consider to extend relationships with other more research centers in order to unite our efforts. More multicentric studies which include large lots of patients are needed to test different types of treatment, in each endocrine disorder.

Another goal of my future career is *to restore endocrinology statute*; the relation of the enhanced knowledge of hormonal molecular mechanisms, leaded to the suggestion that the discipline is becoming a part of the larger field: molecular cell biology. Scientologically, endocrinology was about to be considered as a division of the biomedical sciences.

It is unclear whether this should continue into the future due to its close links with molecular cell biology. Moreover, there are several reasons that endocrinology may continue to exist as a basic science in the future: the presence of orphan receptors and signaling molecules to control critical endocrine functions and unresolved issues that involve whole animal physiology (e.g. growth and puberty).

A new and audacious project of mine leads me to a deeper exploration of the *interventional endocrinology*.

Endocrinology has to manage either oversecretion or undersecretion of hormones. Traditionally, this science has all been about thoughtful science in which we suppress or stimulate a gland's function. But with advent of science, we are looking beyond the traditionally; in cases such as a nonlocalized parathyroid adenoma, a doubtful pituitary adenoma, suspected insulinoma.

Within all these processes of extending our understanding and knowledge, interventional endocrinology plays a pivotal role. The interventions are performed for both therapeutic and diagnostic purpose. They have been a tool to pave our way into the future, taking us beyond what was obvious through blood investigations and structural imaging.

In this extensive domain I am going to focus my activity and research for:

- cavernous sinus sampling, internal jugular vein sampling or pituitary brachytherapy for pituitary disorders.
- genetic analysis for medullary thyroid cancer, percutaneous ablation of thyroid nodule, thyroidectomy for thyroid diseases
- parathyroid venous sampling for localization of parathyroid adenoma and intraoperative localization of parathyroid adenoma with methylene blue, MIBI and USG
- intra-arterial calcium stimulation testing, pancreatic angiography and pancreatic chemoembolization for pancreas insulinomas
- adrenal venous sampling for primary hyperaldosteronism
- genetic analysis for cause of intersex, ovarian cystectomy, sperm retrieval and intracytoplasmic sperm injection, antenatal steroid therapy for congenital adrenal hyperplasia, hormonal therapy in transsexuals
- whole body venous sampling for tumor-induced osteomalacia, osteoporosis therapy
- genetic analysis to characterize the type of multiple endocrine neoplasia, prophylactic thyroidectomy and parathyroid autoimplantation
- continuous glucose monitoring system, genetic analysis for diagnosis of maturity onset diabetes of young and closed loop insulin delivery or insulin pumps.

A huge impact of interventional endocrinology is in cases of multiple endocrine neoplasias (MEN), where the value of endocrine interventions cannot be overstated. The diagnosis of the type of MEN by genetic analysis and thereby being able to do prophylactic surgeries has no parallel in medical science in altering the course of any other disease.

In this domain I have already started the research by participating to HOPE (How Oncogenetics Predicts & Educates) project. The project is about promoters of advanced oncogenetics which open online training and multimedia raise awareness on multidisciplinary assessment of patients and their families at risk of hereditary or familial cancer. It includes in the list of hereditary and familial cancers the endocrine malignancies, especially MEN. The aim of this project is to understand genetic predisposition to cancers and care for persons at risk. Genetic predispositions are often associated with a family history of cancer. However, cancers are very frequent and family histories exist, so a family history of cancer is not synonymous with a genetic predisposition. The purpose of a genetic consultation is to determine the share of family history and possible predisposition. Genetic tests, stills seldom practiced, sometimes confirm a hereditary origin. If an alteration is identified in a family, it can be sought in its relations. This makes it possible to reassure those having no predisposition and following up those at risk.

Selective venous sampling (SVS) is another intervention that has been used extensively in diagnosing and treating a large number of endocrine neoplasias. This works by demonstrating a certain predefined gradient across an endocrine gland, which can confirme the tumorous growth. The technique has been particularly used in defining pituitary tumours, especially corticotrophinomas, where it is performed by cavernous sinus sampling.

Another technique - internal jugular venous sampling - is a further not-so-invasive one and it has been validated for diagnosing adrenocorticotropic hormone (ACTH) producing pituitary adenomas.

To diagnose small and occult pancreatic insulinoma, value of intra-arterial calcium stimulation cannot be overemphasized. SVS may play a major role in diagnosing difficult cases of tumor-induced osteomalacia in the future.

For reproductive endocrinology, replacement of sex steroids in hypogonadal males and females can be as gratifying and fruitful as any surgical intervention in any other disease. In the cases of transsexuals, hormonal therapy has yielded stunning results and has blurred the traditional gender definitions.

Important findings have been made in evaluation and treatment of infertility and are giving hope to infertile couples and could bring them the joy of their life.

Brachytherapy is increasingly being used for pituitary, adrenal, and endocrine tumors with at least as good results as provided by external beam radiotherapy. Nuclear medicine-based radiation therapies are expected in the future and these are about to provide targeted therapy with minimal side effects. Some of these therapies are already available for tumours such as metastatic medullary thyroid carcinoma and metastatic thyroid carcinoma.

Interventional endocrinology plays an important role in oncology. Rescuing ovarian reserve with gonadotropin-releasing hormone (GnRH) agonists during cytotoxic chemotherapy is rapidly becoming a norm rather than being an exception. GnRH agonists are increasingly being used for medical gonadectomy in cases of prostate carcinomas.

Replacement therapies for hormonal deficiencies have always resulted in miraculous results - growth hormone-deficiency, Addisonian patients, hypothyroid patients, and hypogonadal patients. It represents the future reflection of the past.

The term “interventional endocrinology” is used by some scientists for antiaging medicine. Hormonal deficiencies increasing with age is sustain by some authors but the idea is still not proved. If so, this would play a major role in aging process and replacing them might halt or even reverse the aging. We can count for this the growth hormone, insulin-like growth factor 1, dehydroepiandrosterone (DHEA), melatonin, adipocytokines.

Future gene therapy and interventions in hormonal milieu may bring us the elixir of unending youth.

Our studies should address the contribution of sex differences in the behavioural outcomes mediated by endocrine routes; the host gender represents a key variable in stress-related behaviours and, given the hormonal discrepancies present among males and females, a deeper investigation of this aspect is required.

The last new era in neuroendocrinology, at the end of the former millennium, was based on the insight that there is an important input to hypothalamus from other peripheral sources, by peptide hormones. These peptide hormones, in contrast to steroids, were initially not thought to have access to the brain because of the blood-brain barrier. Our present new era deals with leptin, orexin, cannabinoids, hypothalamic networks underlying aggression (important in the world of today), and with mechanisms underlying obesity and anorexia.

This next generation era of neuroendocrinology is based on a new enabling set of technologies, including single cell sequencing and other analyses, optogenetics, clearing methods for 3D reconstruction of brain (CLARITY, iDISCO), advanced viral tracing techniques and the development of numerous strains of genetically modified mice.

All these methods taken together have made it possible to take a large step forward to understanding the basic hypothalamic functions with direct implications for treating neuroendocrine diseases. This includesthe metabolic syndrome, sleep disorders, and others. The ways in which these new efficacious medicines are being designed were presented, offering hopes for large populations of seriously ill patients, and hopefully contributing to a solution to the urgent problem of the ever increasing costs of health care. With all of this progress in basic research and clinical medicine, the meeting provided a relevant and appropriate opportunity to talk about a new era in neuroendocrinology. Extraordinary opportunities, built on pioneering work that came before, abound.

Another future research we have in mind is comparative endocrinology, wherein we aim to *study and describe the repertoire and the evolution of the entire G-protein coupled receptors (GPCR) family*. A number of genomes have recently been sequenced and many more are in progress but there is a huge amount of work to annotate and clarify the ortologues/paralogues relationship of the genes from the recent sequencing project such as the *Fugu*, zebrafish, and chicken which are key species to receive a comprehensive overview of the vertebrate origin of the GPCRs. The findings in the new genomes will bridge some of the long evolutionary distances of the humanaty also, in order to understand the mechanisms of the groups of GPCRs. The study of these proteins is rapidly evolving and we believe that it is important to understand the detailed mechanism that affect the evolution of these groups and contrast them with the mechanisms that seem to have shaped the evolution of genes.

The field of endocrinology continues to be stimulated by clinical issuess that need to be addressed and new treatments and management protocols are coming up velociously.

SECTION III - REFRENCES

Abdel-Rahman O., ElHalawani H., Fouad M., (2016), Risk of endocrine complications in cancer patients treated with immune check point inhibitors: a meta-analysis, Future Oncol, 12, 3, 413-425.

Aberg N.D., Brywe K.G., Isgaard J., (2006), Aspects of growth hormone and insulin-like growth factor-I related to neuroprotection, regeneration, and functional plasticity in the adult brain, Scientific World Journal, 53, 80.

Adamczyk P., Bužga M., Holéczy P., Švagera Z., Zonča P., Sievänen H., Pluskiewicz W., (2015), Body Size, Bone Mineral Density, and Body Composition in Obese Women After Laparoscopic Sleeve Gastrectomy: A 1-Year Longitudinal Study, Horm Metab Res, 47, 12, 873-879.

Aguiar-Oliveira M.H., Bartke A., (2019), Growth Hormone Deficiency: Health and Longevity, Endocr Rev, 40, 2, 575-601.

Al-Delaimy W.K., von Muhlen D., Barrett-Connor E., (2009), Insulinlike growth factor-1, insulinlike growth factor binding protein-1, and cognitive func- tion in older men and women, J Am Geriatr Soc, 57, 8, 1441–1446.

Al-Khawari M., Shaltout A., Qabazard M., Al-Sane H., Elkum N., (2015), Prevalence of thyroid autoantibodies in children, adolescents and young adults with type 1 diabetes in Kuwait, Med Princ Pract, 24, 3, 280-284.

Alanazi A.S., (2013), The role of nutraceuticals in the management of autism, Saudi Pharmaceutical Journal, 21, 233-243.

Alberti A., Pirrone P., Elia M., Waring R.H., Romano C., (1999), Sulphation deficit in "low-functioning" autistic children: a pilot study, Biological Psychiatry, 46, 420- 424.

Albuquerque C.A., Smith K.R., Johnson C., Chao R., Harding R., (2004), Influence of maternal tobacco smoking during pregnancy on uterine, umbilical and fetal cerebral artery blood flows, Early Human Development, 80, 31-42.

Alfred C., Sandison A.T., (1963), The Pharaoh Akhenaten: A Problem in Egyptology and Pathology, 36, 293.

Algun E., Erkoc R., Kotan C., Guler N., Sahin I., Ayakta H., Uygan I., Dilek I., Aksoy H., (2001), Polyserositis as a rare component of polyglandular autoimmune syndrome type II, Int J Clin Pract, 55, 4, 280-281.

Alissa E.M., Ferns G.A., (2012), Functional foods and nutraceuticals in the primary prevention of cardiovascular diseases, Journal of Nutrition and Metabolism, 2012, http://dx.doi.org/10.1155/2012/569486.

Allen D.B., (2006), Growth hormone therapy for short stature: is the benefit worth the burden? Pediatrics,118, 343-348.

Allen D.B., Fost N., (2004), hGH for short stature: ethical issues raised by expanded access, Journal of Pediatrics, 144, 648–652.

Almeida M.Q., Stratakis C.A., (2010), Solid tumors associated with multiple endocrine neoplasias, Cancer Genet Cytogenet, 203, 30–36.

Alonso-Magdalena P., Ropero A.B., Soriano S., Quesada I., Nadal A., (2010), Bisphenol-A: a new diabetogenic factor?,Hormones, 9, 118-126.

Alves V.A.F., Kakudo K., LiVolsi V. et al., (2018), Noninvasive Follicular Thyroid Neoplasm With Papillary-Like Nuclear Features (NIFTP): Achieving Better Agreement By Refining Diagnostic Criteria, Clinics, 73; Article Number: UNSP e576

American Psychiatric Association, (2013), Diagnostic and Statistical Manual of Mental Disorders, 5th ed., American Psychiatric Association, Arlington, VA.

Anand B.K., Brobeck J.R., (1951), Localization of a “feeding center” in the hypothalamus of the rat, Proc Soc Exp Biol Med, 77, 323-324.

Andersen H.R., Cook S.J., Waldbillig D., (2002), Effects of currently used pesticides in assays for estrogenicity, androgenicity, and aromatase activity in vitro, Toxicology and Applied Pharmacology, 179, 1-12.

Angelidou A., Alysandratos K.D., Asadi S., Zhang B., Francis K., V asiadi M., Kalogeromitros D., Theoharides T.C., (2011), Brief report: "allergic symptoms" in children with Autism Spectrum Disorders.More than meets the eye?,Journal of Autism and Developmental Disorders, 41, 1579-1585.

Arthur J.R., Beckett G.J., (1999), Thyroid function, Br Med Bull, 55, 3, 658-668.

Ashwell M., Lambert J.P., Alles M.S., Branca F., Bucchini L., Brzozowska A., et al., (2008), EURRECA Network. How we will produce the evidence-based EURRECA toolkit to support nutrition and food policy, Eur J Nutr, 47, (Suppl 1), 2–16.

Ashwood P., Anthony A., Pellicer A.A., Torrente F., Walker-Smith J.A., Wakefield A.J., (2003), Intestinal lymphocyte populations in children with regressive autism: evidence for extensive mucosal immunopathology, Journal of Clinical Immunology, 23, 504-517.

Ashwood P., Krakowiak P., Hertz-Picciotto I., Hansen R., Pessah I., Van De Water J., (2011), Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome, Brain, Behavior, and Immunity, 25, 40-45.

Atladottir H.O., Pedersen M.G., Thorsen P., Mortensen P.B., Deleuran B., Eaton W.W., Parner E.T., (2009), Association of family history of autoimmune diseases and autism spectrum disorders, Pediatrics, 124, 687- 694.

Atladottir H.O., Thorsen P., Ostergaard L., Schendel D.E., Lemcke S., Abdallah M., Parner E.T., (2010), Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders, Journal of Autism and Developmental Disorders, 40, 1423-1430.

Aycan Z., Berberoglu M., Adiyaman P., Ergur A.T., Ensari A., Eyliyaoglu O., Siklar Z., Ocal G., (2004), Latent autoimmune diabetes mellitus in children (LADC) with autoimmune thyroiditis and celiac disease, J Pediatr Endocrinol Metab, 17, 11, 1565-1569.

Baba T., Mimura J., Nakamura N., (2005), Intrinsic function of the aryl hydrocarbon (dioxin) receptor as a key factor in female reproduction, Molecular and Cellular Biology, 25, 10040–10051.

Backhed F., Fraser C.M., Ringel Y., Sanders M.E., Sartor R.B., Sherman P.M., Versalovic J., Young V., Finlay B.B., (2012), Defining a healthy human gut microbiome: current concepts, future directions, and clinical applications, Cell Host Microbe, 12, 611-622.

Badawi A.F., Cavalieri E.L., Rogan E.G., (2000), Effect of chlorinated hydrocarbons on expression of cytochrome P450 1A1, 1A2 and 1B1 and 2- and 4-hydroxylation of 17β-estradiol in female Sprague-Dawley rats, Carcinogenesis, 21, 1593–1599.

Baeke F., Takiishi T., Korf H., Gysemans C., Mathieu C., (2010), Vitamin D: modulator of the immune system, Current Opinion in Pharmacology, 10, 482-496.

Bailey Byron J., (2001), Head and neck surgery otolaryngology, 3rd. Philadelphia-New York: Ed. Lippincott-Raven,1345- 66.

Balázs C., Kaczur V., (2012), Effect of selenium on HLA- DR expression of thyrocytes, Autoimmune Diseases, ID 374635, 2012, http://dx.doi.org/10.1155/2012/374635.

Balázs C., Rácz K., (2013), The role of selenium in endocrine system diseases, Orvosi Hetilap, 154, 1628- 1635.

Barbieri R.L., Sluss P.M., Powers R.D., McShane P.M., Vitonis A., Ginsburg E., Cramer D.C., (2005), Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing in vitro fertilization, Fertile Steril, 83, 302–308.

Baron-Cohen S., Lombardo M.V., Auyeung B., Ashwin E., Chakrabarti B., Knickmeyer R., (2011), Why are autism spectrum conditions more prevalent in males?,PLOS Biology, 9, e1001081.

Bartholomeo Eustachi: Tabulae anatomicae. Rome, 1783.

Bauer A.Z., Kriebel D., (2013), Prenatal and perinatal analgesic exposure and autism: an ecological link, Environmental Health: A Global Access Science Source, 12, 41.

Baumann E., Fromm E., (1895), Ueber die Thioderivate der Ketone, Ber Dtsch Chem Ges, 28, 895-907.

Becerra T.A., Wilhelm M., Olsen J., Cockburn M., Ritz B., (2013), Ambient air pollution and autism in Los Angeles county, California, Environmental Health Perspectives, 121, 380-386.

Beckers A., (2013), Means, motive, and opportunity: SDH mutations are suspects in pituitary tumors.,J Clin Endocrinol Metab, 98, 2274 –2276.

Beckett G.J., Arthur J.R., (2005), Selenium and endocrine systems, Journal of Endocrinology, 184, 455-465. Bem E.M., (1981), Determination of selenium in the environment and in biological material, Environmental Health Perspectives, 37, 183-200.

Belei O., Brad G.F., Marginean O., (2015), An adolescent suspected by IPEX syndrome: immune dysregulation, polyendocrinopathy, enteropathy X-linked, Acta Endocrinol (Buc), 11, 1, 103-108.

Benedini S, Tufano A, Passeri E, Mendola M, Luzi L, Corbetta S., (2015), Autoimmune polyendocrine syndrome 3 onset with severe ketoacidosis in a 74-year-old woman, Case Rep Endocrinol, 960615.

Beneduci A., Filippelli L., Cosentino K., Calabrese M.L., Massa R., Chidichimo G., (2012), Microwave induced shift of the main phase transition in phosphatidylcholine membranes, Bioelectrochemistry, 84, 18-24.

Benn D.E., Gimenez-Roqueplo A.P., Reilly J.R., et al., (2006), Clinical presentation and penetrance of pheochromocytoma/paraganglioma syndromes, J Clin Endocrinol Metab, 91, 827–836.

Benvenuto A., Moavero R., Alessandrelli R., Manzi B., Curatolo P., (2009),

Syndromic autism: causes and pathogenetic pathways, World Journal of Pediatrics, 5, 169-176.

Berg R.D., (1996), The indigenous gastrointestinal microflora, Trends in Microbiology, 4, 430-435.

Bergenfelz A., Jansson S., Kristoffersson A., et al., (2008), Complications to thyroid surgery: results as reported in a database from a multicenter audit comprising 3,660 patients, Langenbecks Archives Of Surgery, 393, 5, 667-673.

Berger G., Trouillas J., Bloch B., et al., (1984), Multihormonal carcinoid tumor of the pancreas, Secreting growth hormone-releasing factor as a cause of acromegaly, Cancer, 54, 2097–2108.

Bernal A., Jirle R.L., (2010), Epigenomic disruption: the effects of early developmental exposures, Birth Defects Research. Part A, Clinical And Molecular Teratology, 88, 938-944.

Bernard C. Lecons de Physiologie experimentale appliquee a la Medecine. 2 vols. Paris: J.B. Bailliere, 1855–1856.

Bhuyan A.K., Sarma D., Saikia U.K., (2012), Selenium and the thyroid: A close-knit connection, Indian Journal of Endocrinology and Metabolism, 95, 5180-5188.

Biver E., Salliot C., Combescure C., Gossec L., Hardouin P., Legroux-Gerot I., Cortet B., (2011), Influence of adipokines and ghrelin on bone mineral densityand fracture risk: a systematic review and meta-analysis, J Clin Endocrinol Metab, 96, 9, 2703-2713.

Blumenthal N., Byth K., Eastman C.J., (2012), Iodine intake and thyroid function in pregnant women in a private clinical practice in Northwestern Sydney before mandatory fortification of bread with iodised salt, Journal of Thyroid Research, article ID 798963, 6 pages.

Bonfig W., Gärtner R., Schmidt H., (2010), Selenium supplementation does not decrease thyroid peroxidase antibody concentration in children and adolescents with autoimmune thyroiditis, Scientific World Journal, 10, 990-996.

Bonthuis M., van Stralen K.J., Verrina E., Edefonti A., Molchanova E.A., Hokken-Koelega A.C., Schaefer F., Jager K.J., (2012), Use of national and international growth charts for studying height in European children: development of up-to-date European height-for-age charts, PLoS One, 7, 8.

Bowie C.R., Harvey P.D., (2006), Administration and interpretation of the trail making test, Nat Protoc, 1, 5, 2277–2281.

Boyles A.L., Billups A.V., Deak K.L., Siegel D.G., Mehltretter L., Slifer S.H., Bassuk A.G., Kessler J.A., Reed M.C., Nijhout H.F., George T.M., Enterline D.S., Gilbert J.R., Speer M.C., Group N.T.D.C., (2006), Neural tube defects and folate pathway genes: family-based association tests of gene-gene and gene- environment interactions, Environmental Health Perspectives, 114, 1547-1552.

Brain P., (1986), Galen on bloodletting: a study of the origins, development, and validity of his opinions, with a translation of the three works, Cambridge University Press 1. ISBN 0-521-32085-2

Brandt F., Thvilum M., Almind D., Christensen K., Green A., Hegedüs L., Brix T.H., (2013), Morbidity before and after the diagnosis of hyperthyroidism: a nation wide register-based study, PLOS ONE, 8, 6, 66711.

Brantsaeter A.L., Abel M.H., Haugen M., Meltzer H.M., (2013), Risk of suboptimal iodine intake in pregnant Norwegian women, Nutrients, 5, 424-440.

Brauer V.F., Scholz G.H., Neumann S., Lohmann T., Paschke R., Koch C.A., (2004), RET germline mutation in codon 791 in a family representing 3 generations from age 5 to age 70 years: should thyroidectomy be performed? Endocr Pract, 10, 5–9.

Bredfeldt T.G., Greathouse K.L., Safe S.H., Hung M.C., Bedford M.T., Walker C.L., (2010), Xenoestrogen induced regulation of EZH2 and histone methylation via estrogen receptor signaling to PI3K/AKT, Molecular Endocrinology, 24, 993–1006.

Brent A.G., (2010), Environmental exposures and autoimmune, Thyroid, 20, 755-760.

Bretveld R., Zielhuis G.A., Roeleveld N., (2006), Time to pregnancy among female greenhouse workers, Scandinavian Journal of Work, Environment & Health, 32, 359–67.

Bridges C.C., Zalups R.K., (2010), Transport of inorganic mercury and methylmercury in target tissues and organs, Journal of Toxicology and Environmental Health.Part B: Critical Reviews, 13, 385-410.

Brownawell A.M., Caers W., Gibson G.R., Kendall C.W., Lewis K.D., Ringel Y., Slavin J.L., (2012), Prebiotics and the health benefits of fiber: current regulatory status, future research, and goals, Journal of Nutrition, 142, 962-974.Brudnak M.A., (2002), Probiotics as an adjuvant to detoxification protocols, Medical Hypotheses, 58, 382-385.

Brucker-Davis F., Ferrari P., Gal J., Berthier F., Fenichel P., Hieronimus S., (2012), Journal of Thyroid Research, article ID 168764, 6 pages.

Brummelman P., Koerts J., Dullaart R.P., et al., (2012), Effects of previous growth hormone excess and current medical treatment for acromegaly on cognition, Eur J Clin Invest, 42, 12, 1317–1324.

Bruni G., Solimene R., Marzocchella A., Salatino P., Yates J.G., Lettieri P., Fiorentino M., (2002), Self- segregation of high-volatile fuel particles during devolatilization in a fluidized bed reactor, Powder Technology, 128, 11-21.

Buchlis J.G., Irizarry L., Crotzer B.C., Shine B.J., Allen L., MacGillivray M.H., (1998), Comparison of final heights of growth hormone-treated vs. untreated children with idiopathic short stature, J Clin Endocrinol M etab, 83, 1075–1079.

Burek C.L., Talor M.V., (2009), Environmental triggers of autoimmune thyroiditis, Journal of Autoimmunity.,33, 183–189.

Burman P., Hetta J., Wide L., Månsson J.E., Ekman R., Karlsson F.A., (1996), Growth hormone treatment affects brain neurotransmitters and thyroxine [see comment], Clin Endocrinol, 44, 3, 319–324.

Burrow G.N., Fisher D.A., Larsen P.R., (1994), Maternal and fetal thyroid function, New England Journal of Medicine, 331, 1072-1078.Buss R.R., Sun W., Oppenheim R.W., (2006), Adaptive roles of programmed cell death during nervous system development, Annual Review of Neuroscience, 29, 1- 35.

Calderón-Garcidueñas L., Serrano-Sierra A., Torres-Jardón R., Zhu H., Yuan Y., Smith D., Delgado-Chávez R., Cross J.V., Medina-Cortina H., Kavanaugh M., Guilarte T.R., (2013), The impact of environmental metals in young urbanites' brains, Experimental and Toxicologic Pathology, 65, 503-511.

Caliman F.A., Gavrilescu M., (2009), Pharmaceuticals, personal care products and endocrine disrupting agents in the environment – a review, CLEAN – Soil, Air, Water, 37,

277–303.

Campbell N.R.C., Dary O., Cappuccio F.P., Neufeld L.,M., Harding K.B., Zimmermann M.B., (2012), Need for coordinated programs to improve global health by optimizing salt and iodine intake, Pan American Journal of Public Health , 32, 281-285.

Cannell J.J., Hollis B.W., Zasloff M., Heaney R.P., (2008), Diagnosis and treatment of vitamin D deficiency, Expert Opinion on Pharmacotherapy, 9, 107-118.

Careaga M., Van De Water J., Ashwood P., (2010), Immune dysfunction in autism: a pathway to treatment, Neurotherapeutics, 7, 283-292.

Carel J.C., Ecosse E., Bastie-Sigeac I., et al., (2005), Quality of life determinants in young women with Turner's syndrome after growth hormone treatment: results of the StaTur population based cohort study, JClin Endocrinol Metab, 90, 1992–1997.

Carere C., Constantini D., Sorace A., Santuci D., Alleva E., (2010), Birds populations as sentinels of endocrine disrupting chemicals, Annali dell'Istituto Superiore di Sanità, 46, 81-88.

Carling T., Udelsman R., (2005), Thyroid tumors. În: DeVita VT Jr, Hellman S, Rosenberg SA, editors. Cancer: principles and practice of oncology, 7th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2005. p. 1502-19.

Carpenter C.C., Solomon N., Silverberg S.G., Bledsoe T., Northcutt R.C., Klinenberg Jr, Bennett Jr., Harvey A.M., (1964), Schmidt's Syndrome (Thyroid And Adrenal Insufficiency). A review of the literature and a report of fifteen new cases including ten instances of coexistent diabetes mellitus, Medicine (Baltimore), 43, 153-180.

Carrasco F., Basfi-Fer K., Rojas P., Valencia A., Csendes A., Codoceo J., Inostroza J., Ruz M., (2014), Changes in bone mineral density after sleeve gastrectomy or gastric by-pass: relationship with variations in vitamin D, ghrelin, and adiponectin levels, ObesSurg, 24, 6, 877-884.

Casida J.E., (2009), Pest toxicology: the primary mechanisms of pesticide action, Chemical Research in Toxicology, 22, 609-619.

CDC, (2012), Prevalence of autism spectrum disorders-- Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008, MMWR: Morbidity and Mortality Weekly Report, Surveillance Summaries, 61, 1-19.

Čeh K., Majdič G., (2010), Pesticides as endocrine disruptors, Slovenian Veterinary Research, 47, 163- 166.

Chahal H.S., Stals K., Unterlander M., et al., (2011), AIP mutation in pituitary adenomas in the 18th century and today, N Engl J Med, 364, 43–50.

Chakrabarti,S.K.,Ghosh,S.,Banerjee,S.,Mukherjee,S.,Chowdhury, S., (2016), Oxidative stress in hypothyroid patients and the role of antioxidant supplementation, Indian J Endocr Metab, 20, 674-678.

Chen J., (2012), An original discovery: selenium deficiency and Keshan disease (an endemic heart disease), Asia Pacific Journal of Clinical Nutrition, 21, 320-326.

Chang S.H., Stoll C.R., Song J., Varela J.E., Eagon C.J., Colditz G.A., (2014), The effectiveness and risks of bariatric surgery: An updated systematic review and meta-analysis, 2003-2012, JAMA Surg, 149, 3, 275-287.

Cheslack-Postava K., Liu K., Bearman P.S., (2011), Closely spaced pregnancies are associated with increased odds of autism in California sibling births, Pediatrics, 127, 246-

253.

Chettiparamb A. interdisciplinarity: a literature review. 2007, The Interdisciplinary Teaching and Learning Group, Subject Centre for Languages, Linguistics and Area Studies, School of Humanities, University of Southampton, SO17 1BJ Nair KM, Dolovich L, Brazil K, Raina P. It's all about relationships: A qualitative study of health researchers' perspectives of conducting interdisciplinary health research. BMC Health Services Research 2008, 8:110

Chiang S.Y., Swenberg J.A., Weisman W.H., Skopek T.R., (1997), Mutagenicity of vinyl chloride and its reactive metabolites, chloroethylene oxide and chloroacetaldehyde, in a metabolically competent human B-lymphoblastoid line, Carcinogenesis, 18, 31- 36.

Chilimba A.D., Young S.D., Black C.R., Rogerson K.B., Ander E.L., Watts M.J., Lammel J., Broadley M.R., (2011), Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi, Scientific Reports, 1, doi: 10.1038/srep00072.

Choudhury P.R., Lahiri S., Rajamma U., (2012), Glutamate mediated signaling in the pathophysiology of autism spectrum disorders, Pharmacology, Biochemistry and Behavior, 100, 841-849.

Christou N., Mathonnet M., (2013), Complications after total thyroidectomy, J Visc Surg, 150, 249–256.

Ciaranello A.L., Ciaranello R.D., (1995), The neurobiology of infantile autism, Annual Review of Neuroscience, 18, 101-128.

Ciuca A., (2009), Conceptul de “demnitate” a fiintei umane in bioetica si drept, Revista Romana de Bioetica, 7, 2, 5-11.

Clifton V.L., Hodyl N.A., Fogarty P.A., Torpy D.J., Roberts R., Nettelbeck T., Ma G., Hetzel B., (2013), The impact of iodine supplementation and bread fortification on urinary iodine concentration in a mildly iodine deficient population of pregnant women in South Australia, Nutritional Journal, 12:32.

Coates P.S., Fernstrom J.D., Fernstrom M.H., Schauer P.R., Greenspan S.L., (2004), Gastric bypass surgery for morbid obesity leads to an increase in bone turnover and a decrease in bone mass, J Clin Endocrinol Metab, 89, 3, 1061-1065.

Colborn T., (2004), Neurodevelopment and endocrine disruption, Environmental Health Perspectives, 112, 944-949.

Cole T.J., (1990), The LMS method for constructing normalized growth standards, Eur J Clin Nutr, 44, 1, 45-60.

Colon I., Caro D., Bourdony C.J., Rosario O., (2000), Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development, Environmental Health Perspectives, 108, 895– 900.

Comi A.M., Zimmerman A.W., Frye V.H., Law P.A., Peeden J.N., (1999), Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism, Journal of Child Neurology, 14, 388-394.

Cooper D.S., Doherty G.M., Haugen B.R., Kloos R.T., Lee S.L., Mandel S.J., et al., (2009), Rvised american thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer, Thyroid, 19, n11, 1167-1214.

Cooper G.S., Baird D.D., Hulka B.S., Weinberg C.R., Savitz D.A., Hughes C.L. Jr., (1995), Follicle- stimulating hormone concentrations in relation to active and passive smoking, Obstetrics & Gynecology, 85, 407–411.

Corsini E., Liesivuori J., Vergieva T., Van Loveren H., Colosio C., (2008), Effects of pesticide exposure on the human immune system, Human and Experimental Toxicology, 27, 671-680.

Cox C., (2001), Imidacloprid insecticide factsheet, Journal of pesticide reform, 21, 15–21.

Cozzolino M., Gallieni M., Corsi C., et al., (2004), Management of calcium refilling post-parathyroidectomy in end-stage renal disease, Journal Of Nephrology, 17, 1, 3-8.

Craig Z.R., Wang W., Flaws J.A., (2011), Endocrine – disrupting chemicals in ovarian function: effects on steroidogenesis, metabolism and nuclear receptor signaling, Reproduction, 142, 633-646.

Crespo I., Webb S.M., (2014), Perception of health and cognitive dysfunction in acromegaly patients, Endocrine, 46, 3, 365–367.

Crinnion W.J., (2009), Chlorinated pesticides: threats to health and importance of detection, Alternative Medicine Review, 14, 347-359.

Critchfield J.W., Van Hemert S., Ash M., Mulder L., Ashwood P., (2011), The potential role of probiotics in the management of childhood autism spectrum disorders, Gastroenterology Research and Practice, 2011, 161358.

Croen L.A., Grether J.K., Yoshida C.K., Odouli R., Van De Water J., (2005), Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study, Archives of Pediatrics and Adolescent Medicine, 159, 151-157.

Cui Q.X., Kong D.G., Li Z.H., Wang K., Zhang D., Tang J.N., Liao X., Yuan Q.Q., Gong Y., Wu G.S., (2019), Parathyroid autotransplantation at a novel site for better evaluation of the grafted gland function: study protocol for a prospective, randomized controlled trial, TRIALS 20, 1, 96.

Cushing H. The Pituitary Body and its Disorders.Clinical Status Produced by Disorders of the Hypophysis Cerebri. J.B. Lippincott, Philadelphia, PA 1912

Cuttler L., (2005), Safety and efficacy of growth hormone treatment for idiopathic short stature, J Clin Endocrinol Metab, 90, 5502-5504 .

D'amelio M., Ricci I., Sacco R., Liu X., D'agruma L., Muscarella L.A., Guarnieri V., Militerni R., Bravaccio C., Elia M., Schneider C., Melmed R., Trillo S., Pascucci T., Puglisi-Allegra S., Reichelt K.L., Macciardi F., Holden J.J., Persico A.M., (2005), Paraoxonase gene variants are associated with autism in North America, but not in Italy: possible regional specificity in gene-environment interactions, Molecular Psychiatry, 10, 1006-1016.

Dackiw A.P., Cote GJ, Fleming JB, et al., (1999), Screening for MEN1 mutations in patients with atypical endocrine neoplasia, Surgery, 126, 1097–1103.

Dai G., Levy O., Carrasco N., (1996), Cloning and characterization of the thyroid iodide transporter, Nature, 379, 458–460.

Daly A.F., Rixhon M., Adam C., Dempegioti A., Tichomirowa M.A., Beckers A., (2006), High prevalence of pituitary adenomas: a cross-sectional study in the province of Liege, Belgium. J Clin Endocrinol Metab, 91, 4769 – 4775.

Dănulescu R., Goiceanu C., Dănulescu E., Reaboiu K., Bălaceanu G., Borza V., (2010), Nervous system and neuroendocrine effects in long term occupational exposure to microwaves, Environmental Engineering and Management Journal, 10, 481-489.

DeFariasC.R.,CardosoB.R.,DeOliveiraG.M.B.,De MelloGuazzelliI.C.,CatarinoR.M.,Chammas,M.C., Cozzolino S.M.F., KnobelM.J., (2015), A randomized- controlled, double-blind study of the impact of selenium supplementation on thyroid autoimmunity and inflamation with foscus on the GPX1 genotypes, EndocrinolInvest,38,1065-1074.

de Filette J., Andreescu C.E., Cools F., Bravenboer B., Velkeniers B., (2019), A Systematic Review and Meta-Analysis of Endocrine-Related Adverse Events Associated with Immune Checkpoint Inhibitors, Horm Metab Res, 51, 3, 145-156.

De Filippo C., Cavalieri D., Di Paola M., Ramazzotti M., Poullet J.B., Massart S., Collini S., Pieraccini G., Lionetti P., (2010), Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa, Proceedings of the National Academy of Sciences of the United States of America, 107, 14691-14696.

De Iuliis G.N., Newey R.J., King B.V., Aitken R.J., (2009), Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro, PlOS One, 4, e6446.

De Lorenzi D.R.S., Catan L.B., Moreira K., Artico G.R., (2009), Assistência à mulher climatérica: novos paradigmas [Assistance to the climacteric woman: new paradigms]. Rev Bras Enferm, Brasília, 62, 2, 287-293.

de Onis M., Onyango A.W., Borghi E., Siyam A., Nishida C., Siekmann J., (2007), Development of a WHO growth reference for school-aged children and adolescents, Bull World Health Organ, 85, 9, 660-667.

De Theije C.G., Wu J., Da Silva S.L., Kamphuis P.J., Garssen J., Korte S.M., Kraneveld A.D., (2011), Pathways underlying the gut-to-brain connection in autism spectrum disorders as future targets for disease management, European Journal of Pharmacology, 668,, S70-80, doi: 10.1016/j.ejphar.2011.07.013.

Dechanet C., Anahory T., Mathieu Daude J.C., Quantin X., Reyftmann L, Hamamah S., Hedon B., Dechaud H., (2011), Effects of cigarette smoking on reproduction, Human Reproduction Update, 17, 76-95.

Deijen J.B., Arwert L.I., Drent M.L., (2011), The GH/IGF-I Axis and cognitive changes across a 4-year period in healthy adults, ISRN Endocrinol, 2011, 1–6.

Delhanty P.J., van der Eerden B.C., van der Velde M., Gauna C., Pols H.A., Jahr H., Chiba H., van der Lely A.J., van Leeuwen J.P, (2006), Ghrelin and unacylated ghrelin stimulate human osteoblast growth via mitogen-activated proteinkinase (MAPK)/phosphoinositide 3-kinase (PI3K) pathways in the absence of GHS-R1a, J Endocrinol, 188, 37-47.

Dematti S., Branz G., Casagranda G., et al., (2013), Pituitary tumors in SDH mutation carriers, Paper presented at: 12th Annual Meeting of European Network for the Study of Adrenal Tumors ENSAT, Abstract Book, P29.

Deng C.Z., Fons M.P., Rosenblatt J., El-Zein R.A., Abdel- Rahman S.Z., Albrecht T., (2006), Nickel potentiates the genotoxic effect of benzo[a]pyrene in Chinese hamster lung V79 cells, Environmental and Molecular Mutagenesis, 47, 150-161.

Denham J.M., Hill I.D., (2013), Celiac disease and autoimmunity: review and controversies, Curr Allergy Asthma Rep, 13, 4, .347-353.

Delange F., Benker G., Charon Ph., Eber O., et al., (1997), Thyroid volume and

urinary iodine in European schoolchildren: standardization of values for assessment of iodine deficiency, Eur J Endocrinol, 136, 180-187.

Derumeaux H., Valeix P., Castetbon K. et al., (2003), Association of selenium with thyroid volume and echostructure in 35-to 60-year-old French adults, Eur J Endocrinol, 148, 3, 309-315.

Deverman B.E., Patterson P.H., (2009), Cytokines and CNS development, Neuron, 64, 61-78.

Devesa J., Almenglo C., Devesa P., (2016), Multiple Effects of Growth Hormone in the Body: Is it Really the Hormone for Growth?,CliniCal MediCine insights: endoCrinology and diabetes, 9, 1-25.

Devine B.J., (1974), Gentamicin therapy, Drug Intell Clin Pharm, 8, 650-655.

Dharmasena A., (2014), Selenium supplementation in thyroid associated ophthalmopathy: an update, International Journal of Ophthalmology, 7, 365-375.

Diamanti-Kandarakis E., Bourguignon J.-P., Giudice L.C., Hauser R., Prins G.S., Soto A.M., Zoeller R.T., Gore A.C., (2009), endocrine-disrupting chemicals. An endocrine society scientific statement, Endocrine Reviews, 30, 293-342.

Dixon S.L., Gaitens J.M., Jacobs D.E., Strauss W., Nagaraja J., Pivetz T., Wilson J.W., Ashley P.J., (2009), Exposure of U.S.children to residential dust lead, 1999-2004: II.The contribution of lead- contaminated dust to children's blood lead levels, Environmental Health Perspectives, 117, 468-474.

Doberentz E., Preuss-Wossner J., Kuchelmeister K., Madea B., (2011), Histological examination of the pituitary glands in cases of fatal hypothermia, Forensic Sci Int, 207, 46–49.

Dolinska B., Zieliński M., Dobrzański Z., Chojnacka K., Opaliński S., Ryszka F., (2012), Influence of incubation condition on hydrolysis efficiency and iodine enrichment in baker's yeast, Biological Trace Element Research, 147, 354–358.

Dominguez-Bello M.G., Costello E.K., Contreras M., Magris M., Hidalgo G., Fierer N., Knight R., (2010), Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns, Proceedings of the National Academy of Sciences of the United States of America, 107, 11971- 11975.

DrutelA.,ArchambeaudF.,CaronP., (2013), Selenium and the thyroid gland: more good news for clinicians, Clin.Endocrinol.,78, 155-164.

Drutel A., Archambeaud F., Caron P., (2013), Selenium and the thyroid gland: more good news for clinicians, Clinical Endocrinology, 78, 155-164.

Duclos A., Peix J.L., Colin C., Kjaimps J.L., Menegaux F., Pattou F. et al., (2012), Influence of experience on performance of individual surgeons in thyroid surgery a prospective cross sectional multicenter study, BMJ, 344, 8041.

Duntas L.H., (2010), Selenium and the thyroid: a close-knit connection, JClinEndocrMetab,95,12,.5180-5188.

Duntas L.H., (2010), Selenium and the Thyroid: A Close-Knit Connection, J Clin Endocrinol Metab, 95, 12, 5180–5188.

Duntas L.H., Mantzou E., Koutras D.A., (2003), Effects of a six month treatment with selenomethionine in patients with autoimmune thyroiditis, European Journal of Endocrinology, 148, 389-393.

Duntas,L.H., (2015), The Role of Iodine and Selenium in Autoimmune Thyroiditis, HormMetabRes,47,10,721-726.

Dwight T., Mann K., Benn D.E., et al., (2013), Familial SDHA mutation as- sociated with pituitary adenoma and pheochromocytoma/paraganglioma, J Clin Endocrinol Metab, 98, E1103–E1108.

Edge S.B., Compton C.C., (2010), The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM, Ann Surg Oncol, 17, 6, 1471.

Edis A.J., (1977), Surgical treatment for thyroid cancer, Surg Clin North Am, 57, 3, 533-42.

EEA, (2012), The impacts of endocrine disrupters on wildlife, people and their environments, EEA Technical report No 2/2012, European Environment Agency, Copenhagen.

Effraimidis G., Wiersinga W.M., (2014), Mechanisms in endocrinology: autoimmune thyroid disease: old and new players, European Journal of Endocrinology, 170, R241-R252.

EFSA, (2009), Scientific Opinion on the substantiation of health claims related to seleniu and protection of DNA, proteins and lipids from oxidative damage (ID 277, 283, 286, 1289, 1290, 1291, 1293, 1751), function of the immune system (ID 278), thyroid function (ID 279, 282, 286, 1289, 1290, 1291, 1293), function of the heart and blood vessels (ID 280), prostate function (ID 284), cognitive function (ID 285) and spermatogenesis (ID 396) pursuant to Article 13(1) of Regulation (EC) No 1924/20061, European Food Safety Authority Journal, 7, doi: 10.2903/j.efsa.2009.1220

Eidelman A.I., Samueloff A., (2002), The pathophysiology of the fetus of the diabetic mother, Seminars in Perinatology, 26, 232-236.

Eiholzer U., (2005), Deaths in children with Prader-Willi syndrome: a contribution to the debate about the safety of growth hormone treatment in children with PWS, Horm Res, 63, 33–39.

Eisenbarth G.S., Gottlieb P.A., (2003), The immunoendocrinopathy syndromes. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, ed. Williams Textbook of Endocrinology. Tenth edition. Philadelphia: Saunders, 1763-1776.

Eisenbarth G.S., Gottlieb P.A., (2004), Autoimmune Polyendocrine Syndromes, N Engl J Med, 350, 20, 2068-2079.

Eisenhofer G., Pacak K., Maher E.R., Young W.F., de Krijger R.R., (2013), Pheochromocytoma, Clin Chem, 59, 466–472.

El Mehdawi A.F., Quinn C.F., Pilon-Smits E.A., (2011), Selenium hyperaccumulators facilitate selenium-tolerant neighbors via phytoenrichment and reduced herbivory, Current Biology, 21, 1440-1449.

El-Ansary A., Shaker G.H., El-Gezeery A.R., Al-Ayadhi L., (2013), The neurotoxic effect of clindamycin - induced gut bacterial imbalance and orally administered propionic acid on DNA damage assessed by the comet assay: protective potency of carnosine and carnitine, Gut Pathogens, 5, 9.

Elder J.H., Shankar M., Shuster J., Theriaque D., Burns S., Sherrill L., (2006), The gluten-free, casein-free diet in autism: results of a preliminary double blind clinical trial, Journal of Autism and Developmental Disorders, 36, 413-420.

Eliasen M., Tolstrup J.S., Nybo Andersen A.M., Gronbaek M., Olsen J., Strandberg-Larsen K., (2010), Prenatal alcohol exposure and autistic spectrum disorders--a population-based prospective study of 80,552 children and their mothers, International Journal of Epidemiology, 39, 1074-1081.

Ellison T.I., Smith M.K., Gilliam A.C., Macdonald P.N., (2008), Inactivation of the vitamin D receptor enhances susceptibility of murine skin to UV-induced tumorigenesis, Journal of Investigative Dermatology, 128, 2508-2517.

Enderle A., (1998), Dwar~sm and gigantism in historical picture postcards, J R Soc Med, 81, 273–278.

Ergur A.T., Ocal G., Berberoglu M., et al., (2010), Celiac disease and autoimmune thyroid disease in children with type 1 diabetes mellitus: clinical and HLA-genotyping results, J Clin Res Pediatr Endocrinol, 2, 4, 151-154.

EspositoD.,RotondiM.,AccardoG.,ValloneG.,ConzoG,.DocimoG.,SelvaggiF.,CappelliC.,Chiovato L.,GiulianoD., Pasquali D., (2017), Selenium, J Endocrinol Invest, 40, 83.

Evans H.M., Long J.A. Characteristic effects upon growth, oestrus, and ovulation induced by the intraperitoneal administration of fresh anterior hypophyseal substance. Proc Natl Acad Sci 1922;8:38–39

Falandysz J., (2013), Review: On published data and methods for selenium in mushrooms, Food Chemistry, 138, 242-250.

FAO/WHO, (2001), Human vitamin and mineral requirements, Report of a joint FAO/WHO expert consultation, Bangkok, Thailand, On line at: http://www.fao.org/3/a-y2809e.pdf.

Farooq U., Xiong G., Irshad R., Yaqoob S., (2010), Pattern of joints involvement in Kashin-Beck disease: a local osteochondropathy in China, Journal of Ayub Medical College Abbottabad, 22, 97-100.

Fasano A., (2006), Systemic autoimmune disorders in celiac disease, Curr Opin Gastroen, 22, 6, 674-679.

Fernandez A., Karavitaki N., Wass J.A., (2010), Prevalence of pituitary ade- nomas: a community-based, cross-sectional study in Banbury (Ox- fordshire, UK), Clin Endocrinol (Oxf), 72, 377–382.

Ferner R.E., Huson S.M., Thomas N., et al., (2007), Guidelines for the diagnosis and management of individuals with neurofibromatosis, J Med Genet, 44, 81–88.

Fetene D.M., Betts K.S., Alati R., (2017), Maternal thyroid dysfunction during pregnancy and behavioural and psychiatric disorders of children: a systematic review, Eur J Endocrinol, 177, 5, R261-R273.

Filer D.L., Kothiya P., Setzer R.W., Judson R.S., Martin M.T., (2017), tcpl: the ToxCast pipeline for high-throughput screening data, Bioinformatics, 33, 4, 618-620.

Filipek P.A., Accardo P.J., Baranek G.T., Cook E.H., Jr., Dawson G., Gordon B., Gravel J.S., Johnson C.P., Kallen R.J., Levy S.E., Minshew N.J., Ozonoff S., Prizant B.M., Rapin I., Rogers S.J., Stone W.L., Teplin S., Tuchman R.F., Volkmar F.R., (1999), The screening and diagnosis of autistic spectrum disorders, Journal of Autism and Developmental Disorders, 29, 439-484.

Filipic M., Hei T.K., (2004), Mutagenicity of cadmium in mammalian cells: implication of oxidative DNA damage, Mutation Research, 546, 81-91.

Finegold S.M., (2011), Desulfovibrio species are potentially important in regressive autism, Medical Hypotheses, 77, 270-274.

Finegold S.M., Dowd S.E., Gontcharova V., Liu C., Henley K.E., Wolcott R.D., Youn E., Summanen P.H., Granpeesheh D., Dixon D., Liu M., Molitoris D.R., Green J.A., 3rd, (2010), Pyrosequencing study of fecal microflora of autistic and control children, Anaerobe, 16, 444-453.

Finkelstein B.S., Imperiate T.F., Speroff T., Marrero U., Radcliffe D.J., Cuttler L., (2002), Effect of growth hormone therapy on height in children with idiopathic short stature, Arch Pediatr Adolesc M ed 156, 230-40.

Fisch A., Pichard E., Prazuck T., Sebbag R., Torres G., Gemez G., Gentilini M., (1993), American Journal of Public Health, 83, 540-545.

Fitzgerald P.A., (2013), Thyroid cancer. In: Papadakis MA, editor; McPhee SJ, editor. , eds. Current Medical Diagnosis & Treatment. 52nd ed.US: McGraw-Hill Companies, 1126–1134.

Flint H.J., Bayer E.A., Rincon M.T., Lamed R., White B.A., (2008), Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis, Nature Reviews: Microbiology, 6, 121-131.

Flohe S.B., Bruggemann J., Herder C., Goebel C., Kolb H., (2002), Enhanced proinflammatory response to endotoxin after priming of macrophages with lead ions, Journal of Leukocyte Biology, 71, 417-424.

Folli F., Sabowitz B.N., Schwesinger W., Fanti P., Guardano- Mendoza R., Muscogiuri G., (2012), Bariatric surgery and bone disease: from clinical perspective to molecular insights, Int J Obes (Lond), 36, 11, 1373-1379.

Fong P., (2011), Thyroid iodide efflux: a team effort?,J Physiol, 24 , 5929–5939.

Forster E., Bock H.H., Herz J., Chai X., Frotscher M., Zhao S., (2010), Emerging topics in Reelin function, European Journal of Neuroscience, 31, 1511-1518.

Franco R., Sanchez-Olea R., Reyes-Reyes E.M., Panayiotidis M.I., (2009), Environmental toxicity, oxidative stress and apoptosis: menage a trois, Mutation Research, 674, 3-22.

Franklin S.L., Geffner M.E., (2009), Growth hormone: The expansion of available products and indications, Endocrinol Metab Clin North Am, 38, 587–611.

Frederiksen M., Vorkamp K., Thomsen M., Knudsen L.E., (2009), Human internal and external exposure to PBDEs--a review of levels and sources, International Journal of Hygiene and Environmental Health, 212, 109-134.

Fredriks A.M., Buuren S.V., Jeurissen S.E.R., Decker F.W., Verloove-Vanhorick S.P., Wit J.M., (2003), Height, weight, body mass index and pubertal development reference values for children of Turkish origin in the Netherlands, Eur J Pediatr, 162, 11, 788-793.

Fried M., Yumuk V., Oppert J.M., Scopinaro N., Torres A., Weiner R., Yashkov Y., Fruhbeck G., (2014), International Federation for Surgery of Obesity and Metabolic Disorders – European Chapter (IFSA-EC); European Association for the Study of Obesity (EASO); European Association for the Study of Obesity. Obesity Management Task Force (EASO OMTF), Interdisciplinary European guidelines on metabolic and bariatric surgery, Obes Surg, 24, 1, 42-55.

Friedrich M.J., (2017), Global Obesity Epidemic Worsening, JAMA, 318, 7, 603

Frost H.M., (1987), Bone ‘mass’ and the ‘mechanostat’: a proposal, Anat Rec, 219, 1–9.

Frye R.E., Huffman L.C., Elliott G.R., (2010), Tetrahydrobiopterin as a novel therapeutic intervention for autism, Neurotherapeutics, 7, 241- 249.

Fukuzawa N.H., Ohsako S., Wu Q., (2004), Testicular cytochrome P450scc and LHR as possible targets of 2,3,7,8- tetrachlorodibenzo-p-dioxin (TCDD) in the mouse, Molecular and Cellular Endocrinology, 221, 87–96.

Fuortes L., Clark M.K., Kirchner H.L., Smith E.M., (1997), Association between female infertility and agricultural work history, American Journal of Industrial Medicine, 31, 445–51.

Gabbianelli R., Falcioni M.L., Cantalamessa F., Nasuti C., (2009), Permethrin induces lymphocyte DNA lesions at both Endo III and Fpg sites and changes in monocyte respiratory burst in rats, Journal of Applied Toxicology, 29, 317-322.

Gadad B.S., Hewitson L., Young K.A., German D.C., (2013), Neuropathology and animal models of autism: genetic and environmental factors, Autism Res Treat, 2013, 731935.

Gagnon C., Schafer A.L., (2018), Bone Health After Bariatric Surgery, JBMR Plus, 2, 3, 121-133.

Galaris D., Evangelou A., (2002), The role of oxidative stress in mechanisms of metal-induced carcinogenesis Critical Reviews in Oncology/Hematology, 42, 93- 103.

Gardener H., Spiegelman D., Buka S.L., (2009), Prenatal risk factors for autism: comprehensive meta-analysis, British Journal of Psychiatry, 195, 7-14.Gavrilescu M., (2005), Fate of pesticides in the environment and its bioremediation, Engineering in Life Sciences, 5, 497–526.

GartnerR.,GasnierB.C.,DietrichJ.W.J., (2002), Selenium supplementation in patients with autoimmune thyroiditis decreases thyroid peroxidase antibodies concentrations,ClinEndocrand Metab, 87, 1687-1691.

Garza C., de Onis M., (2004), Rationale for developing a new international growth reference, Food Nutr Bull, 25, (Suppl 1), S5-S14.

Gavrilescu M., (2005), Fate of pesticides in the environment and its bioremediation, Engineering in Life Sciences, 5, 497-526

Gavrilescu M., Demnerova K., Aamand J., Agathos S., Fava F., (2015), Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation, New Biotechnology,32, 147-156.

Gehrer S., Kern B., Peters T., Christoffel-Courtin C., Peterli R., (2010), Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after laparoscopic RouxY-gastric bypass (LRYGB)-a prospective study, Obes Surg, 20, 447–453.

Gentile I., Zappulo E., Militerni R., Pascotto A., Borgia G., Bravaccio C., (2013), Etiopathogenesis of autism spectrum disorders: fitting the pieces of the puzzle together, Medical Hypotheses, 81, 26-35.

Georgieff M.K., (2006), The effect of maternal diabetes during pregnancy on the neurodevelopment of offspring, Minnesota Medicine, 89, 44-47.

Georgieff M.K., (2008), The role of iron in neurodevelopment: fetal iron deficiency and the developing hippocampus, Biochemical Society Transactions, 36, 1267-1271.

Germak J.A., (1996), Growth hormone therapy in children with short stature: is bigger better or achievable? Indian J Pediatr, 63, 591-597.

Gill A.J., Benn D.E., Chou A., et al., (2010), Immunohistochemistry for SDHB triages genetic testing of SDHB, SDHC, and SDHD in paraganglioma-pheochromocytoma syndromes, Hum Pathol, 41, 805– 814.

Gill A.J., Toon C.W., Clarkson A., et al., (2014), Succinate dehydrogenase deficiency is rare in pituitary adenomas, Am J Surg Pathol, 38, 560 –566.

Gimenez-Roqueplo A.P., Dahia P.L., Robledo M., (2012), An update on the genetics of paraganglioma, pheochromocytoma, and associated hereditary syndromes, Horm Metab Res, 44, 328–333.

Girirajan S., Johnson R.L., Tassone F., Balciuniene J., Katiyar N., Fox K., Baker C., Srikanth A., Yeoh K.H., Khoo S.J., Nauth T.B., Hansen R., Ritchie M., Hertz- Picciotto I., Eichler E.E., Pessah I.N., Selleck S.B., (2013), Global increases in both common and rare copy number load associated with autism, Human Molecular Genetics, 22, 2870-2880.

Goebel C., Flohe S.B., Kirchhoff K., Herder C., Kolb H., (2000), Orally administered lead chloride induces bias of mucosal immunity, Cytokine, 12, 1414-1418.

Goines P., Van De Water J., (2010), The immune system's role in the biology of autism, Current Opinion in Neurology, 23, 111-117.

Goines P.E., Ashwood P., (2013), Cytokine dysregulation in autism spectrum disorders (ASD): possible role of the environment, Neurotoxicology and Teratology, 36, 67-81.

Gonnelli S., Caffarelli C., Del Santo K., Cadirni A., Guerriero C., Lucani B., Franci B., Nuti R., (2008), The relationship of ghrelin and adiponectin with bone mineral density and bone turnover markers in elderly men, Calcif Tissue Int, 83, 55-60.

Gonzalez A., Stombaugh J., Lozupone C., Turnbaugh P.J., Gordon J.I., Knight R., (2011), The mind-body- microbial continuum, Dialogues in Clinical Neuroscience, 13, 55-62.

Gore A.C., (2010), Neuroendocrine targets of endocrine disruptors, Hormones (Athens), 9, 16-27.

Goth S.R., Chu R.A., Gregg J.P., Cherednichenko G., Pessah I.N., (2006), Uncoupling of A TP-mediated calcium signaling and dysregulated interleukin-6 secretion in dendritic cells by nanomolar thimerosal, Environmental Health Perspectives, 114, 1083-1091.

Gottehrer A., Roa J., Stanford G.G., Chemow B., Sahn S.A.(1990), Hypothyroidism and pleural effusions, Chest, 98, 5, 1130-1132.

Gotyo N., Hiyama M., Adachi J., Watanabe T., Hirata Y., (2010), Respiratory failure with myxedema ascites in a patient with idiopathic myxedema, Inter Med, 49, 18, 1991-1996.

Goyer R.A., (1997), Toxic and essential metal interactions, Annual Review of Nutrition, 17, 37-50.

Grabrucker A.M., (2012), Environmental factors in autism, Front Psychiatry, 3, 118.

Grandjean P., (1995), Individual susceptibility in occupational and environmental toxicology, Toxicology Letters, 77, 105-108.

Gregoraszczuk E., (2002), Dioxin exposure and porcine reproductive hormonal activity, Cadernos de Saúde Pública, 18, 453–462

Gu C., Goodarzi M., Yang X., Bian Y., Sun C., Jiang X., (2012), Predictive insight into the relationship between AhR binding property and toxicity of polybrominated diphenyl

ethers by PLS-derived QSAR, Toxicology Letters, 208, 269-274.

Guney M., Ozguner F., Oral B., Karahan N., Mungan T., (2007), 900 MHz radiofrequency-induced histopathologic changes and oxidative stress in rat endometrium: protection by vitamins E and C, Toxicology and Industrial Health, 23, 411-420.

Gunnarsdottir I., Dahl L., (2012), Iodine intake in human nutrition: a systematic literature review, Food & Nutrition Research, 56.

Guo Z., Yu P., Liu Z. et al., (2013), Total thyroidectomy vs bilateral subtotal thyroidectomy in patients with Graves' diseases: a meta-analysis of randomized clinical trials, Clinical Endocrinology, 79, 5, 739-746.

Gupta S., Aggarwal S., Rashanravan B., Lee T., (1998), Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism, Journal of Neuroimmunology, 85, 106-109.

Guyda H.J., (1999), Four decades of growth hormone therapy for short children: what have we achieved? J Clin Endocrinol M etab, 84, 4307–4316.

Hahr J.Y., (2013), Iatrogenic autism, Medical Hypotheses, 81, 251-252.

Hall J.A., Bobe G., Hunter J.K., Vorachek W.R., Stewart W.C., Vanegas J. A., Estill C. T., Mosher W.D., Pirelli G.J., (2013), Effect of feeding selenium-fertilized alfalfa hay on performance of weaned beef calves, PLoS One, 8, e58188, doi:10.1371/journal.pone.0058188.

Hallberg M., Nyberg F., (2012), Growth hormone receptors in the brain and their potential as therapeutic targets in central nervous system disorders, Open Endocrinol J, 6, 1, 27–33.

Hallmayer J., Cleveland S., Torres A., Phillips J., Cohen B., Torigoe T., Miller J., Fedele A., Collins J., Smith K., Lotspeich L., Croen L.A., Ozonoff S., Lajonchere C., Grether J.K., Risch N., (2011), Genetic heritability and shared environmental factors among twin pairs with autism, Archives of General Psychiatry, 68, 1095-1102.

Halpern J.P., Boyages S.C., Maberly G.F. et al., (1991), The neurology of endemic cretinism - a study of 2 endemias, Brain, 114, 2, 825-841.

Harding B., Lemos M.C., Reed A.A., et al., (2009), Multiple endocrine neoplasia type 1 knockout mice develop parathyroid, pancreatic, pituitary and adrenal tumours with hypercalcaemia, hypophosphataemia and hypercorticosteronaemia, Endocr Relat Cancer, 16, 1313–1327.

Hardy J., (1969), Transphenoidal microsurgery of the normal and pathological pituitary, Clin Neurosurg, 16, 185–217.

Harper J., Madan A.K., Ternovits C.A., Tichansky D.S., (2007), What happens to patients who do not follow-up after bariatric surgery? Am Surg, 73, 2, 181-184.

Hatch E.E., Nelson W.J., Stahlhut W.R., Webster F.T., (2010), Association of endocrine disruptors and obesity: perspectives from epidemiologic studies, International Journal of Andrology, 33, 324 – 332.

Haugen B.R., Sawka A.M., Alexander E.K., Bible K.C. et al.,(2017), American Thyroid Association Guidelines on the Management of Thyroid Nodules and Differentiated Thyroid Cancer Task Force Review and Recommendation on the Proposed Renaming of Encapsulated Follicular Variant Papillary Thyroid Carcinoma Without Invasion to Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features, Thyroid, 27, 4, 481-483.

Heflin L.H., Laluz V., Jang J., Ketelle R., Miller B.L., Kramer J.H., (2011), Let's inhibit our excitement: the relationships between Stroop, behavioral disinhibi- tion, and the frontal lobes, Neuropsychology, 25, 5, 655–665.

Heinlein C.A., Chang C., (2002), Androgen Receptor (AR) coregulators: An overview, Endocrine Reviews, 23, 175–200.

Heinlen J.E., Buethe D.D., Culkin D.J., Slobodov G., (2011), Multiple endocrine neoplasia 2a presenting with pheochromocytoma and pituitary macroadenoma, ISRN Oncol, 73, 2452.

Henderson J. A life of Ernest Starling, New York: American Physiological Society by Oxford University Press 2005, ISBN 978-0-19-517780-0

Henderson LJ. Blood. A Study in General Physiology. Yale University Press, New Haven, and Humphrey Milford, Oxford University Press, London, 1928

Henley V.D., Korach S.K., (2010), Physiological effects and mechanisms of action of endocrine disrupting chemicals that alter estrogen signaling, Hormones, 9, 191-205.

Hennessey J.V., (2015), Historical and current perspective in the use of thyroid extracts for the treatment of hypothyroidism, Endocr Pract, 21, 10, 1161-70.

Herbert M.R., Sage C., (2013), Autism and EMF? Plausibility of a pathophysiological link - Part I, Pathophysiology, 20, 191-209.

Herbstman J.B., Sjodin A., Kurzon M., Lederman S.A., Jones R.S., Rauh V., Needham L.L., Tang D., Niedzwiecki M., Wang R.Y., Perera F., (2010), Prenatal exposure to PBDEs and neurodevelopment, Environmental Health Perspectives, 118, 712-719.

Herder W.W., (2009), Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). Pituitary, 12, 236-244.

Herder W.W., (2016), The history of Acromegaly, Neuroendocrinology, 103, 7-17.

Herincs M., Owusu-Antwi S., Chahal H.S., et al., (2013), Prevalence of familial isolated pituitary adenomas, Endocrine Abstracts, 31, 258.

Hermanussen M, Stec K, Aßmann C, Meigen C, Van Buuren S., (2016), Synthetic growth reference charts, Am J Hum Biol, 28, 1, 98- 111.

Hermanussen M., Assmann C., Tutkuviene J., (2010), Statistical agreement and cost-benefit: Comparison of methods for constructing growth reference charts, Ann Hum Biol, 37, 1, 57-69.

Hertz-Picciotto I., Park H.Y., Dostal M., Kocan A., Trnovec T., Sram R., (2008), Prenatal exposures to persistent and non-persistent organic compounds and effects on immune system development, Basic & Clinical Pharmacology & Toxicology, 102, 146-154.

Hetzel B.S., (1983), Iodine deficiency disorders (idd) and their eradication, Lancet, 2, 8359, 1126-1129.

Heusinkveld H.J., Westerink R.H., (2012), Organochlorine insecticides lindane and dieldrin and their binary mixture disturb calcium homeostasis in dopaminergic PC12 cells, Environmental Science & Technology, 46, 1842-1848.

Hlihor R.M., Apostol L.C., Smaranda C., Pavel L.V., Căliman F.A., Robu B.M., Gavrilescu M., (2009), Bioavailability processes for contaminants in soils and their use in risk assessment, Environmental Engineering and Management Journal, 8, 1199-1206.

Holick M.F., (2005), The vitamin D epidemic and its health consequences, Journal of

Nutrition, 135, 2739S- 2748S.

Holmes G., (2001), Celiac disease and type 1 diabetes mellitus – the case for screening, Diabet Med, 18, 3, 169-177.

Horoupian D.S., (1980), Large mitochondria in a pituitary adenoma with hyperprolactinemia, Cancer, 46, 537–542.

Horvath E., Kovacs K., Singer W., et al., (1981), Acidophil stem cell adenoma of the human pituitary: clinicopathologic analysis of 15 cases, Cancer, 47, 761–771.

Housley S.L., Lindsay R.S., Young B., et al., (2010), Renal carcinoma with giant mitochondria associated with germ-line mutation and somatic loss of the succinate dehydrogenase B gene, Histopathology, 56, 405– 408.

Hu G.-H., Lian Q.Q., Ge R.-S., Hardy D.O., Li X.-K. Li, (2009), Phthalate-induced testicular dysgenesis syndrome: Leydig cell influence, Trends in Endocrinology and Metabolism, 20, 139–145.

Hu V.W., (2013), From genes to environment: using integrative genomics to build a "systems-level" understanding of autism spectrum disorders, Child Development, 84, 89-103.

Hu V.W., Nguyen A., Kim K.S., Steinberg M.E., Sarachana T., Scully M.A., Soldin S.J., Luu T., Lee N.H., (2009), Gene expression profiling of lymphoblasts from autistic and nonaffected sib pairs: altered pathways in neuronal development and steroid biosynthesis, PlOS One, 4, e5775.

Hurst R., Siyame E.W., Young S.D., Chilimba A.D., Joy E.J., Black C.R., Ander E.L., Watts M.J., Chilima B., Gondwe J., Kang'ombe D., Stein A.J., Fairweather- Tait S.J., Gibson R.S., Kalimbira A.A., Broadley M.R., (2013), Soil-type influences human selenium status and underlies widespread selenium deficiency risks in Malawi, Scientific Reports, 3, ID1425, doi:10.1038/srep01425.

Hustad S., Midttun O., Schneede J., Vollset S.E., Grotmol T., Ueland P.M., (2007), The methylenetetrahydrofolate reductase 677C-->T polymorphism as a modulator of a B vitamin network with major effects on homocysteine metabolism, American Journal of Human Genetics, 80, 846-855.

Hvidtjorn D., Grove J., Schendel D., Schieve L.A., Svaerke C., Ernst E., Thorsen P., (2011), Risk of autism spectrum disorders in children born after assisted conception: a population-based follow-up study, Journal of Epidemiology and Community Health, 65, 497-502.

Ibrahimpasic T., Ghossein R., Shah J.P., Ganly I., (2019), Poorly Differentiated Carcinoma of the Thyroid Gland: Current Status and Future Prospects, Thyroid, 29, 3, 311-321.

Ilhan A., Gurel A., Armutcu F., Kamisli S., Iraz M., Akyol O., Ozen S., (2004), Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain, Clinica Chimica Acta, 340, 153-162.

Institutul Național de Sănătate Publică Centrul Național de Evaluare și Promovare a Stării de Sănătate. Evaluarea stării de nutriţie a copiilor din ciclul primar prin participarea la proiectul “European Childhood Obesity Surveillance Initiative (COSI)”. Institutul Național de Sănătate Publică, Centrul Național de Evaluare și Promovare a Stării de Sănătate; 2013. Available from: http://insp. gov.ro/sites/cnepss/wp-content/uploads/2014/12/COSI-2013.pdf .

Institutul National de Statistica. Recensamantul Populatiei si al Locuintelor 2011.

[Internet]. 2011 [cited 2015 Nov 23]. Available from: http://www.recensamantromania.ro/noutati/volumul-ii-populatia- stabila-rezidenta-structura-etnica-si-confesionala/.

Iovino M., Messana T., De Pergola G., Iovino E., Dicuonzo F., Guastamacchia E., Giagulli V.A., Triggiani V., (2018), The Role of Neurohypophyseal Hormones Vasopressin and Oxytocin in Neuropsychiatric Disorders. Endocr Metab Immune Disord Drug Targets Volume, 18, 4, 341-347.

Ishikawa T., Miyaishi S., Tachibana T., Ishizu H., Zhu B.L., Maeda H., (2004), Fatal hypothermia related vacuolation of hormone-producing cells in the anterior pituitary, Leg Med (Tokyo), 6, 157–163.

Iversen K., (1952), Acromegaly associated with phaeochromocytoma, Acta Med Scand, 142, 1–5.

Jakovcevski M., Akbarian S., (2012), Epigenetic mechanisms in neurological disease, Nature Medicine, 18, 1194-1204.

James S.J., Melnyk S., Jernigan S., Pavliv O., Trusty T., Lehman S., Seidel L., Gaylor D.W., Cleves M.A., (2010), A functional polymorphism in the reduced folate carrier gene and DNA hypomethylation in mothers of children with autism, American Journal of Medical Genetics.Part B: Neuropsychiatric Genetics, 153B, 1209-1220.

Jamilloux Y., Favier J., Pertuit M., et al., (2014), A MEN1 syndrome with a paraganglioma, Eur J Hum Genet, 22, 283–285.

Jamshidi A., Hunter S., Hazrati S., Harrad S., (2007), Concentrations and chiral signatures of polychlorinated biphenyls in outdoor and indoor air and soil in a major U.K.conurbation, Environmental Science & Technology, 41, 2153-2158.

Jensen M.D., Ryan D.H., Apovian C.M., Ard J.D., Comuzzie A.G., Donato K.A., et al., (2013), American College of Cardiology/American Heart Association Task Force on Practice Guidelines; Obesity Society, AHA/ ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines and The Obesity Society. Circulation 2014; 129(25 Suppl 2):S102- 138.

Jetten M.J., Gaj S., Ruiz-Aracama A., De Kok T.M., Van Delft J.H., Lommen A., Van Someren E.P., Jennen D.G., Claessen S.M., Peijnenburg A.A., Stierum R.H., Kleinjans J.C., (2012), 'Omics analysis of low dose acetaminophen intake demonstrates novel response pathways in humans, Toxicology and Applied Pharmacology, 259, 320-328.

Ježek P., Škarpa T., Lošák J., Hlušek M., Elzner J., Elzner P., (2012), Selenium – An Important Antioxidant in Crops Biofortification, In: Antioxidant Enzyme, Mohammed Amr El-Missiry M.A. (Ed.), InTech, Rijeka, Croatia, 343-368.

Jiang H.Q., Powers H.J., Rossetto G.S., (2019), A systematic review of iodine deficiency among women in the UK, Public Health Nutr, 22, 6, 1138-1147.

Jin P., Huang G., Lin J., Yang L., Xiang B., Zhou W., Zhou Z., (2011), High titre of antiglutamic acid decarboxylase autoantibody is a strong predictor of the development of thyroid autoimmunity in patients with type 1 diabetes and latent autoimmune diabetes in adults, ClinEndocrinol (Oxf), 74, 5, 587-592.

Jinga M., Jurcut C., Vasilescu F., Balaban V.D., Maki M., Popp A., (2014), Celiac gluten sensitivity in an adult woman with autoimmune thyroiditis, Acta Endocrinol (Buc), 10,

1, 128-133.

Johnson C.C., Fordyce F.M., Stewart A.G., (2003), Environmental controls in iodine deficiency disorders-project summary report, Nottingham, UK, British Geological Survey, 20.

Johnson K., Heger N., Boekelheide K., (2012), Of mice and men (and rats): phthalate-induced fetal testis endocrine disruption is species-dependent, Toxicological Sciences, doi: 10.1093/toxsci/kfs206.

Jonklaas J., Danielsen M., Wang H., (2013), A Pilot Study of Serum Selenium, Vitamin D, and Thyrotropin Concentrations in Patients with Thyroid Cancer, Thyroid, 23, 9, 1079–1086.

Joy E.J., Broadley M.R., Young S.D., Black C.R., Chilimba A.D., Ander E.L., Barlow TS., Watts M.J., (2015), Soil type influences crop mineral composition in Malawi, Science of the Total Environment, 505, 587–595.

Juutilainen J., Kumlin T., Naarala J., (2006), Do extremely low frequency magnetic fields enhance the effects of environmental carcinogens? A meta-analysis of experimental studies, International Journal of Radiation Biology, 82, 1-12.

Jyonouchi H., (2010), Autism spectrum disorders and allergy: observation from a pediatric allergy/immunology clinic, Expert Review of Clinical Immunology, 6, 397-411.

Kabata-Pendias, A., Mukherjee, A.B., Trace Elements from Soil to HumanBerlin: Springer, 1, 2007, p.401.

Kahaly G.J., (2009), Polyglandular autoimmune syndromes, Eur J Endocrinol, 161, 11-20.

Kalkbrenner A.E., Daniels J.L., Chen J.C., Poole C., Emch M., Morrissey J., (2010), Perinatal exposure to hazardous air pollutants and autism spectrum disorders at age 8, Epidemiology, 21, 631-641.

Kang J.H., Le Q.A., (2017), Effectiveness of bariatric surgical procedures. A systematic review and network meta-analysis of randomized controlled trials, Medicine Baltimore, 96, 46, e8632.

Kapil U., (2007), Health consequences of iodine deficiency, Sultan Qaboos University Medical Journal, 7, 267-272.

Karavanaki K., Kakleas K.., Paschali E, Kefalas N., Konstantopoulos I., Petrou V., Kanariou M., Karayianni C., (2009), Screening for associated autoimmunity in children and adolescents with type 1 diabetes mellitus, Horm Res, 71, 4, 201-206.

Kassebaum N.J., Arora M., Barber R.M. et al., Group Author(s): GBD 2015 Daly & Hale. Global, regional, and national disability-adjusted life-years (DALYs) for 315 diseases and injuries and healthy life expectancy (HALE), 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 2016;388(10053): 1603-1658

Kazaure H.S., Sosa J.A., (2018), Endocrinology And Metabolism Clinics Of North America, 47, 4, 783.

Keech B., Crowe S., Hocking D.R., (2018), Intranasal oxytocin, social cognition and neurodevelopmental disorders: A meta-analysis, Psychoneuroendocrinology, 87, 9-19.

Kemp S.F., Kuntze J.., Attie KM., (2005), Efficacy and safety results of long-term growth hormone treatment of idiopathic short stature, J Clin Endocrinol Metab, 90, 5247-5253.

Kempuraj D., Asadi S., Zhang B., Manola A., Hogan J., Peterson E., Theoharides T.C., (2010), Mercury induces inflammatory mediator release from human mast cells, Journal of Neuroinflammation, 7, 20.

Kim J., Brethauer S., (2015), On behalf of the ASMBS Clinical Issues Committee, American Society for Metabolic and Bariatric Surgery Clinical Issues Committee, Position Statement. Metabolic bone changes after bariatric surgery, Surg Obes Relat Dis, 11, 2, 406–411.

Kinney D.K., Barch D.H., Chayka B., Napoleon S., Munir K.M., (2010), Environmental risk factors for autism: do they help cause de novo genetic mutations that contribute to the disorder?,Medical Hypotheses, 74, 102-106.

Kisakol G., Kaya A., Gonen S., et al., (2003), Bone and calcium metabolism in subclinical autoimmune hyperthyroidism and hypothyroidism. Endocrine Journal, 50, 6, 657-661.

Klijanienko J., Lagace R., (2011), Particular aspects. In: Klijanienko J, Lagace R, eds. Soft Tissue Tumors: A Multidisciplinary Decisional Diagnostic Approach. Hoboken, NJ: John Wiley Blackwell; 2011:121–411.

Kodavanti P.R., (2005), Neurotoxicity of persistent organic pollutants: possible mode(s) of action and further considerations, Dose-Response: A Publication of International Hormesis Society, 3, 273-305.

Koenig J.E., Spor A., Scalfone N., Fricker A.D., Stombaugh J., Knight R., Angenent L.T., Ley R.E., (2011), Succession of microbial consortia in the developing infant gut microbiome, Proceedings of the National Academy of Sciences of the United States of America, 108, 4578-4585.

Kohrle J., Jakob F., Contempre B., Dumont J.E., (2005), Selenium, the thyroid, and the endocrine system, Endocrine Review, 26, 944-984.

Kong A., Frigge M.L., Masson G., Besenbacher S., Sulem P., Magnusson G., Gudjonsson S.A., Sigurdsson A., Jonasdottir A., Jonasdottir A., Wong W.S., Sigurdsson G., Walters G.B., Steinberg S., Helgason H., Thorleifsson G., Gudbjartsson D.F., Helgason A., Magnusson O.T., Thorsteinsdottir U., Stefansson K., (2012), Rate of de novo mutations and the importance of father's age to disease risk, Nature, 488, 471-475.

Konturek P.C., Brzozowski T., Konturek S.J., (2011), Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options, Journal of Physiology and Pharmacology, 62, 591-599.

Korbonits M., Storr H., Kumar A.V., (2012), Familial pituitary adenomas— who should be tested for AIP mutations? Clin Endocrinol (Oxf), 77, 351–356.

Krakowiak P., Walker C.K., Bremer A.A., Baker A.S., Ozonoff S., Hansen R.L., Hertz-Picciotto I., (2012), Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders, Pediatrics, 129, e1121-1128.

Krassas G.E., Pontikides N., Tziomalos K., Tzotzas T., Zosin A., Vlad M., Luger A., Gessl A., Marculescu R., Toscano V., Morgante S., Papini E., Pirags V., Konrade I., Hybsier S., Hofmann P.J., Schomburg L., Kohrle J., (2014), Selenium status in patients with autoimmune non-autoimmune thyroid diseases from four European countries, Expert Review of Endocrinolology and Metabolism, 9, 685-692.

Krassas,G.E.,Pontikides,N.,Tziomalos,K.,Tzotzas,T.,

Zosin,I.,Vlad,M.,Luger,A.,Gessl,A.,Marculescu,R.,
Toscano,V.,Morgante,S.,Papini,E.,Pirags,V.,Konrade,I.,
Hybsier,S.,Hofmann,PJ.,Schomburg,L.,Kohrle,J., (2014), Selenium status in patients with autoimmune and non-autoimmune thyroid diseases from four Europeabn countries. Expert RevEndocrinolMetab,9,685.

Kreier F, Swaab DF. Handbook of Clinical Neurology, Vol. 95 (3rd series) History of Neurology. Chapter 23: history of neuroendocrinology "the spring of primitive existence". Elsevier BV 2010;95: 335-360

Kroemer G, (2006), Mitochondria in cancer, Oncogene, 25,4630– 4632.

Kuehn B.M., (2007), CDC: autism spectrum disorders common, Journal of the American Medical Association, 297, 940.

Kunzler L.S., Naves L.A., Casulari L.A., (2018), Cognitive-behavioral therapy improves the quality of life of patients with acromegaly, Pituitary, 21, 3, 323–333.

Kupper C., Carpentere L.J., McFiggansf G.B., Palmere C,J., Waite T.J., Boneberg E-M., Woitsch S., Weiller M., Abela R., Grolimund D., Potin P., Butler A., Luther IIIh G.W, Kroneck P.M.H., Meyer-Klaucke W, Feiters M.C., (2008), Iodide accumulation provides kelp with an inorganic antioxidant impacting atmospheric chemistry, Proceedings of the National Academy of Sciences, 105, 6954-6958.

Kurelac I., MacKay A., Lambros M.B., et al., (2013), Somatic complex I disruptive mitochondrial DNA mutations are modifiers of tumorigenesis that correlate with low genomic instability in pituitary adenomas, Hum Mol Genet, 22, 226–238.

Kwon H., Kim J.K., Lim W., Moon B.I., Paik N.S., (2019), Increased risk of postoperative complications after total thyroidectomy with Graves' disease, Head And Neck-Journal For The Sciences And Specialties Of The Head And Neck, 41, 2, 281-285.

La Barre S., Potin P., Leblanc C., Delage L., (2010), The Halogenated metabolism of brown algae (Phaeophyta), its biological importance and its environmental significance, Marine Drugs, 8, 988-1010.

Lai Z, Roos P, Zhai O, et al., (1993), Age-related reduction of human growth hormone-binding sites in the human brain, Brain Res, 621, 2, 260–266.

Lammer C, Weimann E., (2008), Early onset of type 1 diabetes mellitus, Hashimoto's thyroiditis and celiac disease in a 7-yr-old boy with Down's syndrome, Pediatr Diabetes, 9, 423-425.

Lander R.L., Enkhjargal T., Batjargal J., Bailey K.B., Diouf S., Green T.J., Skeaff C.M., Gibson R.S., (2008), Multiple micronutrient deficiencies persist during early childhood in Mongolia, Asia Pacific Journal of Clinical Nutrition, 17, 429-440.

Landini M., Gonzali S., Kiferle C., Tonacchera M., Agretti P., Dimida A., Vitti P., Alpi A., Pinchera A., Perata P., (2012), Metabolic engineering of the iodine content in Arabidopsis, Scientific Reports, 2, 338.

Landis J.D., Hamm N.T., Renshaw C.E., Dade W., Magilligan F.J., Gartner J.D., (2012), Surficial redistribution of fallout 131iodine in a small temperate catchment, Proceedings of the National Academy of Sciences, 109, 4064-4069.

Langer P., Cupisti K., Bartsch D.K., et al., (2002), Adrenal involvement in multiple endocrine neoplasia type 1, World J Surg, 26, 891– 896.

Langeveld W.T., Meijer M., Westerink R.H., (2012), Differential effects of 20 non-

dioxin-like PCBs on basal and depolarization-evoked intracellular calcium levels in PC12 cells, Toxicological Sciences, 126, 487- 496.

Larsson M., Weiss B., Janson S., Sundell J., Bornehag C.G., (2009), Associations between indoor environmental factors and parental-reported autistic spectrum disorders in children 6-8 years of age, Neurotoxicology, 30, 822-831.

Lasalle J.M., (2011), A genomic point-of-view on environmental factors influencing the human brain methylome, Epigenetics, 6, 862-869.

Lasalle J.M., (2013), Autism genes keep turning up chromatin, OA Autism, 1, 14.

Lee M.J., Davis M.M., Clark S.J., Hofer T.P., Kemper A.R., (2006), Estimated cost-effectiveness of growth hormone therapy for idiopatic short stature, Arch Pediatr Adolesc Med, 160, 263-269.

Lee MJ, Appugliese D, Coleman SM, Kaciroti N, Corwyn RF, Bradley RH, Sandberg DE, Lumeng JC. Short stature in a population-based cohort: social, emotional and behavioral functioning. Pediatrics. 2009; 124:903-910.

Lema S.C., Dickey J.T., Schultz I.R., Swanson P., (2008), Dietary exposure to 2,2',4,4'-tetrabromodiphenyl ether (PBDE-47) alters thyroid status and thyroid hormone-regulated gene transcription in the pituitary and brain, Environmental Health Perspectives, 116, 1694-1699.

Leon-Carrion J, Martin-Rodriguez JF, Madrazo-Atutxa A, et al., (2010), Evidence of cognitive and neurophysiological impairment in patients with untreated naive acromegaly, J Clin Endocrinol Metab, 95, 9, 4367–4379.

Leschek E.W., Rose S.R., Yanovski J.A., et al., (2004), Effect of growth hormone treatment on adult height in peripubertal children with idiopathic short stature: a randomized, double-blind, placebo-controlled trial, J Clin Endocrinol Metab, 89, 3140–3148.

Levitt P., Campbell D.B., (2009), The genetic and neurobiologic compass points toward common signaling dysfunctions in autism spectrum disorders, Journal of Clinical Investigation, 119, 747-754.

Li Q., (2007), New mechanism of organophosphorus pesticide-induced immunotoxicity, Journal of Nippon Medical School, 74, 92-105.

Li S., Bañuelos G.S., Wu L., Shi W., (2014), The changing selenium nutritional status of Chinese residents, Nutrients, 6, 1103-1114.

Li X., Chauhan A., Sheikh A.M., Patil S., Chauhan V., Li X.M., Ji L., Brown T., Malik M., (2009), Elevated immune response in the brain of autistic patients, Journal of Neuroimmunology, 207, 111-116.

Li X., Zou H., Brown W.T., (2012), Genes associated with autism spectrum disorder, Brain Research Bulletin, 88, 543-552.

Libbey J.E., Sweeten T.L., Mcmahon W.M., Fujinami R.S., (2005), Autistic disorder and viral infections, Journal of Neurovirology, 11, 1-10.

Liesenkötter K.P., Kiebler A., Stach B., Willgerodt H., Grüters A., (1997), Small thyroid volumes and normal iodine excretion in Berlin schoolchildren indicate full normalization of iodine supply, Exp Clin Endocrinol Diabetes, 105, (suppl.4), 46-50.

Lifshitz F.,(1998), Considerations related to the use of recombinant human growth hormone in children, GGH, 14, 8-10.

Limke T.L., Otero-Montanez J.K., Atchison W.D., (2003), Evidence for interactions

between intracellular calcium stores during methylmercury-induced intracellular calcium dysregulation in rat cerebellar granule neurons, Journal of Pharmacology and Experimental Therapeutics, 304, 949-958.

Lindholm J., (2006), Growth hormone: historical notes, Pituitary, 9, 1, 5-10.

Lindsay R.S., Toft A.D., (1997), Hypothyroidism, Lancet, 349, 413-417.

Lingner S., (2011), Science, interdisciplinarity, and the society, Poiesis Prax, 7, 221–223.

Lintas C., Altieri L., Lombardi F., Sacco R., Persico A.M., (2010), Association of autism with polyomavirus infection in postmortem brains, Journal of Neurovirology, 16, 141-149.

Linz K.W., Von Westphalen C., Streckert J., Hansen V., Meyer R., (1999), Membrane potential and currents of isolated heart muscle cells exposed to pulsed radio frequency fields, Bioelectromagnetics, 20, 497-511.

Livingston R.J., Von Niederhausern A., Jegga A.G., Crawford D.C., Carlson C.S., Rieder M.J., Gowrisankar S., Aronow B.J., Weiss R.B., Nickerson D.A., (2004), Pattern of sequence variation across 213 environmental response genes, Genome Research, 14, 1821-1831.

Loke K.Y., (2005), Making sense of growth hormone therapy for normal short children, Annals Academy of Medecine, 34, 657-659 .

Lombardi F.A., Fiore E., Tonacchera M., Antonangeli L., Rago T., Frigeri M., Provenzale A.M., Montanelli L., Grasso L., Pinchera A., Vitti P., (2013), The Effect of voluntary iodine prophylaxis in a small rural community: the Pescopagano survey 15 years later, Journal of Clinical Endocrinology and Metabolism, 98, 1031-1039.

Lopez-Bellido Garrido F.J., Lopez Bellido L., (2013), Selenium and health; reference values and current status of Spanish population, Nutricion Hospitaliaria, 28, 1396-1406.

Lopez-Jimenez E., de Campos J.M., Kusak E.M., et al., (2008), SDHC mutation in an elderly patient without familial antecedents, Clin Endocrinol (Oxf), 69, 906–910.

Lotter V., (1966), Epidemiology of autistic conditions in young children: Some characteristics of the parents and children, Social Psychiatry, 124–137.

Lovekamp-Swan T., Davis B.J., (2003), Mechanisms of phthalate ester toxicity in the female reproductive system, Environmental Health Perspectives, 111, 139– 145.

Lu Y., Wang N., , Zhu L. , Wang G. ,Wu H., Kuang L. , Zhu W., (2005), Investigation of iodine concentration in salt, water and soil along the coast of Zhejiang, China, Journal of Zhejiang University Science B,6, 1200-1205.

Lugli F., Leone E., Iacovazzo D., et al., (2012) FMTC and prolactinoma: a casual association or a new genetic syndrome? Paper presented at: 13th International Workshop on Multiple Endocrine Neoplasia Final Program and Abstract Book, 77, 43.

Lukas J., Drabek J., Lukas D., Dusek L., Gatek J., (2012), The epidemiology of thyroid cancer in the Czech Republic in comparison with other countries, BiomedicalPapers of the Medical Faculty of the University Palacky Olomouc Czech Republic, http://biomed.papers.upol.cz/.

Lyall K., Schmidt R.J., Hertz-Picciotto I., (2013), The environment in autism spectrum disorders, In: The Neuroscience of Autism Spectrum Disorders, Buxbaum, J.D., Hof, P .R.(Eds.), Academic Press, Elsevier, Oxford, United Kingdom, 203-214.

Maccarinelli G., Sibilia V., Torsello A., Raimondo F., Pitto M., Giustina A., Netti C., Cocchi D., (2005), Ghrelin regulates proliferation and differentiation of osteoblastic cells, J Endocrin, 184, 249- 256.

Maghrabi A.H., Wolski K., Abood B., et al., (2015), Two-year outcomes on bone density and fracture incidence in patients with T2DM randomized to bariatric surgery versus intensive medical therapy, Obesity (Silver Spring), 23, 12, 2344-2348.

Mahaffey K.R., Gartside P.S., Glueck C.J., (1986), Blood lead levels and dietary calcium intake in 1- to 11-year- old children: the Second National Health and Nutrition Examination Survey, 1976 to 1980, Pediatrics, 78, 257-262.

Majumdar S., Friedrich C.A., Koch C.A., Megason G.C,. Fratkin J.D., Moll G.W., (2010), Compound heterozygous mutation with a novel splice donor region DNA sequence variant in the succinate dehydrogenase subunit B gene in malignant paraganglioma, Pediatr Blood Cancer, 54, 473– 475.

Makovey J., Naganathan V., Seibei M., Sambrook P., (2007), Gender differences in plasma ghrelin and its relations to body composition and bone – an opposite-sex twin study, Clin Endocrinol (Oxf), 66, 4, 530-537.

Malaviya M., Husain R., Seth P.K., Husain R., (1993), Perinatal effects of two pyrethroid insecticides on brain neurotransmitter function in the neonatal rat, Veterinary and Human Toxicology, 35, 119-122.

Marie P., (1886), Sur deux cas d'acromégalie; hypertrophie singulière non congénitale des extrémités supérieures, inférieures et céphalique, Rev Med Liège, 6, 297-333.

Mariussen E., Fonnum F., (2006), Neurochemical targets and behavioral effects of organohalogen compounds: an update, Critical Reviews in Toxicology, 36, 253- 289.

Martin J., (2013), Human dignity: several interpretations with different consequences, Revista Romana De Bioetica, 9, 1, 63-64.

Martín-Rodríguez J.F., Madrazo-Atutxa A., Venegas-Moreno E., et al., (2013), Neurocognitive function in acromegaly after surgical resection of GH-secreting adenoma versus naïve acromegaly, PLoS One, 8, 4, e60041.

Marty M.S., Carney W.E., Rowlands J.C., (2011), Endocrine Disruption: Historical Perspectives and Its Impact on the Future of Toxicology Testing, Toxicological Sciencies 120, 93-108.

Matta M.P., Couture E., Cazals L., Vezzosi D., Bennet A., Caron P., (2008), Impaired quality of life of patients with acromegaly: control of GH/IGF-I excess improves psychological subscale appearance, Eur J Endocrinol, 158, 3, 305–310.

Mazzaglia P.J., (2012), Hereditary pheochromocytoma and paraganglioma, J Surg Oncol, 106, 580–585.

McAllister J.E., Dhurandhar V.N., Keith W.S., Aronne J.L., Barger J., Baskin M., Benca M.R., Biggio J., Boggiano M.M., Eisenmann C.J., Elobeid M., Fontaine R.K., Gluckman P ., Hanlon C.E., Katzmarzyk P., Petrobelli A., Redden T.D., Ruden M.D., Wang C., Waterland A.R., Wright M.S., Allison B.D., (2010), Ten putative contributors to the obesity epidemic, Critical Reviews in Food Science and Nutrition, 49, 868 – 913.

McCally M., (2002), Life Support: The Environment and Human Health, MIT Press.

McKinlay R, Plant J.A., Bell J.N.B., Voulvoulis N., (2008), Calculating human exposure to endocrine disrupting pesticides via agricultural and non-agricultural exposure

routes, Science of the Total Environment, 398, 1-12.

Mccanlies E.C., Fekedulegn D., Mnatsakanova A., Burchfiel C.M., Sanderson W.T., Charles L.E., Hertz- Picciotto I., (2012), Parental occupational exposures and autism spectrum disorder, Journal of Autism and Developmental Disorders, 42, 2323-2334.

Mehdi Y., Hornick J.L., Istasse L., Dufrasne I., (2013), Selenium in the environment, metabolism and involvement in body functions, Molecules, 18, 3292- 3311.

Mehdi,Y.,Hornick,J.L.,Istasse,L.,Dufrasne,I., (2013), Selenium in the enviroment, metabolism and involvement in body functions, Molecules,18, 3, 3292.

Melmed S., (2009), Acromegaly pathogenesis and treatment. J Clin Invest. 2009;119(11):3189–3202.

Mense S.M., Sengupta A., Lan C., Zhou M., Bentsman G., Volsky D.J., Whyatt R.M., Perera F.P., Zhang L., (2006), The common insecticides cyfluthrin and chlorpyrifos alter the expression of a subset of genes with diverse functions in primary human astrocytes, Toxicological Sciences, 93, 125-135.

Messaaoui A., Tenoutasse S., Van der Auwera B., Mélot C., Dorchy H., (2012), Autoimmune thyroid, celiac and Addison's diseases related to HLA-DQ types in young patients with type 1 diabetes in Belgium, Open Journal of Endocrine and Metabolic Diseases, 2, 4, 70-73.

Midtvedt T., (2012), The gut: a triggering place for autism - possibilities and challenges, Microbial Ecology in Health and Disease, 23, doi:10.3402/mehd.v23i0.18982.

Millan M.J., (2013), An epigenetic framework for neurodevelopmental disorders: from pathogenesis to potential therapy, Neuropharmacology, 68, 2-82.

Miller J., Silverstein J., Shuster J,. Driscoll D.J., Wagner M., (2006), Short term effects of growth hormone on sleep abnormalities in Prader-Willi syndrome, J Clin Endocrinol Metab, 91, 413–417.

Milman N., Byg K.E., Hvas A.M., Bergholt T., Eriksen L., (2006), Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women, European Journal of Haematology, 76, 200-205.

Minkowski O., (1887), Über einem Fall von Akromegalie. Berliner Klin Wochensch, 21, 371–4

Mirmiran P., Golzarand M., Serra-Majem L., Azizi F., (2012), Iron, iodine and vitamin A in the Middle East; a systematic review of deficiency and food fortification, Iranian Journal of Public Health, 41, 8-19.

Mishra K.P., (2009), Lead exposure and its impact on immune system: a review, Toxicology in Vitro, 23, 969-972.

Mnif W., Hassine A.I.H, Bouaziz A., Bartegi A., Thomas O., Roig B., (2011), Effect of endocrine disruptor pesticides: A review, International Journal of Environmental Research and Public Health, 8, 2265- 2303.

Mohammadi M., Azizi F., Hedayati M., (2018), Iodine deficiency status in the WHO Eastern Mediterranean Region: a systematic review, Environ Geochem Health, 40, 1, 87-97.

Moley J.F., DeBenedetti M.K., (1999), Patterns of nodal metastases in palpable medullary thyroid carcinoma: recommendations for extent of node dissection, Ann Surg, 229, 6, 880-7.

Moosa A., Shu H., Sarachana T., Hu V.W., (2018), Are endocrine disrupting compounds environmental risk factors for autism spectrum disorder? Horm Behav, 101 Special Issue: SI: 13-21. Nemec J. Witchcraft and Medicine. National Institutes of Health 1974, DHEW Pub. No. (NIH) 76-636.

Mouradian M., Abourizk N., (1983), Diabetes mellitus and thyroid disease, Diab Care, 6, 5, 512-520.

Mpalaris V., Anagnostin P., Anastasilakis A.D., Goulis D.G., Doumas A., Iakovou I., (2016), Serum leptin, adiponectin and ghrelin concentrations in post-menopausal women: Is there an association with bone mineral density? Maturitas, 88, 32-36.

Muller CD, Newlands S, Quinn FB Jr, Ryan MW, (2002), Neck dissection: classification, indications and techniques. Grand Rounds Presentation, UTMB (The University of Texas Medical Branch), Dept. of Otolaryngology January 16, 7-18.

Mumby C., Davis J.R., Trouillas J., Higham C.E., (2014), Phaeochromocytoma and acromegaly: a unifying diagnosis. Endocrinol Diabetes Metab Case Rep, 2014, 140036.

Muñoz Ladrón de Guevara C., Fernández-Serrano M.J., Reyes del Paso G.A., Duschek S., (2018), Executive function impairments in fibromyalgia syndrome: relevance of clinical variables and body mass index, PLoS One, 13, 4, e0196329.

Munz C., Lunemann J.D., Getts M.T., Miller S.D., (2009), Antiviral immune responses: triggers of or triggered by autoimmunity?,Nature Reviews: Immunology, 9, 246-258.

Muraoka Y., Itoh F., (1980), Sex difference of mercuric chloride-induced renal tubular necrosis in rats--from the aspect of sex differences in renal mercury concentration and sulfhydryl levels, Journal of Toxicological Sciences, 5, 203-214.

Mutoh J., Taketoh J., Okamura K., (2006), Fetal pituitary gonadotropin as an initial target of dioxin in its impairment of cholesterol transportation and steroidogenesis in rats, Endocrinology, 147, 927–936.

Nadal A., Ropero A.B., Laribi O., Maillet M., Fuentes E., Soria B., (2000), Nongenomic actions of estrogens and xenoestrogens by binding at a plasma membrane receptor unrelated to estrogen receptor α and estrogen receptor β, Proceedings of the National Academy of Sciences U S A, 97, 11603–11608.

Napoli F., Olivieri G., Russo M.E., Marzocchella A., Salatino P ., (2012), Continuous lactose fermentation by Clostridium acetobutylicum-Assessment of energetics and product yields of the acidogenesis, Enzyme and Microbial Technology, 50, 165-172.

Napoli N., Pedonec C., Pozzilli P., Lauretani F., Bandinelli S., Ferruccif L., Incalzic R.A., (2011), Effect of ghrelin on bone mass density: The InChianti study, Bone, 49, 2, 257–263.

Natelson B.H., (2013), Brain dysfunction as one cause of CFS symptoms including difficulty with attention and concentration, Frontiers in Physiology, 4, 109.

National Institutes of Health Consensus Development Panel, (1991), Bariatric surgery for morbid obesity: health implications for patients, health professionals, and third party payers, Ann Intern Med, 115, 956–961.

Nazemi L., Nazmara S., Eshraghyan M.R., Nasseri S., Djafarian K., Yunesian M., Sereshti H., Moameni A., Shahtaheri S.J., (2012), Selenium status in soil, water and essential

crops of Iran, Journal of Environmental Health Sciency & Engineering, 9, doi:10.1186/1735-2746-9-11.

NCD Risk Factor Collaboration (NCD-RisC). A century of trends in adult human height. Elife. 2016; 5.

Necchi V., Sommi P., Vanoli A., Manca R., Ricci V., Solcia E., (2011), Proteasome particle-rich structures are widely present in human epithelial neoplasms: correlative light, confocal and electron microscopy study, PLoS One, 6, e21317.

Negro R., (2008), Selenium and thyroid autoimmunity, Journal of Biologics, 2, 265-273.

Nesin V., Bowman A.M., Xiao S., Pakhomov A.G., (2012), Cell permeabilization and inhibition of voltage-gated Ca(2+) and Na(+) channel currents by nanosecond pulsed electric field, Bioelectromagnetics, 33, 394- 404.

Neufeld M., Maclaren N., Blizzard R., (1980), Autoimmune polyglandular syndromes, Pediatr Ann, 9, 4, 154-162.

Newbold R.R., (2010), Impact of environmental endocrine disrupting chemicals on the development of obesity, Hormones, 9, 206-217.

Newton K.E., Leonard M., Delves H.T., Bowen-Jones D., (2005), A problem with her lead weight, Annals of Clinical Biochemistry, 42, 145-148.

Nguycn Q.T., Lee E.J., Huang M.G., Park Y.I., Khullar A., Plodkouski R.A., (2015), Diagnosis and Treatment of Patients wilh thyroid Cancer, Am Health Drug Benefits, 8, 1, 30-40.

Niazi A.K.., Kalra S, Irfan A., Islam A., (2011), Thyroidology over the ages, Indian J Endocrinol Metab, 15, 121–126.

Niculescu D.A., Baciu I.F., Capatina C., et al., (2017), Acromegaly treatment in Romania, How close are we to disease control? Endokrynol Pol, 68, 5, 519–523.

NIEHS, (2010), Endocrine Disruptors, On line at: http://www.niehs.nih.gov/health/materials/endocrine_ disruptors_508.pdf.

Nittby H., Grafstrom G., Eberhardt J.L., Malmgren L., Brun A., Persson B.R., Salford L.G., (2008), Radiofrequency and extremely low-frequency electromagnetic field effects on the blood-brain barrier, Electromagnetic Biology and Medicine, 27, 103-126.

Nixon I.J., Ganly I., Shah J.P., (2013) Thyroid cancer: surgery for the primary tumor. Oral Oncol. 2013; 49: 654–658.

Nordio M., Pajalich R., (2013), Combined treatment with Myo-inositol and selenium ensures euthyroidism in subclinical hypothyroidism patients with autoimmune thyroiditis, Journal of Thyroid Research, ID 424163, doi:org/10.1155/2013/424163.

Nyberg F., (2000), Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance, Front Neuroendocrinol, 21, 4, 330–348.

Nyenwe E.A., Dagogo-Jack S., (2009), Iodine deficiency disorders in the iodine-replete environment, American Journal of Medical Sciences., 337, 1-11.

O'roak B.J., Vives L., Girirajan S., Karakoc E., Krumm N., Coe B.P., Levy R., Ko A., Lee C., Smith J.D., Turner E.H., Stanaway I.B., Vernot B., Malig M., Baker C., Reilly B., Akey J.M., Borenstein E., Rieder M.J., Nickerson D.A., Bernier R., Shendure J., Eichler E.E., (2012), Sporadic autism exomes reveal a highly interconnected protein network of de novo

mutations, Nature, 485, 246-250.

Occhi G., Regazzo D., Trivellin G., et al., (2013), A novel mutation in the upstream open reading frame of the CDKN1B gene causes a MEN4 phenotype, PLoS Genet, 9, e1003350.

Oh K.W., Lee W.Y., Rhee E.J., et al., (2005), The relationship between serum resistin, leptin, adiponectin, ghrelin levels and bone mineral density in middle-aged men, Clin Endocrinol (Oxf), 63, 2, 131-138.

Ohsako S., Miyabara Y., Nishimura N., (2001), Maternal exposure to a low dose of 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD) suppressed the development of reproductive organs of male rats: dose-dependent increase of mRNA levels of 5α-reductase type 2 in contrast to decrease of androgen receptor in the pubertal ventral prostate, Toxicological Sciences, 60, 132–143.

Ohshima A., Yamashita H., Noguchi S., Uchino S., Watanabe S., Toda M., et al., (2000), Indications for bilateral modified radical neck dissection in patients with papillary carcinoma of the thyroid, 135, 10, 1194-8; discussion 1199.

Oliveira F.R., Ferreira J.R., Dos Santos C.M., Macedo L.E., De Oliveira R.B., Rodrigues J.A., Do Nascimento J.L., Faro L.R., Diniz D.L., (2006), Estradiol reduces cumulative mercury and associated disturbances in the hypothalamus-pituitary axis of ovariectomized rats, Ecotoxicology and Environmental Safety, 63, 488-493.

Olujimi1 O.O., Fatoki O.S., Odendaal J.P., Okonkwo J.O., (2010), Endocrine disrupting chemicals (phenol and phthalates) in the South African environment: a need for more monitoring, Water SA, 36, 671-682.

Ong K.R., Woodward E.R., Killick P., Lim C., Macdonald F., Maher E.R., (2007), Genotype-phenotype correlations in von Hippel-Lindau disease, Hum Mutat, 28,143–149.

Owens M., Stals K., Ellard S., Vaidya B., (2009), Germline mutations in the CDKN1B gene encoding p27 Kip1 are a rare cause of multiple en- docrine neoplasia type 1, Clin Endocrinol (Oxf), 70, 499 –500.

Ozen S., Darcan S., (2011), Effects of environmental endocrine disruptors on pubertal development, Journal of Clinical Research in Pediatric Endocrinology, 3, 1- 6.

Ozogul B., Akcay M.N., Akcay G., Bulut O.H., (2014), Factors affecting hypocalcaemia following total thyroidectomy: a prospective study, Eurasian J Med, 46, 1, 15–21.

Pandav C.S., (2012), Economic evaluation of iodine deficiency disorder control program in Sikkim: a cost – benefit analysis, Indian Journal of Public Health, 56, 214-222.

Papadakis M., Manios A., Schoretsanitis G., Trompoukis C., (2015), Landmarks in the history of adrenal surgery, Hormones (Athens), 15, 1, 136-41.

Papathomas T.G., Gaal J., Corssmit E.P., et al., (2014), Non-pheochromocytoma (PCC)/paraganglioma (PGL) tumors in patients with succinate dehydrogenase-related PCC-PGL syndromes: a clinicopathological and molecular analysis, Eur J Endocrinol, 170, 1–12.

Parent A.S., Naveau E., Gerard A., Bourguignon J.P., Westbrook L.G., (2011), Early developmental actions of endocrine disruptors on the hypothalamus, hippocampus and cerebral cortex, JournalSeek entry for Journal of Toxicology and Environmental Health Part B: Critical Reviews, 14, 328 – 345.

Park J.H.,Yoon J.H., (2019), Lobectomy in patients with differentiated thyroid cancer: indications and follow-up, Endocr Relat Cancer, 26, 7, R381-R393.

Parker S.K., Schwartz B., Todd J., Pickering L.K., (2004), Thimerosal-containing vaccines and autistic spectrum disorder: a critical review of published original data, Pediatrics, 114, 793-804.

Parker W., (2012), BPA metabolite findings may reveal real estrogenic culprit, On line at: http://www.scienceagogo.com/news/20120904234802 data_trunc_sys.shtml.

Parkman H., Hultberg H., (2002), Occurrence and effects of selenium in the environment- a literature review, report of the IVL Swedish Environmental Research Institute, On line at: http://www3.ivl.se/rapporter/pdf/B1486.pdf.

Parracho H.M., Bingham M.O., Gibson G.R., Mccartney A.L., (2005), Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children, Journal of Medical Microbiology, 54, 987-991.

Patel K., Curtis L.T., (2007), A comprehensive approach to treating autism and attention-deficit hyperactivity disorder: a prepilot study, Journal of Alternative and Complementary Medicine, 13, 1091-1097.

Pathak A., Sinha R.A., Mohan V., Mitra K., Godbole M.M., (2011), Maternal thyroid hormone before the onset of fetal thyroid function regulates reelin and downstream signaling cascade affecting neocortical neuronal migration, Cerebral Cortex, 21, 11-21.

Patisaul B.H., Jefferson W., (2010), The pros and cons of pthyroestrogens, Frontiers in Neuroendocrinology, 31, 400-419.

Pavel V.L., Sobariu D.L., Diaconu M., Stătescu F., Gavrilescu M., (2013), Effects of heavy metals on Lepidium sativum germination and growth, Environmental Engineering and Management Journal, 12, 727-733.

Pearce J.M.,(2000), Harvey William Cushing (1869-1939). J Neurol, 247, 397-398.

Pekar, J., Skolarczyk, J., Malecka-Massalska, T., Skorzynska-Dziduszko A., (2017), Effect of selenium supplementation in thyroid gland diseases, J Elem, 22, 1, 91.

Pereira A.M., (2015), Neuropsychological functioning in acromegaly: towards identification of modifiable factors to improve long-term care after remission, Endocrine, 50, 3, 523–525.

Perez K.M., Patel N.J., Lord J.H., et al., (2017), Executive function in adolescents with Type 1 diabetes: relationship to adherence, glycemic control, and psychosocial outcomes, J Pediatr Psychol, 42, 6, 636–646.

Pessah I.N., Cherednichenko G., Lein P.J., (2010), Minding the calcium store: Ryanodine receptor activation as a convergent mechanism of PCB toxicity, Pharmacol Ther, 125, 260-285.

Pessah I.N., Lein P.J., (2008), Evidence for environmental susceptibility in autism what we need to know about gene x environment interactions, In: Autism: current theories and evidence, Zimmerman, A.W.(Ed.), Humana Press, Towata, NJ, 409–428.

Peterson Myers J., Zoeller E.T., Vom Saal S.F., (2009), A clash of old and new scientific concepts in toxicity, with important implications for public health, Environmental Health Perspectives, 117, 1652 – 1655.

Peyvand A., Cahill F., Wadden D. et al., (2013), Beneficial association of serum ghrelin and peptide YY with bone mineral density in the Newfoundland population, BMC

Endocrine Disorders, 13, 35.

Pirola,I.,Gandossi,E.,Agosti,B.,Delbarba,A.,Cappelli, C., (2016), Selenium, Endokynol Pol, 67, 6, 567.

Pistea I., Rosu C., Martonos I., Ozunu A., (2013), Romanian surface water quality: Tarnava Mare River between Medias and Copsa Mica case study , Environmental Engineering and Management Journal , 12, 283-291.

Pizzorno L., (2016), Bariatric Surgery: Bad to the Bone, Part 2, Integr Med (Encinitas), 15, 2, 35-46.

Posavec M., Timinszky G., Buschbeck M., (2013), Macro domains as metabolite sensors on chromatin, Cellular and Molecular Life Sciences, 70, 1509-1524.

Prader A., Largo R.H., Molinari L., Issler C., (1989), Physical growth of Swiss children from birth to 20 years of age. First Zurich longitudinal study of growth and development, Helv Paediatr Acta Suppl, 52, 1, 125.

Price C.S., Thompson W.W., Goodson B., Weintraub E.S., Croen L.A., Hinrichsen V.L., Marcy M., Robertson A., Eriksen E., Lewis E., Bernal P., Shay D., Davis R.L., Destefano F., (2010), Prenatal and infant exposure to thimerosal from vaccines and immunoglobulins and risk of autism, Pediatrics, 126, 656-664.

Prummel M.F., Strieder T., Wiersinga W.M., (2004), The environment and autoimmune diseases, European Journal of Endocrinology, 150, 605-618.

Psaras T., Honegger J., Gallwitz B., Milian M., (2011), Are there gender-specific differences concerning quality of life in treated acromegalic patients? Exp Clin Endocrinol Diabetes, 119, 5, 300–305.

Qiao L., Zheng L., Cai D., (2007), Study on the di-n-butyl phthalate and di-2-ethylhexyl phthalate level of girl serum related with precocious puberty in Shanghai, Wei Sheng Yan Jiu, 36, 93–5.

Quigley C.A., Gill A.M., Crowe B.J., (2005), Safety of growth hormone treatment in pediatric patients withidiopathic short stature, J Clin Endocrinol Metab, 90, 5188-5196.

Radcliffe B., Payne J.E.., Porteous H, Johnston S.G., (2007), “Failure to thrive” or failure to use the right growth chart? Med J Aust, 186, 12, 660-661.

Ralston N.V.C., Raymond L.J., (2010), Dietary selenium’s protective effects against methylmercury toxicity, Toxicology, 278, 112-123.

Rasalam A.D., Hailey H., Williams J.H., Moore S.J., Turnpenny P.D., Lloyd D.J., Dean J.C., (2005), Characteristics of fetal anticonvulsant syndrome associated autistic disorder, Developmental Medicine and Child Neurology, 47, 551-555.

Rattenberry E., Vialard L., Yeung A., et al., (2013), A comprehensive next generation sequencing-based genetic testing strategy to improve diagnosis of inherited pheochromocytoma and paraganglioma, J Clin Endocrinol Metab, 98, E1248–E1256.

Rauch M., Lynch S.V., (2012), The potential for probiotic manipulation of the gastrointestinal microbiome, Current Opinion in Biotechnology, 23, 192-201.

Razagui I.B., Ghribi I., (2005), Maternal and neonatal scalp hair concentrations of zinc, copper, cadmium, and lead: relationship to some lifestyle factors, Biological Trace Element Research, 106, 1-28.

Rebordosa C., Zelop C.M., Kogevinas M., Sorensen H.T., Olsen J., (2010), Use of acetaminophen during pregnancy and risk of preeclampsia, hypertensive and vascular

disorders: a birth cohort study, Journal of Maternal-Fetal&NeonatalMedicine,23,371-378.

Redlefsen T., Commentz J., Meigen C., Hermanussen M., (2007), Reference values for height, weight and body mass index of German born Turkish children, Anthropol Anz, 65, 3, 263-274.

Reik W., Dean W., (2001), DNA methylation and mammalian epigenetics, Electrophoresis, 22, 2838- 2843.

Report of the Committee on Growth Hormone Use of the Lawson Wilkins Pediatric Endocrine Society (PES), May 1985. Degenerative neurologic disease in patients formerly treated with human growth hormone. J Pediatr 1985;107:10–2.Boguszewski CL. Update on GH therapy in adults. F1000Res. 2017;6:1-13

Revanasiddappa H.D., Kumar T.N., (2001), A facile spectrophotometric method for the determination of selenium, Analytical Sciences, 17, 1309-1312.

Reynolds P., Von Behren J., Gunier R.B., Goldberg D.E., Hertz A., Harnly M.E., (2002), Childhood cancer and agricultural pesticide use, an ecologic study in California, Environmental Health Perspectives, 110, 319-324.

Riesco-Eizaguirre G., Rodríguez I., De la Vieja A., Costamagna E., Carrasco N., Nistal M., Santisteban P., (2009), The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer, Cancer Res, 69, 21, 8317–25.

Riley W.J., Maclaren N.K., Lezotte D.C., Spillar R.P., Rosenbloom A.L., (1981), Thyroid autoimmunity in insulin-dependent diabetes mellitus: the case for routine screening, J Pediatr, 99, 3, 350-354.

Risher J.F., Keith S., (2009), Iodine and inorganic iodides: Human health aspects, WHO- Library.

Roberts A.L., Lyall K., Hart J.E., Laden F., Just A.C., Bobb J.F., Koenen K.C., Ascherio A., Weisskopf M.G., (2013), Perinatal air pollutant exposures and autism spectrum disorder in the children of Nurses' Health Study II participants, Environmental Health Perspectives, 121, 978-984.

Roberts E.M., English P.B., Grether J.K., Windham G.C., Somberg L., Wolff C., (2007), Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the California Central Valley, Environmental Health Perspectives, 115, 1482-1489.

Robins C.J., Marsit J.C., Padbury F.J., Sharma S.S., (2011), Endocrine disruptors, environmental oxygen, epigenetics and pregnancy, Frontiers in Bioscience, 3, 690 – 700.

Robu B.M., Căliman F.A., Beţianu C., Gavrilescu M., (2007), Methods and procedures for environmental risk assessment, Environmental Engineering and Management Journal, 6, 573-592.

Rokita S.E., Adler J.M., McTamney P.M., Watson Jr. J.A., (2010), Efficient use and recycling of the micronutrient iodide in mammals, Biochimie, 92, 1227–1235.

Rooney A.A., Matulka R.A., Luebke R.W., (2003), Developmental atrazine exposure suppresses immune function in male, but not female Sprague-Dawley rats, Toxicological Sciences, 76, 366-375.

Ross J.L., Sandberg D.E., Rose S.R., et al., (2004), Psychological adaptationin children with idiopathic short stature treated with growth hormone or placebo, J Clin

Endocrinol Metab, 89, 4873–4878.

Ross J.L., Sandberg D.E., Rose S.R., Leschek E.W., Baron J., Chipman J., et al., (2004), Psychological adaptation in children with idiopathic short stature treated with growth hormone or placebo, J Clin Endocrinol M etab, 89, 4873-8.

Rossi E.D., Faquin W.C., Baloch Z., Fadda G., Thompson L., Larocca L.M., Pantanowitz L., (2019), Noninvasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFTP): Update and Diagnostic Considerations-a Review, Endocr Pathol, 30, 2, 155-162.

Rossignol D.A., Frye R.E., (2012), A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures, Molecular Psychiatry, 17, 389-401.

Rossignol D.A., Genuis S.J., Frye R.E., (2014), Environmental toxicants and autism spectrum disorders: a systematic review, Translational Psychiatry, 4, e360.

Rowley B., Monestier M., (2005), Mechanisms of heavy metal-induced autoimmunity, Molecular Immunology, 42, 833-838.

Ruiz-Tovar J., Oller I., Priego P., Arroyo A., Calero A., Diez M., Zubiaga L., Calpena R., (2013), Short- and mid-term changes in bone mineral density after laparoscopic sleeve gastrectomy, Obes Surg, 23, 7, 861-866.

Saalu L.C., Osinubi A.A., (2009), Environmental endocrine disruptors of testicular function, African Journal of Endocrinology and Metabolism, 8, 13-23.

Sage C., Carpenter D.O., (2009), Public health implications of wireless technologies, Pathophysiology, 16, 233- 246.

Saito T., Miura D., Taguchi M., Takeshita A., Miyakawa M., Takeuchi Y., (2010), Coincidence of multiple endocrine neoplasia type 2A with acromegaly, Am J Med Sci, 340, 329 –331.

Salford L.G., Nittby H., Persson B.R., (2012), Effects of EMF from wireless communication upon the blood– brain barrier, In: BioInitiative 2012: A Rationale for a Biologically-Based Public Exposure Standard for Electromagnetic Fields (ELF and RF), Sage, C.(Ed.). Sanders A.P., Flood K., Chiang S., Herring A.H., Wolf L., Fry R.C., (2012), Towards prenatal biomonitoring in North Carolina: assessing arsenic, cadmium, mercury, and lead levels in pregnant women, PloS One, 7, e31354.

Salthouse T.A., (2011), What cognitive abilities are involved in trail-making performance? Intelligence, 39, 4, 222–232.

Sandberg D.E., Bukowski W.M., Fung C.M., Noll R.B., (2004), Height and social adjustment: are extremes a cause for concern and action? Pediatrics, 114, 744–750.

Sandberg D.E., Colsman M., (2005), Growth hormone treatment of short stature: status ofthe quality of life rationale, Hormone Research, 63, 275–283.

Sandell E.B., Kolthoff I.M., (1934), Chronometric catalytic method for the determination of micro quantities of iodine, J Am Chem Soc, 56, 6, 1426-1426.

Saranac L., Zivanovic S., Bjelakovic B., Stamenkovic H., Novak M., Kamenov B., (2011), Why is the thyroid so prone to autoimmune disease?,Hormone Ressearch in Paediatrics, 75, 157-165.

Sassolas G., Chayvialle JA, Partensky C, et al., (1983), Acromegaly, clinical

expression of the production of growth hormone releasing factor in pancreatic tumors, Ann Endocrinol (Paris), 44, 347–354.

Schmidt R.J., Hansen R.L., Hartiala J., Allayee H., Schmidt L.C., Tancredi D.J., Tassone F., Hertz- Picciotto I., (2011), Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism, Epidemiology, 22, 476-485.

Schmidt R.J., Tancredi D.J., Ozonoff S., Hansen R.L., Hartiala J., Allayee H., Schmidt L.C., Tassone F., Hertz-Picciotto I., (2012), Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case- control study, American Journal of Clinical Nutrition, 96, 80-89.

Schönbeck Y, van Dommelen P, HiraSing RA, van Buuren S., (2015), Trend in Height of Turkish and Moroccan Children Living in The Netherlands, PLoS One, 10, 5, e0124686.

Schouten J.P., McElgunn C.J., Waaijer R., Zwijnenburg D., Diepvens F., Pals G. , (2002), Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification, Nucleic Acids Res, 30,e57.

Sells C.J., Carpenter R.L., Ray C.G., (1975), Sequelae of central-nervous-system enterovirus infections, New England Journal of Medicine, 293, 1-4.

Senti F., (1974), Soy protein foods in U.S. assistance programs, Journal of the American Oil Chemists' Society, 51, 138–140.

Severinski S., Banac S., Severinski N.S., Ahel V., Cvijovic K., (2009), Epidemiology and clinical characteristics of thyroid dysfunction in children and adolescents with type 1 diabetes, Coll Antropol, 33, 1, 273-279.

Shafer T.J., Meyer D.A., Crofton K.M., (2005), Developmental neurotoxicity of pyrethroid insecticides: critical review and future research needs, Environmental Health Perspectives, 113, 123-136.

Shaltout A.A., Castilho I.N., Welz B., Carasek E., Martens I.B., Martens A., Cozzolino S.M., (2011), Method development and optimization for the determination of selenium in bean and soil samples using hydride generation electrothermal atomic absorption spectrometry, Talanta, 85, 1350-1356.

Shanle K.E., Xu W., (2011), Endocrine disrupting chemicals targeting estrogen receptor signaling: Identification and mechanisms of action, Chemical Research in Toxicology, 24, 6-19.

Sheaves R., (1999), A History of Acromegaly, Pituitary, 2, 7–28

Sheka A.C., Wirth K.M., Ikramuddin S., (2018), Preventing macrovascular events with bariatric surgery, JAMA, 320, 15, 1545-1547.

Shelton J.F., Hertz-Picciotto I., Pessah I.N., (2012), Tipping the balance of autism risk: potential mechanisms linking pesticides and autism, Environmental Health Perspectives, 120, 944-951.

Shetty P.K., Galeffi F., Turner D.A., (2012), Cellular Links between Neuronal Activity and Energy Homeostasis, Frontiers in Pharmacology, 3, 43.

Shore S., Waghorn A.J.W., (2011), Thyroidectomy, Surgery (Oxf), 29, 446–450.

Sievers C, Sämann PG, Pfister H, et al., (2012), Cognitive function in acromeg- aly: description and brain volumetric correlates, Pituitary, 15, 3, 350–357.

Sievers C., Sämann P.G., Dose T., et al., (2009), Macroscopic brain architecture changes and white matter pathology in acromegaly: a clinicoradiological study, Pituitary, 12, 3, 177–185.

Silins I., Hogberg J., (2011), Combined toxic exposures and human health: biomarkers of exposure and effect, International Journal of Environmental Research and Public Health, 8, 629 – 647.

Silva-Pereira L.C., Cardoso P.C., Leite D.S., Bahia M.O., Bastos W.R., Smith M.A., Burbano R.R., (2005), Cytotoxicity and genotoxicity of low doses of mercury chloride and methylmercury chloride on human lymphocytes in vitro, Brazilian Journal of Medical and Biological Research, 38, 901-907.

Sitges-Serra A., Ruiz S., Girvent M. et al., (2010), Outcome of protracted hypoparathyroidism after total thyroidectomy, British Journal Of Surgery, 97, 11, 1687-1695

Sjostrom L., Narbro K., Sjostrom C.D., Karason K., et al., (2007), Swedish Obese Subjects Study. Effects of bariatric surgery on mortality in Swedish obese subjects, N Engl J Med, 357, 741–752.

Skeaff S.A., Thomson C.D., Wilson N., Parnell W.R, (2012), A comprehensive assessment of urinary iodine concentration and thyroid hormones in New Zealand schoolchildren: a cross-sectional study, Nutrition Journal, 11, 1-4.

Skinner K.M., Manikkam M., Guerrero – Bosagna C., (2010), Epigenetic transgenerational actions of environmental factors in disease etiology, Trends in Endocrinology & Metabolism, 21, 214-222.

Smith PE. The disabilities caused by hypophysectomy and their repair. J Am Med Ass 1927;88:158–161

Soderlund D.M., (2012), Molecular mechanisms of pyrethroid insecticide neurotoxicity: recent advances, Archives of Toxicology, 86, 165-181.

Solomon G.M., Schettler T., (2000), Environment and health: 6. Endocrine disruption and potential human health implications, Canadian Medical Association Journal, 63, 1471-1476.

Somosy Z., Thuroczy G., Kovacs J., (1993), Effects of modulated and continuous microwave irradiation on pyroantimonate precipitable calcium content in junctional complex of mouse small intestine, Scanning Microscopy, 7, 1255-1261.

Speranza A., (2010), Into the world of steroids. A biochemical “keep in touch” in plants and animals, Plant Signaling & Behaviour, 5, 940 – 943.

Srigley J.R., Delahunt B., Eble J.N., et al., (2013), The International Society of Urological Pathology (ISUP) Vancouver Classification of Renal Neoplasia, Am J Surg Pathol, 37, 1469–1489.

Srirangalingam U., Khan F., Gunganah K., et al., (2014), SDHB surveillance regime: a single UK institution experience, Endocrine Abstracts, 34, 188.

Stamou M., Streifel K.M., Goines P .E., Lein P .J., (2013), Neuronal connectivity as a convergent target of gene x environment interactions that confer risk for Autism Spectrum Disorders, Neurotoxicology and Teratology, 36, 3-16.

Starling EH. Principles of human physiology (I ed.). London: J. & A. Churchill 1912.

Statisches Bundesamt. Bevölkerung mit Migrationshintergrund - Ergebnisse des Mikrozensus - Fachserie 1 Reihe 2.2. [Internet]. 2014 [cited 2015 Nov 20]. Available from:

https://www. destatis.de/DE/Publikationen/Thematisch/Bevoelkerung/MigrationIntegration/Migrationshintergrund2010220137004. pdf?__blob=publicationFile

Steffenburg S., Gillberg C., Hellgren L., Andersson L., Gillberg I.C., Jakobsson G., Bohman M., (1989), A twin study of autism in Denmark, Finland, Iceland, Norway and Sweden, Journal of Child Psychology and Psychiatry and Allied Disciplines, 30, 405-416.

Stein E.M., Silverberg S.J., (2014), Bone loss after bariatric surgery: causes, consequences and management. Lancet Diabetes Endocrinol, 2, 165-174.

Stojanovic S.S., Arsenijevic N.A., Djukic A., et al., (2018), Adiponectin as a Potential Biomarker of Low Bone Mineral Density in Postmenopausal Women with Metabolic Syndrome, Acta Endo (Buc), 14, 2, 201-207.

Stratakis C.A., (2005), Applications of genomic medicine in endocrinology and post-genomic endocrine research, Hormones (Athens), 4, 1, 38-44.

Stubbs E.G., (1976), Autistic children exhibit undetectable hemagglutination-inhibition antibody titers despite previous rubella vaccination, Journal of Autism and Childhood Schizophrenia, 6, 269-274.

Suchy Y., (2009), Executive functioning: overview, assessment, and research issues for non-neuropsychologists, Ann Behav Med, 37, 2, 106–116.

Sumner JY. Human growth hormone: Current status of availability and usefulness. Pediatrics 1969;44:766–7.

Svechnikov K., Izzo G., Landreh L., Weisser J., Soder O., (2010), Endocrine disruptors and Leydig cell function, Journal of Biomedicine and Biotechnology, ID 684504, 1-10, doi:10.1155/2010/684504.

Swedenborg E., Rüegg J., Mäkelä S., Pongratz I., (2009), Endocrine disruptive chemicals: mechanisms of action and involvement in metabolic disorders, Journal of Molecular Endocrinology, 43, 1-10.

Szcześniak D.M., Jawiarczyk-Przybyłowska A., Matusiak L., et al., (2017), Is there any difference in acromegaly and other chronic disease in quality of life and psychiatric morbidity? Endokrynol Pol, 68, 5, 524–532.

T'sjoen G., Bex M., Maiter D.,Velkeniers B., Abs R., (2007), Health-relatedquality of life in acromegalic subjects: data from AcroBel, the Belgian registry on acromegaly, Eur J Endocrinol, 157, 4, 411–417.

Tadevosyan-Leyfer O., Dowd M., Mankoski R., Winklosky B., Putnam S., Mcgrath L., Tager-Flusberg H., Folstein S.E., (2003), A principal components analysis of the Autism Diagnostic Interview-Revised, Journal of the American Academy of Child and Adolescent Psychiatry, 42, 864-872.

Takser L., Mergler D., Baldwin M., De Grosbois S., Smargiassi A., Lafond J., (2005), Thyroid hormones in pregnancy in relation to environmental exposure to organochlorine compounds and mercury, Environmental Health Perspectives, 113, 1039-1045.

Tan L., Sang Z. N., Shen J., Wu Y.T., Yao Z.X., Zhang J. X., Zhao N., Zhang W.Q., (2013) Selenium supplementation alleviates autoimmune thyroiditis by regulating expression of TH1/TH2 cytokines, Biomedical Environmental Science, 26, 920-925.

Tanriverdi F., Yapislar H., Karaca Z, Unluhizarci K, Suer C, Kelestimur F., (2009), Evaluation of cognitive performance by using P300 auditory event related potentials (ERPs) in patients with growth hormone (GH) defi- ciency and acromegaly, Growth Horm IGF Res,

19, 1, 24–30.

Taylor A.J., Vom Saal S.F., Welshons V.W., Drury B., Rottinghaus G., Hunt A.P., Toutain P.L., Laffont M.C., VanderVoort A.C., (2011), Similarity of bisphenol a pharmacokinetics in rhesus monkeys and mice: relevance for human exposure, Environmental Health Perspectives, 119, 422 – 430.

Theoharides T.C., (1990), Mast cells: the immune gate to the brain, Life Sciences, 46, 607-617.

Theoharides T.C., (2013), Is a subtype of autism an allergy of the brain?,Clinical Therapeutics, 35, 584-591. Theoharides T.C., Angelidou A., Alysandratos K.D.,

Thomas J.M., Lutz S.F., (2001), Soy protein lowers fat and saturated fat in school lunch beef and pork entrees, Journal of the American Dietetic Association, 101, 461–463.

Thomusch O., Machens A., Sekulla C. et al., (2003), The impact of surgical technique on postoperative hypoparathyroidism in bilateral thyroid surgery: A multivariate analysis of 5846 consecutive patients, Surgery, 133,.2, 180-185 .

Tian Y., Green P.G., Stamova B., Hertz-Picciotto I., Pessah I.N., Hansen R., Y ang X., Gregg J.P ., Ashwood P ., Jickling G., Van De Water J., Sharp F.R., (2011), Correlations of gene expression with blood lead levels in children with autism compared to typically developing controls, Neurotoxicity Research, 19, 1-13.

Tiemann U., (2008), In vivo and in vitro effects of the organochlorine pesticides DDT, TCPM, methoxychlor, and lindane on the female reproductive tract of mammals: a review, Reproductive Toxicology, 25, 316–326.

Tiemensma J, Biermasz NR, van der Mast RC, et al., (2010), Increased psychopathology and maladaptive personality traits, but normal cognitive functioning, in patients after long-term cure of acromegaly, J Clin Endocrinol Metab, 95, 12, 365–367.

Tiihonen K., Ouwehand A.C., Rautonen N., (2010), Human intestinal microbiota and healthy ageing, Ageing Research Reviews, 9, 107-116.

Toescu E.C., (2000), Mitochondria and Ca(2+) signaling, Journal of Cellular and Molecular Medicine, 4, 164- 175.

Tonacchera M., Dimida A., De Servi M., Frigeri M., Ferrarini E., De Marco G., Grasso L., Agretti P., Piaggi P., Aghini-Lombardi F., Perata P., Pinchera A., Vitti P., (2013), Iodine fortification of vegetables improves human iodine nutrition: in vivo evidence for a new model of iodine prophylaxis, Journal of Clinical Endocrinology and Metabolism, 98, no. 4 E694-E697.

Tonmukayakul U., Velasco P.R., Tantivess S., Teerawattananon Y., (2012), Lessons drawn from research utilization in the maternal iodine supplementation policy development in Thailand, BMC Public Health, 12, 1-4.

Triggiani V., Tafaro E., Giagulli V.A., Sabbà C., Resta F., Licchelli B., Guastamacchia E., (2009), Role of iodine, selenium and other micronutrients in thyroid function and disorders, Endocr Metab Immune Disord Drug Targets, 9, 3, 277-94.

Trimboli P., Crescenzi A., Castellana M., Giorgino F., Giovanella L., Bongiovanni M., (2019), Italian consensus for the classification and reporting of thyroid cytology: the risk of malignancy between indeterminate lesions at low or high risk, A systematic review and meta-analysis, Endocrine, 63, 3, 430-438.

Tudorancea A., Francois P., Trouillas J., et al., (2012), Von Hippel-Lindau disease

and aggressive GH-PRL pituitary adenoma in a young boy, Ann Endocrinol (Paris), 73, 37–42.

Turker O., Kumanlioglu K., Karapolat I., Dogan I., (2006), Selenium treatment in autoimmune thyroiditis: 9- month follow-up with variable doses, Journal of Endocrinology, 190, 151-156.

TurkerO.,KumanliogluK.,Karapolat I.,DoganI.J., (2006), Selenium treatment in autoimmune thyroiditis: 9-month follow-up with variable doses, Endocrinol, 190, 1, 151-156.

USEPA, (2006), An inventory of sources and environmental releases of dioxin-like compounds in the United States for the years 1987, 1995, and 2000. National Center for Environmental Assessment, EPA/600/P-03/002F, Washington, DC, On line at: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid= 159286.

Valentino M., Rapisarda V., Santarelli L., Bracci M., Scorcelletti M., Di Lorenzo L., Cassano F., Soleo L., (2007), Effect of lead on the levels of some immunoregulatory cytokines in occupationally exposed workers, Human and Experimental Toxicology, 26, 551-556.

Valko M., Morris H., Cronin M.T., (2005), Metals, toxicity and oxidative stress, Current Medicinal Chemistry, 12, 1161-1208.

Valles Y., Gosalbes M.J., De Vries L.E., Abellan J.J., Francino M.P., (2012), Metagenomics and development of the gut microbiota in infants, Clinical Microbiology and Infection, 18, 21-26.

Van Eijsden M., Smits L.J., Van Der Wal M.F., Bonsel G.J., (2008), Association between short interpregnancy intervals and term birth weight: the role of folate depletion, American Journal of Clinical Nutrition, 88, 147-153.

Van Voorhis B.J., Syrop C.H., Hammitt D.G., Dunn M.S., Snyder G.D., (1992), Effects of smoking on ovulation induction for assisted reproductive techniques, Fertility and Sterility, 58, 981–985.

Vancassel S., Durand G., Barthelemy C., Lejeune B., Martineau J., Guilloteau D., Andres C., Chalon S., (2001), Plasma fatty acid levels in autistic children, Prostaglandins, Leukotrienes, and Essential Fatty Acids, 65, 1-7.

Vanderpas J.B., Moreno-Reyes R., (2017), Historical aspects of iodine deficiency control, Minerva Med, 108, 2, 124-135.

Vandevijvere S., (2012), Sodium reduction and the correction of iodine intake in Belgium: policy options, Archives of Public Health, 70, lth.com/content/70/1/10.

Vandevijvere S., Coucke W., Vanderpas J., Trumpff C., Fauvart M., Meulemans A., Marie S., Vincent M-F., Schoos R., Boemer F., Vanwynsberghe T., Phillips E., Eyskens F., Wuyts B., Selimaj V., Van Overmeire B., Kirkpatrick C., Van Oyen H., Moreno-Reyes R., (2012), Neonatal thyroid-stimulating hormone concentrations in Belgium: a useful indicator for detecting mild iodine deficiency ?, Plos One, 7, www.plosone.org

Varo P., Alfthan G., Ekholm P., Aro A., Koivistoinen P., (1988), Selenium intake and serum selenium in Finland: effects of soil fertilization with selenium, American Journal of Clinical Nutrition, 48, 324-329.

Varsavsky M., Sebastian-Ochoa A., Torres Vela E., (2013), Coexistence of a pituitary macroadenoma and multicentric paraganglioma: a strange coincidence, Endocrinol Nutr, 60, 154–156.

Verma S., Hoffmann F.W., Kumar M., Huang Z., Roe K., Nguyen-Wu E., Hashimoto A. S., Hoffmann P. R., (2011), Selenoprotein K knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses, Journal of Immunology, 186, 2127-2137.

Vilarrasa N., de Gordejuela A.G., Gómez-Vaquero C., Pujol J., et al., (2013), Effect of bariatric surgery on bone mineral density: comparison of gastric bypass and sleeve gastrectomy, Obes Surg, 23, 12, 2086-91.

Volk H.E., Lurmann F., Penfold B., Hertz-Picciotto I., Mcconnell R., (2013), Traffic-related air pollution, particulate matter, and autism, JAMA Psychiatry, 70, 71-77.

Volta U., Tovoli F., Caio G., (2011), Clinical and immunological features of celiac disease in patients with type 1 diabetes mellitus, Expert Rev Gastroenterol Hepatol, 5, 4, 479-487.

Voss L.D., (2000), Growth hormone therapy for the short normal child: who needs it and who wants it? Journal of Pediatrics, 136, 103–110.

Voss L.D., (2006), Is short stature a problem? The psychological view, European Journal of Endocrinology, 155, 39-45.

Voutilainen P.E., Multanen M.M., Leppäniemi A.K., Haglund C.H., Haapiainen R.K., Franssila K.O., (2001), Prognosis after lymph node recurrence in papillary thyroid carcinoma depends on age, Thyroid, 11, 10, 953-957. Thyroid. In: American Joint Committee on Cancer.: AJCC Cancer Staging Manual, 6th ed. New York: Springer; 2002. p. 77-87.

Waddington C.H., (1942), The epigenotype, Endeavour, 1, 18-20.

WajnerS.M.,RohenkohlH.C.,SerranoT.,MaiaA.L., (2015), Sodium selenite supplementation does not fully restore oxidative stress-induced deiodinase dysfunction: Implications for the nonthyroidal illness syndrome, Redox Biol, 6, 436-445.

Wakefield A.J., Murch S.H., Anthony A., Linnell J., Casson D.M., Malik M., Berelowitz M., Dhillon A.P., Thomson M.A., Harvey P., Valentine A., Davies S.E., Walker-Smith J.A., (1998), Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children, Lancet, 351, 637- 641.

Waldman M., Nicholson S., Adilov N., Williams J., (2008), Autism prevalence and precipitation rates in California, Oregon, and Washington counties, Archives of Pediatrics and Adolescent Medicine, 162, 1026-1034.

Walker A.W., Ince J., Duncan S.H., Webster L.M., Holtrop G., Ze X., Brown D., Stares M.D., Scott P., Bergerat A., Louis P., Mcintosh F., Johnstone A.M., Lobley G.E., Parkhill J., Flint H.J., (2011), Dominant and diet-responsive groups of bacteria within the human colonic microbiota, ISME Journal, 5, 220-230.

Waly M.I., Hornig M., Trivedi M., Hodgson N., Kini R., Ohta A., Deth R., (2012), Prenatal and Postnatal Epigenetic Programming: Implications for GI, Immune, and Neuronal Function in Autism, Autism Research and Treatment, 2012, 190930.

Wang J., Hallinger D.R., Murr A.S., Buckalew A.R., Simmons S.O., Laws S.C., Stoker T.E., (2018), High-Throughput Screening and Quantitative Chemical Ranking for Sodium-Iodide Symporter Inhibitors in ToxCast Phase I Chemical Library, Environ Sci Technol, 52, 9, 5417-5426

Wang L., Christophersen C.T., Sorich M.J., Gerber J.P., Angley M.T., Conlon M.A., (2011), Low relative abundances of the mucolytic bacterium Akkermansia muciniphila and Bifidobacterium spp.in feces of children with autism, Applied and Environmental

Microbiology, 77, 6718-6721.

Wang L., Christophersen C.T., Sorich M.J., Gerber J.P., Angley M.T., Conlon M.A., (2013), Increased abundance of Sutterella spp.and Ruminococcus torques in feces of children with autism spectrum disorder, Molecular Autism, 4, 42.

Wang X., Li L., Zhu C., Gao J., Qu S., (2018), Alterations of Bone Mineral Density Differs Between Genders in Obese Subjects After Laparoscopic Sleeve Gastrectomy: Bone Morphogenetic Protein 4 May Count, Obes Surg, doi: 10.1007/s11695-018-3298-5.

Wang X., Zhang W., Chen H., Liao N., Wang Z.,Zhang X., Hai C., (2014), High selenium impairs hepatic insulin sensitivity through opposite regulation of ROS, Toxicol. Lett, 224, 1, 16-23.

Waye A., Trudeau L.V., (2011), Neuroendocrine disruption: more than hormones are upset, Journal of Toxicology and Environmental Health, Part B, 14, 270 – 291.

Webb S.M., Badia X., (2007), Quality of life in growth hormone deficiency and acromegaly, Endocrinol Metab Clin North Am, 36, 1, 221–232.

Webb S.M., Badia X., Surinach N.L., (2006), Spanish AcroQol Study Group. Validity and clinical applicability of the acromegaly quality of life questionnaire, AcroQoL: a 6-month prospective study, Eur J Endocrinol, 155, 2, 269–277.

Weeks B.S., Hanna M. S., Cooperstein D., (2012), Dietary selenium and selenoprotein function, Medical Science Monitor, 18, 127-132.

Weetman A.P., Yateman M.E., Ealey P.A., et al., (1990), Thyroid-stimulating antibody-activity between different immunoglobulin-g subclasses, Journal Of Clinical Investigation, 86, 3, 723-727.

Weiss L.A., Langenberg C., Barrett-Connor E., (2006), Ghrelin and bone: is there an association in older adults?: the Rancho Bernardo study, J Bone Miner Res, 21, 5, 752-757.

Wémeau J.L., Proust-Lemoine E., Ryndak A., Vanhove L., (2013), Thyroid autoimmunity and polyglandular endocrine syndromes, Hormones, 12, 1, 39-45.

WHO (World Health Organisation). Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee.Technical Report Series no. 854. World Health Organisation, Geneva, 1995.

Wiersinga, W.M., (2016), Clinical Relevance of Environmental Factors in the Pathogenesis of Autoimmune Thyroid Disease, Endocrinol Metab, 31, 2, 213-222.

Wilhelm S.M., Wang T.S., Ruan D.T. et al., (2016), The American Association of Endocrine Surgeons Guidelines for Definitive Management of Primary Hyperparathyroidism, Jama Surgery, 151, 10, 959-968.

Williams B.L., Hornig M., Buie T., Bauman M.L., Cho Paik M., Wick I., Bennett A., Jabado O., Hirschberg D.L., Lipkin W.I., (2011), Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism and gastrointestinal disturbances, PloS One, 6, e24585.

Williams B.L., Hornig M., Parekh T., Lipkin W.I., (2012), Application of novel PCR-based methods for detection, quantitation, and phylogenetic characterization of Sutterella species in intestinal biopsy samples from children with autism and gastrointestinal disturbances, MBio, 3, doi: 10.1128/mBio.00261-11.

Williams D.T., Dann S., Wheeler M.H., (2003), Phaeochromocytoma - views on

current management, Eur J Surg Oncol, 29, 10, 933-933.

Williams J.G., Higgins J.P., Brayne C.E., (2006), Systematic review of prevalence studies of autism spectrum disorders, Archives of Disease in Childhood, 91, 8-15.

Williams M.K., Rundle A., Holmes D., Reyes M., Hoepner L.A., Barr D.B., Camann D.E., Perera F.P., Whyatt R.M., (2008), Changes in pest infestation levels, self- reported pesticide use, and permethrin exposure during pregnancy after the 2000-2001 U.S.Environmental Protection Agency restriction of organophosphates, Environmental Health Perspectives, 116, 1681-1688.

Willing B.P., Vacharaksa A., Croxen M., Thanachayanont T., Finlay B.B., (2011), Altering host resistance to infections through microbial transplantation, PloS One, 6, e26988.

Windham G.C., Zhang L., Gunier R., Croen L.A., Grether J.K., (2006), Autism spectrum disorders in relation to distribution of hazardous air pollutants in the san francisco bay area, Environmental Health Perspectives, 114, 1438-1444.

Winneke G., (2011), Developmental aspects of environmental neurotoxicology: lessons from lead and polychlorinated biphenyls, Journal of the Neurological Sciences, 308, 9-15.

Winther K.H., Watt T., Bjørner J.B., Cramon P., Feldt-Rasmussen U., Gluud C., Gram J., Groenvold M., Hegedüs L., Knudsen N., Rasmussen Å.K., Bonnema S.J., (2014), The chronic autoimmune thyroiditis quality of life selenium trial (CATALYST): study protocol for a randomized controlled trial, Trials, 115, doi: 10.1186/1745-6215-15-115.

Winther,K.H.,Bonnema,S.J.,Cold,F.,Debrabant,B.,Nybo, M.,Cold,S.,Hegedus,L., (2015), Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population, EurJEndocrinol,172,6, 657.

Wit JM, Lyset TM, Rekers-Mombarg, Cutler GB, Crowe B, Beck TJ, et al., (2005), Growth hormone (GH) treatment to final height in children with idiopathic short stature: evidence for a dose-effect, J Pediatr, 146, 45-53.

World Health Organization. Growth reference data for 5-19 years. [Internet]. 2007 [cited 2015 Nov 10]. Available from: http:// www.who.int/growthref/en/ .

Wozniak K., Blasiak J., (2004), Nickel impairs the repair of UV- and MNNG-damaged DNA, Cellular & Molecular Biology Letters, 9, 83-94.

Wu Y., Li X., Chang S., Liu L., Zou S., Hipgrave D.B., (2012), Variable iodine intake persists in the context of universal salt iodization in China, Journal of Nutrition, 142, 1728-1734.

Wuttke W., Jarry H., Seidlovo-Wuttke D., (2010), Definition, classification and mechanism of action of endocrine disrupting chemicals, Hormones, 9, 1- 15

Wygold T., (2002), Psychosocial adaptation to short stature – an indication for growth hormone therapy? Hormone Research, 58 (Suppl 3), 20–23.

Xekouki P., Pacak K., Almeida M., et al., (2012), Succinate dehydrogenase (SDH) D subunit (SDHD) inactivation in a growth-hormone-pro- ducing pituitary tumor: a new association for SDH? J Clin Endo- crinol Metab, 97, E357–E366.

Xekouki P., Stratakis C.A., (2012), Succinate dehydrogenase (SDHx) mutations in pituitary tumors: could this be a new role for mitochondrial complex II and/or Krebs cycle defects? Endocr Relat Cancer, 19, C33–C40.

Xu L.M., Li J.R., Huang Y., Zhao M., Tang X., Wei L., (2012), AutismKB: an

evidence-based knowledgebase of autism genetics, Nucleic Acids Research, 40, D1016-1022.

Yan J., Wang F., Qin H., Chen G., Eviatar N., Fahima T., Cheng J., (2011), Natural variation in grain selenium concentration of wild barley, Hordeum spontaneum, populations from Israel, Biological Trace Element Research, 142, 773-786.

Yanovski S.Z., Yanovski J.A., (2014), Long-term Drug Treatment for Obesity: A Systematic and Clinical Review, JAMA, 311, 1, 74-86.

Yarrington C., Pearce E.N., (2011), Iodine and pregnancy, Journal of Thyroid Research, Article ID 934104, 8.

Yasuda H., Tsutsui T., (2013), Assessment of infantile mineral imbalances in autism spectrum disorders (ASDs), International Journal of Environmental Research and Public Health, 10, 6027-6043.

Yedinak CG, Fleseriu M., (2014), Self-perception of cognitive function among patients with active acromegaly, controlled acromegaly, and nonfunctional pituitary adenoma: a pilot study, Endocrine, 46, 3, 585–593.

Zaman G., Goschin Z., (2010), Multidisciplinaritate, interdisciplinaritate şi transdisciplinaritate: abordări teoretice şi implicaţii pentru strategia dezvoltării durabile postcriză [Multidisciplinarity, interdisciplinarity and transdisciplinarity: theroretical abord ans implications for the strategy of post-crisis sustainable strategy development]. Economie teoretică şi aplicată, 12, 553, 3-20.

Zaim M., Jambulingam P., (2007), Global Insecticide Use for Vector-Borne Disease Control, Docont D. (Ed.), 3rd ed, World Health Organization, Geneva.

Zama A.M., Uzumcu M., (2010), Epigenetic effects of endocrine – disrupting chemicals on female reproduction: An ovarian perspective, Frontiers in Neuroendocrinology, 31, 420 – 439.

Zbranca E., Galusca B., Mogos V., (2008) Tiroida in Ghid de diagnostic si tratament in bolile endocrine, Polirom Ed, Iasi, 125-131.

Zerbo O., Iosif A.M., Delwiche L., Walker C., Hertz- Picciotto I., (2011), Month of conception and risk of autism, Epidemiology, 22, 469-475.

Zewdie T., Smith C.M., Hutcheson M., West C.R., (2010), Basis of the Massachusetts reference dose and drinking water standard for perchlorate, Environmental Health Perspective, 118, 42-48.

Zhan X., Desiderio D.M., (2010), Signaling pathway networks mined from human pituitary adenoma proteomics data, BMC Med Genomics, 3, 13.

Zhang B., Asadi S., Francis K., Toniato E., Kalogeromitros D., (2012), Mast cell activation and autism, Biochimica et Biophysica Acta, 1822, 34-41.

Zhang H., (2014), Advances in research on the mechanisms of Selenium–Mercury interactions and health risk assessment, In: Impacts of Selenium on the biogeochemical cycles of Mercury in terrestrial ecosystems in Mercury mining areas, Zhang H. (Ed), Springer, 17-34.

Zimmermann M.B., (2007), The adverse effects of mild-to- moderate iodine deficiency during pregnancy and childhood: a review, Thyroid, 17, 829-835.

Zoeller R.T., (2010), Environmental chemicals targeting thyroid, Hormones, 9, 28-40.

Zygmunt A., Adamczewski Z., Wojciechowska-Durczynska K., Cyniak-Magierska A., Krawczyk-Rusiecka K., Zygmunt A., Karbownik-Lewinska M., Lewinski A., (2012),

Evaluation of efficacy of iodine prophylaxis in Poland based on the examination of schoolchildren living in Opoczno town (Lodz Voivodship), Thyroid Research, 5, 1-23.

Zygmunt A., Adamczewski Z., Wojciechowska-Durczynska K., Cyniak-Magierska A., Krawczyk-Rusiecka K., Zygmunt A., Karbownik-Lewinska M., Lewinski A., (2012), Evaluation of efficacy of iodine prophylaxis in Poland based on the examination of schoolchildren living in Opoczno town (Lodz Voivodship), Thyroid Research, 5, 1-23.