PEDIATRIC ENDOCRINOLOGY AND DIABETES

2017 UPDATE

Editors:

Iulian P. VELEA
Corina PAUL
Stuart J. BRINK
Ciril KRŽIŠNIK

EDITURA MIRTON

PEDIATRIC
ENDOCRINOLOGY AND DIABETES
2017 UPDATE

Authors: Iulian P. VELEA,
Corina PAUL,
Stuart J. BRINK,
Ciril KRŽIŠNIK

Descrierea CIP a Bibliotecii Naţionale a României
Pediatric endocrinology and diabetes 2017 update /
ed.: Iulian P. Velea, Corina Paul, Stuart J. Brink, Ciril Krzisnik.
- Timişoara : Mirton, 2017

ISBN 978-973-52-1727-3

I. Velea, Iulian (ed.)
II. Paul, Corina (ed.)
III. Brink, Stuart J. (ed.)
IV. Krzisnik, Ciril (ed.)

616

PEDIATRIC ENDOCRINOLOGY AND DIABETES 2017 UPDATE

Authors: Iulian P. VELEA,
Corina PAUL,
Stuart J. BRINK,
Ciril KRŽIŠNIK

Editura Mirton
Timişoara
2017

Authors:

Stuart J. BRINK, MD
Senior Endocrinologist, New England Diabetes and Endocrinology Center (NEDEC), Waltham MA, USA
Associate Clinical Professor of Pediatrics, Tufts University School of Medicine, Boston MA, USA

Ciril KRŽIŠNIK MD, PhD
Pediatrician endocrinologist, senior councillor
Professor emeritus, University of Ljubljana,
Faculty of Medicine, Ljubljana, Slovenia

Corina PAUL MD, PhD
Senior Pediatrician, Specialist Endocrinology, Clinic II Pediatrics, "Pius Branzeu" Clinical County Hospital, Timisoara, Romania
Lecturer, Department of Pediatrics, University of Medicine and Pharmacy "Victor Babeş" Timişoara.

Iulian P. VELEA, MD, PhD
Associate Clinical Professor of Pediatrics at the UMPh "V. Babeş"
Timisoara, Romania
Senior Pediatrician at Clinic II Pediatrics, "Pius Branzeu" Clinical County Hospital Timisoara, Romania

Preface

Endocrine problems encountered in childhood are diverse, presenting a broad spectrum of pathologies that are substantially different from those encountered in the adult and elderly population, confirming that the smallest of the patients are not miniaturized adults.

According to the stated goal of increasing the professional training of doctors, the Romanian Society for Diabetes, Nutrition and Endocrinology also publishes in the "Yearbook of ENDOPED" a few of the lectures presented at the Congress.

We hope that this habit of providing the interested people with a written piece of material that will be able to find it "in need" will become a certainty of the "ENDOPED" manifestations, and will always rise to the expectations of all colleagues.

Iulian Velea

President ENDOPED

Contents

PEDIATRIC AND ADOLESCENT CHRONIC THYROID AUTOIMMUNE DISEASE

Stuart J. Brink

Introduction

Autoimmune thyroid disease (AITD) encompasses the spectrum of disorders including **Hashimoto's thyroiditis** as well as **Graves disease**.

It can be a congenital problem reflecting intrauterine exposure usually from maternal sources and transplacental passage of such processes, but, more commonly, is an acquired problem affecting the thyroid gland and associated with cell-mediated autoimmune effects.[1] The consequences on the thyroid gland can be minimal, sometimes with a small or large goiter, sometimes with cyst or nodule formation while sometimes there is minimal effect on the gland except for positive antibodies present on blood sampling. The effects of such thyroiditis can, however, also be more dramatic with interference with thyroid function itself as well as other autoimmune phenomenon whereby binding in either positive or negative fashion can change thyroid functionality and feedback loops. Thus hyperthyroidism, hypothyroidism or variants are associated with autoimmune thyroid disease.

Families can have one ore another type of AITD and there seems to be a predominantly but not solely autosomal dominant pattern very commonly.

AITD is seen in children and adults although with more frequency in adolescent girls and young adult women than in their

male counterparts. The postpartum period of time has also been shown to be a particularly vulnerable time period especially in women already diagnosed with type 1 diabetes, another autoimmunopathy.

Thyroid dysfunction, then, can include acquired hypothyroidism, complete or compensated forms, hyperthyroidism (Graves Disease) with or without concomitant eye dysfunction, Hashimoto's thyroiditis (symptomatic or asymptomatic with or without thyroid gland enlargement), Hashitoxicosis, a variant which presents with hyperthyroid findings but then "changes" to either a euthyroid state or a hypothyroidism variant thereafter.

More rarely, AITD can be associated with solitary or multiple thyroid nodules, thyroid cysts and thyroid cancers.

Congenital Autoimmune Thyroid Disease

Congenital autoimmune thyroid disease can be hypothyroid or hyperthyroid.

A history of autoimmune thyroid disease in the family and specifically in the mother raises suspicion of the possibility of a transient congenital hypothyroid picture perhaps related to transplacental factor (s) transmission and/or autoantibodies crossing the placenta but having some effect in the fetus or newborn. This may result in goiters as well as abnormal thyroid function. Even more rarely, transplacental passage of stimulatory antibodies or other factors may produce congenital hyperthyroidism with or without a goiter. Generally these are transitory phenomenon but detailed family history, obstetrician and neonatologist/pediatrician awareness of such family histories allows key focusing on obtaining the necessary thyroid hormone tests and decisions about treatment or close surveillance to help decide if this is a permanent or transitory phenomenon, whether supportive or more definitive treatment is needed and for how long.

Transient congenital hypothyroidism occurs in about 5-10% of newborn screening programs and is usually related to babies born small for gestational age or prematurely but this does not usually have much to do with AITD. Most such transient congenital hypothyroidism involves elevated TSH and normal or only minimally low free T4 or total T4 levels. In some studies, autoantibodies and transplacental passage account for about 1-2% of such cases. Endemic iodine deficiency, maternal iodine ingestion, anti-thyroid drug ingestion are other associated explanations and most such

cases resolve spontaneously with only observation and periodic thyroid function testing required.

Acquired Hashimoto's Thyroiditis: Chronic Lymphocytic Autoimmune Thyroid Disease

Fig.1.1. – Dr. Hakaru Hashimoto

Acquired Hashimoto's thyroiditis, chronic lymphocytic autoimmune thyroid inflammation, was not known until **Dr Hakaru Hashimoto**, a Japanese surgeon, first described this in the literature in 1912[2].

His descriptions of chronic inflammatory changes in enlarged thyroid glands were not well accepted at first but have proven to be correct and AITD carries Hashimoto's eponym around the world as a result of his unique observations: goiters with similar diffuse plasma cell infiltrates, lymphocyte infiltration, fibrosis and parenchymal atrophy.

Hashimoto's thyroiditis

Is an organ-specific autoimmune problem that is the most common instigator of thyroid disorders[3].

It is believed that the basic underlying defect in Hashimoto's thyroiditis is an abnormality involving suppressor T- lymphocytes that promotes helper T lymphocytes to interact with specific antigens that are directed again the thyroid tissue itself.

There is a known genetic predisposition associated with HLA antigens (HLA DR3, DR5, B8, for instance) and there is an higher association with other types of autoimmunopathies[4] (for instance, type 1 diabetes mellitus[5], Addison's disease[6], pernicious anemia, hypogonadism, vitiligo[7], alopecia, systemic lupus erythematosis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, celiac disease as well as more commonly seen in Turner Syndrome[8], Noonan syndrome[9], Down Syndrome [10], Klinefelter Syndrome [11] among other specific genetic disorders)[12].

The onset of thyroid disease associated with Hashimoto's thyroiditis is often insidious but often with **positive family history** of thyroid dysfunction as a potential "warning signal".

The thyroid gland itself does not automatically have to be enlarged (goiter) and with increasing awareness in the medical community as well as in families known to have thyroid disease, sometimes there are no or only minimal symptoms; those with very large goiters are not as common as in the past when medical attention is available and affordable.

AITD is considered to be the most common cause of nonendemic goiter and of hypothyroidism in children and teenagers[13].

Prevalence and distribution of autoimmune thyroid disease are presented in *Table 1* with studies all around the world describing more common findings in females than males, more common findings in adults than in adolescents or children and worldwide distribution[14].

Table 1.1.

Hashimoto's Thyroiditis and Graves Disease General Statistics

		ADULTS	**ADULTS**
AUTOIMMUNE HASHIMOTO'S THYROIDITIS	**Incidence**	0.3-1.5 cases/1000/year Peak: 45-65 y/o	Probably lower Peak: early to mid puberty
	Prevalence	8 cases/1000 (46/1000-NHANES III)	0.55-0.8 % (USA,UK)
	F/M	10:1-20:1	4:1-8:1
GRAVES' DISEASE	**Incidence**	21/100,000/year Peak: 40-60 y/o	0.1-3/100,000 Peak :10-15 y/o
	Prevalence	0.5 %	0.02 %
	F/M	5:1-10:1	3:1-6:1

The thyroid gland in Hashimoto's thyroiditis is often but not always diffusely enlarged, symmetric, firm and nontender.

Over time and without any treatment, the gland can enlarge as its ability to produce thyroid hormone is diminished but the enlargement is "driven" by increases in pituitary-derived TSH (thyroid stimulating hormone). Yet the gland cannot make the needed hormone because of the autoimmune attack present, and so the "feedback" loop continues to make more TRH, thyroid releasing hormone, from the hypothalamus, more TSH from the pituitary and around and round the cycle goes making the gland grow but still unable to make the needed hormone. Eventually T4 and T3 levels fall further as TSH levels increase further and more symptoms may

occur. Sometimes, there can be a compensatory effect with some total T4 and free T4 levels rising but only at the expense of the gland being continuously stimulated by TSH (and growing).

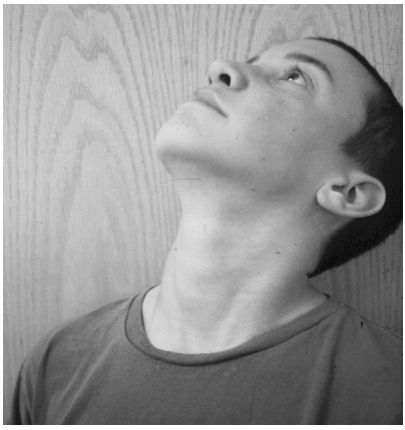
Rarely, is there pain or tenderness associated with such goiters and usually the whole process is a very slow one over months and sometimes over years before coming to medical attention.

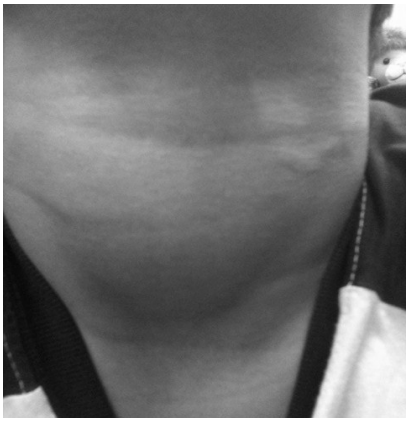
The gland can be smooth or pebbly.

Sometimes cysts or nodules can also be present but this too is highly variable.

Blood testing should include measurement of **total T4** as well as **free T4**, **total T3** and **TSH**. The single most important blood test, however, would likely be a TSH, since this would detect primary hypothyroidism with elevated TSH levels, compensated or partial hypothyroidism with somewhat elevated TSH as well as suppressed TSH levels in hyperthyroid conditions.

If all four thyroid function tests are available, then this presents more ideal information for more subtle differential diagnostic results. As indicated below, when hyperthyroidism is a consideration, either overt hyperthyroid Graves Disease or Hashitoxicosis and its variants, then more sophisticated antibodies including **thyroid receptor, thyroid inhibitory immunoglobulins and thyroid stimulatory immunoglobulin** testing can also provide excellent additional information to help figure out an exact diagnosis and treatment strategy as well as follow for ongoing surveillance, recurrence and compliance issues.





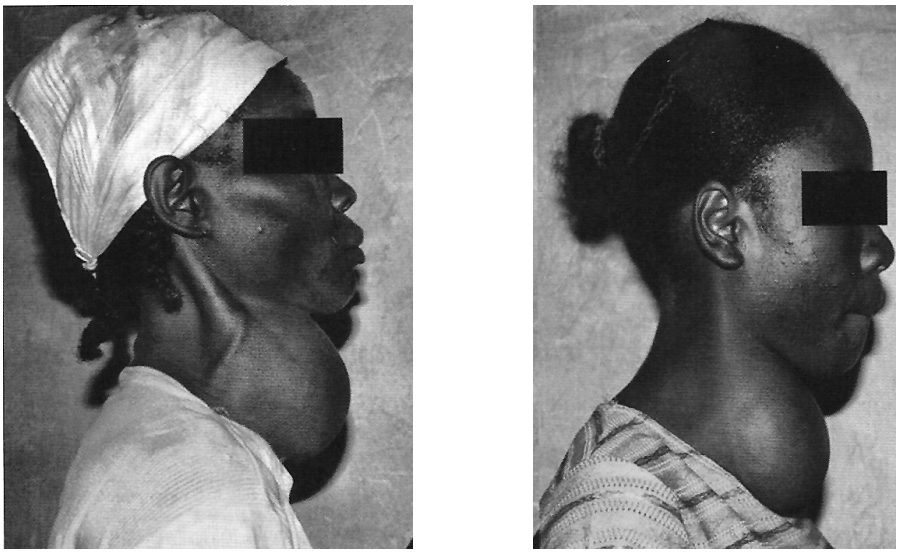


Figure 1.2. - Small goiter and medium-sized goiter with relatively short duration of symptoms vs enormous goiters in mother and daughter with long duration of symptoms and no medical care

Blood testing for the two most common general Hashimoto's thyroiditis antibodies, **thyroglobulin antibodies** (thyroglob) and **thyroid microsomal/peroxidase (TPO) antibodies** are also increasingly available and when positive, these antibodies confirm suspicion of Hashimoto's lymphocytic thyroiditis as the "causative finding"[15]. Sometimes both are positive, sometimes only one and sometimes they go back and forth over time for reasons still not well understood. De Luca and colleagues reported on 608 children and adolescents and found that 52% were euthyroid at presentation and diagnosis while 41% had overt or subclinical hypothyroidism. Another 7% were considered to have Hashitoxicosis more on the basis of blood work than overt symptoms but with some overlap. Another study [16] attempted to ascertain other risk factors and reported that the younger the child, the more severe was the thyroid function abnormalities implying that such youngsters had more symptoms because they had more severe disease per se early on. If there was an associated genetic diagnosis (ie. (Down Syndrome, Turner Syndrome, Noonan Syndrome, for instance), then such youngsters had more risk. They also reported earlier diagnosis if there were associated other autoimmunopathies present as well as

less eye disease with Graves Disease in kids or teens compared to adults.

A separate entity of **Hashimoto's thyroiditis associated with encephalopathy** is quite rare in children and adolescents and much more common in older aged adults. In such cases, the thyroid symptoms can be absent or quite subtle, and presentation in a more elderly cohort involves neurologic findings: acute or subacute encephalopathy, seizures, stroke-like symptoms, myoclonus, tremors and/or cognitive impairments. Exactly what is different about such patients and whether or not the thyroid antibodies themselves are the precipitating culprit is still not known. Of importance, is that there is an impressive response to corticosteroids for the diagnosis of Hashimoto's associated encephalopathy. Unrelated to such encephalopathy is the still confusing issues as to whether or not positive thyroid antibodies in and of themselves also can cause any symptoms or signs separate from what is occurring in the thyroid gland function itself; this too remains not completely settled.

Acquired hypothyroidism

Hypothyroidism[17] secondary to Hashimoto's thyroiditis, as mentioned previously, is the most common associated finding but it can be euthyroid, compensated hypothyroidism or frank hypothyroidism.

The symptoms can be severe or very subtle and may change depending upon the sophistication of the medical community involved, how long symptoms have been present and whether or not the child or adolescent can report what they have been experiencing. Similarly, depending upon family members sophistication and observation skills, as well as what specific signs and symptoms are present in any given individual, what is reported by patient and family may also be highly variable. At its most extreme, there can be **myxedematous coma**, usually not in children or adolescents but more frequently in the elderly with cardiac or neurologic/circulatory co-morbidities although the 3 year old pictures in *Figure 1.3.* presented with significant myxedema – but not coma, just extreme lethargy.

Table 1.2. includes a list of such findings highlighting weakness and fatigue as well as unexplained abnormal growth patterns (decreased height velocity associated with excess weight) for children while any of the others myriad thyroid related findings

can be present to variable degrees and depends upon length of hypothyroidism as well as vagaries of presentation itself.

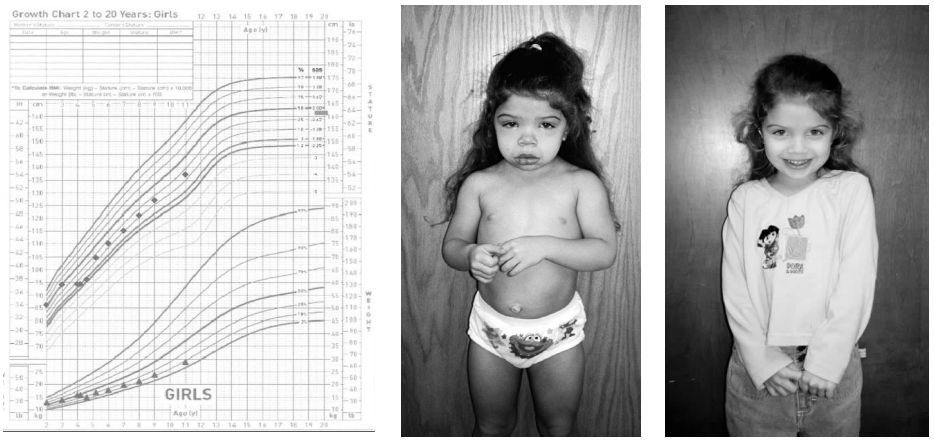


Figure 1.3. - Myxedema and growth failure in 3 year old with primary acquired hypothyroidism secondary to Hashimoto's thyroiditis and follow-up growth chart and photograph after 18 months replacement treatment with normalization of all TFTs

Table 1.2.

Acquired hypothyroidism

Thyroid gland	painless usually, rarely tender
	enlarged/goiter, rarely defined nodule(s) or cyst(s)
	symmetrical or unilateral
General	weight gain without increased calories
	cold intolerance
Growth	slowed height progression, short stature
Skin	dry, coarse skin
Hair	dry, coarse or sparse hair
Nails	not growing
Brain	fatigue, depression, decreased concentration
	general lack of interest
Heart	slowed pulse, decreased BP, decreased cardiac output
Gastrointestinal	constipation, decreased appetite
Kidneys	mild decreased function, rarely fluid retention
Breasts	galactorrhea, delayed puberty if prolonged
Reproductive	amenorrhea or irregular menses
	pubertal delay or cessation of progress males/females
	decreased libido, erectile dysfunction, decreased fertility
Neurologic	absent or decreased DTRs
	muscle weakness
Other lab tests	hyperlipidemia, nonspecific anemia

Parents and other family members as well as primary care providers should always investigate unexplained weakness and fatigue as well as abnormal growth patterns to eliminate easily treated hypothyroidism as a cause.

As with all other chronic conditions, detailed medical questioning will often bring out a variety of other problems not necessarily recognized by patient and/or family on their own. Goiters may be present, can be unilateral or bilaterally enlarged or maybe absent. Cysts and nodules as well as tenderness are less common but may also be present at diagnosis.

In places around the world where medical care is not available, goiters associated with hypothyroidism can be enormous whereas in most parts of the world with reasonable access to medical care, small to modest goiter may be present. The rest of the hypothyroid patients who do not present with a goiter, however, cannot be readily explained at present.

Thyroiditis produces interference with thyroid gland hormone production so that T4 and T3 as well as their free T4 and T3 counterparts are low. Because this is a primary hypothyroid situation, the hypothalamic TRH and pituitary TSH systems "register" this decreased hormone production from the thyroid "factory" and because the primary autoimmune attack is most commonly aimed at the thyroid gland, TSH (and if one could easily measure it, also TRH) levels are elevated.

Elevated TSH in association with low levels of total T4, free T4 and total T3 are confirmatory of primary acquired hypothyroidism. With positive thyroglobulin and/or thyroid peroxidase antibodies, the diagnosis of Hashimoto's thyroiditis with acquired hypothyroidism can be confirmed.

While there are autoimmune pituitary and hypothalamic disorders, that is not the focus of this presentation and antibodies directed against these parts of the endocrine systems are difficult to obtain and expensive. Secondary hypothyroidism would involve disorders of the pituitary while tertiary (hypothalamic) hypothyroidism would involve disorders of the hypothalamic and higher central nervous system and vicinity with both secondary and tertiary hypothyroidism sometimes more subtle, usually without goiters (since there is less central stimulation effects from lack of TRH and/or TSH).

Diagnosing hypothyroidism like many other aspects of medicine requires some detective work, focus on family and personal medical history of the chief complaints and any associated

disorders as well as physical examination, palpation of the thyroid gland and appropriate laboratory testing to assess thyroid function and antibody determinations.

Biopsies of the thyroid gland and thyroid technetium or iodine uptake nuclear scans well as ultrasonography and CT scans/MRI imaging usually are not needed because the laboratory testing is sufficient with history, index of suspicion and physical examination parameters to make the diagnosis.

The exception would be when there are specific cysts or nodules present and there is some suspicion that there may be malignancy involved or if there is a defined asymmetric or unilateral presentation. This may simply mean that there is thyroid gland hypoplasia so that what appears to be asymmetric is in reality involving the entire thyroid gland, only in such circumstances, it was previously unknown that some of the gland was not present previously.

Compensated acquired hypothyroidism

Another variety of acquired hypothyroidism with more subtle or no symptoms at all can be called compensated acquired hypothyroidism. This may also be with or without any goiter or thyroid enlargement. Usually this occurs when there has not been such a long time of symptoms as well as when there is only partial decreased thyroid functioning even though positive antibodies have been involved.

The classic situation would involve normal total T4 and free T4 but elevated TSH levels that persists on repeat testing. Sometimes this can be present for months or years, sometimes this produces higher or lower levels that meander up and down depending on circumstances of the moment and sometimes there will be a steady progression with slowly worsening and lowering of the T4 and T3 levels while the TSH levels slowly increase.

Antibody levels can be very elevated or also go up and down under such circumstances as well. Exactly why these variations occur is also not known.

Sometimes there are family patterns where similar circumstances have occurred with other family members and this provides the clue for thyroid investigations.

Acquired hypothyroidism treatment

Treatment goals after making the diagnosis for acquired hypothyroidism do not vary whether or not there is positive antibodies.

Sometimes there are negative antibodies and only partial or compensated hypothyroidism while other times there are classical presentations, recognized or unrecognized symptoms and signs but confirmatory laboratory results with low T4 and T3 levels, high TSH levels and positive antibodies. Nevertheless, elimination of hypothyroid signs and symptoms in a slow and steady fashion should be a primary goal with thyroid home replacement designed to normalize all the thyroid blood function tests.

At the same time, if there is a small or large goiter, such treatment should ideally slowly and steadily reduce the size of the goiter so that no goiter is palpable clinically.

The longer the symptoms and signs of hypothyroidism, the longer might be expected for it to take for thyroid hormone replacement treatment to reverse these findings clinically and biochemically.

Antibody abnormalities generally remain positive but these also can wax and wane over time. Unless there is significant medication noncompliance (or malabsorption), resolution is fairly predictable. Mostly, levothyroxine is used alone to boost the low serum T4 levels. Under most circumstances the body can convert total T4 provided in this fashion orally once-a-day to the active free T4 and free T3 forms needed by the body. When this occurs, the hypothalamic and pituitary "controlling sites", reduce their production of TRH and TSH accordingly so that the measurable blood thyroid functions tests normalize and stay normal.

Children's doses and those of very small adolescents and/or adults, need to be somewhat less than doses for those of higher weight.

Pubertal doses are often significantly higher and change dramatically especially if there is also concomitant obesity.

If there is associated celiac disease with some malabsorption, there may also be some difficulty until gluten-free meal planning is instituted.

Most pharmaceutical preparations for levothyroxine are quite stable with small dose incremental tablets available for fine-tuning. There is some controversy over manufacturing practices and inconsistencies so that some thyroid specialists, including this

author, prefer certain brands because of such consistency of manufacturing production. This occurs because of the sensitivity of current TSH assays and the secondary goal to optimally normalize TSH to the 1-3 μgm/ml range rather than just getting "close" to normal ranges. With some generic preparations, there is sufficient inconsistency, that this becomes a difficult task unless higher quality standard of production are met. How much such problems exist because of patient noncompliance rather than pharmaceutical production inconsistencies, remains debatable.

A further theoretical benefit of maintaining more idealized (and lower) TSH levels is the reduction or elimination of TSH-stimulated future thyroid malignancy in a cohort of patients with Hashimoto's thyroiditis.

Monitoring blood T4, free T4, TSH and total T3 levels provides surveillance information about dosing compliance, dose needs as does detailed follow-up examinations of the thyroid gland itself, complete physical examination focusing on parameters known to be involved with subtle or overt hypothyroidism and its treatment (galactorrhea, pulse and blood pressure, weight and height plotted for children and adolescents, skin and hair exam, reflexes etc).

If growth was abnormal (weight excess and/or height deceleration),one would expect as the thyroid functions improve, that the weight and height abnormalities would also correct over several months again depending upon the degree and duration of abnormalities at diagnosis.

Lipids abnormalities as well as anemia that might have occurred with unrecognized initial hypothyroidism also would self-correct over several months too.

Thyroxine Replacement Dosage

For very young children, usually starting with 25-50 μg/day of levothyroxine is sufficient. If there are older children, adolescents or adults with significant myxedema or other hypothyroid-related symptoms, then starting with such small doses also maybe quite reasonable to avoid "jolting" the body too quickly.

The ultimate goal is to slowly and steadily normalize and bring down the elevated TSH, while doing the same but slowly and steadily bringing the total and free T4 as well as total T3 levels into the high-normal range.

Then keeping these blood result values in those categories thereafter. The more abnormal the initial values, the longer might be expected for this to take place with typical dose adjustments every 2-8 weeks according to interim history, exam findings and actual thyroid function test results upon which the next dose adjustments are based.

Typical late childhood or early adolescent doses would be 75-100 μg/day while older adolescents and adults may need the doses further increased to 112-150 μg/day. There are those who have individualized higher dose needs just as there are about 20-30% in this author's experience who cannot be maintained at normal TSH levels without adding a small dose of liothyronine (T3 replacement) under these assumption that not all patients convert synthetic levothyroxine optimally to normalize all these functions. Sometimes, there are also those who need a very small dose of levothyroxine and this small dose does not require upward adjustments over time. There are no dogmatic dose decisions but all should be adjusted according to symptoms, goiter responsivity and, most importantly, actual sequential thyroid function test results as the basis for dose decisions.

The issues of compliance with daily medications is especially difficult for some adolescents but also for some families, children and adults as well. Especially the blood T3 levels are lower than expected while the Total T4 and free T4 levels are high-normal range yet still there is elevated TSH, this would be the classical description of when to add a small dose of T3 to the T4 prescription. In addition, there are those patients who just seem to need significantly higher doses of levothyroxine and doses in the 150-250 ugm/day tablet range meet these needs based on close history, compliance discussions and lab results. This group tends to be the heaviest by weight with an assumption by this author that this is a weight-based requirement and not automatically noncompliance.

Levothyroxine is a relatively long-lasting once-a-day medications and it does not matter if it is given at any particular time of the day. Ideally, it should be before food so that there is no food interference with absorption. Likewise, in patients taking multiple minerals or calcium supplements, sometimes these can interfere with medication absorption and so the timing should be discussed.

There is some controversy about specific brands of levothyroxine and whether or not generic thyroid medications are adequately controlled at the manufacturing steps to be consistent in

terms of balancing out individual needs. This author has often found that generic medications do not always meet this therapeutic criteria and that much time and money is wasted bringing patients back to the office, having to do more blood testing and follow-up when the real answer gets itself corrected by using the somewhat more expensive but more predictable brand-name preparations thus saving medical staff time, patient and family time and requiring fewer laboratory follow-up tests accordingly. In such circumstances, brand names such as Synthroid or Unithroid which cost more money per pill actually end up being better choices because they save time and money in the long run. Each practitioner will have to learn and decide if this is an issue for their practice environment. Usually it takes about 6 weeks for elevated TSH levels to respond to a dose of levothyroxine and small dose adjustments of 12.5 – 25 μg of T4 helps make such adjustments without risks of overtreatment.

Eisenbarth Adapted Model of Autoimmune Hashimoto's Disease

When thinking about autoimmune endocrine disorders such a s type 1 diabetes mellitus or Addison's disease, Eisenbarth presented a beautiful model[18] to ponder as herewith adapted by this author. The theoretical construct of such autoimmunopathies would suggest that the development of Hashimoto's Disease begins with a stage of potential development because of genetic predisposition, modified by whatever environmental or other triggers or present to subclinical stages that might be detected by positive antibodies but normal thyroid functioning of the gland itself and the controlling TRH and/or TSH levels.

Thereafter, it could stay dormant and only involve positive antibodies but no further destruction for some time or could slowly or more rapidly, for reasons not yet fully understood, progress to mild but compensated hypothyroidism and then symptomatic hypothyroidism all the way to overt myxedema – with or without goiters, nodules or cysts developing. While myxedema coma can result in death if prolonged duration and lack of treatment is possible, this is less lethal than comparable adrenal insufficiency situations or even type 1 diabetes mellitus because of more severe insulin deficiency, metabolic ketoacidosis and death from both of these conditions left unrecognized and/or untreated. Exact environmental triggering factors are usually not apparent or known and pathogenic immunological factors include cytotoxic T

lymphocytes, lymphokines and perhaps blocking antibodies as well for any of these conditions.
Diagnosis of thyroid abnormalities, unless there is a huge goiter or obvious signs and symptoms, involves a high index of suspicion based on medical history, family history and physical exam paying particular attention to other autoimmune diseases that may co-exist.

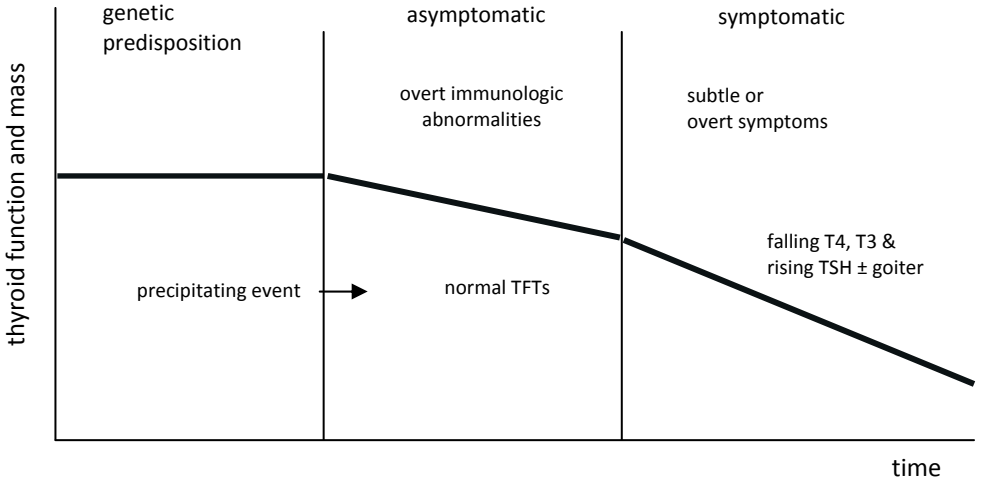


Figure 1.4. - Brink modified Eisenbarth Thyroiditis model

If available and affordable, thyroid antibody determinations may allow risk assessment and decisions regarding further more definitive thyroid function testing (T4, free T4, TSH, total T3) as well as another specific immunologic markers according to findings. This should allow earlier diagnosis, less morbidity and earlier institution of appropriate treatment. TRH and/or TSH stimulation testing is usually not required but there are times when this would also be indicated just as most imaging studies are not required with modern lab testing, but there are exceptions to that as well. Genetic testing is likely to improve in the coming years but at present this remains a research tool not a clinical tool under most circumstances.

Autoimmune Polyglandular Syndromes[19, 20]

APS-1 is also called **AIRE** because its genetic cause is associated with changes in the **A**uto**I**mmune **RE**gulatory gene mutations. APS-1 classical tried includes mucocutaneous

candidiasis, primary hypoparathyroidism particularly in children less than 10 years of age and then autoimmune AI in somewhat older children, adolescents or adults. The AIRE mutations in genes of chromosome 21 are felt to be autosomal recessive genes.

APS-1 is also known as **Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED)**. There also are some associations with type 1 diabetes mellitus, primary hypothyroidism/Hashimoto's thyroiditis, vitiligo, pernicious anemia, alopecia, malabsorption, keratitis and hepatitis as well as ovarian and testicular failure so absolute differentiation with APS-2 is not always possible. Iranian Jews, Sardinians and Finns seem to have higher prevalence in some reports.[21]

APS-2 is significantly more common than APS-1 and involves genetic predisposition of the HLA-DR3 and HLA-DR4 regions associated with not only AI and autoimmune adrenalitis, adrenal lymphocytic infiltration and positive antibodies but also type 1 autoimmune diabetes mellitus and Hashimoto's thyroiditis as well as Grave's Disease.

Euthyroid goiters, compensated hypothyroidism with or without small goiters can also be commonly seen. All these can be present at diagnosis or there can be subsequent development of one after the other but the time course of presentation is quite variable.

More females than males are seen with APS-2 for reasons not well understood.

Some environmental trigger is also postulated and may include common childhood viruses, endocrine disruptor chemicals as well as early wheat/gluten exposure. The association of Addison's Disease (AI) with thyroid disease also has been named **Schmidt's Syndrome**. If type 1 diabetes is also present, this has been named **Carpenter's Syndrome**.

While celiac disease is also associated with other autoimmunopathies, notably type 1 diabetes mellitus and various thyroid disorders, it has not been "formally" placed into APS-2 categories and this author includes celiac disease in the **APS-4** category of "other conditions" also associated with autoimmune AI.

Vitiligo may fit into either APS-3 or APS-4 categories with more of all kinds of autoimmunopathies associated in patients with vitiligo.

Turner Syndrome and Down Syndrome are also associated with increased frequency of type 1 diabetes mellitus as well as increased frequency of autoimmune thyroid disorders, particularly hypothyroidism, but not necessarily adrenalitis.

Noonan Syndrome and Klinefelter's Syndrome are known to have more common thyroid dysfunctions associated with them but not particularly more adrenalitis/AI; Klinefelter's patients, particularly those with associated obesity, are thought to have more diabetes but it is not clear whether or not this is type 1, type 2 diabetes or LADA.

Exactly whether or not these specific genetic syndromes would also be sub-classified in one of the APS subtypes remains a topic for further research and consensus.

With Turner and Down syndromes, there may also be more celiac disease while in the other syndromes mentioned, there is not necessarily more celiac diseases recognized. In all of these syndromes, however, AI is extremely rare compared to the autoimmune diabetes and thyroid diagnoses.

APS-3 includes autoimmune thyroid disease with other autoimmune diseases (ie. pernicious anemia with positive gastroparietal antibodies) but not diabetes and not adrenal insufficiency so APS-3 has some overlap in categorization with APS-4.

Exact guidelines for screening in those suspected of autoimmune polyglandular syndromes remains somewhat controversial. It is clear that the astute clinician will not only have heightened awareness of the potential for other autoimmune dysfunctions under such circumstances and at NEDEC we follow the following clinical testing guidelines:

- Increased awareness of possible other autoimmunopathies when primary diagnosis is:
    - type 1 diabetes mellitus
    - Hashimoto's Disease
    - Graves Disease
    - goiter
    - vitiligo
    - adrenal insufficiency or adrenalitis/Addison's Disease
    - pernicious Anemia
    - chronic active hepatitis
    - lupus
    - Sjogren Syndrome
    - celiac disease
    - Turner Syndrome
    - Noonan Syndrome
    - Down Syndrome
    - Klinefelter Syndrome …
- Get detailed family history of autoimmunopathies to help prioritize risk

    - Consider annual screen with available and affordable antibodies at least for the first few years after diagnosis
- Check at least baseline thyroid functions, iron and ferritin levels
- If consistently negative, consider less screening
- If vague but suspicious symptoms or signs occur, early lab testing and antibody checks
- The more positive antibodies that exist, the more other antibodies should also be added to the screening panel
- When there are two clinical autoimmune diagnoses, increase screening frequency and more specific/detailed testing
- When there are three or more clinical autoimmune diagnoses in the same patient or family, further screening in an effort to detect abnormalities before serious symptoms or signs occur

Hyperthyroid Graves Disease

Hyperthyroidism involves the overproduction of thyroid hormones causing metabolism to speed up, exactly the opposite of hypothyroidism. Elevated total T4, free T4 and total T3 occurs in association with suppressed TSH levels.

Graves' Disease, sometimes called Basedow's Disease, is not as common as autoimmune-based hypothyroidism but the hypothyroid, euthyroid and hyperthyroid variations of chronic thyroiditis require identification and treatment by trained medical personnel.

All can occur in the same family and any form of thyroid dysfunction can be associated with the same list of other autoimmunopathies as already listed. It is estimated that pediatric and adolescent Graves' disease occurs in about 1-15% of thyroid disorders in patients less than 18 years of age.[22] With medical treatment, only about 20-30% of children achieve remission lasting more than 2 years.[23, 24] Remission rates are even lower in prepubertal than pubertal children and we still do not have accurate ways to identify those who will achieve long term remission.[25] The Eisenbarth autoimmune model applies to hyperthyroidism just as it applies to autoimmune hypothyroidism or to simple goiters.

The differential diagnosis of hyperthyroidism also must rule out autonomous hyperfunctioning nodules or areas and so thyroid scans and uptakes may also be helpful if routine exam and thyroid function tests as well as antibody determinations still do not allow a specific diagnosis.

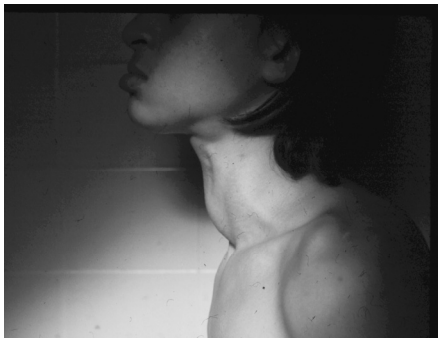
Table 1.3.

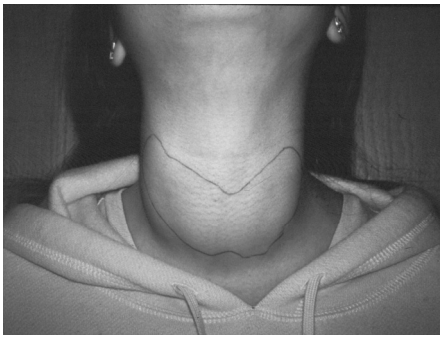
Acquired hyperthyroidism

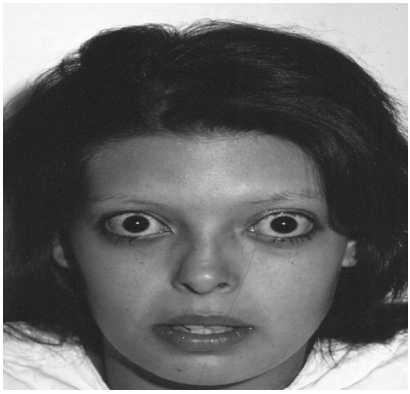
Thyroid gland	painless usually, rarely tender
	enlarged/goiter, rarely defined nodule(s) or cyst(s)
	symmetrical or unilateral
General	unintentional weight loss despite insatiable appetite
	hotter than usual, heat intolerance
Growth	slowed height progression, short stature
Skin	sweaty, moist palms; velvety skin
Hair	thinning or sparse hair
Brain	fatigue, anxiety or nervousness, irritability
	change in school performance
	poor sleep
Heart	rapid or irregular heart beat, palpitations, higher BP
Gastrointestinal	frequent, looser bowel movements
Reproductive	amenorrhea or irregular menses, male/female infertility
	excessive vomiting in pregnancy
	gynecomastia w/higher T & E2 levels/conversion
Neuromuscular	brisk or hyperactive DTRs
	tremors
	osteopenia, muscle weakness & atrophy
Eyes	bulging, protruding eyes, lid lag, conjunctival irritation

History and physical exam are usually sufficient to make one think of hyperthyroidism particularly if there is also exophthalmos, red, irritated conjunctivae or interference with extraocular eye movements or bringing the eyelids fully downward.

With a goiter or nodule, tachycardia, hypertension, tremors, heat intolerance, loose bowel movements, unexplained weight loss, not much else that would explain all of these findings.







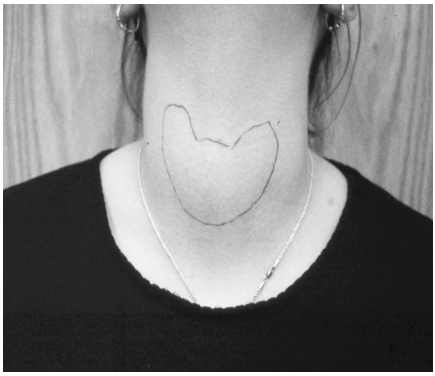


Figure 1.5.- Hyperthyroid goiters, nodules and exophthalmos/bulging eyes

If more subtle hyperthyroidism, however, this can sometimes be a difficult diagnosis to make particularly if only a few abnormalities reported.

Blood should be obtained for total T4, free T4, TSH and total T3. Sometimes also for free T3 levels particularly when only subtle or minimal clinical hyperthyroidism occurs with a condition called T3 thyrotoxicosis.

Thyroglobulin and thyroid microsomal/peroxidase antibody determinations also should be obtained and consideration for thyroid binding immunoglobulins, thyroid stimulating or receptor antibodies or the entire panel depending on degree of suspicion.

Positive thyroid stimulating immunoglobulin markers are confirmatory if positive for Graves' disease.

TSH receptor antibodies are positive also in about 90% of Grave's patients. There also are times when a radioiodine or technetium thyroid scan will help better define the degree of hyperthyroidism particularly if medication is not working to produce expected hyperthyroid blockade or when consideration for alternative treatment must occur, questions about hyperfunctioning nodules or other diagnoses must be considered.

HLA-DR3 and some other genetics markers are thought to confer increased risk for Graves' Disease but still are considered mostly research tools at present.

The simple approach to hyperthyroid treatment[1,7,12,14] is to provide symptom management and to block the overproduction of the thyroid hormones causing the hyperthyroidism symptoms. This

can be accomplished with one of several oral medications as well as with radioactive ablation of the hyperthyroid gland or with surgical excision of the overactive thyroid gland. If there is significant cardiac hyperactivity (hypertension, tachycardia), then beta blockers can also be provided in the earliest phases of such treatment to provide symptomatic relief. Usually the tachycardia is supraventricular but atrial fibrillation may be superimposed on the underlying heart disease or secondary to the hyperthyroidism itself. Sometimes flow murmurs also co-exist. With the enlargement of the hyperthyroid gland itself, there is often increased thyroid blood flow and a thyroid bruit may also be present.

Treatment Approaches for Hyperthyroidism[26]

a. Oral antithyroid medication

For years, the mainstay of hyperthyroidism treatment has been oral medication using either propylthiouracil (PTU) or methimazole (MMI).

Each thyroid specialist had their own preference but more recently, with concerns about liver side effects utilizing propylthiouracil [27] especially in children and adolescents, most specialists have switched to favoring methimazole. PTU can still be used for short term especialy if MMI side effects prohibit its use or while awaiting alternative therapeutic approaches to hyperthyroidism treatment. Both medications come in tablet form and require medications to be given from one to three times a day but usually one starts with a single dose each morning and then a second dose can be added based on lab responsivity and symptoms. Adult doses for MMI are 5-20 mg/day with divided doses either twice-a-day or sometimes every 8 hours. On a weight basis, MMI is generally provided at 0.1-1 mg/kg/day.

The most serious side effects involve liver irritation but there also can be agranulocytosis, leukopenia, thrombocytopenia or aplastic anemia, although relatively rarely. Rashes, hives, nausea and vomiting as well as dyspepsia, arthralgia, myalgia, headache and edema also have been reported. PTU generally comes in 50 mg tablet forms and initial doses maybe as high as 300-400 mg/day divided into three doses. Often this dosage can be decreased down to 100-150 mg/day also in divided dosage. PTU dose is approximately 5-7 mg/kg/day. The same list of potential side effects exists for propylthiouracil as for MMI with some concern of more serious and more frequent liver abnormalities than with MMI. Both

PTU and MMI need to be continued for many months and sometimes for years with periodic reassessment based on thyroid function test result and symptom management. Both MMI and PTU inhibit oxidation and organic binding of thyroidal iodine while propylthiouracil also inhibit the peripheral conversion of T4 to T3 as well. MMI is about 10-20 times more potent than PTU with a longer half life so the actual mg doses for MMI is significantly lower. An alternative available in some places is carbimazole at doses closer to MMI; carbimazole is converted to MMI and the carbimazole dose is 0.5-0.7 mg/kg/day. MMI also has potential teratogenic effects so that PTU is the preferred oral medication during pregnancy. Side effects can occur up to 20% in treated children with oral anti-thyroid medications and, of course, compliance issues for several years of therapy can be daunting especially with adolescents.

There are two general methods for dosing with oral medications: **titration** vs **block and replace**. "**Titration**" involves adjustment of doses to achieve euthyroidism, starting with smaller doses in an effort to minimize any side effects and then titrating upwards until full blockade occurs. Thereafter reverse titration of dosage to maintain the blockade or until remission occurs of the hyperthyroidism itself.

The alternative is called "**block and replace**." In this system, full suppression of thyroid overactivity is started at diagnosis associated with supplementation with levothyroxine to normalize circulating T4 and TSH levels. There may, however, be more sided effects until the doses are balanced for the individual patient but some authors report higher probability of inducing a remission of the hyperthyroidism itself because of the more aggressive, early hormone blockade from the higher doses utilized. There is no definitive preference for one or the other methodology and individual practitioner experience usually dictates which method is followed.

Remission rates after 1-2 years of antithyroid management generally are about 20-25%. Remission rates are more likely with smaller gland size, decreased T3 to T4 ratio, lower radio-iodine uptake initially, and lower thyroid stimulating immunoglobulin or other antibody levels. Low change of remission if the thyroid gland remains more than 2-5x normal size as well as if the hyperthyroidism occurs in someone who is a child instead of n adolescent. Non-Caucasians also are less likely to go into remission of their hyperthyroidism.

If there is significant tachycardia, hypertension and anxiety, sometimes it is also prudent to add a beta blocker for several weeks

until the original very high thyroid function results are brought back downward; any such beta blocker can be utilized including propranolol, labetolol and/or atenolol.

Propranolol dosage is 1-2 mg/kg/day and atenolol dose 0.5-1.2 mg/kg/day. In patients with known significant cardiac pathology, eg. Down Syndrome or Turner Syndrome patients, cardiology consultation may also be required to assist with medication options.

Hyperthyroid medication treatment goals remain to lower and normalize all abnormalities of thyroid function tests. This may take a long time to take place, and, for some patients, TSH suppression persists for months or years even when other functions normalize. Similarly, thyroid stimulating immunoglobulins and thyroid binding immunoglobulins may remain abnormal for many years post-oral medications (or even post-radioiodine or post-surgery) because of whatever immunologic effect have been established.

b. Radioiodine antithyroid ablation

A second option for hyperthyroidism treatment utilizes radioiodine I^{131}. This can be considered now as a primary not just a secondary options because long term studies have documented no future malignancy risks that were of original concern to pediatric endocrinologists. In addition, long term spontaneous remission of Graves' disease occurs in les than 30% of children and there is little evidence that use of antithyroid oral medications beyond a few years increases the likelihood of spontaneous, long term remissio[28].

If there is allergy to any oral anti-thyroid medications, if abnormal liver functions or side effects occur to oral anti-thyroid medications, or if there is documentation of inability to normalize hyperthyroid function tests clinically and/or biochemically, then radioiodine ablation is an excellent good option. In addition, if there is recurrence of hyperthyroidism or if remission does not occur after several months, it may be prudent to use radioiodine ablation of the hyperthyroid gland to eliminate any hyperthyroid risks.

In most adult endocrinology referral centers and in a few pediatric endocrinology centers [29] (ie. Massachusetts General Hospital, Yale Medical School, University of Florida, University of Iowa among others), radioiodine has become the treatment of choice for acquired hyperthyroidism because of its ability to induce hypothyroidism relatively quickly and because of its safety profile without worries of future malignancy as well as the low risks of recurrence or compliance problems with hyperthyroid oral

medication treatment regimens. The goal of radioablation of the hyperthyroid gland is the same as with oral medications: induce normal thyroid functioning. Remission rates generally exceed 95% with radioiodine treatment utilizing the Quimby-Marinelli dosage equation to supply[30] sufficient microcuries of I^{131} based on thyroid gland weight/mass[31].

Children as young as one year of age have been treated successfully with I^{131}. The goal should be to not only stop the hyperthyroidism but also to prevent future recurrence and, in order to accomplish this goal, essentially, to create a hypothyroid situation biochemically. While this will necessitate and one can expect to prescribe levothyroxine supplementation to keep the thyroid functions normal, this is far better than recurrence hyperthyroidism and easier to manage with a once-a-day levothyroxine dosage compared to what is required for hyperthyroidism itself. Typically if sufficient radioiodine is supplied, hypothyroidism will develop. When this was initially started, relatively low dose of radioiodine were thought to be preferable but it turns out that this actually increases the risks of benign thyroid neoplasms and other thyroid cancers whereas does >150 mCi/gram do not have such risks at all. There is also no evidence of increased risk of birth defects in the offspring of individuals treated with I^{131} as a child[32]. Usually biochemical as well as clinical hypothyroidism develops within 2-3 months post-radiation as the gland loses its ability to make T4 and/or T3 and there is no response to endogenous TSH or any of the immune modulators that might mimic TSH effects. If hyperthyroidism persists after 4-6 months despite radioiodine, then a second course of radioiodine treatment would be indicated with the assumption that the original dose was too low to accomplish its goal.

<10% of children complain of mild tenderness over the thyroid in the first week post radioiodine treatment. No long term genetic damage has been seen in several follow-up studies[24]. Utilizing >150 uCi of I^{131} (higher rather than lower dose calculations) provides better efficacy against recurrent hyperthyroid, helps induce hyperthyroid remission more often and minimizes ay thyroid tissue residua and future cancer risks. Earlier studies utilizing lower doses of radioiodine, often required retreatment but as the doses were increased, this occurred less frequently. In these studies, there was no thyroid cancer or leukemia risks with average length of follow-up of 36 years post treatment[33].

c. Surgical thyroidectomy

The third option at one time was the only option for hyperthyroidism before medications or radioiodine ablation became available. The Nobel Prize in Physiology and Medicine was awarded to Kocker in 1909 work in this area with a much more rapid resolution of the hyperthyroid state post-surgery compared to post-radioiodine utilizing total and not subtotal thyroidectomy[34,35]. Acute complications after thyroid surgery include hypocalcemia from interference with the parathyroid hormone system, hematoma, infection and recurrent laryngeal nerve paralysis whereas long-term complications post-operatively can include persistence of these same problems in addition to neck scarring as well as the expense of the surgical procedure and hospital stay itself. The experience of the surgical center and the surgeon are of key importance with thyroidectomy complication rates suggested to be significantly less when more experienced centers and surgeons are involved but there is little comparative data on thyroidectomy issues in children or adolescents published[28]. Thyroidectomy also has a place in thyroid glands that are exceptionally large since remissions rates in such cases are also much lower than in smaller goiters.

In addition, in those who have failed medical antithyroid therapy for any reason (side effects, noncompliance) and who refuse radioiodine (usually because of cancer fears associated with radioactive iodine), surgery also may be considered. Preoperatively, there are several options also to consider to help achieve euthyroidism and minimize any adverse outcomes. In addition to consideration of the experience and skills of the surgeon, per se, Lugol solutions, 3-5 drops or 50-150 mg/dose three times a day has been advised for 7-19 days presurgery as have short term use of antithyroid oral medications and beta-adrenergeic blockers. During surgery itself, rapid PTH testing at the end of the procedures has helped some predict the occurrence of post-operative hypogcalcemia even when specific attention to identifying all four parathyroid glands has been accomplished.

Acute complications post-thyroidectomy can be minimized with the post-operative staff aware of these issues and ongoing surveillance particularly of calcium issues. Anti-thyroid medication may need to be continued with "release" of some of the gland's thyroid factors or as results of the preoperative release of these factors and the half-lives of clearance from the body. Close surveillance will help to decrease such medications started preoperatively with the expectation that total thyroidectomy will

purposefully produce a state of hypothyroidism and thyroid hormone supplementation will be needed indefinitely with a goal of maintaining TSH suppression so that any minimal thyroid tissue still present will not have the option of stimulation by TSH.

Graves Eye Disease

Opthalmopathy affects about 25% of patients with Graves' Disease but can also (rarely) occur in patients with Hashimoto's thyroiditis but no elevated thyroid function levels. Significant eye findings, however, only occur in about 1-5% of Graves' patients. The main findings are proptosis, conjunctival redness and irritation, ocular discomfort, double vision and periorbital edema as well as lid lag. Such eye problems are least likely in the youngest children but increase somewhat in adolescents and young adults. The pathogenesis is thought to be retro-orbital inflammatory changes induced by cytokines associated with Graves' disease, per se. CT and MRI scans of the orbit show thickened eye muscles that may impinge on the optic nerve and impair vision. There is some evidence that the TSH receptor may be the orbital antigen that has role in triggering such ophthalmopathy. Treatment is not entirely satisfactory but most of the time there is spontaneous resolution even though complete resolution is not always possible. Corticosteroid therapy in high dose has sometimes been tried to reduce the inflammation and direct consultation with an experienced ophthalmologist will be needed. Orbital decompression has also sometimes been necessary. Complete thyroidectomy[36] has been associated with improvement and radioiodine therapy may worsen Graves' eye disease so recommendations when there is co-existing eye disease is that antithyroid medications or surgery may be preferable to radioiodine under those circumstances until the clinical situation stabilizes.

Hashitoxicosis

Hashitoxicosis [37] is another variant of autoimmune thyroid dysfunction which involves classical but sometimes inconsistent hyperthyroid symptoms associated with no, large or small goiters, sometimes hyperfunctioning cysts or nodules and without any true predictive findings. Positive thyroid antibodies are present, thus the Hashimoto's-like nomenclature. But, in contrast to classical Graves'

disease and hyperthyroidism, this hyperthyroid picture is a relatively short-lived effect lasting several days to weeks or months and then spontaneous change to either a euthyroid Hashimoto's picture, with or without goiter staying the same, or evolving over time to hypothyroidism that requires hypothyroid hormone replacement therapy. The entire time course is very variable and very unpredictable and, in general, there may be mild biochemical hyperthyroid functional abnormalities without so many classical findings. It is believed to result from unregulated release of stored thyroid hormone during inflammatory-mediated destruction of the thyroid gland. Hashitoxicosis can also be associated with thyroid stimulating immunoglobulins, thyroid binding immunoglobulin and these may stay positive for much longer time even when the initial hyperthyroid state has gone away.

Differential diagnosis included toxic adenoma, multinodular goiter, exogenous ingestion of thyroid hormone, McCune Albright syndrome and subacute thyroiditis but it may sometimes be difficult to make a definitive diagnosis depending on circumstances I^{123}. Thyroid scan results that shows low uptake (compared to high uptake in Graves' disease) may provide some initial clues. If classical hyperthyroidism has been treated with antithyroid oral medications, then these may need to be decreased or terminated. With ongoing surveillance, if hypothyroidism intervenes, then levothyroxine supplementation will need to be started and doses titrated acceding to standard clinical and biochemical laboratory protocol. Having staff be aware of the possibility of Hashitoxicosis, its unpredictable clinical course and the importance of sequential follow-up and ongoing surveillance, remains one of the important recommendations so that appropriate medication adjustment can occur to help minimize side effects. Long term follow-up studies have not been available but it is presumed that the eventual hypothyroid state that occurs will be permanent and require thyroid hormone supplements indefinitely.

Thyroid Storm

Thyroid storm is a severe and life-threatening presentation of thyrotoxicosis with an exaggeration of the usual symptoms of hyperthyroidism but the degree of hyperthyroidism lab testing abnormalities is not a criterion for diagnosis.

Thyroid storm is present associated with an acute onset of hyperthermia and tachycardia that may be precipitated by a new

infection, diabetic ketoacidosis or may occur during surgical or radioiodine therapy to treat the hyperthyroidism *per se*. Symptoms include high fever, sweating, tachycardia, reduced mental state ranging from confusion to coma.

Specific treatment [38] should include consideration of the following: beta blockers such as propranolol should be started urgently at a starting dose of 2-3 mg/kg/day divided into to doses every 6 hours to control the adrenergic symptoms of thyroid storm. Propranolol can also be given intravenously if oral administration is not possible at doses of 0.01 to 0.1 mg/kg over 10-15 minutes by an experienced intensive care team and utilizing an intra-atrial pacing catheter as a precaution.

Dexamethasone in a dose of 1-2 mg every 6 hours can also help decrease conversion of T4 to T3; high dose intravenous or hydrocortisone doses can also be used instead.

Intravenous sodium iodine in a dose of 125-250 mg/day up to 1-2 gram/day may decrease the release of thyroid hormone from the thyroid gland; if the patient is conscious, Lugol's solution in a dose of 5 drops every 8 hours orally can be given if the patient is conscious. A cooling blanket can also help control the hyperpyrexia as can acetaminophen but aspirin use is not recommended in children.

Propylthiouracil and methimazole can also be provided but the effects will not be obvious for several days: 6-10 mg/kg/day up to 200-300 mg propylthiouracil divided into doses every 6 hours around the clock or methimazole at a dose of 0.6-0.7 mg/kg/day as an alternative. Fluid management should also be optimized with particular attention to avoiding high output cardiac failure as well as attention to renal status, electrolyte levels and oxygenation.

Hashimoto's thyroiditis and associated cancer risk

Papillary thyroid carcinoma risk has been thought to have some association with chronic Hashimoto's thyroiditis. These risks are variable with reports ranging from 10-58% associated with positive thyroid antibodies but up to as much as 300% increase in some other studies[39]. Thyroid nodules are more unusual in children and adolescent compared to adult but also more often maybe malignant compared to adults. Hardness, immobility, associated lymphadenopathy, male vs female sex, solid vs cystic appearance on thyroid ultrasonography scans are all consideration for need to have fine-needle aspiration or biopsy and then consideration for subtotal

or total thyroidectomy with life-long TSH suppression with levothyroxine in an attempt to minimize recurrence. The exact relationship of the Hashimoto's thyroiditis to such malignancies is not fully understood.

Hashimoto's thyroiditis and pregnancy

Reproductive complications have been associated with chronic lymphocytic Hashimoto's thyroiditis before, during and/or after pregnancy in women of all ages[40]. Specifically, these risks are associated with positive and higher tissue titers for thyroid peroxidase antibodies. The increased risks include miscarriage, infertility, fetal death, pre-eclampsia, maternal hypertension, preterm delivery, placental abruptio, low birth weight infants and lower eventual IQ of babies so exposed in addition to slower mental development of these babies. After delivery, mother's with Hashimoto's thyroiditis even without overt thyroid dysfunction proven have been reported to have more postpartum ongoing thyroiditis, depression, postpartum anemia but long term studies about future actual thyroid lab abnormalities is not well known. Exactly what is the association of positive thyroid antibodies and their interaction with the pregnant women and her fetus/future child remains a topic of ongoing research and study. If there is a positive family history of thyroid disease or if there is known positive thyroid antibodies, ongoing thyroid function testing should be done several times throughout the pregnancy with an objective of identifying who might require additional studies and/or thyroid supplementation to decrease these associated complications.

References

1. Winter WE. Autoimmune endocrinopathy, in: Lifshitz F, ed. Pediatric Endocrinology. Fifth Edition, Volume 2. Informa Healthcare, New York, London, 595-616.
2. Hashimoto H. Kenntnisderlymphomatosen verandereung der schilddruse. Archiv für klinische Chirurgie, Berlin. 1912:97:219-248.
3. Singer PA. Thyroiditis, in: Lavin, N, ed. Manual of Endocrinology and Metabolism, Fourth Edition. Wolters Kluwer/Lippincott Williams & Wilkins, Baltimore, 431-2.
4. MacLaren NK. Autoimmune polyglandular syndromes, in: DeGroot LJ, Jameson J., eds. Endocrinology. Fifth edition. Elsevier-Saunders: Philadelphia, 2006; 819-835.
5. Brink SJ. Thyroid dysfunction in youngsters with IDDM. Ped Endocrin 1985; 1:181-4.

6. Brink SJ. Adrenal insufficiency in children, adolescents and young adults, in: Velea I, Paul C, Brink SJ. (eds). Pediatric Endocrinology and Diabetes 2016 Update, 2016: Timişoara, Romania: Editura Mirton, 11-47.
7. Rossi WC, Caplin N, Alter CA. Thyroid disorders in children, in: Moshang T, ed. Pediatric Endocrinology. The Requisites in Pediatrics. St Louis, Missouri. Elsevier Mosby. 2005; 171-190.
8. Brink SJ. Turner Syndrome: Diagnosis and Endocrine Dilemmas. In: Velea, I, Paul C, Brink SJ (eds). Pediatric Endocrinology Clinical Update 2011, Timişoara, Romania: Mirton, 74-102.
9. Brink SJ. Noonan Syndrome, in: Velea I, Paul C, Brink SJ (eds). Clinical Issues in Pediatric Endocrinology, 2012, Timişoara, Mirton, 101-122.
10. Cutler AT, Benezra-Obeiter R, Brink SJ. Thyroid function in young children with Down syndrome. Am J Dis Child 1986: 140; 479-
11. Brink SJ. Klinefelter's Syndrome, in: Velea I, Paul C, Brink SJ (Eds). Theoretical and Practical Approach in Pediatric Endocrinology 2013, Timişoara, Romania: Mirton, 1-48.
12. Fisher DA, Grueters A. Thyroid disorders in childhood and adolescence. In: Sperling M, ed. Pediatric Endocrinology; Philadelphia, Saunders, 2008: 227-253.
13. Rallison M, Dobyns BM, Keating Fr. Chronic lymphocytic thyroiditis in children. J Pediatr 1975; 86: 675-82.
14. Castro MR, Gharib H. Thyroid disorders. In: Camacho PM, Gharib H, Sizemore GW, eds. Evidence-Based Endocrinology. Second Ed. 2007: 34-38.
15. De Luca F, Santucci S, Corica D, Pitrolo E, Romeo M, Aversa T. Hashimoto's thyroiditis in childhood: presentation modes and evolution over time. Ital J Pediatr 2013; 39:8-11
16. Wasniewska M, Corrias A, Salerno M, Mussa A, Capalbo D, Messina MF, Aversa T, Bombaci S, De Luca F, Valenzise M. Thyroid function patterns at Hashimoto's thyroiditis presentation in childhood and adolescence are mainly condition by patients; age. Horm Res Paediatr 2012; 78:232-6.
17. Rivkees S. Hypothyroidism and hyperthyroidism in children, in: Pescovitz OH, Eugster EA, eds. Pediatric Endocrinology. Mechanisms, Manifestations and Management. Philadelphia, Lippincott Williams & Wilkins, 2004:508-521.
18. Eisenbarth GS, Gottlieb PA. Autoimmune polyendocrine syndromes. N Engl J Med 2004; 350: 2068-79.
19. Brown RS. The autoimmune polyglandular syndromes. Curr Rev Pediatr Endocrinol Serono Symposia International. 2003; 51-58.
20. Shatz DA, Winter WE. Autoimmune polyglandular syndrome II: Clinical syndrome and tratment. Endocrinol Metab Clin N Am 2002;31:937-949.
21. Haller M, Winter W, Schatz D. Autoimmune polyglandular syndromes, in: Sperling M, ed. Pediatric Endocrinology; Philadelphia, Saunders, 2008: 770-787.
22. Bauer AJ. Approach to the pediatric patient with Graves' Disease: when is definitive therapy warranted? J Clin Endocrinol Metab 2011; 96:580-588.
23. Hamburger JI. Management of hyperthyroidism in children and adolescents. J Clin Metab Endocrinol 1985; 60:1019-1024.
24. Glaser NS, Styne DM. Predictors of early remission of hyperthyroidism in children. J Clin Endocrinol Metab 1997; 82:1719-1726.
25. Lazar L, Kalter-Leibovici O, Pertzelan A, Weintrob N, Josefsberg Z, Phillip M. Thyrotoxicosis in prepubertal children compared with pubertal and postpubertal patients. J Clin Endocrinal Metab 2000; 85:3678-36872.

26. Brook CGD, Brown RS. Handbook of Clinical Pediatric Endocrinology 2008, Blackwell Publ, 84-88.
27. Cooper DS, Rivkees SA. Putting propylthiouracil in perspective. J Clin Endocrinol Metab 2009; 94:1881-82.
28. Rivkees SA, Dinauer C. Controversy in clinical endocrinology. An optimal treatment for pediatric Graves' disease is radioiodine. J Clin Endocrinol Metab 2007; 92:797-800.
29. Rivkees S. Radioactive iodine use in childhood Graves' disease: time to wake up and smell the I-131. J Clin Endocrinol Metab 2004;89: 4227-8.
30. Leslie WD, Ward L, Salamon EA, Ludwig S, Rowe RC, Cowden EA. A randomized comparison of radioiodine doses in Graves' Hyperthyroidism. J Clin Endocrinol Metab 2003; 88:978-983.
31. Kalinyak JE, McDougall IR. How should the dose of iodine-131 be determined in the treatment of Graves' Hyperthyroidism? J Clin Endocrinol Metab 2003; 88:975-977.
32. Rivkees SA, Sklar C, Freemark M. Clinical review 99: the management of Graves' disease in children with special emphasis on radioiodine treatment. J Clin Endocrinol Metab 1998; 83:3767-3776.
33. Read CH, Tansey MJ, Menda Y. A 36 year retrospective analysis of the efficacy and safety of radioactive iodine in treating young Graves' Patients. J Clin Endocrinol Metab 2004; 89:4229-4233.
34. Lal G, Ituarte P, Kebebew E, Siperstein A, Duh QY, Clark OH. Should total thyroidectomy become the preferred procedure for surgical management of Graves' disease. Thyroid 2005; 15:569-574.
35. Altman RP. Total thyroidectomy for the treatment of Graves' disease in children. J Pediatr Surg 1973; 8:295-300.
36. Menconi F, Marino M, Pinchera A. Effects of total thyroid ablation versus near total thyroidectomy alone on mild to moderate Graves' orbitopathy treated with intravenous glucocorticoids. J Clin Endocinol Metab 2007; 92:1653-1658.
37. Nabhan ZM, Kreher NC, Eugster EA. Hashitoxicosis in children: clinical features and natural history. J Pediatr 2005; 146:533-56.
38. Styne DM. Pediatric Endocrinology. Philadelphia. Lippincott Williams & Wilkins, 2004: 299-300.
39. Huang SA. Thyroid, in: Kappy MS, Allen DB, Geffner ME, eds. Pediatric Practice Endocrinology McGraw Hill Medical, New York. 2010:107-130.
40. Stagnaro-Green, A. Thyroid antibodies and miscarriage. Where are we at a generation later? J Thyroid Res 2011 May 12; 2011: 841949.

STUART J. BRINK, MD
Senior Endocrinologist, New England Diabetes and Endocrinology Center (NEDEC), Waltham MA, USA
Associate Clinical Professor of Pediatrics, Tufts University School of Medicine, Boston MA, USA
Address: New England Diabetes and Endocrinology Center (NEDEC), 40 Second Avenue, Suite #170, Waltham MA 02451-1136 USA.
E-mail: stuartbrink@gmail.com

February 2017

HEREDITARY DWARFISM

Ciril Kržišnik

INTRODUCTION

It has been generally accepted that children and adolescents whose height is below the 3rd percentile are short, while a typical defining characteristic of short stature or dwarfism in adults is the height of less than 147 cm.

Dwarfism could be caused by more than 300 medical conditions, many of them are hereditary [1, 2]

We present hereditary dwarfism on the island Krk in the North Eastern part of the Adriatic sea which has been known for more than 150 years. The affected individuals originated from two closely located villages: Baščanska Draga and Jurandvor. They were called “Mali ljudi” (short people) by the Croatian inhabitants.

The surface area of Krk is 428 km2 and the population is approximately 15 000. In the late 1980s the population of Baščanska Draga was 509 and that of Jurandvor 175. (*Figure.2.1*) The first recorded patient with dwarfism was born in 1864 in Baščanska Draga, the second 13 years later in the neighboring village of Jurandvor. By the end of the 19th century seven affected individuals had been born and currently there are 25 that have been documented .All affected patients are related and belong to two clans (*Figure 2*).

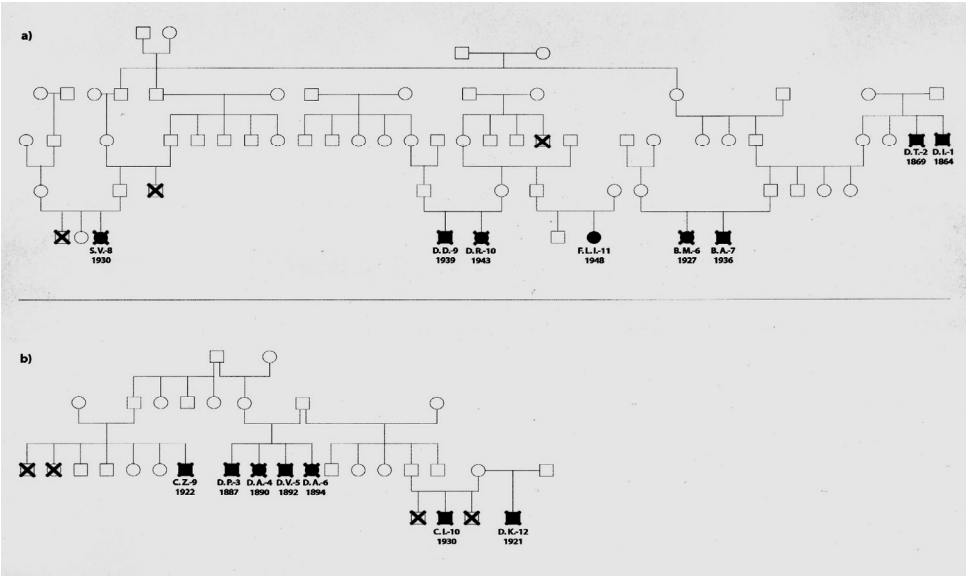


Figure 2: Pedigrees of some dwarfed patients from the villages Baščanska Draga (a) and Jurandvor (b). The patients for whom clinical and laboratory data are available are shown in black.

The first description of dwarfism on the island of Krk was made by the Austrian psychiatrist Wagner Von Jauregg in 1906.[3] He was followed 18 years later by the geneticist Hanhart from Switzerland, who described ten dwarfed patients. Hanhart also investigated hereditary dwarfism in Switzerland. Following his reports the hereditary dwarfs from Switzerland and the island of Krk were called "Hanhart dwarfs".[4] Investigations of 14 patients were performed in 1938 by Vojska from Munich, Germany, within the framework of his doctoral thesis. Consanguinity between the families was reported and a hereditary recessive dysfunction of the

hypothalamo – mesencephalic system was suspected to be the etiology of the dwarfism.[5] Further studies were performed by Fraser in 1964[6] and Zergollern in 1971[7] who studied a 40 year-old dwarf and his aunt and concluded that they suffered from an autosomal recessive type of panhypopituitarism. The first hormone determinations were performed by Kopajtić et al. in 1972.[8-11] These studies concluded that the patients suffer from an inherited form or panhypopituitarism due to absent anterior pituitary function.

In 1988 C. Kržišnik, Z. Kolacio, and Z. Laron visited the island and 6 of 13 living patients agreed to be examined and investigated.[12] Some of the investigations were repeated in 1990, 1997 and 2000. Two patients agreed to be interviewed again in 2007; few additional data were obtained in Outpatient clinic Baška in 2010 and 2014. Table 1 lists the 25 known patients from Krk with multiple pituitary hormone deficiencies (MPHD). The youngest patient (a-13, Table 1) was born in 1996.

Table 1a

Pertinent clinical data of the 25 known hypopituitary patients from the island Krk originating in the two villages of Baščanska Draga (a)

Patients No.	Sex	Years of birth	Age at height measurement		Comment	Ref. no.	Examined in 1988	Visited in 2007
			years	cm				
Village Baščanska Draga								
1	M	1864	42	106	Deceased	1		
2	M	1869	55	117,5	Deceased	2		
3	F	1877	38	112,4	Deceased	2		
4	F	1927	11	114	Sibling of pt. a-5	3		
5	M	1930	8	110,6	Sibling of pt. a-4	3		
6	F	1927	45	131	Sibling of pt. a-7	6		
7	M	1930	42	142	Sibling of pt. a-6	6		
8	F	1930	58	120	Died in 2010 at age 80 yr		x	
9	M	1939	49	139	Sibling of pt. a-10, died in 2009 at age 70 yr		x	
10	F	1943	47	152	Sibling of pt. a-9, died in 2004 at age 61 yr		x	
11	F	1948	25	142		7		
12	F	1976	17	164	Irregularly treated by T_4 and hGH		x	
13	M	1996	-	-	Treated by T_4 and hGH			

Table 1b

Pertinent clinical data of the 25 known hypopituitary patients from the island Krk originating in the two villages of village Jurandvor (b)

Patients No.	Sex	Years of birth	Age at height measurement		Comment	Ref. no.	Examined in 1988	Visited in 2007
			years	cm				
Village Jurandvor								
1	M	1877	47	105,9	Deceased	3		
2	M	1880	44	122,1	Deceased	2		
3	M	1886	37	126	Died in1969* at age 83 yr	2		
4	F	1890	34	133,2	Died in 1981* at age 91 yr	2		
5	M	1892	32	128,5	Died in 1969* at age 77 yr	2		
6	F	1894	30	130,2	Died in 1962* at age 68 yr	3		
7	M	1891	-	-	Deceased	6		
8	F	1894	12	115	Deceased	1		
9	M	1922	68	132	Died in 2009 at age 87 yr		x	x
10	M	1930	58	135	Died in 2009 at age 79 yr		x	x
11	M	1957	15	141		6		
12	M	1921			Moved to USA, track lost	6		

During our visit we examined the patients in the Outpatient clinic in Baška where also blood sampling was done. The records of the patients at the local clinic and in the hospital of Rijeka were also reviewed.

The clinical examination of the patients revealed dwarfism, obesity, dry and wrinkled skin and lack of secondary sexual characteristics. The results of hormonal testing showed absence of growth hormone to insulin hypoglycemia and GHRH, the absence of luteinizing hormone and follicle stimulating hormone unresponsive to gonadotropin releasing hormone and absence of thyrotropin stimulating hormone unresponsive to TRH. These findings indicate defects in the anterior pituitary. Basal levels of prolactin were low but cortisol levels were normal.[12] (*Table 2*).

Table 2
Hormone determinations in five hypopituitary patients from Krk

Patient No.	**Thyroid functions**			**GH**					**Sex hormones**					**Cortisol**
	TT_4[a] µg/dl	T_3 mg/dl	TSH Basal Peak[b]	hGH ng/ml Basal	Peak[c]	Peak[d]	IGF-I nm/l	PRL ng/ml	LH IU/ml Basal	Peak[e]	FSH IU/ml Basal	Peak[e]	E_2 Pg/ml	µg/dl
a-9	8.0*	-	3.1 3.1	0.2		0.2	3	0	<1.5		<1.5			10.7
a-10	7.2*	118	3.1 3.1	0.5		0.5	0	0	0.01	0.01	0.1	0.1	<10	8.5
a-12	8.0*	158	0.8	0.1		0.5	7	5	0.5	0.8	1.2	1.2	<10	16.2
b-9	7.6*	118	1.4	0.9	2.6	0.4	4.5	0	0.03	0.2	0.16	0.14		14.4
b-10	5.4*	-	0.47	0.5			0	5						26.3

Analysis of leukocytes from two patients (a-12 and b-9, Table 1) revealed homozygosity for deletion of an A from codon 50 of exon 2 of the Prop-1 gene. This mutation introduces a frame shift and results in a premature translational stop signal at codon 164. The truncated protein lacks the DNA binding and transcriptional activation domain (*Figure 3*).[12]

Thus, the etiology of dwarfism in the island Krk was clarified. PROP-1 is a pituitary specific transcription factor that is required for the embryologic development of the pituitary cells that produce GH, PRL, TSH and FSH/LH postnatally. Multiple pituitary hormone deficiency (MPHD) is due to a mutation in the PROP-1 gene described.

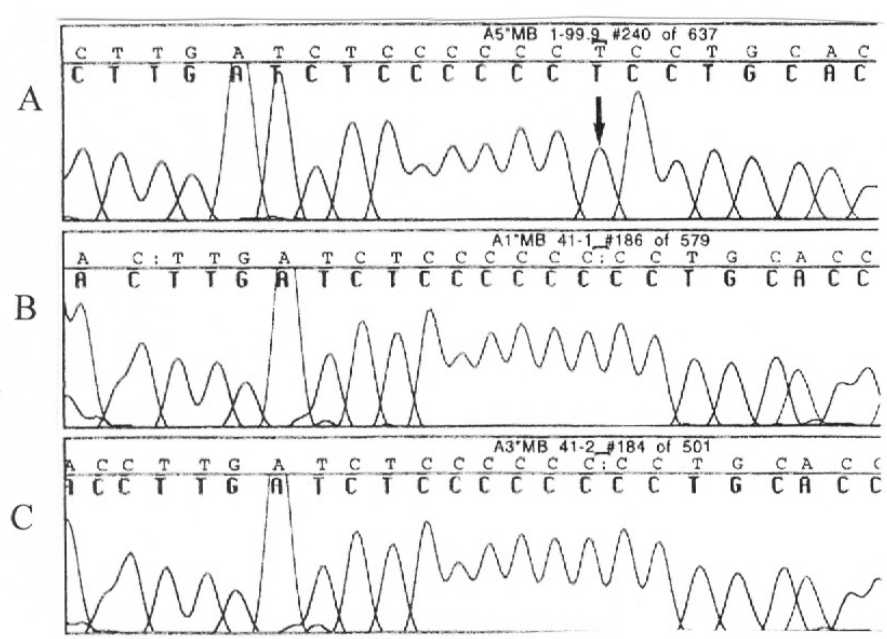


Fig. 3: Antisense sequences of exon 2 of Prop-1. Panel A: Control; Panel B: Patient a-12; Panel C: Patient b-9. A homozygous deletion of an antisense T in codon 50 generates a repeat containing 8 C and introduces a frame shift.

SOME CLINICAL CHARACTERISTICS AND DATA OF INVESTIGATED PATIENTS

We got the most information about history and life from patients Z. Č, born 1923 and I. Č born 1930 (*Figure 4*)

Fig. 4: Patients I. Č. aged 58 (left) and Z. Č. aged 68 (right). Behind dr Laron (left), dr Kržišnik (middle), dr Kolacio (right).

Patient Z. Č. male, (*b-9, Table 1*) *(Fig. 4 right*) was born December 1922. His parents were of normal height. Short stature was diagnosed at age 7 when he started school. At age 16 he measured 113.7 cm.[5] He completed eight years of elementary school and spoke besides Croatian a fluent German and Italian. He was employed as an accountant until he retired.

The patient noted that he was sensitive to cold, always sleepy and suffered from osteoporosis. When examined at age 50, GH deficiency, secondary hypocortisolism, secondary hypothyroidism and hypogonadotropic hypogonadism were reported.[7] He was treated with thyroxine but took the drug irregularly.

At examination in 1988 he was 66 years old, measured 132 cm and weighed 37 kg. Besides short stature, general obesity and high-pitched voice were registered. The skin was dry and wrinkled and the nails were soft. Skinfold measurements were: triceps and subscspular 14 mm, suprailiac 13mm. Pseudogynecomastia measured 6 x 6 cm. The patient had never shaved, and had no hair in the axillary region or on the extremities. There was scanty pubic hair and scrotal rugae were absent. The testes were atrophic and soft. The volume of the left testis was 1ml, and of the right 1.5 ml.

The length of the stretched penis was 6.5 cm, its width 1.5 cm. The clinical evaluation was consistent with panhypopituitarism.

When visited in 2000, he was 78 years old, he declared that he had no significant problems except hypertension and osteoporosis what was also evident from documents of his Outpatient clinic. He has been taking irregularly also thyroxine and statins due to hypercholesterolemia. His mental and physical conditions were good except for mild paresis of the right arm and hand due to a cerebrovascular lesion diagnosed by CT one month before our last visit. He died 2 years later at the age of 87 years due to another cerebrovascular accident. (Figure 5)

Fig. 5a: Patient Z. Č. aged 78 years

Fig. 5b: Patient Z. Č. aged 85 years

Patient V. Š. (a-8, Table 1) was born January 1930. She had a brother and sister of normal height. Birth weight was 4000g and length was not recorded. Retarded growth was observed at age 4 years. When she was 8 years old, she measured 84 cm[7]. She had learning problems and could not pass more than four grades of elementary school. She lived alone and could take care of herself. When examined at her home in Baščanska Draga, she was 58 years old, height 120 cm and weight 30 kg. (*Figure 6*)

Fig. 6: The height of 58 years old patient was 120 cm

She complained of always being sleepy and sensitive to cold. She had never menstruated. Her voice was high-pitched, the skin dry and wrinkled. There was no gray hair. There was no hair on the extremities and axillary region. There was some atrophic mammary tissue. She refused further examination, and blood testing. At another opportunity her serum total T4 was in hypothyroid range. In February 2010 her neighbours found her dead at age 80 years.

Patient D. D. (a-9, Table 1) was born October 1939. Parents were of normal height. His sister is pt. a-10. Birth weight was reported as 4000g, birth length unknown. Retarded growth was recorded at 4 years of age. He passed elementary school and four grades of technical school. His intelligence was recorded as normal. He has always been sensitive to cold and sleepy, and sometimes had edema of the eyelids. At age 33 years, deficiency of GH, TSH, LH and FSH were reported.[8] When examined in 1988 he was 49 years old, measured 137 cm and weighed 57 kg. Besides short stature and high-pitched voice, obesity was registered. Pseudogynecomastia was present. Head circumference was 53.5 cm. Teeth were partially defective. The hair on the skull was sparse and brown. No body hair was observed. Eyebrows were also sparse. There was no axillary hair, but scanty pubic and scrotal hair. Genitalia were hypoplastic. The length of the penis was 3 cm, width 2 cm. Testes were soft, 1.5 ml in volume. (*Figure 7*)

Fig. 7: Patient D.D.

Analysing his medical records in Outpatient clinic Baška in 2007 we stated that he developed hypertension which was treated with beta blockers, besides it was documented that he was smoking and drinking too much. In November 2005 at the age of 66 years he was operated due to planocellular carcinoma of the nasal septum.

Besides plastic surgery he received radiation therapy. He died in 2009 aged 70 years.

Female patient R. D. (a-10, Table 1) born December 1943 had older brother who was diagnosed as a pituitary dwarf (pt. a-9). She was estimated to be short at four years. Due to learning difficulties she completed only four years of elementary school. When no secondary sexual characteristics appeared at the expected time of puberty, she was examined at the University Medical center in Ljubljana. The patient was placed on estrogens and later on estrogen-progesteron therapy. In 1972, deficiencies of GH, LH, FSH and TSH were diagnosed and the patient was placed on L-thyroxine.[8]

When we examined her she was 46 years old, 152 cm tall and weighed 56 kg. Her skin was dry and pale, axillary and pubic hair was scanty and only minimal mammary tissue was observed. She refused further examinations. In 2004 she fell down the steps of her house and died due to a subdural hematoma at the age of 61 years.

Patient I. Č. (b-10, Table 1) (Figure. 4, left and 8) was born October 1930. His twin brother who had normal weight and length died at delivery. The patient has been short since birth but this became more evident when he entered school. At 8 years of age he measured 92 cm (5). He finished elementary school and he was employed as a book-keeper until retirement. He noted that he was always sleepy. Panhypopituitarism was diagnosed when he was 42 years old (8). A lateral skull X-ray done at age 40 had shown a regularly shaped but markedly small sella turcica. [7]

When examined in 1988 he was 58 years old, his height was 135 cm, weight 47 kg. General obesity was present. He was myopic and wore eyeglasses. Skinfold measurements were: triceps 24 mm, subscapular 25 mm, suprailiac 34 mm. Pseudogynecomastia measured 8 x 9 cm. The skin was very dry and the face very wrinkled (*Figure 8*).

Fig. 8: Patient I.Č. aged 58 years

The patient had brown hair with very few white hairs, a saddled nose, crowded and neglected teeth and a hoarse voice. He had no beard and had never shaved. He had no axillary hair but had scattered pubic hair. The scrotum was rugated, the testes hypoplastic, very soft, the right testis was less than 1ml in volume, the left 1 ml. The stretched length of the penis was 6 cm, the width 1 cm. I revisited the patient in 2007 when he was 77 years old. He was living in the Institution for old people in the town Krk. He became more obese than before, his hair became completely white-grey and his face extremely wrinkled and the voice very hoarse. This time he did not allow me to take his photo. He explained that he was smoking a lot in last decade but he stopped to smoke few years before my visit due to mild heart infarction. He died two years later, in 2009, due to a new heart infarction when he was 79 years old.

Patient E. H (a-12, Table 1) was born May 1976. Her paternal grandmother was short-145 cm-and she was probably related to pt.a-11. Birth length was 47 cm, weight 3015 g. Short stature was registred at age 3.5 years. Growth hormone and TSH deficiencies were diagnosed at the Department of Pediatrics in Rijeka. Beginning at age 4, when she measured 87 cm, she was treated with L-thyroxine (50 mcg/day) and rh GH (Genotropin Kabi). According to the local doctor she took the drugs irregularly. At age 10 she measured 122 cm (height SDS: -2.3).

When examined in 1988 she was 14 years old, measured 142 cm (height SDS: -2.85) and weighed 45.5 kg. Besides short stature, moderate obesity and delayed puberty were registered. Skinfold measurements were: triceps 21 mm, suprailiac 20 mm and subscapular 21 mm. Axillary and pubic hair were scored as "+" and breasts were Tanner stage 2. (*Figure 9*)

Fig 9: Patient E.H. aged 14 years

Analysing medical records in Outpatient clinic it was stated she was treated by thyroxine and growth hormone since 4 years of age until age 17 years when she reached her final height of 164 cm. Secondary sexual characteristics appeared at 13 years, menarche at 16 years. Menses were irregular and absent for long periods. She is now 41 years old, she did not marry and lives alone.

Patient M. H. (a-13, Table 1) was born in 1996. He has the same family as one of the previous patient (a-12, Table 1). Growth hormone deficiency, secondary hypothyroidism and hypogonadism have been diagnosed at the age of 8 years in 2004 when he started to be treated by rhGH and tyroxine at the Department of Pediatrics, University Medical Center Rijeka. The patient has visited the Outpatient clinic in Baška few times due to acute respiratory infection.

A visit to the cemetery of Jurandvor revealed the graves of the deceased patients. The tombstones located on the tombhouse of pt. b-3 and pt.b-5 revealed that despite a 6 year difference in age, the two brothers died one day after the other. Notably, the tombstones also carried photographs of the patients. The appearance is consistent with hypopituitarism (*Figure 10 and 11*)

Figure 10: The tombhouse of patients b-3 and b-5 (Table 1) in the cemetery of Jurandvor

Figure 11: The photo and inscription of two dwarfed brothers (b-3, b-5, Table 1) from Jurandvor.

DISCUSSION

Though the hereditary dwarfism of Krk has been known since 1864 and several investigators had found that these patients have been suffering from an autosomal recessive type of MPHD, it was

our investigation that showed that their dwarfism and pituitary insufficiencies were due to a mutation of the PROP-1 transcription factor required for embryologic development of the pituitary cells producing GH, PRL, TSH and FSH/LH (12). Besides PROP-1 many other transcription factors which could influence morphogenesis and differentiation of pituitary cells like HESX1, LHX3, LHX4, PIT-1, SIX6, SOX2, SOX3, GLI2 and OTX2 were described (13, 14, 15, 16, 17). Their mutations could be involved in pituitary hormones insufficiency.

Different PROP-1 mutations, also the same as described in patients from the island Krk have been found in numerous countries.[18, 19, 20, 21] Similar mutations of PROP-1 genes were found in humans and dwarfed Ames mice who also suffer of hormone deficits caused by loss of function of pituitary cells. Brown-Borg HM noticed that these mice had normal dimensions at delivery but later retarded growth was registered and adult specimens can reach only one third of length and weight of non-mutant animals. She reported also that Ames mouse live 49% -69% longer (males and females, respectively) than their normal counterparts [22, 23]

We also observed that majority of patients with MPHD from Krk had normal or even high birth weight and normal or small birth length. In many patients retarded growth was observed at four years or when they entered school. Similar pattern of growth was described in other patients suffering of PROP-1 mutations.[24] Due to data of prolonged life span in Ames mice with PROP-1 mutation we were interested also in longevity of patients from Krk with the same mutation. It is why I visited the patients also after our first investigation, trying to get some new data about their life, morbidity, therapy and maybe mortality reasons.[25]

We succeeded to analyse some data of patients which we examined in Outpatient clinic in Baška 25 years before.

Only two the youngest patients were treated regularly by thyroxine and growth hormone since the age 4 and 8 respectively, while two other older patients were taken thyroxine prescribed irregularly or not at all.

All the patients refused sex hormone therapy except one patient (a-10, Table 1) who was receiving substitutional estrogen-progesteron therapy and reached the highest final height of 152 cm. In other untreated adult patients the final height was reported 106cm to 142 cm in males and 112cm to 133 cm in females. Until the early 70ies the deficiencies of the anterior pituitary hormones had not been treated as growth hormone was not available. In

untreated adult patients with GH deficiency the lifespan was reported to be 10 years shorter than in healthy persons.[26] In dwarfed patients from Krk the life span was not shortened , in the contrary, many patients lived long, not proportionally so much as mice with the same mutation, but some of them lived longer than 80 years , even 90 years. This age is more than the mean reported for the Croatian population: 71.7 years for males and 79.1 for females.[27] Some patients had shortened life due to accidents or malignancies what was the cause of death of 70 years old patient with risky pattern of life. All other patients died due to neurovascular and cardiovascular diseases what use to be the most frequent cause of deaths in adults.

We found out that in last 50 years only two patients with MPHD due to PROP-1 mutation were born in the island. In 19th century and in the beginning of 20 th century the migration of population in the island was negligible, it is why consanguinity was very common. Visiting the cemetery of Jurandvor where majority of patients with MPHD are buried, we observed only 3 or 4 family names on tombstones. In last decades consanguinity became less frequent ,maybe due to increased mobility, maybe it is also the reason why there are not many new patients suffering of MPHD due to Prop-1 mutations in the island. As the MPHD due to PROP-1 mutation is autosomal recessive, new cases of the disease are possible in future as carriers of this disorder are clinically healthy.

CONCLUSION

Using modern molecular genetic techniques we succeeded to clarify the etiology of hereditary dwarfism which has been known for150 years. We found out that the consanguineous population of MPHD patients on Krk due to a PROP-1 mutation could have a long life span despite their untreated congenital absence of growth and sex hormones, and irregular treatment of thyroid hormones.

REFERENCES

1. Rimoin DL, Horton WA. Short stature. J Pediatr 1978; 92: 697-704.
2. Rimoin DL, Borochowitz Z, Horton WA. Short stature-physiology and patology. West J Med 1986; 144: 710-21.
3. Wagner von Jauregg. Uber marinen Kretenismus.Wiener Klin Wchschr 1906; 19: 1273-1278.

4. Hanhart E. Uber heredodegenerativen Zwergwuchs mit Dystrophia adiposo-genitalis. Arch Klaus Stift 1925; 1: 235-253.
5. Vojska V. Beitrag zur Erforschung des Zwergwuchs-problems. Arch Rassen U Ges Biol 1940; 34: 433-469.
6. Fraser GR. Studies in isolates. J Genet Hum (Paris) 1964; 13: 32-46.
7. Zergollern LJ. A follow up on Hanhart's dwarfs of Krk. Birth Defects Original Article Series 1971; 7: 28-32.
8. Kopajtić B, Brozović Z, Dujmović M, Lolić V. Hereditary dwarfism in the island Krk. Proceedings Third Yugoslav Endocrinological Scientific Meeting, Sarajevo, Yugoslavia 1972; 221-227 (in Croatian).
9. Jerkunica I, Kopajtić B, Novak V. First results of pituitary hormone determination in dwarfs from island of Krk. Proceedings First Yugoslav Congress Nuclear Medicine, Split, Yugoslavia, 1974; 117-119 (in Croatian).
10. Kopajtić B, Kolacio Z, Brozović Z, Smokvina D, Dujmović M. Hereditary dwarfism in the island Krk. Proceedings First Yugoslav Endocrinological Congress, Ljubljana, Yugoslavia, 1975; 209-214 (in Croatian).
11. Kopajtić B, Kolacio Z. Hereditary dwarfism of island Krk. First European Congress Endocrinology, Copenhagen, Denmark, 1987; 159 (Abstr #21-607).
12. Kržišnik C, Kolacio Z, Battelino T, Brown M, Parks JS, Laron Z. The "little people" of the island of Krk revisited. Etiology of hypopituitarism revealed. J End Gen 1999; 1: 9-19.
13. Cohen LE. Genetic disorders of the pituitary. Cur Opin Endocrinol Diabetes Obes 2012; 19: 33-9.
14. Pfaffle R, Klammt J. Pituitary transcription factors in the aetiology of combined pituitary hormone deficiency. Best Pract Res Clin Endocrinol Metab 2011; 25: 43-60.
15. Prince KL, Walvoord EC, Rhodes SJ. The role of homeodomain transcription factors in heritable pituitary disease. Nat Rev Endocrinol 2011; 7: 727-37.
16. Tajima Z, Ishizu K, Nakamura A. Molecular and clinical findings in patients with LHX4 and OTX2 mutations. Clin Pediatr Endocrinol 2013; 22: 5-23.
17. De Graaff LC, Argente J, Veenma DC et al. PROP1, HESX, POU1F1, LHX3 and LHX4 mutation and deletion screening and GH 1 P89L and IVS3+1/+2 mutation screening in a Dutch nationwide cohort of patients with combined pituitary hormone deficiency. Horm Res Paediatr 2010; 73: 363-71.
18. Obermannova B, Pfaeffle RW, Zygmunt-Gorska A et al. Mutations and pituitary morphology in a series of 82 patients with PROP 1 gene defects. Horm Res Paediatr 2011; 76: 348-54.
19. Kandemir N, Vuralli D, Taskiran E et al. Frequency of mutations in PROP-1 gene in Turkish children with combined pituitary hormone deficiency. The Turkish Journal of Pediatrics 2012; 54: 570-75.
20. Dusatkova P, Pffafle R, Kržišnik C et al. Genesis of two most prevalent PROP1 gene variants causing combined pituitary hormone deficiency in 21 populations. Eur J Hum Genet 2016; 24: 415-20.
21. Navardauskaite R, Dusatkova P, Obermanova P et al. High prevalence of PROP 1 defects in Lithuania; phenotypic findings in an ethnically homogenous cohort of patients with multiple pituitary hormone deficiency. J Clin Endocrinol Metab 2014; 99: 299-306.

22. Brown-Borg HM, Bartke A. GH and IGF-1 roles in energy metabolism and long-living GH mutant mice. J Gerontol A Biol Sc 2012; 6: 652-60.
23. Brown-Borg HM, Borg KE, Meliska CJ, Bartke A. Dwarf mice and the ageing process. Nature 1996; 384: 33-33.
24. Lebl J, Vosahlo J, Pfaeffle RW et al. Auxological and endocrine phenotype in a population-based cohort of patients with PROP 1 gene defects. European Journal of Endocrinology 2005; 153: 389-96.
25. Kržišnik C, Grgurić S, Cvijović K, Laron Z. Longevity of the hypopituitary patients from the island Krk: a follow-up study. Pediatr Endocrinol Rev 2010; 7: 357-62.
26. Besson A, Salemi S, Galalti S et al. Reduced longevity in untreated patients with isolated growth hormone deficiency. J Clin Endocrinol Metab 2003; 88: 3664-7.
27. CIA World Factbook. Croatia 2009. Retrieved April 2, 2010 from the World Wide Web: http://www.cia.gov/library/publications/the-world-factbook/geos/hr.html.

CIRIL KRŽIŠNIK, MD, PHD
PEDIATRICIAN ENDOCRINOLOGIST, SENIOR COUNCILLOR
PROFESSOR EMERITUS
UNIVERSITY OF LJUBLJANA, FACULTY OF MEDICINE
VRAZOV TRG 2, 1000 LJUBLJANA, SLOVENIA
E-MAIL: CIRIL.KRZISNIK@MF.UNI-LJ.SI

FEBRUARY 2017

BORN SMALL FOR GESTATIONAL AGE - LONG-TERM CONSEQUENCES

Corina Paul, Iulian P. Velea

Introduction

Children born small for gestational age (SGA), present disturbances of growth and pubertal development and are, also, at risk to develop cardiovascular and metabolic diseases like obesity or diabetes.

Definition

According to the SGA Consensus Conference in 2007, a newborn baby should be considered SGA if birth weight and/or birth length is less than -2 DS for the gestational age, using population-specific standards.

This definition, accepted both in Europe and USA, includes a large spectrum of patients, from short normal babies (constitutional smallness) to IUGR (intrauterine growth restriction) infants.[1] Based on this definition (birth length and/or birth weight), approximately 5% of the newborn babies will be labeled as SGA. Up to 90 % of these, will exhibit rapid and early catch-up growth, leading to normal weight and length for age, while, the other 10 % SGA children, who do not exhibit catch-up growth during the first 2 years of life, will remain short throughout childhood, and, have an increased risk to remain short as adults [2,3] The percentage of non catch-up growth children, varies between countries and, seem to be

higher in the Third World countries, because of the low socio-economic status and the high frequency of the gastrointestinal infections. [4]

Causes of SGA

Concerning the etiology of SGA, numerous factors, either physiological or pathological, may be involved.

The most important physiological factors that influences birth weight and length include: maternal factors: maternal height and weight (pre-pregnancy), age of the mother (<16 or >35 y), parity, ethnicity or history of SGA at previous pregnancies, and, also, paternal and fetal factors.[2]

The social conditions and the pathology of the mother may also contribute to an insufficient growth of the fetus. A low socio-economic status of the pregnant woman, associated with malnutrition, will create conditions for SGA. Of the environmental factors, cigarette smoking is the most important cause of restricted intrauterine growth.

Data from literature are showing a linear correlation between the number of cigarettes smoked per day and the degree of growth retardation.[5]

The effects of coffee consumption on the fetus have also been studied, but the results are controversial. Some recent data are showing that a high caffeine intake during the last trimester of pregnancy may be a risk factor for fetal growth retardation, in particular if the fetus is a boy. [6]

Maternal diseases cause restriction of fetal growth. Infections like rubella, cytomegalovirus, toxoplasmosis or herpes virus causes SGA. Pregnancy associated hypertension, eventually complicated with preeclampsia is the most important.

Other pathological conditions of the mother, associated with SGA babies include: severe chronic infections/diseases, malignancies, anemia, abnormalities of the uterus, abnormalities of the placenta etc. [2]

Fetal causes of SGA include genetic syndromes and chromosomal disorders, hormonal deficiencies (caused by mutations in the GH–IGF1 axis, multiple gestations and infections.[7]

Short stature, GH secretion and puberty in SGA born children

Children who are born SGA may catch-up growth and recover the growth deficit until adulthood, whereas others will not recover, ending up short as teenagers and, later, as adults. [2]

SGA born children do not, usually, associate GH deficiency but the literature data show that they may present either low secretion of growth hormone or reduced sensitivity to GH. [4]

Being born SGA has a significant impact on the timing and progression of puberty. These children seem to present more often with precocious pubarche/adrenarche and, sometimes with an earlier onset of puberty, compared with AGA born children, age comparable. Insulin seems to have an important role in the pubertal tempo and pubertal height gain in SGA born girls.[8]

Despite their GH secretion/sensitivity status, most of the short SGA born children treated with growth hormone have a satisfying response to treatment.

Pathology associated in children born SGA

Being born SGA not only influences growth, but also influences health throughout adulthood, with the possible occurence of obesity, type 2 diabetes mellitus, polycystic ovary syndrome or cardiovascular diseases (CVD).

The increase in SGA infant recovery has been associated with subsequent obesity. As obesity is associated with insulin resistance and all connected elements-hypertension, dyslipidemia and low glucose metabolism, the observed relationship between SGA at birth and the subsequent metabolic syndrome, as a precursor of CVD, can be mainly mediated by obesity. [9-12]

Aiming to analyze the long-term consequences of being born SGA, a french study was conducted in adults to evaluate the risk of unhealthy metabolic obesity in patients born SGA compared to those born AGA.[13] The study found that the risk of belonging to the unhealthy metabolic phenotype is higher in young SGA- born obese adults, compared to their AGA-born counterparts. Results from this study (the Hagenau study) showed that being born SGA, in these patients, contributed significantly to the risk of metabolic syndrome, and, also to a higher level of IR in these patients. This greater likelihood of developing metabolic syndrome for individuals

born SGA, could be related to both weight gain (catching-up process) and the fetal programming it self. [14,15]

Many other studies have shown that children born SGA had a higher insulin resistance than AGA infants. There is a compensatory increase in insulin secretion in SGA patients and the relationship between insulin sensitivity and insulin secretion is best described by a hyperbolic function.[16,17] When insulin sensitivity varies, a proportional and reciprocal change in insulin secretion is required to maintain a constant glucose tolerance.[18] If insulin secretion does not change properly, a low glucose tolerance will develop and, ultimately, type 2 diabetes mellitus. [19] In addition to the decreasing insulin sensitivity, in SGA children and adolescents, there also have been reported higher systolic blood pressure and, most of the time, hypercholesterolemia. [20-22]

Epidemiological studies conducted over the years have shown that type 2 diabetes, hypertension and obesity occur more frequently in adults who were born with low birth weight. Insulin is believed to play a key role in the pathogenesis of this syndrome and insulin sensitivity and risk factors for cardiovascular disease have been investigated in short prepubertal children born small for gestational age (SGA).

One study that analyzed almost 30 children born SGA, by multiple glucose tolerance tests and compared results with a control group of gestational age-appropriate weight short children (AGA), revealed that in children with SGA, the mean insulin sensitivity (Si) in children with SGA was significantly reduced in almost 40 %. The mean acute insulin (AIR) response was significantly higher in SGA children compared to short AGA controls. Although metabolic syndrome has been described in adulthood, this study has shown that the risk factors for the development of type 2 diabetes and cardiovascular disease are already present in childhood in prepubertal children born SGA, suggesting a type 2 diabetes mellitus. [23]

Further studies are needed to evaluate a possible relationship between the prior SGA status and insulin resistance, regardless of weight. It was been hypothesized that AGA-born obese children improved their insulin resistance during weight loss, more effectively, compared to obese children who had SGA status.

A one-year follow-up study was conducted in the primary care system in Germany, where 341 obese children (8% SGA, mean age 10.5 ± 0.1 years, body mass index (BMI) 27,7 ± 0.2, Standard Deviation BMI (SDS) (2.47 ± 0.02] were included in the study. The

Insulin Resistance Index (HOMA), blood pressure(BP), glucose, and insulin were monitored in all children before and after 1 year of treatment. [24]

The decrease in body weight predicted the change in HOMA index in the obese children participating in a lifestyle change programme. HOMA index changes have also been predicted by the former SGA status, that influences insulin resistance. [24]

Recent reports indicate that prematurity itself could have negative consequences for the cardiovascular risk factors. Compared with those born on term, preterm infants became young adults that present higher blood pressure and higher glucose levels, whether or not they were born SGA.[25]

In contrast, other authors reported that among children born preterm, only those who were born SGA had an increased systolic blood pressure and pulse wave velocity, which is a measure for arterial stiffness. [26]

One study aiming to investigate whether prematurity has an independent influence on insulin sensitivity (Si), β-cells function and other cardiovascular risk factors, was conducted in a large population of SGA children. The authors assumed that premature birth-SGA is associated with a worse cardiovascular risk profile, than that of a full term- SGA born baby. To test this hypothesis, preterm born SGA children with full-term born SGA children were compared. The study group included 479 prepubertal short children, born SGA, divided into two groups, on the basis of their gestational age: 1) preterm (gestational age < 36 wk), and 2) at term (gestational age ≥ 36 wk).

Preterm SGA children had a significantly higher systolic and diastolic blood pressure (BP)-SDS than term SGA children and this remained so after adjustment for possible confounding factors (age, sex, ethnicity, birth weight SDS, birth length SDS, BMI SDS, and height SDS). In addition, the percentage of children with a high systolic BP was higher in preterm SGA children (26.3%) compared with term SGA children (16.8%).

The conclusions of the above mentioned study were that premature SGA born children, present a significantly higher systolic and diastolic pressure and a lower percentage of SDS body fat. Serum lipid levels, muscle mass and body fat distribution were comparable for preterm SGA and full term SGA children. [27]

Notably, more than 25% of all premature SGA children had high systolic BP according to the modified Adult Treatment Panel III criteria for children.[28] An elevated blood pressure in childhood is

known to be associated with an increased risk for the development of hypertension in adulthood. [29] The conclusion of the mentioned study was that premature birth of SGA children has significant effects on several cardiovascular risk factors. While premature SGA children seem to have higher systolic and diastolic blood pressure, they present a lower percentage of body fat and higher insulin secretion than SGA children- born at an appropriate gestational age.

Conclusions

SGA children are more predisposed to present adult height deficit possibly related also to an earlier onset and faster progression of puberty.

Being born SGA has a significant impact on the future metabolic status in adulthood. A higher insulin resistance and also, an increased BMI have been demonstrated in prepubertal chidren who were born SGA. Obesity, type 2 diabetes mellitus or cardiovascular diseases (CVD) may occur in later life in the SGA born children.

Premature SGA born children, present a significantly higher systolic and diastolic pressure and a lower percentage of SDS body fat than the children born SGA full term.

References

1. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A: Management of the child born small for gestational age through to adulthood: a consensus statement of the international societies of pediatric endocrinology and the growth hormone research society. J Clin Endocrinol Metab 2007;92: 804–810
2. Wolmann HA Children born small for gestational age : definition and etiology in Kiess W, Chernausek SD, Hokken Kolega ACS (eds): small for gestational age. Causes and consequences Pediatr Adolesc Med Basel, Karger 2009, vol13, pp 1-10
3. Karlberg J, Albertsson-Wikland K: Growth in full-term small-for-gestational-age infants:from birth to final height. Pediatr Res 1995;38: 733–739.
4. Dahlgren J Management of short stature in Small for Gestational Age children in Kiess W, Chernausek SD, Hokken Kolega ACS (eds): Small for gestational age. Causes and consequences Pediatr Adolesc Med Basel, Karger 2009, vol13, pp 116-126
5. Stillman RJ, Rosenberg MJ, Sachs BP: Smocking and reproduction . Fertil Steril 1986: 46; 545-566.

6. Vik T, Bakketaeig, Trygg KU et al High Caffeine consumption in the thrid trimester of pregnancy: gender specific effects on fetal growth. Pediatr Perinat Epidemiol2003: 17: 324-331.
7. Clarke WL Born Small for Gestational Age- A lifelong Disorder in Clinical issues in pediatric Endocrinology, Ed Mirton 2012, 35-41
8. Paul C ,Velea IP Growth and puberty in children born SGA In Velea IP, Paul C,Brink JS(eds) Theoretical and practical update in Pediatric Endocrinology and Diabetes, Ed Mirton 2014 ;217-226
9. Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth. *Diabetologia* 2005;48:849–55.
10. Ibanez L, Ong K, Dunger DB, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91:2153–2158
11. Reinehr T, Andler W, Denzer C, Siegried W, Mayer H, Wabitsch M.Nutr Metab Cardiovasc Dis. 2005 Jun;15(3):181-7
12. Ebbeling CB, Pawlak DB, Ludwig DS. Childhood obesity: publichealth crisis, common sense cure. Lancet. 2002;360:473-82
13. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB.BMJ. 2000 Apr 8;320(7240):967-71. Erratum in: BMJ 2000 May 6;320(7244):1244.
14. Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"? Am J Hum Genet 1962;**14**:353.
15. Cutfield WS, Jackson WE, Jefferies C, et al. 2003 Reduced insulin sensitivity during growth hormone therapy for short children born small for gestational age. J Pediatr I42:II3-6
16. Arends NJ, Boonstra VH, Duivenvoorden HJ, Hofman PL, Cutfield WS, Hokken-Koelega AC 2005 Reduced insulin sensivity and the presence of cardiovascular risk factors in short prepubertal children born small for gestational age (SGA). Clin Endocrinol (Oxf) 62:44-50
17. Kahn SE 2003 The relative contribution of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 46:3-19
18. Cnop M, Vidal J, Hull RL, et al. 2007 Preogressive loss of beta-cell function leads to worsening glucose tolerance in first-degree relative of subjects with type 2 diabetes. Diabetes Care 30:677-82
19. Sas T, Mulder P, Hokken-Hoelega A 2000 Body composition, blood presure, and lipid metabolism before and during long-termn growth hormone (GH) treatment in children with short stature born small for gestational age either with or without GH deficiency. J Clin Endocrinol Metab 85:3786-92
20. Leon DA, Johansson M, Rasmussen F 2000 Gestational age and growth rate of fetal mass are inversely associated with systolic blood presure in yound adolts: an epidemiologic study of 165,136 Swedish men aged 18 years. Am J Epidemiol 152:597-604
21. Tenhola S, Martikainen A, Rahiala E, Herrgard E, Halonen P, Voutilainen R 2000 Serum lipid concentration and growth characteristics in 12-years-old children born small for gestational age. Pediatr Res 48:623-8
22. N. J. T. Arends, V. H. Boonstra, H. J. DuivenvoordeP. L. Hofman, W. S. Cutfield, A. C. S. Hokken-Koelega Reduced insulin sensitivity and the presence of cardiovascular risk factors in short prepubertal children

born small for gestational age (SGA http://onlinelibrary.wiley.com/doi/10.1111/j.1365 2265.2004.02171.x/full

23. Thomas Reinehr, Michaela Kleber, Andre Michael Toschke Former small for gestational age (SGA) status is associated to changes of insulin resistance in obese children during weight loss. http://onlinelibrary.wiley.com/doi/10.1111/j.1399-5448.2009.00624.x/abstract;jsessionid=5AF0B79E4A0E6EDAB1CA7D89E8072D37.f02t02
24. Lopez-Bermejo A, Casano-Sancho P, Fernandez-Real JM, et al. 2004 Both intrauterine growth restriction and postnatal growth influence childhood serum concentrations of adiponectin. Clin Endocrinol (Oxf) 61:339-46
25. Van Dijk M, Willemsen RH, Mulder PGH, van Toorenenbergen AW, de RijkeYB, Hokken-Koelega ACS 2007 Changes in insulin sensitivity are associated with change in adiponectin levels during 24 months of growth hormone (GH) treatment in short children born small for gestational age (SGA)(Chapter 4 in thesis) Pediatric Endocrinology. Erasmus University, Rotterdam, pp72-83
26. Ester W, Bannink EM, van Dijk M, Willemsen RH, van der Kaay DC, de Ridder M, Hokken-Koelega ACS 2007 Subclassification of small for gestational age children with persistent short stature: growth patterns and response to GH treatment. *Horm Res* 6:89–98
27. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S 2004 Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 350:2362–2374
28. Bao W, Threefoot SA, Srinivasan SR, Berenson GS 1995 Essential hypertension predicted by tracking of elevated blood pressure from childhood to adulthood: the Bogalusa Heart Study. *Am J Hypertens* 8:657–665

Corina Paul MD, PhD
Lecturer, University of Medicine and Pharmacy "Victor Babes" Timişoara,
Senior Pediatrician, Clinic II Pediatrics, "Pius Branzeu" Clinical County Hospital, Timişoara, Romania.
Address: 1 - 3, Evlia Celebi str, 300226, Timişoara, Romania
E-mail: paulcorina@yahoo.com

March 2017

NON-CLASSICAL EFFECTS OF THE VITAMIN D IN PEDIATRIC PATHOLOGY

Iulian P. Velea, Corina Paul

Introduction

For more than a century it has been known that vitamin D is the main regulator of phosphocalcic homeostasis, intervening in the renal, intestinal, bone, and parathyroid glands. In these target organs the intervention is carried out by the active form of vitamin D (calcitriol) acting upon binding to a specific receptor belonging to the steroid / thyroid hormone receptor family.

How Vitamin D acts at the cellular level through cellular receptors has led to the idea that vitamin D behaves more like a hormone than a vitamin.

Recent years have brought more and more evidence of the effects of vitamin D and its metabolites in a large number of tissues. Experimental animal models have led to the involvement of vitamin D in neurological functions (thus contributing to improved sleep, decreasing anxiety and depressive episodes).[1] It also has metabolic, cardiovascular[2], endocrine[3] effects especially in relation to the immune system[4] by increasing the body's defense capacity (*Table 5.1.*).

In the same sense, it has been shown that when vitamin D levels increase, muscle strength increases, suggesting a possible association with muscle function.[5,6]

All these effects support the idea that the vitamin D hormonal function is a "highly evolutionary" endocrine function, whereas a more primitive role than that of a cytokine capable of protecting the host of bacterial invasion is apparently responsible for certain

immune functions of vitamin D.[5] The best evidence is the dietary supplements based on "fish oil" received by children from the early days of life, more than half a century ago in the regions of Norway, and which practically rarely fell ill.

Table V.1.

Non-classical 1a,25-dihydroxyvitamin D3 target tissues in VDR null mice models [12]

Tissue	Feature
Skin	Alopecia Abnormal hair cycling Anti-proliferative and pro-differentiative effect on keratinocytes
Muscle	Smaller muscle fibers Persistent expression of early markers of myogenic differentiation
Cardiovascular system	High renin hypertension Cardiac hypertrophy
Immune system	Impaired macrophage function Abnormal Th1 macrophage-induced formation Increased number of immature dendritic cells Predisposition to autoimmune diseases such as type 1 diabetes Decreased monocytic differentiation and antibacterial activity of monocytes
Pancreas beta cells	Normal glucose tolerance or mild glucose intolerance Is a target tissue with discrepancy between effects of the vitamin D ligand and the VDR it self
Brain	Abnormal behavior, especially muscle and motor behavior
Cell proliferation / cancer	Hyperproliferation of colonic cells Dysregulated growth of alveolar and ductal mammary gland cells Enhanced susceptibility to chemocarcinogen-induced leukemia
Reproductive system	Uterine hypoplasia Impaired ovarian folliculogenesis Male infertility

Vitamin D Metabolism

It is known that vitamin D is found in two forms: vitamin D3 and vitamin D2, known as ergocalciferol and cholecalciferol, respectively. Vitamin D3, synthesized in the skin under the action of ultraviolet rays, is the most important source of the vitamin D in the body and is also called "*sun vitamin*".

Researchers have estimated that in most country, vitamin D is in majority (80-90%) from endogenous sources. However, this percentage varies among people around the world due to different exposure times to ultraviolet radiation (UVB). But, in the other

hand, the amount of UVB radiation varies depending on latitude, altitude, hour, air quality, month, year.

The exogenous vitamin D source from food is either vitamin D3 (derived from food of animal origin: fish oil, egg yolk, liver, etc.) or D2 (ergocalciferol) found in some plants.

Regardless of origin (endogenous or exogenous), both forms of vitamin D reach the liver where the vitamin D binding protein (DBP) is present. At this level, under the action of 25-hydroxylase, both forms are converted (hydroxylated) into 25 (OH) D3 and 25 (HO) D2 circulating linked transport protein (DBP)[7] respectively.

Having an active period of 2-3 weeks, the serum level of 25(HO)D is basically the indicator of the vitamin D status in the body for which reason the current value of this intermediate product is used. 25(OH)D undergoes a second hydroxylation on the kidneys and results the active form of $1,25(OH)_2D3$ acting as an endocrine modulator of Ca and P homeostasis. Once released into circulation, it is transported by DBP to the bones parathyroid, liver, intestine.[8,9]

Some evidence suggests that vitamin D2 could be metabolized faster than vitamin D3 [10], but with regular daily use, they can be considered bioequivalent to maintain serum vitamin D (HO) levels.[11]

Thus, vitamin D is more of a pro-hormone than a real vitamin, the characteristics of the active metabolite $1,25(OH)_2D$ being those of a hormone. On the one hand, the structure of $1,25(OH)_2D$ is similar to the other steroid hormones and $1,25(OH)_2D$ interacts with a single vitamin D receptor (VDR) to realise biological functions.

All this behavior supports the statements of those who consider vitamin D as "hormone D".

The vitamin D receptor (VDR)

VDR is a phosphoprotein that is part of the nuclear receptor superfamily and functions as a ligand-dependent transcription factor. VDR binds to the retinoid X receptor also found in the regulatory regions of several target genes such as osteocalcin, osteopontin, calbindin-D28K, TGF-β2, 24-hydroxylase.

It is also worth noting that a number of factors such as glucocorticoids, retinoid estrogens can modulate VDR levels.[13]

Prevalence of vitamin D deficiency in children and adolescents

The American Society of Endocrinology, in agreement with the Vitamin D Expert Group, defined vitamin D *deficiency* as the 24-hour serum vitamin D level of less than 20 ng / ml, vitamin D *insufficiency* when the serum level ranges from 20 And 30 ng / ml and the *optimal level* when the serum level is greater than 30 ng / ml.[14] Studies conducted in the U.S. in children and adolescents shows that the cumulative percentage of deficient and insufficient cases exceeds 50% of the pediatric population.

Obtaining and maintaining minimum levels of vitamin D

In recent years, more and more voices recognize that hypovitaminosis D is a problem that affects most countries. Although the guidelines recommend supplementing vitamin D consumption during pregnancy and after birth throughout the first year of life, sometimes up to 2 years of age, the results of studies have shown the existence of a large number of children with hypovitaminosis D. In these conditions, the main question which is to be *"how to obtain and, most importantly, maintain the optimum minimum level of vitamin D?*" There are also questions about the rhythm of the supplement: daily? weekly? monthly? or even less? Unfortunately, to date, these questions have not led to a widely accepted response because there is no universal guide to how to supplement vitamin D. Common recommendations involve administering 400-1000 IU / day (10-25 mg / day) during the first year of life, 600-1000 IU / day (15-25 mg / day) in children over 1 year and 1500-2000 IU / day (from 37.5 to 50 mg / Day) over 18 years of age and adults.[15]

Another problem that occurs is the correction of hypovitaminosis D, namely the rapid normalization of vitamin D levels (25HO vitamin D as well as vitamin D deposits). There are authors recommending rapid correction using high doses of "stoss" of 300,000-600,000 i.e. The argument in favor of this option is that although vitamin D HO 25 serum increases rapidly, the PTH level decreases more slowly. [16,17]

Other authors support the idea of using the accepted alternative regimen only if there is low adherence to treatment, in most cases the daily doses of 800-2000 IU / day (20-50 mg / day)

are useful. Long-term supplements are recommended in risk groups because a daily dose of 600 IU in case of insufficient or no sun exposure and consumption of vitamin D by diet are not able to maintain a level of 25(OH)D above 20 ng / ml.[18,19]

In accordance with the recommendations of the Clinical Guide developed by the Endocrinology Society, the treatment of hypovitaminosis D should take into account the age of the patient.[20]

Carrier protein vitamin D

The transport of any form of vitamin D (D3 or D2) is performed using a carrier protein (DBP) belonging to the alpha-fetoprotein family, a protein encoded by the GC gene (also known as GBP, GRD3, VDBG, VDBP, DBP / GC, NCBI gene) located on chromosome 4 (4q12-13). In the case of the GC gene, two polymorphisms of rs4588 (T436K) and rs7041 (D432E) have been identified, which combine to produce three major isoforms of the DBP serum, namely GC-1f, GC-1s, GC-2. The prevalence of the 25 (HO) D deficiency in children and adolescents is supposed to be that the GC gene polymorphisms are associated with an increased sensitivity to the deficiency of 25 (HO) D.[21]

Vitamin D and immune cell modeling [22]

The first question is: is there a relationship between vitamin D (circulating levels) and autoimmune diseases? This is a problem where more and more studies provide evidence for the link between vitamin D and a range of autoimmune diseases such as type 1 diabetes, multiple sclerosis, inflammatory bowel disease, SLE and rheumatoid arthritis.

Vitamin D directly influences T cells and B and, therefore, their response to activation mode.

The effect of $1.25(OH)_2D3$ in the presence of a specific antigen is to inhibit T cell proliferation. Addition of vitamin D to CD4 inhibits Th1 cell proliferation and cytokine production (decreases IL-2 and IFNγ secretion). The relationship with IL-4 is nevertheless controversial. Some studies have shown that IL-4 production (associated with Th2 cytokine) increases in vivo after treatment with vitamin D, while other authors have negated this effect.

In B cells, vitamin D inhibits the secretion and production of antibodies.

On the other hand, VDR receptor immunity has been demonstrated. After activating vitamin D, the VDR receptor regulates cellular expression of genes in a variety of sensitive tissues having a role in calcium homeostasis as well as in the release of immunomodulatory effects.

Experiments performed on Knock-out mice revealed that VDR needed $1.25(OH)_2D3$ to induce bone marrow cell precursor differentiation into monocytes/macrophages, although their differentiation can also be performed in the absence of VDR.[23] The results of these studies come to encourage future clinical trials to confirm the effect of vitamin D on the immune system, which most likely occurs at multiple levels and has a number of different mechanisms.

Vitamin D and type I diabetes

Type I diabetes is secondary to almost complete destruction of pancreatic β cells. From an etiopathogenic point of view, theories claim that people with a strong genetic predisposition, under the influence of certain environmental factors such as infectious agents, trigger chemical reactions to release a cell-mediated cellular response. This response is directed against pancreatic β cells or their components as autoantigens, a phenomenon that ultimately results in the destruction of cells but at variable rates (velocities).

Over time, various animal studies with autoimmune diabetes have been performed spontaneously. These, along with information gathered over the years that reveal the important role of HLA, have led to the development of the current etiopathogenic concept that shows that the genetic element along with environmental factors can favor the onset of this disease. The onset of type 1 diabetes is the result of initiating an autoimmune process whose target is the pancreatic β cell. In terms of glucose homeostasis, vitamin D has been shown to affect the proliferation and in particular the life of pancreatic beta cells, and is also involved in the etiopathogenesis of type 1 diabetes. Although controversial, studies support the idea that the vitamin D status affects the incidence T1DM and increased prevalence. Analysis of the data obtained in numerous Northern population studies suggests that vitamin D, at optimal concentrations, provides protection against type 1 diabetes or autoantibody development. An example of the results published by Sorensen et al (2012) shows that vitamin D levels in the mother

during pregnancy influence the risk of developing type 1 diabetes in children. Maternal children with low vitamin D had twice as much risk of developing type 1 diabetes compared to mothers with high vitamin D. In another study in northern Finland, Hypponen (2001) found that vitamin D supplementation in childhood reduced the risk of type 1 diabetes by 80% compared to the non-supplemented group. Similar results were obtained in sub-study 2 of the EURODIAB group.

Mohr et al (2010) support the same theory of the protective effect of vitamin D supplements in childhood based on the proportional relationship observed between the progressive decrease in vitamin D dose in children from 2000 IU / day in 1964 to 400 IU / Day in 1992 and the brutal increase in the number of new detected T1D cases.

Polymorphism of the VDR gene may be associated with the risk of type 1 diabetes, although the results of the studies seem to be contradictory.[12]

However, there is experimental evidence that supports the idea of Vitamin D protection against Type 1 diabetes.[12] Thus, administering 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] to non-obese mice (NOD) in pharmacological doses prevents the development of diabetes. This finding is supported by another study in which marked NOD mice with marked vitamin D deficiency developed diabetes at a lower age compared to non-deficient NOD mice. Although the results of experimental studies support the concept that: Supplements of vitamin D administered can prevent Type 1 diabetes, the daily dose that can be administered without side effects has not been established with certainty.

Deficiency of vitamin D and the risk of chronic complications

Once diagnosed, type 1 diabetes in children raises new problems in achieving and maintaining glycemic and metabolic balance and eliminating the risk of degenerative chronic complications.

Greer et al. (2013) showed that in children with type 1 diabetes, serum levels of 25 HO vitamin D were lower compared to the control group consisting of children with type I diabetes mellitus and a group of children with other infectious pathology). The group did not have side effects of hippocampus D, which is why this study

can not determine whether the low level of vitamin D is the result of type 1 diabetes, and in these conditions, vitamin D consumption should be supplemented to prevent complications (osteopenia or increased susceptibility to other diseases) or is a risk factor, requiring supplementation with vitamin D in patients at high risk for type 1 diabetes.[25]

Although lately more and more effective diets and insulin delivery devices (including the insulin pump) have removed the risk of installing chronic complications, their spectrum continues to lie on children and their families. For these reasons, the research was focused on the possible preventive effect of chronic complications. Although vitamin D deficiency is associated with elevated levels of inflammatory markers, no significant differences in vitamin D levels have been found in patients with microangiopathy compared to those without.[26] It is accepted that vitamin D provides protection against inflammation but appears to have an effect on the renin-angiotensin system with an antiproliferative effect on endothelial cells (Tergher G et al., Quoted by [27]). In an experiment on mice with ischemic retinopathy, administration of $1,25(HO)_2D3$ inhibited neovascularization of the retina[28], and in cell cultures inhibited proliferation of endothelial cells[29]. It has also been shown that persistent microalbuminuria is associated with a low level of 25 (HO) D in patients with type 1 diabetes compared to the control group.

The effect of vitamin D supplements on metabolic control

Low levels of 25 (HO) D found in patients with diabetes and in patients with chronic degenerative complications have highlighted potential effects on metabolic balance during disease progression. One such study was focused on analyzing the effect of supplementation with vitamin D at a dose of 0.25 mcg compared to the addition of nicotinamide on the maintenance of beta cell functional reserve. After four weeks no improvement was achieved either in the C-peptide level or in the HbA1c value, but the insulin requirement was lower in the vitamin D supplementary group.

Vitamin D and type 2 diabetes

There is now a lot of evidence suggesting the main role of modified Ca and vitamin D homeostasis in setting DZ type 2.[30]

It is unanimously recognized that insulin secretion is a Ca-dependent process, and therefore any abnormal Ca flux has effects on β-cell secretion. The most plausible explanation would be that inadequate calcium / vitamin D intake can alter the balance between intracellular and extracellular calcium levels.

Likewise, vitamin D has implications for insulin resistance syndrome by stimulating the insulin receptor.[31]

Type 2 diabetes is known to be a disease associated with systemic inflammation and elevated levels of cytokines involved in beta-cell dysfunction leading to apoptosis. Thus, vitamin D, improving insulin sensitivity, can contribute to the survival of β cells by modulating the effects of cytokines. Perhaps the strongest link between type 2 diabetes and vitamin D is metabolic syndrome that includes: obesity, insulin resistance, hypertension and dyslipidemia.[32] Although not all studies confirm a significant association between vitamin D and glucose metabolism, they suggest a relationship between the level of 25(OH)D and HbA1c levels.[33]

The "turntable" between insulin resistance and vitamin D is PTH. The argument in favor of this assertion is that vitamin D deficiency causes a compensatory increase in PTH secretion, and high concentrations of PTH are known to inhibit the synthesis and secretion of insulin from β cells.[34] Observational studies have shown that a daily intake of 500 IU vitamin D reduces the risk of developing Type 2 diabetes by 13% compared to an intake of less than 200 IU / day.

Another theory argues that inflammatory processes are involved in the pathophysiology of various chronic diseases. Based on this, the strong link between type 2 diabetes and the incidence of subclinical inflammation, which supports the concept that diabetes is partly an inflammatory disease, has been demonstrated.[35] On the other hand, many studies have provided evidence that in type 2 diabetes the relationship between Vitamin D and chronic inflammation, together with the insulin resistance phenomenon, can be partially mediated through the immune modulatory properties of $1,25(OH)_2D3$ to regulate the production of proinflammatory cytokines (especially IL-6 and TNFα) [36] but also to modulate the inflammatory response of macrophages and monocytes.[37] The proven link between type 2 diabetes and vitamin D leads to the idea that diabetes mellitus can be considered a risk factor for lowering bone density and even the occurrence of osteoporotic fractures as early as type 1 diabetes can lead to the

onset of osteopenia suggesting the association of hypovitaminosis D with early complications of diabetes.[38]

Vitamin D and increased blood pressure

The analysis of data from children aged 1-21 using the NHANES database 2001-2004 revealed a significantly higher prevalence of high blood pressure and diastolic blood pressure in the vitamin D deficient group (<15 ng / ml). Hypertension was 2.5 times higher than those with vitamin D deficiency (<15 ng / ml) than those with vitamin D 25HO above 30 ng / ml.[39]

All pediatric studies are cross-sectional analyzes that have highlighted associations but causality is not yet proven.

Regarding the effect on cardiovascular disease, some studies have attempted to evaluate the efficacy of the monthly doses of vitamin D versus daily or weekly dosages. The results, although not conclusive, suggest that monthly doses of vitamin D are less effective in preventing disease than daily or weekly doses.[40]

However, this topic can not be closed in the light of the descriptive epidemiological findings on the reverse association between sun radiation and CVD diseases in terms of season, latitude and altitude.[41,42]

Vitamin D and lipid fractions

In addition to insulin resistance and hypertension, vitamin D deficiency has also been associated with a modified lipid profile, so at risk for cardiovascular disease.

Vitamin D is essential for maintaining adequate levels of ApoA-I, a major component of HDL cholesterol. People with high concentrations of 25 (OH) D have the highest serum levels of ApoA-I, which supports a positive correlation between 25 (OH) D and the serum HDL cholesterol level.[43]

In a study in 217 obese children, low levels of HDL cholesterol were associated with a low level of 25 (OH) D. Analysis of NHANES 2001-2004 data showed that in children with 25 (OH) D deficiency <15 ng / ml) had low HDL cholesterol compared to those with levels below 30 ng / ml.

Vitamin D and obesity

In recent years, obesity has become a major health problem worldwide, being associated with the development of cardiovascular disease, hypertension, diabetes and various forms of cancer. As with adults, childhood obesity is a chronic, complex metabolic disease that occurs as a result of the imbalance between consumption and increased caloric intake. It is known that certain environmental factors and food behaviors when appearing on a predisposing genetic plot lead to obesity. At present, there are studies suggesting that 25-hydroxy-vitamin D is associated with obesity, [44] although the mechanisms by which vitamin D deficiency is involved in body weight are only partially recognized.

Some experiments have suggested that vitamin D deficiency may result in greater adiposity by increasing PTH levels [45]. On the other hand, it is known that leptin plays a very important role in the development of obesity, and vitamin D is an essential factor for the production of leptin. Thus, depletion of vitamin D can cause obstructions of leptin synthesis, increases appetite for obesity [46].

Although the results are not conclusive, it is clear from these studies that there is a positive relationship between vitamin D deficiency and obesity, but the relationship: Supplementing vitamin D consumption to low adiposity requires assessments and future studies.

A new direction for the participation of vitamin D in the development of insulin resistance was possible after the discovery of FGF, especially FGF23, and its endocrine action [47], which may play a key role in the early phase of low insulin sensitivity in obese adolescents.[49] However, the exact mechanisms of FGF23 intervention in changes in insulin metabolism in obese patients remain unclear.

In this respect, although there are studies demonstrating the existence of low FGF23 values in people with vitamin D deficiency, not all of these values are confirmed in larger groups.[50]

On the contrary, other recent studies lead to the conclusion that there is a simple correlation between vitamin D levels and insulin resistance in obese adolescents. They show that low levels of vitamin D are most likely the result of obesity and not an independent risk factor for insulin resistance.[51, 52]

Bronchial asthma and vitamin D

Bronchial asthma, a chronic disease whose prevalence in the pediatric population is about 8-10%. Although asthma is a disease that can be controlled by medication, optimal management is not always achieved in all patients. Observational and even randomized studies [53] suggested that vitamin D could play a role in managing asthma.

Based on this idea, studies conducted on groups of children with asthma have highlighted that serum vitamin D levels in children with asthma were lower than those of urine suggesting that there is a correlation between serum vitamin D And asthma in the baby. [54,55,56,57] as low levels of serum vitamin was associated with a higher frequency of respiratory tract infection and more severe asthma.[58]

Another approach to the relationship of vitamin D asthma followed the exacerbation rate. Several observational studies support the idea of a correlation between vitamin D levels and exacerbations of asthma.[59]

The many proposed mechanisms have raised the anti-inflammatory effects of vitamin D as well as the possibility of improving the anti-inflammatory properties of corticosteroids by reducing inflammation and obstruction of the airways.[60]

All of these arguments appear to be viable arguments for supplementing with vitamin D as a potential target in improving the management of asthma in children.

Although many southern people have arguments in favor of the vitamin D relationship - the risk of exacerbating asthma, there is still uncertainty about practical applicability. More studies are needed to determine if there is a difference in the severity of asthma, the initial vitamin D status, and the doses that need to be administered.

References

1. Slinin Y, Paudel ML, Taylor BC, Fink HA, Ishani A, Canales MT, Yaffe K, Barrett-Connor E, Orwoll ES, Shikany JM et al. 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. Neurology 2010 74 33–41. (doi:10.1212/ WNL.0b013e3181c7197b)
2. Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J & Balk EM. Vitamin D and cardiovascular outcomes. Annals of Internal Medicine 2010 152 307–314. (doi:10.7326/0003-4819-152-5-201003020-00009)

3. Dusso A, Gonzalez EA & Martin KJ. Vitamin D in chronic kidney disease. Best Practice & Research. Clinical Endocrinology & Metabolism 2011 25 647–655. (doi:10.1016/j.beem.2011. 05.005)
4. Hewison M. An update on vitamin D and human immunity. Clinical Endocrinology 2012 76 315–325. (doi:10.1111/j.1365-2265.2011.04261.x)
5. (Clinical issues in pediatric endocrinology – Stuart Brink, pag. 123)
6. Holick MF. Vitamin D deficiency. New England Journal of Medicine 2007 357 266–281. (doi:10.1056/NEJMra070553)
7. Elisabetta Romagnoli, Jessica Pepe, Sara Piemonte, Cristiana Cipriani and Salvatore Minisola - Value and limitations of assessing vitamin D nutritional status and advised levels of vitamin D supplementation. European Journal of Endocrinology (2013) :169, R59–R69
8. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B-„Estimation of optimal serum concentrations of 25-hydroxyvitaminD for multiple health outcomes", AM J Clin Nutr 2006;84:18-28.
9. Lips P, Hosking D, Lippuner K, et al- „The prevalence of vitamin D inadequacy amongst women with osteoporosis:an international epidemiological investigation", J Intern Med 2006;260:245-54.
10. Armas LA, Hollis BW, Heaney RP. Vitamin D2 is much less effective than vitamin D3 in humans. J Clin Endocrinol Metab. 2004; 89(11):5387-5391.
11. Holick MF, Biancuzzo RM, Chen TC, et al. Vitamin D2 is as effective as vitamin D3 in maintaining circulating concentrations of 25-hydroxyvitamin D. J Clin Endocrinol Metab. 2008; 93(3):677-681.
12. Patricia Muszkat, Marilia Brasilio Rodrigues Camargo1, Luiz Henrique Maciel Griz, Marise Lazaretti-Castro - Evidence-based non-skeletal actions of vitamin D. - Arq Bras Endocrinol Metab. 2010; 54 / 2, p.110 – 117.
13. Reichrath J. Vitamin D and the skin: an ancient friend, revisited. Experiment Dermatol. 2007;16: 618-25.
14. Rovner, A. J., & O'Brien, K. O. (2008). Hypovitaminosis D among healthy children in the united states: A review of the current evidence. Archives of Pediatrics & Adolescent Medicine, 162, 513–519.)
15. Slinin Y, Paudel ML, Taylor BC, Fink HA, Ishani A, Canales MT, Yaffe K, Barrett-Connor E, Orwoll ES, Shikany JM et al. 25-Hydroxyvitamin D levels and cognitive performance and decline in elderly men. Neurology 2010 74 33–41. (doi:10.1212/ WNL.0b013e3181c7197b)
16. Cipriani C, Romagnoli E, Scillitani A, Chiodini I, Clerico R, Carnevale V, Mascia ML, Battista C, Viti R, Pileri Met al. Effect of a single oral dose of 600,000 IU of cholecalciferol on serum calciotropic hormones in young subjects with vitamin D deficiency: a prospective intervention study. Journal of Clinical Endocrinology and Metabolism 2010 95 4771–4777. (doi:10.1210/jc.2010-0502)
17. Ilahi M, Armas LA & Heaney RP. Pharmacokinetics of a single, large dose of cholecalciferol. American Journal of Clinical Nutrition 2008 87 688–691.
18. Heaney RP & Holick MF. Why the IOM recommendations for vitamin D are deficient. Journal of Bone and Mineral Research 2011, 26 455–457. (doi:10.1002/jbmr.328)
19. Holick MF. The D-batable Institute of Medicine Report: a D-lightful perspective. Endocrine Practice 2011 17 143–149. (doi:10.4158/EP.17.1.143)

20. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. Jun 6 2011;[Medline].
21. Santos BR, Mascarenhas LPG, Boguszewski MCS, Spritzer PM. Variations in the Vitamin D-Binding Protein (DBP) Gene Are Related to Lower 25-Hydroxyvitamin D Levels in Healthy Girls: A Cross-Sectional Study, Horm Res Paediatr 2013;79:162-168 (DOI:10.1159/000348847)
22. Yoav Arnson, Howard Amital, Yehuda Shoenfeld. Vitamin D and autoimmunity: new aetiological and therapeutic considerations. Ann Rheum Dis 2007; 66:1137–1142 (doi: 10.1136/ard.2007.0698)
23. O'Kelly J, Hisatake J, Hisatake Y, Bishop J, Norman A, Koeffler HP. Normal myelopoiesis but abnormal T lymphocyte responses in vitamin D receptor knockout mice. J Clin Invest 2002;109:1091–9.
24. Giulietti, A., Gysemans, C., Stoffels, K., van Etten, E., Decallone, B., Overbergh, L., Bouillon, R. & Mathieu, C. (2004) Vitamin D deficiency in early life accelerates type 1 diabetes in non-obese diabetic mice. Diabetologia 47: 451–462.
25. Greer RM, Portelli SL, Hung BS, Cleghorn GJ, McMahon SK, Batch JA, Conwell LS. Serum vitamin D levels are lower in Australian children and adolescents with type 1 diabetes than in children without diabetes. Pediatr Diabetes. 2013 Feb;14(1):31-41. doi: 10.1111/j.1399-5448.2012.00890
26. S. Devaraj, J. M. Yun, C. R. Duncan-Staley, and I. Jialal, "Low vitamin d levels correlate with the proinflammatory state in type 1 diabetic subjects with and without microvascular complications," American Journal of Clinical Pathology, vol. 135,no. 3, pp. 429–433, 2011.
27. Harleen Kaur, Kim C. Donaghue, Albert K. Chan, Paul Benitez -Aguire, Stephen Hing, Margaret Lloyd, Janine Cusumao, Alison Pryke, Maria E. Craig. Vitamin D Deficiency Is Associated With Retinopathy in Children and Adolescents With Type 1 Diabetes, Diabetes Care 2011, 34:1400–1402.
28. Albert DM, Scheef EA, Wang S, et al. Calcitriol is a potent inhibitor of retinal neovascularization. Invest Ophthalmol Vis Sci 2007;48:2327–2334
29. Mantell DJ, Owens PE, Bundred NJ, Mawer EB, Canfield AE. 1 alpha,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. Circ Res 2000; 87:214–220
30. Sheena Kayaniyil, ravi Retnakaran, Stewart B. Harri8s, reinhold Vieth, julia A. Knight, Hertzel c. Gerstein, bruce A. Perkins, Bernard Zinman, Anthony J. Hanley-„Prospective Associations of Vitamin D with β-Cell Function and Glycemia- The PROspective Metabolism and Islet cell Evaluation (PROMISE) Cohort Study", Diabetes 60:2947-2953, 2011
31. Anastassios G. Pittas , Joseph Lau, Frank HU, Bess Dawson-Hughes- „The Role of Vitamin D and Calcium in type 2 diabetes. A systematic Review and Meta-Analysis", J Clin Endocrinol Metab, 2007 June;92(6):2017-2029
32. National Cholesterol Education Program (NCEP) Expert Panel on detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment panel III): Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143-3421
33. T. Mezza, G. Muscogiuri, G.P. Sorice, A. Prioletta, E. Salomone, A. Pontecorvi, A. Giaccari-„Vitamin D Deficiency: A New Risk Factor for Type 2 Diabetes", Ann Nutr Metab 2012;61:337-348

34. Jung Re Yu, Sang Ah Lee, Jae-Geun Lee, Gil Myeong Seong, Seong Joo Ko, Gwanpyo Koh, Mi-Hee Kong, Keun-Young Park, Byung-Joon Kim, Dong-Mee Lim, Dae Ho Lee-„Serum Vitamin D Status and its Relationship to Metabolic Parameters in Patients with Type 2 Diabetes Mellitus", Chonnam Med J 2013;48:108-115
35. Pradhan A-„Obesity, Metabolic Syndrome, and Type 2 Diabetes: Inflammatory Basis of Glucose Metabolic Disorders", Nutr. Rev. 2007, 65, S152-S156
36. Flores M-„A role of vitamin D in low-intensity chronic inflammation and insulin resistance in type 2 diabetes mellitus?"Nutr. Res. Rev. 2005,18, 175-182
37. Borges MC, Martini LA, Rogero MM-„Current perspectives on vitamin D, immune system, and chronic diseases", Nutrition 2011,27, 399-404
38. Atsushi Suzuki, Motoko Kotaye, Yasunaga Ono, Taiya Kato, Naohisa Oda, Nobuki Hayakawa, Shuji Hashimoto, Mitsuyasu Itoh-„Hypovitaminosis D in Type 2 Diabetes mellitus: Association with Microvascular Complications and Type of Treatment", Endocrine Journal 53:503-510, 2006
39. Kumar J, Muntner P, Kaskel FJ, Hailpern SM, Melamed ML. Prevalence and associations of 25- hydroxyvitamin D deficiency in US children: NHANES 2001–2004. Pediatrics. 2009
40. Robert Scragg, Alistair W. Stewart, Debbie Waayer, Carlene M.M. Lawes, Les Toop, John Sluyter, Judy Murphy, Kay-Tee Khaw, Carlos A. Camargo Jr. – Effect ofMonthly High-Dose Vitamin D Supplementation on Cardiovascular Disease in the Vitamin D Assessment Study A Randomized Clinical Trial. JAMA Cardiol. doi:10.1001/jamacardio.2017.0175
41. Marti-Soler H, Gonseth S, Gubelmann C, et al. Seasonal variation of overall and cardiovascular mortality: a study in 19 countries from different geographic locations. PLoS One. 2014;9(11):e113500.
42. BurtscherM. Effects of living at higher altitudes onmortality: a narrative review. Aging Dis. 2013;5(4): 274-280.
43. Carbone LD, Rosenberg EW, Tolley EA, et al. 25-Hydroxyvitamin D, cholesterol, and ultraviolet irradiation. Metabolism 2008;57(6):741–748. [PubMed: 18502255]
44. Yingshui Yao, Lijun Zhu, Lianping He, Ying Duan, Wei Liang, Zhonghua Nie, Yuelong Jin, Xiaolu Wu, Yun Fang - A meta-analysis of the relationship between vitamin D defciency and obesity. Int J Clin Exp Med 2015;8(9):14977-14984 www.ijcem.com /ISSN:1940-5901/IJCEM0011016
45. Hjelmesaeth J, Hofso D, Aasheim ET, Jenssen T, Moan J, Hager H, Roislien J and Bollerslev J. Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. Cardiovasc Diabetol 2009; 8: 7.
46. Kremer R, Campbell PP, Reinhardt T and Gilsanz V. Vitamin D status and its relationship to body fat, fnal height, and peak bone mass in young women. J Clin Endocrinol Metab 2009;94: 67-73.
47. Wojcik M, Janus D, Dolezal-Oltarzewska K, Drozdz D, Sztefko K, Starzyk JB. et al. The association of FGF23 levels in obese adolescents with insulin sensitivity. J Pediatr Endocrinol Metab. 2012; 25: 687-90.
48. Wojcik M, Dolezal-Oltarzewska K, Janus D, Drozdz D, Sztefko K, Starzyk JB. et al. FGF23 contributes to insulin sensitivity in obese adolescents - preliminary results. Clin Endocrinol (Oxf). 2012; 77: 537-40

49. Razzaque MS, Sitara D, Taguchi T, St-Arnaud R, Lanske B. Premature aging-like phenotype in fibroblast growth factor 23 null mice is a vitamin D-mediated process. FASEB J. 2006; 20: 720-2.
50. Dai B, David V, Martin A, Huang J, Li H, Jiao Y, et al. A comparative transcriptome analysis identifying FGF23 regulated genes in thekidney of a mouse CKD model. PLoS One. 2012; 7: e44161.
51. Creo AL, Rosen JS, Ariza AJ, Hidaka KM, Binns HJ. Vitamin D levels, insulin resistance, and cardiovascular risks in very young obese children. J Pediatr Endocrinol Metab. 2013; 26: 97-104.
52. Reyman M, Verrijn Stuart AA, van Summeren M, Rakhshandehroo M, Nuboer R, de Boer FK, et al. Vitamin D deficiency in childhood obesity is associated with high levels of circulating inflammatory mediators, and low insulin sensitivity. Int J Obes (Lond). 2013; 10.1038/ijo.2013.75.
53. P. Majak, M. Olszowiec-Chlebna, K. Smejda, and I. Stelmach,"Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection," Journal of Allergy and Clinical Immunology,vol.127,no.5,pp.1294–1296, 2011.
54. Freishtat RJ, Iqbal SF, Pillai DK, Klein CJ, Ryan LM, Benton AS, et al. High prevalence of vitamin D deficiency among inner-city African American youth with asthma in Washington, DC. J Pediatr 2010;156(6):948-952.
55. Litonjua AA. Vitamin D deficiency as a risk factor for childhood al-lergic disease and asthma. Curr Opin Allergy Clin Immunol 2012;12(2):179-185.
56. Alyasin S, Momen T, Kashef S, Alipour A, Amin R. The relation-ship between serum 25 hydroxy vitamin d levels and asthma in children. Allergy Asthma Immunol Res 2011;3(4):251-255.
57. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? J Allergy Clin Immunol 2007;120(5):1031- 1035.
58. Ginde AA, Mansbach JM, Camargo CA Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infec-tion in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009;169(4):384-390.
59. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. (2010) Serum vitamin D levels and severe asthma exacerbations in the Childhood AsthmaManagement Programstudy. Journal of Allergy and Clinical Immunology 126: 52–58.e55. doi: 10.1016/j.jaci.2010.03.043 PMID:
60. Banerjee A, Damera G, Bhandare R, Gu S, Lopez-Boado Y, Panettieri R Jr., et al. (2008) Vitamin D and glucocorticoids differentially modulate chemokine expression in human airway smooth muscle cells. Br. J Pharmacol 155: 84–92. doi: 10.1038/bjp.2008.232 PMID: 18552877

Iulian P. Velea, MD, PhD
Associate Clinical Professor of Pediatrics
University of Medicine and Pharmacy "V. Babeş" Timisoara, Romania
Senior Pediatrician, Clinic II Pediatrics, Clinical County Hospital
Timisoara, Romania
E-mail : ivelea56@yahoo.com

April, 2017

Insulin Pumps and Continuous Glucose Monitoring Systems en route to the Artificial Pancreas 2017

Stuart J. Brink

Introduction

Insulin pumps were first utilized in the late 1970s, were somewhat crude and bulky and modeled after hospital based small intravenous infusion pumps to deliver insulin subcutaneously to type 1 diabetes patients[1]. Over the decades the pumps have gotten smaller, more sophisticated, better computerized and able to deliver basal (background) insulin as well as modifiable bolus insulin to control meal as well as between meal and overnight glycemic values [2] . Over more than 30 years the newest continuous subcutaneous insulin infusion (CSII) devices have color screens, microcomputers, a reservoir for about 2-3 days of fast-acting insulin analogs delivered via subcutaneous catheter either via a pod without tubing or via a thin plastic delivery tube connected to a subcutaneous delivery port[3]. Precision micro-drop insulin delivery allows such manual, semi-automatic adaptation of insulin based on capillary blood glucose monitoring and/or continuous (every 5 minute) glucose monitoring with feedback to the patient (and his or her family). The original models [4] were all manually controlled while the newest models can be preprogrammed to deliver the same or different background/basal insulin and the timing and methods of insulin delivery can be changed for faster or slower bolus delivery as

well as able to compensated for the dawn phenomenon, exercise or illness effects etc. Insulin pumps also have bolus wizard calculators to help sort out the proper amount of insulin for different amounts and types of carbohydrate, varying amounts of protein and fat which have an impact on insulin needs (extended boli: square or dual wave delivery). In addition these wizards use the information entered about individualized insulin:carbohydrate ratios (ie. 1 unit covers 15 grams of carbohydrates) as well as insulin correction dosage (ie. 1 unit corrects ~50 mg/dl (~3 mmol) blood glucose levels based on target blood glucose desired (ie. 100mg/dl (~5-6 mmol/l)[5].

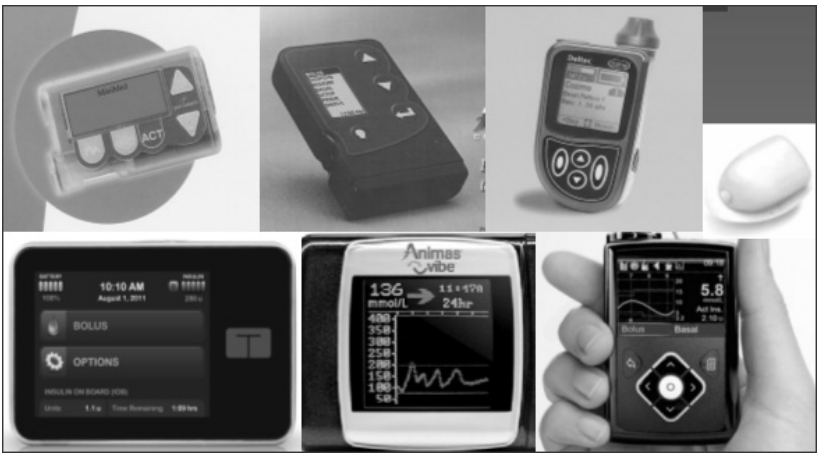


Figure 5.1: various insulin pumps available

Further insulin dose flexibility occurs with 0.1 unit dose increments for better fine-tuning in addition to “extended” bolus doses called square waves or dual waves for high fat-carbohydrates containing foods such as hamburgers, cheeseburgers, eggs, pizza or even bagels. The three variations of bolus delivery (*Figure 5.2*) are a major benefit of insulin pump over syringe or pen delivered boluses with MDI (multidose insulin) regimens since they allow maximum matching of insulin to glycemic excursion needs while at the same time minimizing mis-matching of insulin:food needs.

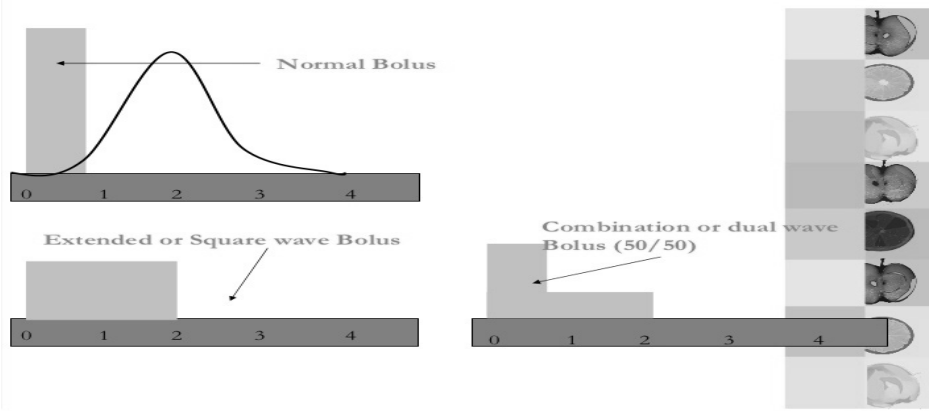


Figure 5.2: Standard, extended and dual waves bolus

The net benefit is fewer high BG values at the same time there is less likelihood of hypoglycemia occurrence. The principles of MDI as delivered by basal-bolus insulin pump therapy are also presented (*Figure 5.3*).

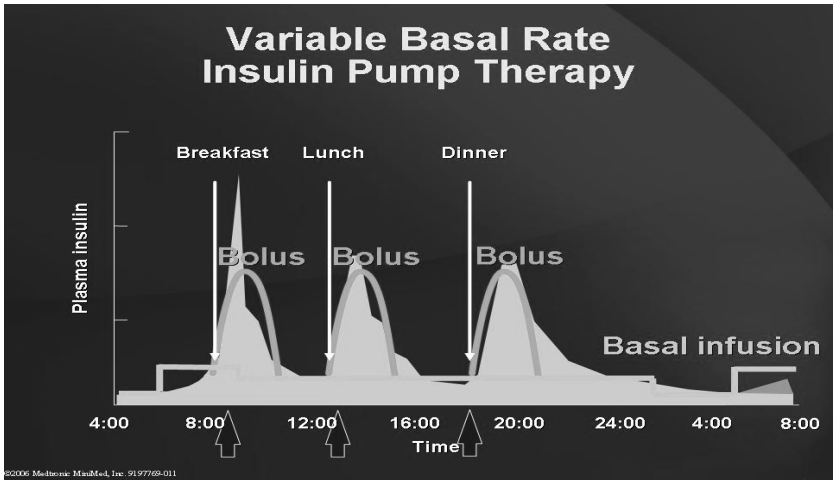


Figure 5.3: Basal-Bolus CSII concept

Insulin pumps have the benefits of fewer injections compared to syringes and pen dosage, greater flexibility of dosing as a result of potential better matching of activity and food needs as well as overall decreased hypoglycemia[6]. (*Figure 5.4, 5.5, 5.6*). Many studies have documented lessened hypoglycemia particularly when all the tools of the insulin pump and now with the continuous glucose

monitoring system are actually in place and being utilized. This is true for adults as well as adolescents and pediatric patients [7] [8] [9] [10]. It goes without saying that non-use of these systems, does not provide any significant benefit.

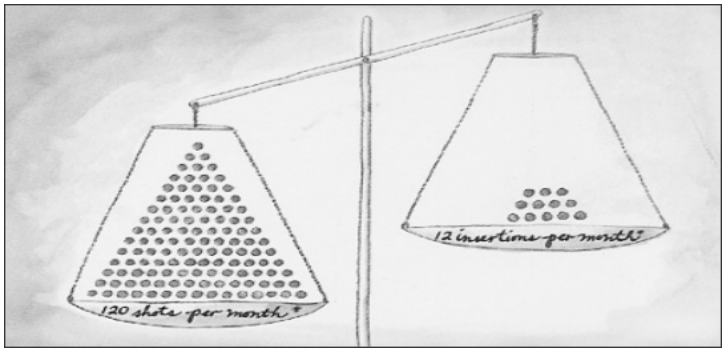


Figure 5.4: Fewer shots

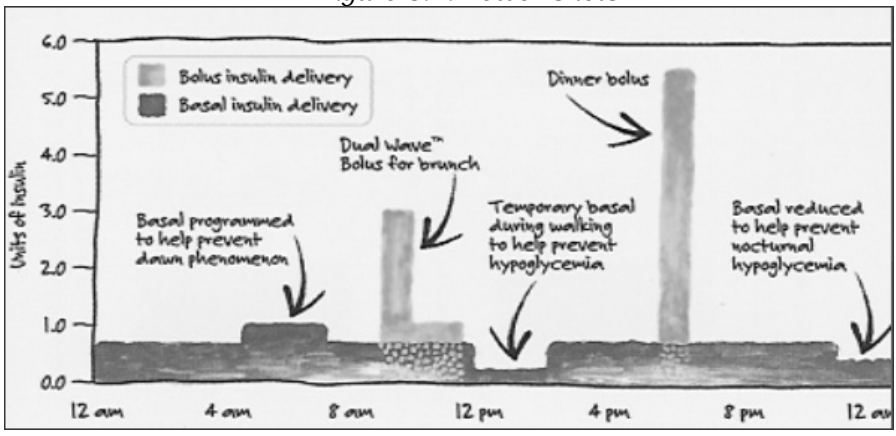


Figure 5.5: Improved insulin flexibility

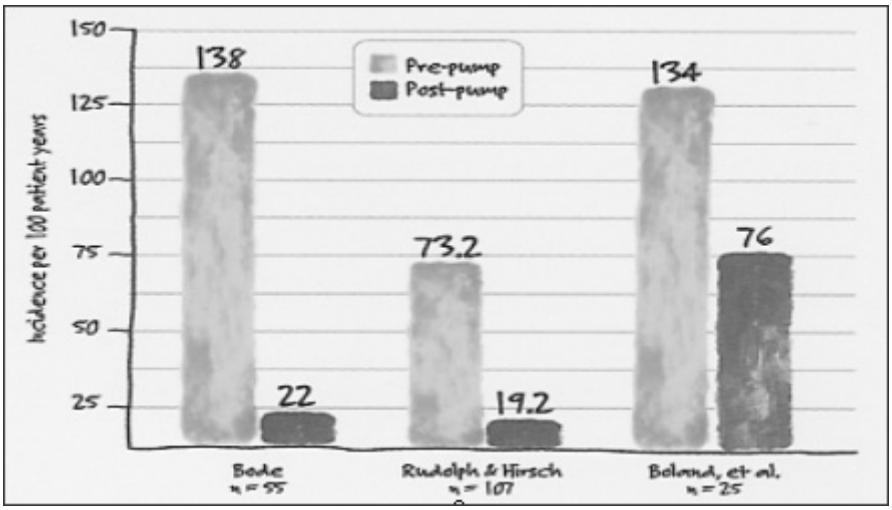


Figure 5.6: Decreased hypoglycemia

While not everyone feels comfortable thinking about wearing an insulin pump and "announcing to the world" that they have something different about them (diabetes), the potential benefits of improved quality of life, fewer hypoglycemic episodes, fewer hyperglycemic episodes and better overall control and long term health would seem to outweigh the downside to using such expensive technology for many. Little children can use insulin pump as can school age, middle age, teenage children and adults [11]. Pumps can be utilized safely during pregnancy and the newest pump versions are more and more waterproof. With contact sports, however, insulin pumps usually are temporarily disconnected.

As with most electromechanical biomedical devices, there are some special issues with insulin pump that must be addressed. Battery source power must be ensured and occasional mechanical or electrical failure occurs but there are ways to minimize such disruptions and to become more aware of when they are likely to occur. If insulin delivery is disrupted, most of the time it is not because of the pump malfunction itself but rather the attachment sites of the catheters to the skin or disconnections or blockage in the delivery catheters/tubes more so than lack of proper pumping itself.

Because there is no basal insulin being provided except that which the pump provides, rapid increase in hyperglycemia need to be identified and methods taught to ensure safety so that diabetic

ketoacidosis does not occur with prolonged disruptions. This too is rather rare but appropriate educational efforts to increase awareness and setting up protocols for identifying when to do extra blood glucose checks, when to check for ketones and how to deliver insulin under such circumstances with back up syringe or pen remain important tools to ensure safety for the patient.

Some pumps communicate directly with blood glucose meters via bluetooth technology and some also can communicate with mobile cell phones and home computers. These can be set to send information to parents, spouses and friends as well as to medical personnel for review and awareness. Graphs, summary statistics, averages and patterns can be identified and response acknowledged for younger patients and also for those patients who do not always remember to do such tasks themselves[12].

Examples of such data analysis are presented in *Figure 5.7* which show overall patterns as well as color-coded day-by-day comparative analysis.

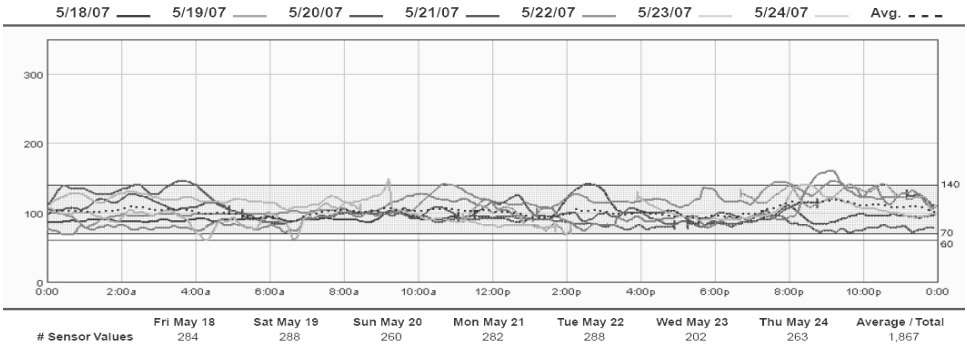


Figure 5.7: Carelink Sensor Data

For this author, one of the more potent graphs for home review as well as in-office review is the standard or modal day pattern to see if there are outliers. If these can be explained and learned, then it should be possible to utilize such information to be proactive and therefore prevent future occurrences more and more often. In this example in Figure 7, for instance, there are several days of post-dinner hyperglycemia that would suggested either poor carbohydrate potion control not being covered by sufficient prandial insulin, lack of use of the extended bolus function for high fat foods at this meal or just not timing the insulin bolus correctly without allowing sufficient time prior to insulin administration to adequately

cover the meal quantities. Because of the 24 hour time period for these several days, the "correct" answer in this instance was not utilizing the square wave bolus function for high fat-carbohydrate foods on those three days; just making this correction, "solved" the problem on future evenings quite readily.

Alternative programs have alternative graphic displays and some are easier to use for individual patients so that whatever works is the program that should be utilized, of course. Some will identify averages but not individual day-by-day readings of either home blood glucose readings or CGMS data. Newer pump statistical downloads allow identification of hypoglycemic episodes, meal time analysis vs overnight analysis as well as more detailed statistics including averages, percentage hyperglycemia vs hypoglycemia; total average insulin dose, basal:bolus ratios and differences between sensor glycemia and blood glucose capillary data.

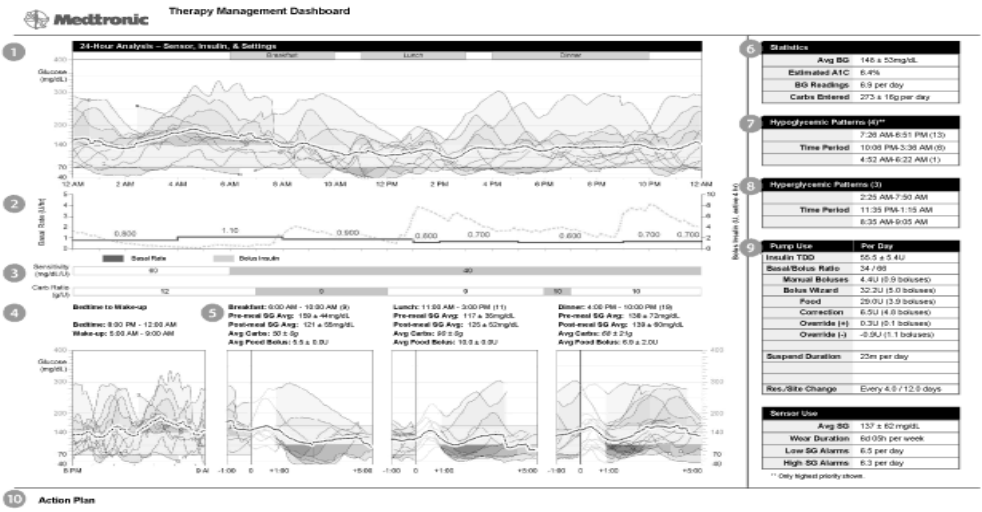


Figure 5.8: Pump CGMS data analysis graphics

A key issues for the medical care team is to have a unified approach to recommendations for how often downloading at home should occur, whether or not it should take place only at the office visits or between visits with this author recommended optimal downloading twice-a-week so that specific aberrations or variance can be identified and remembered in an effort to become more proactive and less reactive. Both reactivity to high and low values as well as being more preventive (proactivity) are very important to

learn and practice so that improved glycemic control, lowered glycemic variability and ultimately better quality of life and fewer short and long term complications may occur. Some patients can learn this quite effectively and others who have math or language difficulties may take longer to learn to respond, if at all. Sometimes other members of the family must be called to assist under such circumstances. Practice in the office and practice at home between visits need to be encouraged and assistance with computer problems from pump and CGMS manufactures also may be required to help with home systems.

In the past few years, the CGMS have improved dramatically[13] so that less efforts is needed for daily calibration and many of the newest CGMS will be factory calibrated in the near future. Three major sensors now are available DexCom®, Enlite® and Libre®. Most provide round-the-clock every 5 minute automatic subcutaneous glucose data on a continuous basis and most sensors last from 4 up to 14 days depending upon the particular system. The DexCom® sensors transmit their information to their own receivers as well as download to computers and phones. More recently, they also transmit such information to several insulin pump systems but the pumps do not yet have the capability of "responding" to such data automatically. The patient, however, can manually respond and make basal and/or bolus dose adjustments accordingly. There are experimental versions of DexCom CGMS where the pump have the capacity to respond with computerized mathematical formulas and such systems seem to work well for reducing hypoglycemic events, reducing severity of hypoglycemia and even reducing hyperglycemia as well as glycemic variability[14]. This is true for Omnipod®, Roche®, Animas® and T:Slim pump systems[15]

With the initial Medtronic pump systems coupled with Enlite ® sensors, there was some more variability and the DexCom sensors seemed to be more reliable and more accurate; over recent years, the Enlite sensors have improved dramatically so that there is virtually no difference in accuracy or specificity any longer with virtually identically MARD values of 0.4[16]. The biggest advantage currently of the Medtronic pumps is not only that the Enlite sensors are more accurate and reliable but also that the Enlite sensors send information to mobile phones and home computers as well as directly to Medtronic insulin pumps acting as information receivers. The screens of the Medtronic 530G insulin pump and more recently the 630, 640 and 670G Medtronic pumps, in color, now show every

5 minute actual blood glucose readings from the sensor, graphic analysis (24 hour, 12 hour, 6 hour and 3 hour updated curves) looking at trend analysis and arrows that highlight upward or downward trends for easier identification. Connectivity of the sensors as well as battery power and insulin reserve are also provided on most pump screens as well.

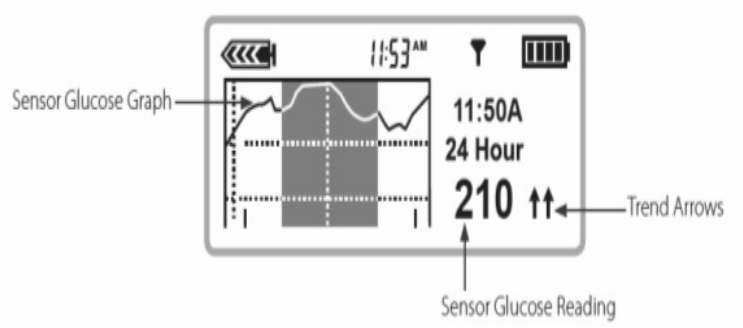


Figure 5.9: Medtronic 530G screenshot

Figure 5.10: Medtronic 630G screenshot

Other systems are available for those who prefer raw mathematical data identifying amount of time in range, above or below individually identified target glycemic goals and pie-charts for graphic representation looking at overall time periods as well as specifically identified hours of the day or night. *Figure 5.11* shows one such pie chart analysis.

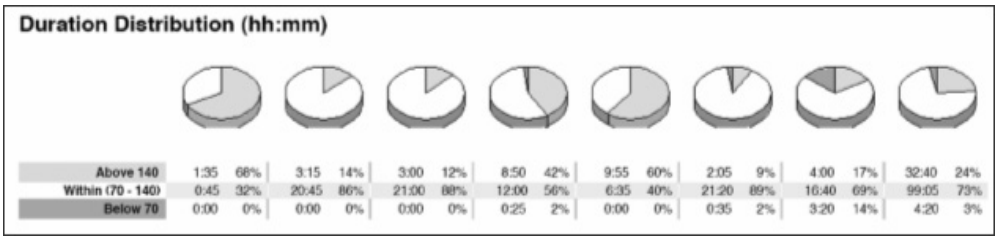


Figure 5.11: Pie chart CGMS downloaded data analysis

As older patients and family members get more comfortable with analysis of their own pump and CGMS data, not only the modal day and summary data is important but better problem solving can take place when reviewing the individual daily summary downloads. These provide specific information to verify the accuracy or inaccuracy of the sensors graphs compared to capillary blood glucose calibration information since the graphic display overlays this quite easily for review. Insulin delivery and use (or lack of use of square and dual waves) as well as manual or automatic low glucose suspension features of the pumps also is demonstrable. Carbohydrate counting errors, missed information, exercise and any other events so code also can help assess glycemic responses. These daily response graphs are most dramatic when people believe that the sensors are inaccurate yet their own data can help convince them under what circumstances this is valid or misconstrued information. *Figure 5.12* shows such a daily summary download chart showing such features.

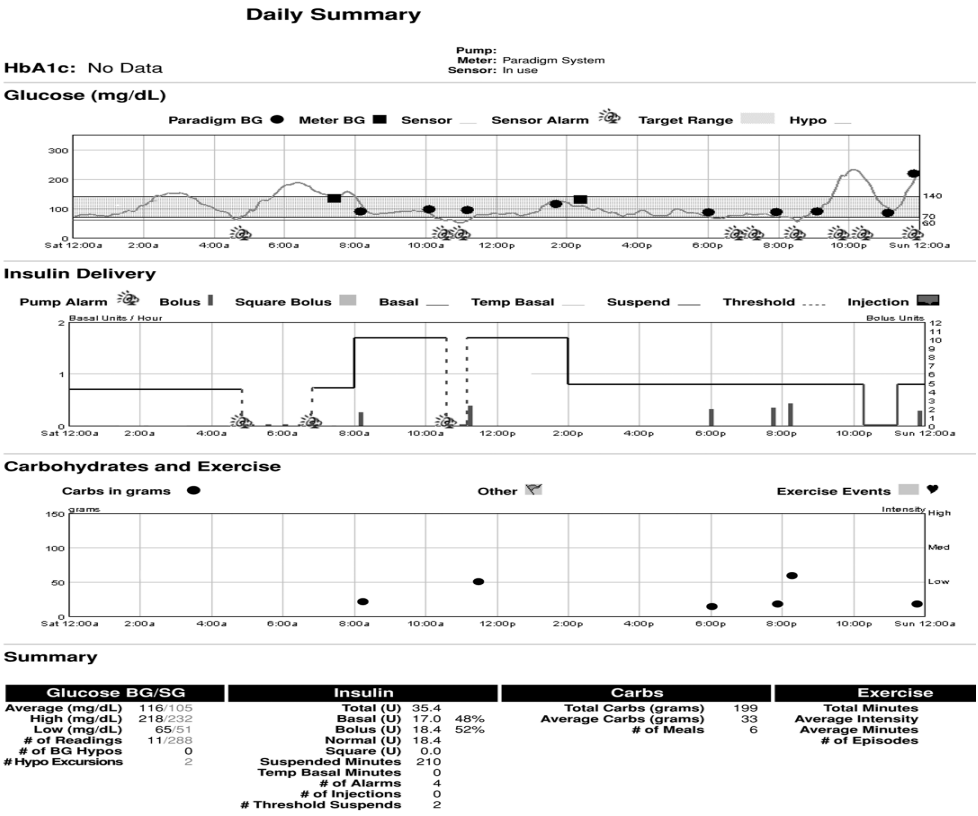


Figure 5.12: Carelink ® Daily Combination CSII & CGMS Daily Summary Sheet

A demonstration of the DexCom G4 sending information to the DexCom receiver and the newer DexCom G4 sending information to a mobile phone ap are presented in Figure 13A and Figure 13B has a demonstration of two weeks of DexCom sensor data and the superb response of the patient showing remarkable improvement in glycemic variability on the second week of DexCom sensor use.

Figure 5.13a: DexCom G4 sensor and receiver and DexCom G4 sensor and phone ap.

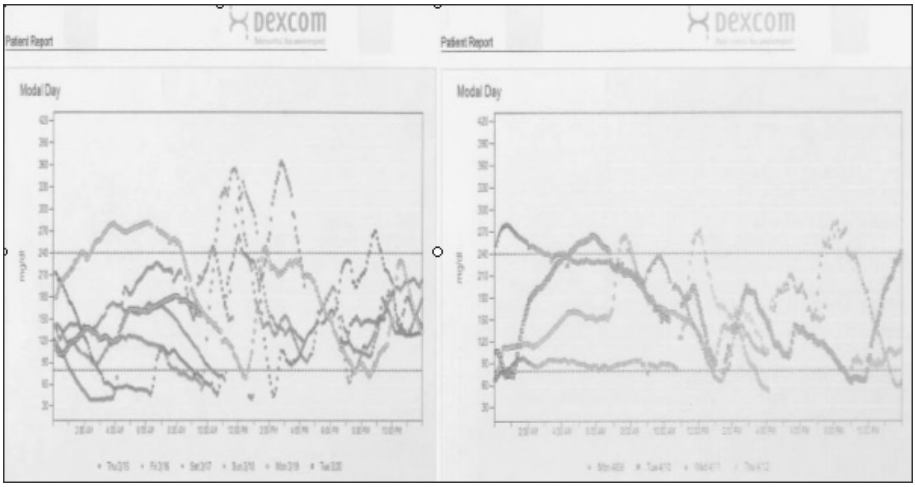


Figure 5.13b: DexCom week 1 data analysis and then follow-up week 2 data response

Much work by all of the insulin pump and sensor manufacturers as well as academic diabetologists around the world has continued in close cooperation with statisticians, mathematicians and computer engineers to improve the situation with insulin pup and with sensor accuracy.[17] In 2013, Medtronic® received permission to market the 530G insulin pump which added a major safety advance: coupling the Enlite ® sensor improvements to the pump and the mobile phone ap but also established an automatic low glucose suspend safety features. This feature not

only alarms and identifies trends of blood glucose rising and blood glucose falling, as do most of the CGMS systems available but also, if there is no operator or family response to the preset low glucose suspend, automatically stops all insulin delivery for 2 hours. This has been called **threshold suspend** or **LGS**: **low glucose suspend.** It has amazingly documented up to several hours of hypoglycemic occurring without the patient being aware in many instances and especially so nocturnally, the most risky and potentially dangerous type of severe and prolonged hypoglycemia for diabetes patients to encounter [18] . Diabetic ketoacidosis (DKA) does not occur from temporarily suspending insulin basal delivery for up to 2 hours and the LGS can function throughout the days and the night, can repeat such suspension as often as is necessary for repetitive low enough glycemic and continues to alarm the patient and others around them. he Aspire In-Clinic Study [19] , the Aspire In-Home study [20] and the Star 3 Study [21] had a run-in phase when the sensors were worn in blinded fashion to documented baseline glycemia and demonstrated a 38% reduction in nocturnal low sensor glucose events when the suspend feature was operational compared to the control group. (*Figure 5.14*)

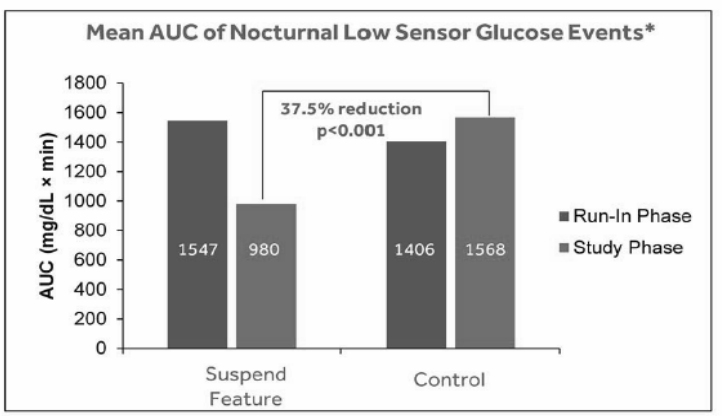


Figure 5.14: Aspire In-Home Study 2013

Data presented by Kaufman et al utilizing centralized CareLink downloaded data, showed a 66% decreased amount of time with hypoglycemia when the SmartGuard feature was actually turned on and operational compared to sensor use but Smart Guard feature turned off for BG values <50 mg/dl, a 36% reduction when the cutoff analysis levels was <80 and no statistically significant

elevation of BGs >300 mg/dl in the same study group (See *Figure 5.15*)

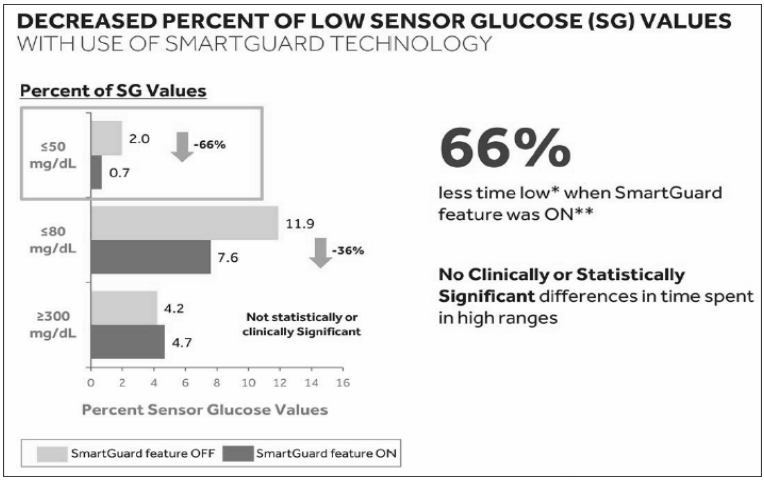


Figure 5.15: Carelink SmartGuard Reduced Hypoglycemia 2016

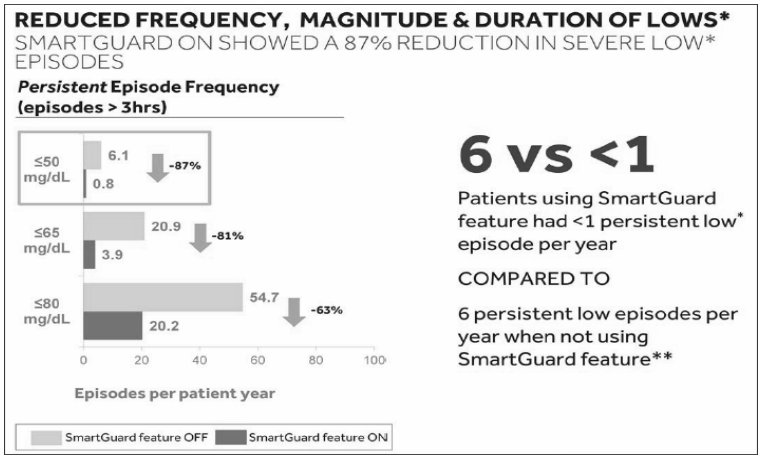


Figure 5.16: Carelink Smart Guard Reduced Frequency, Magnitude & Duration of Hypoglycemia 2016

In the same Carelink study, Kaufman et al also presented a 6x decreased in reduced frequency, magnitude and duration of hypoglycemia for hypoglycemia episodes that persisted for greater than 3 hours: 87% reduction if BG <50 mg/dl, 81% reduction if BG <65 mg/dl and 53% reduction if BG <80 mg/dl were used for

analysis when SmartGuard features were available and actually turned on and utilized.

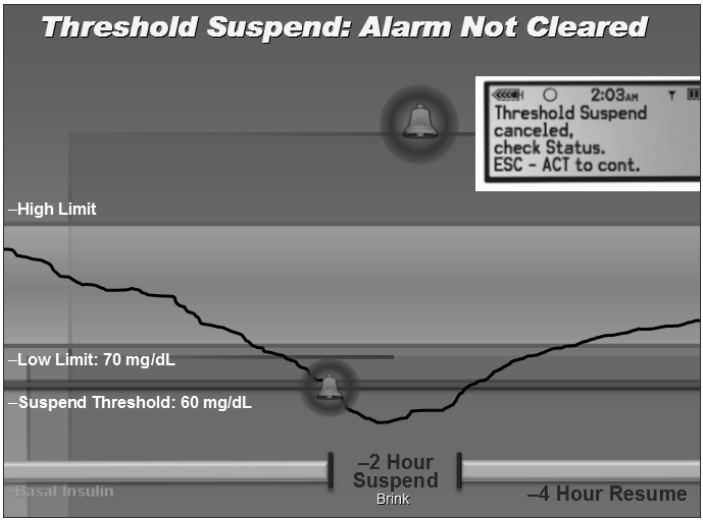


Figure 5.17: Threshold Suspend diagram documenting actual CGMS glycemic results with 2 hour LGS after no response to decelerating glycemia (black line), no response from 70 down to 60 mg/dl (blue to purple lines) suspend threshold (shown with cessation of gold basal insulin bar) and then the automatic 2 hour suspend and resumption of insulin delivery thereafter.

For those with the most severe types of hypoglycemia unawareness syndrome and recurring hypoglycemic convulsions, the LGS has virtually been a "game-changer" because it has almost certainly markedly decreased or eliminated such events. One of my patients with severe diabetes induced autonomic neuropathy with recurring hypoglycemic convulsions associated with severe diabetic gastroparesis has had such convulsions end completely using the 530G pump with LGS Enlite CGMS.

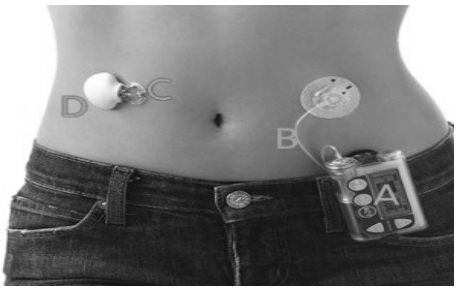


Figure 5.18: 530G Medtronic® pump (A), catheter insertion tubing, (B), Enlite® sensor (C) and transmitter/battery source (D) in situ

The STAR3 study [22] published in 2013 documented significant lowering of A1c at the same time there was significant reduction in hypoglycemia however defined (nocturnal, seizure related, unconscious reactions) when the pump and CGMS were actually used by patients. Not surprisingly, if one has an insulin pump but does not wear it, if one has a CGMS but does not put it on or connect it or utilize the data generated, the results do not take place. These results were especially supportive for those with the most severe issues: hypoglycemia unawareness or hypophobia. More importantly, the improvement took place with 21% reduction in those BG levels M55 mg/dl in addition to a 23% reduction in those BG levels <240 mg/dl and all the improvement showed up in values subsequently in the goal range of 81-140 mg/dl.

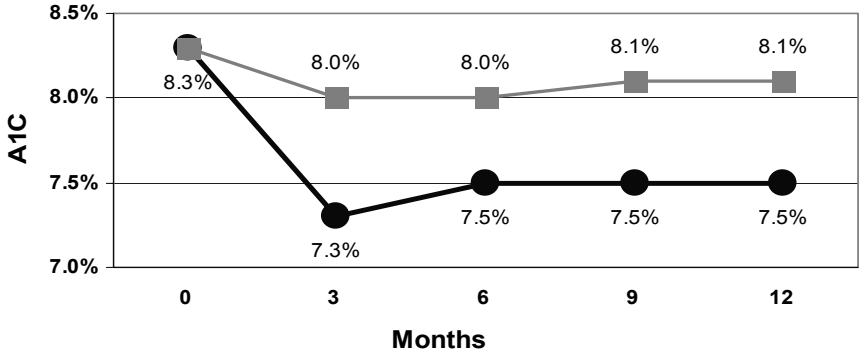


Figure 5.19: Star3 Study A1c lowering; CSII & CGMS

In late 2016 and early 2017 the European Medicine Agency as well as the US Food and Drug Administration approved the DexCom G5 ® sensor system as well as the Animas Vibe® system for use without calibration because of dramatically improved sensor accuracy so that insulin dosing decisions can safely be made under these circumstances without automatic calibration. Studies with

the multiple CGMS systems also have documented comparable results but with some different degrees of accuracy and inaccuracy[23]. Vibe® systems also were presented and published which documented similar automatic response to insulin pumps to what has been presented by the Medtronic 530G [24], 640G [25], and now the newest approved 670G systems.

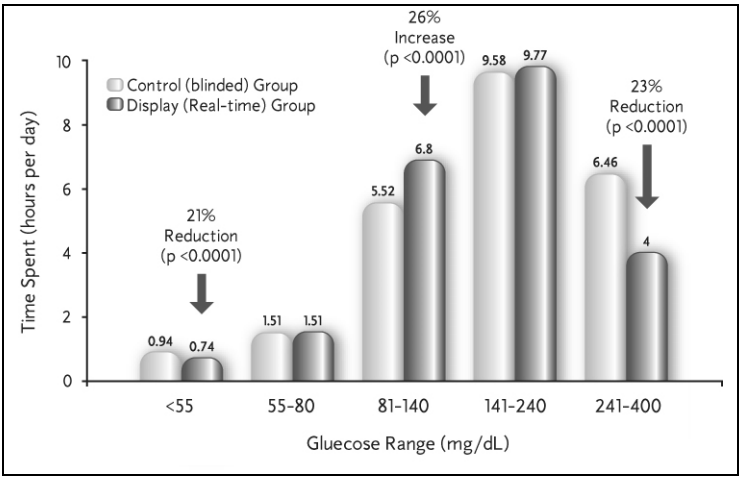


Figure 5.20 – Star 3 Study Actual Lowered wery low BG, very high BG and movement to target goal range

Nakamura in 2015 presented comparative data with the DexCom standalone sensor documenting an average of 0.4 hours/night of unrecognized hypoglycemia in a study of 300 patients between 8 pm and 8 am (using CGM levels <55 mg/dl as the cutoff). Using the same methodology, but with more than 13,000 patients, Minimed 530G with Enlite smart Guard technology turned off, obtained the exact same estimate of hypoglycemia, 0.4 hours/night. When the Smart Guard technology was actually turned on in more than 34,000 reporting, there was a reduction of 50% to less than 0.2 hours/night. Studies by Buckingham et al [26] documented

The Abbott Freestyle ® Libre ® system

A third sensor has been developed by Abbott Laboratories with data presented in 2016 [27] where there is no calibration required by the patients. glucose levels were automatically recorded every 15

minutes instead of every 5 minutes but where the data is blinded from the patient in real time.

Data downloading lasts for 14 days and can be analyzed afterwards with the receiver as well as sent to an Android mobile phone.

Figure 5.22: Abbott Free Style Libre Pro ®

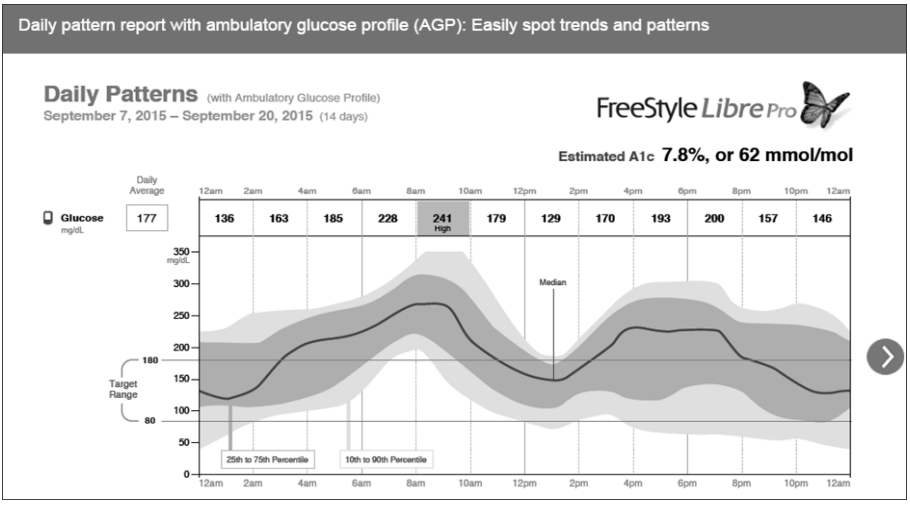


Figure 5.23: FreeStyle Libre Pro® ambulatory glucose daily pattern profile

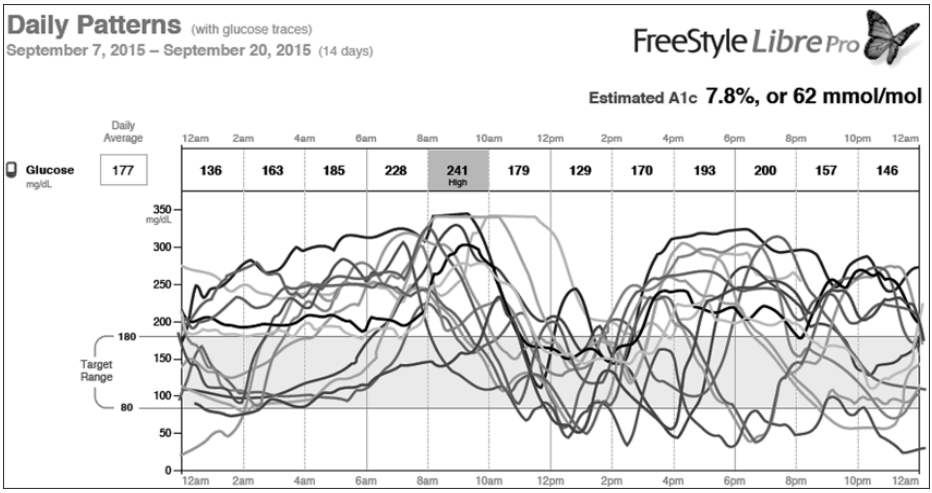


Figure 5.24: FreeStyle Libre Pro® 14 day daily patterns with glucose traces

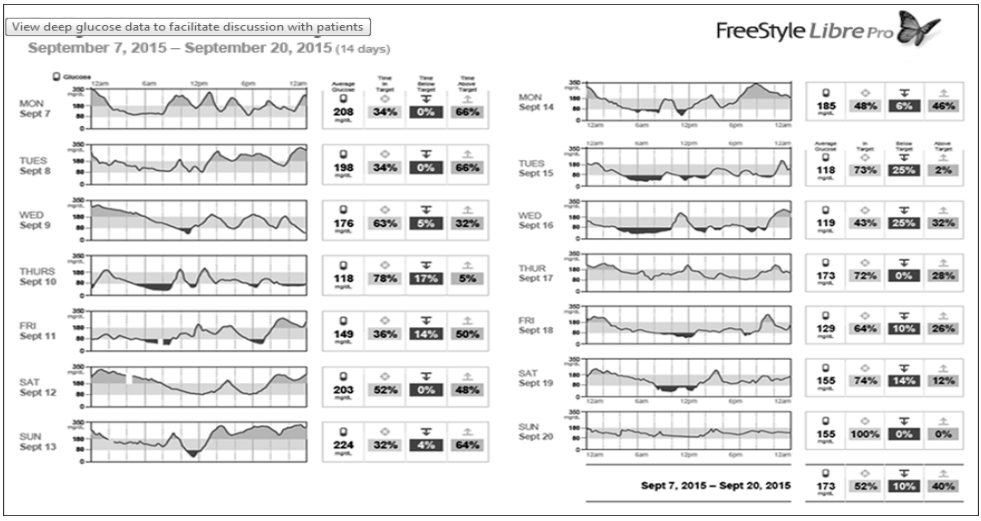


Figure 5. 25: FreeStyle Libre Pro ® daily glucose averages and % time at, below & above target each day

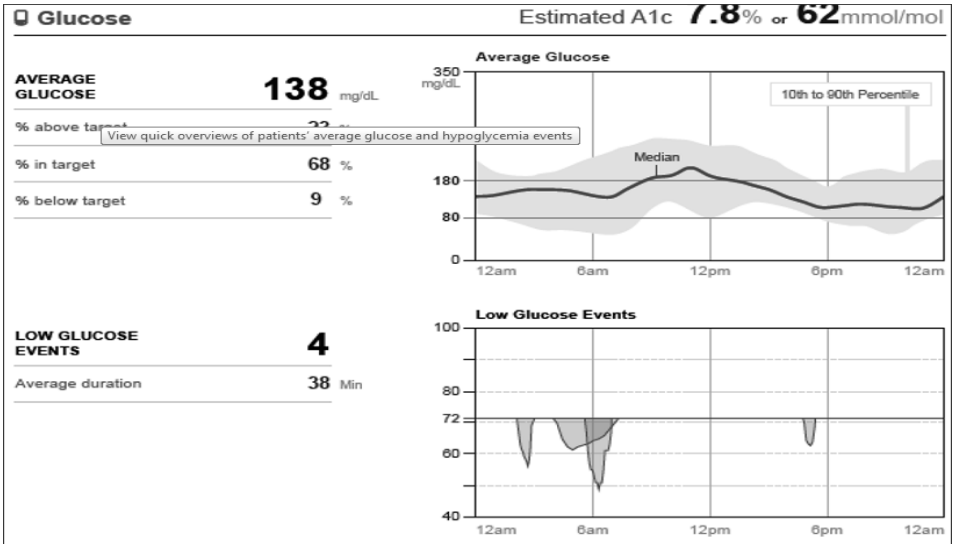


Figure 5.26: FreeStyle Libre Pro ® snapshot modal day averages and low glucose events

Figure 5.23 – 5.26 demonstrate Abbott sample data analyses available from this European study involving adults in five countries named the IMPACT International Trial. These 252 patients were utilizing CGMS vs SMBG in a cross national study in Austria, Germany, Netherlands, Spain and Sweden with a similar study ongoing in a pediatric cohort due to be completed in 2017. There was a 38% reduction in overall hypoglycemia <70 mg/dl, a 40% reduction in nocturnal hypoglycemia, a 50% reduction in severe hypoglycemia defined as levels <55 mg/dl and no A1c increase over the six months of study.

Data scanning increased throughout the day and routine fingerstick BGs were eliminated by 91% of the participants since no calibration was required.

The latest advance in the march towards the artificial pancreas was approved by the US FDA in September 2016 and is called the "hybrid" artificial pancreas as presented by Medtronic utilizing the Medtronic 670G pump. It involves a color screen, is waterproof and included the LGS (low glucose suspension) automatic features of the 530G and 640G pumps.

In addition, it keeps the Enlite CGMS integrated with bidirectional communication to the 670 pump. It has an additional

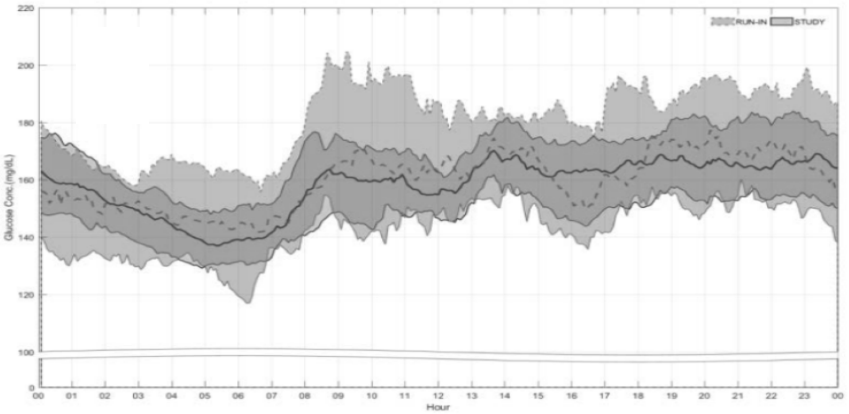
"hybrid closed loop" (HCL) [28] that automatically adjusted basal rates trending upwards if there is a pattern of increasing glycemia and, in addition, automatically also adjusts basal rates trending downward – before LGS initiates, to attempt to prevent more severe hypoglycemia.

Two levels of personalization with this 670G "hybrid" artificial pancreas: suspend before low option and auto mode option which adjusted basal insulin delivery every 5 minutes based on defined sugar levels to maintain improved target range glycemia day and night without operator activity[29]. Data presented in adolescents and adults for 3 months of HCL use saw a 0.5% reduction in A1c moving initial A1c from 7.4% to 6.9%, 44% reduction in time spent in low blood glucose (defined as levels <70 mg/dl), a 40% decline in time spent in dangerous hypoglycemia (<50 mg/dl) and an 11% decline in time spent over 180 mg/dl concordant with an 8% improvement in time-in-range (71-180 mg/dl).

Studies organized by Damiano, Russell and colleagues [30] in Boston have over the past several years been utilizing a T-Slim adapted insulin pump with DexCom sensors and appropriate bidirectional communication with the pump, sensors, phones and computer systems.

The unique aspect of this system was the use of an insulin pump as well as a glucagon pump to be modified according to a CGMS defined parameters with the idea that this may be a more physiologic approach to artificial pancreas technology and closer to the way the pancreas should be working.

Adolescents on the MiniMed 670G (pink) vs. Open Loop (gray). The range of glucose values was much narrower overall at all time of day, particularly after breakfast. Unlike the adults, the adolescents saw a lower average glucose on the 670G at many times of day.



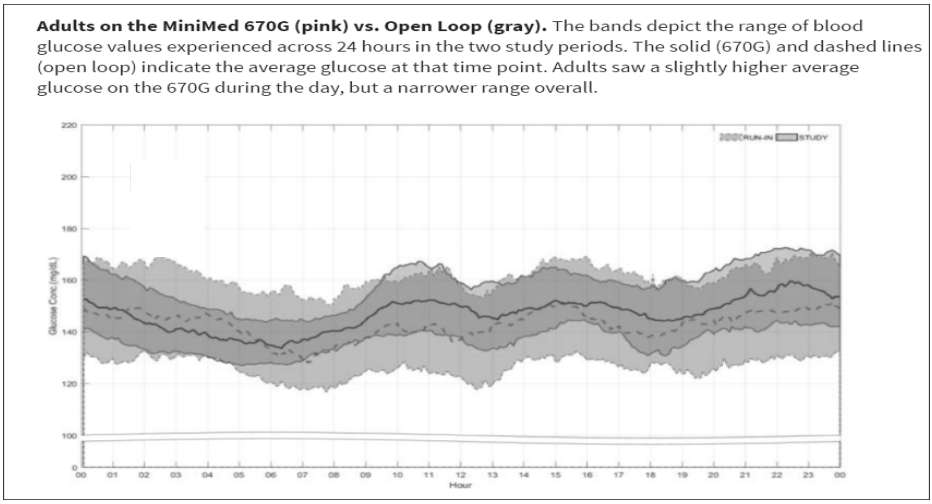


Figure 5.27: Adolescents and adults in the Hybrid Closed Loop 670G study, downloaded CGMS data across 24 hours.

While this is more complicated (and more expensive) and had to overcome stability issues with glucagon (successfully accomplished), data suggests it can also achieve reasonable glycemic control although still with large glycemic bursts post-prandially as shown in *Figure 28.* A more recent study compared bihormonal combination insulin and glucagon pumps with conventional insulin-only sensor driven pumps in adults with type 1 diabetes in a multicentered, randomized clinical trial[31].

There were no restrictions on food or home activities and two glucose targets were compared: 130 mg and 145 mg/dl. No significant differences in CGM glucose levels and time less than 60 mg/dl were seen in the two groups although both did not meet the actual target glucose levels set. The amount of insulin delivered was similar between groups and there was no less hypoglycemia documented in the target group of 145 mg/dl compared to the target group of 130 mg/dl.

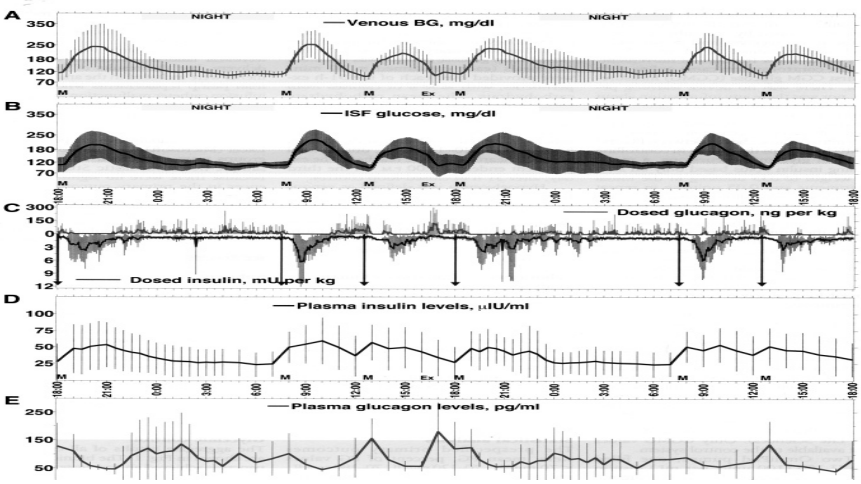


Figure 28. Bihormonal insulin and glucagon "double" pump data

The JDRF has set its "Road to Fully Automated Closed-Loop Pumps as presented by Kowalski[32] in *Figure 5.29:*

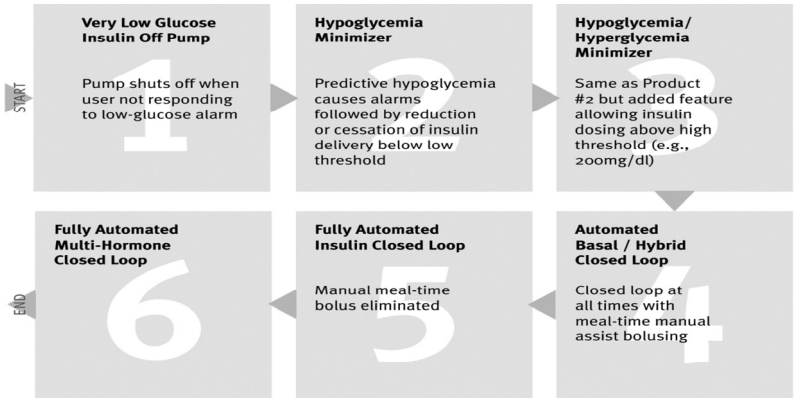


Figure 29: JDRF Road to Closed-Loop Pump

We have moved through phases 1,2,3 and are now "officially" in phase 4 with the introduction of the hybrid closed loop 670G Medtronic pump. We started back in the 1970's with proposed models of what the artificial mechanical pancreas would require and actually saw some initial developments in that regard *Figure 5.30, 5.31* and the first "small and portable" blood glucose monitor, *Figure 5.32.*

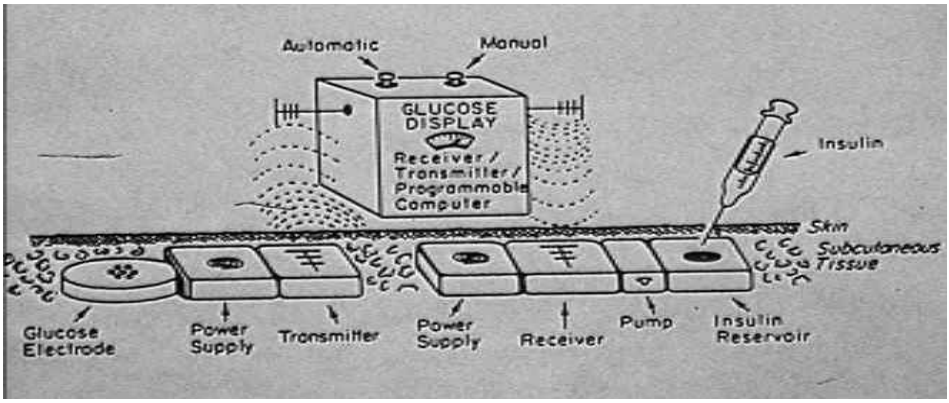


Figure 5.30 - Dr J. Stuart Soeldner's model of the future artificial mechanical pancreas

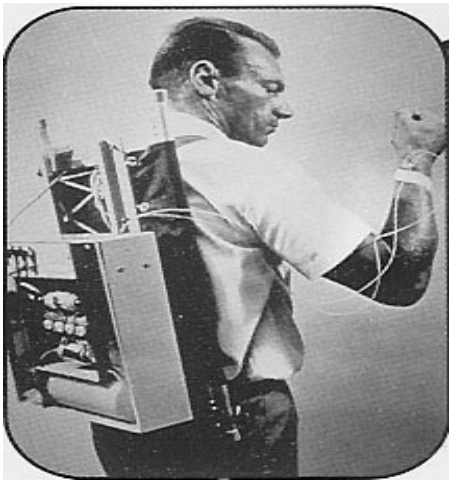


Figure 5.31 - first artificial mechanical pancreas

The very latest development over the past few years has also been the introduction of parents working with computers, opening up pumps and CGMS software to allow communication with each other, with their children and spouses and also to talk to mobile phones in a more facilitated fashion.

Figure 5.31: Ames Biostator: first "portable" intravenous monitor and insulin delivery system ~1974

Figure 5.32: First Ames glucometer (the "brick"):

This has not happened with official government or industry sanction and it has opened up some intriguing ownership, legal and medial quandaries about who owns the software rights, computer maneuvering rights and data when someone has "purchased" an insulin pump, blood glucose meter or continuous glucose monitor.

Nevertheless, the work has continued around the world with the internet serving as a vehicle for communications, sharing and improvement in software and hardware. Open Source, OpenAp and NightScout are some of the groups operating in this environment to bring improvements such as advanced waterproofing, better communication possibilities, better alarm notification and improved

quality of life for all utilizing these new developments in artificial intelligence, insulin pumps, monitoring and access. Stay tuned.

Summary

Pumps are here. CGMS is here. Communication between pumps, CGMS, computers, mobile phones, family members, medical staff are all here and improving rapidly but expensively. With an educated and motivated patient and family, reasonable and honest medical care insurance systems, and attention to the behavioral as well as medical aspects of a chronic disease like diabetes, the ability to adjust and adapt food, carb counting and glycemic indexing allows for optimizing and analysis of this data using these new systems, allows the ability to add flexibility and to be reactive as well as proactive utilizing insulin, timing and activity to better mimic natural pancreatic function while at the same time minimizing hypoglycemia[33]. Knowledge that is more automatically generated and with added sensitivity and specificity can be added to these devices to improve quality of life, immediate short term and long term diabetes outcomes and hopefully to decrease short and long term complications of living with diabetes for all.

References

1. Tamborlane WV, Sherwin RS, Genel M, Felig P. Reduction to normal of plasma glucose in juvenile diabetes by subcutaneous administration of insulin with a portable infusion pump. N Engl J Med 1979; 300:578-581.
2. Fredrickson L, ed. The Insulin Pump Therapy Book. Insights from the Experts. Minimed Technologies, Sylmar CA. 1995.
3. Kaufman FR with Westfall E. Insulin Pumps and Continuous Glucose Monitoring. A User's Guide to Effective Diabetes Management. American Diabetes Association, New York. 2012.
4. Walsh J, Roberts R. Pumping Insulin 4th edition. Torrey Pines Press, San Diego. 2006.
5. Chase HP and Messer L. Understanding Insulin Pumps and Continuous Glucose Monitors. 2nd Edition. Children's Diabetes Foundation at Denver, Colorado. 2011.
6. Hanas R. Type 1 Diabetes. A Guide for Children, Adolescents, Young adults – and Their Caregivers. Marlowe & Company, New York. 2005.
7. Bode BW, Streed RD, Davidson P. Reduction in severe hypoglycemia with long term continuous subcutaneous insulin infusion in type 1 diabetes. Diabetes Care 1996; 19:324-327.
8. Hirsch IB, Abelseth J, Bode BW. Sensor augmented insulin pump therapy: results of the first randomized treat-to-target study. Diab Technol Ther 2008; 10:377-383.
9. Boland EA, Monsod T, Delucia M, Brandt CA, Fernando S, Tamborlane WV. Limitations of conventional methods of self-monitoring of blood glucose:

lessons learned from 3 days of continuous glucose sensing in pediatric patients with type 1 diabetes. Diabetes Care 2001; 24:1858-1862.
10. Deiss D, Bolinder J, Riveline JP, Battelino T, Bosi E, Tubiana-Rufi N, Kerr D, Phillip M. Improved glycemic control in poorly controlled patients with type 1 diabets using real-time continuous glucose monitoring. Diabetes Care 2006; 29:2730-2732.
11. Mauras N, Beck R, Xing DY, Ruedy K, Buckingham B, Tansey M, White NH, Weinzimer SA, Tamborlane W, Kollman C, DirectNet Study Group. A randomized clinical trial to assess the efficacy and safety of real-time continuous glucose monitoring in the management of type 1 diabetes in young children aged 4 to <10 years. Diabetes Care 2012; 35:204-210.
12. Ahern JAH, Boland EA, Tamborlane WV. The formula for successfulimplementation of insulin pump therapyin children. In: Brink SJ, Serban V, eds. Pediatric and Adolescent Diabetes, Revised Edition, 2004; Brumar, Timisoara, Romania, 145-164.
13. Phillip M, Danne T, Shalitin S, Buckingham B, Laffel L, Tamborlane W, Battelino T for the Consensus Forum Participants. Use of continuous glucose monitoring in children and adolescents. Pediatric Diabetes 2012; 13:215-228.
14. Kovatchev B, Anderson S, Heinemann L, Clarke W. Comparison of the numerical and clinical accuracy of four continuous glucose monitors. Diabetes Care 2008; 31:1160-1164.
15. Garg SK, Smith J, Beatson C, Lopez-Baca B, Voelmle M, Gottlieb PA. Comparison and accuracy of the SEVEN and the Navigator continuous glucose monitoring systems. Diabetes Technol Ther 2009; 11:65-72.
16. Kaufman F. Nakamura 2015 Medtronic in-house Carelink data
17. Klonoff DC, Buckingham B, Christiansen JS, Montori VM, Tamborlane WV, Vigersky RA, Wolpert H. Continuous Glucose Monitoring: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2011; 96:2968-2979.
18. Ly TT, Nicholas JA, Retterath A, Davis EA, Jones TW. Analysis of glucose responses to automated insulin suspension with sensor-augmented pump therapy. Diabetes Care 2012; 35:1462-1465.
19. Garg S, Brazg RL, Bailey TS. Reduction in duration of hypoglycemia by automatic suspension of insulin delivery: the in-clinic ASPIRE study. Diabetes Technol Ther 2012; 14:205-209.,
20. Bergenstal RM, RM, Klonoff DC, Garg SK. ASPIRE In-Home Study Group. Threshold based insulin pump interruption for reduction of hypoglycemia. N Engl J Med 2013; 369:224-232.
21. Bergenstal RM, Tamborlane WV, Ahmann A, Buse JB, Davis SN, Perkins BA, Willi SM, Wood MA for the Star 3 Study Group. Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes. N Engl J Med 363; 311-320..
22. Bergenstal RM, Tamborlane WV, Ahmann A, Buse JB, Dailey G, Davis SN, Joyce C, Perkins BA, Welsh JB, Willi SM, Wood MA for the Star 3 Study Group. Sensor-augmented pump therapy for A1c reduction (STAR3)Study. Results from the 6 month continuation phase. Diabetes Care 2011; 34:2403-2405.
23. Luijf YM, Mader JK, Doll W, Pieber T, Farret A, Place J, Renard E, Bruttomesso D, Filippi A, Avogaro A, Arnolds S, Benesch C, Heinemann L, DeVries JH on behalf of the AP @home consortium. Accuracy and

reliability of continuous glucose monitoring systems: a head-to-head comparison. Diab Technol Ther. 2013; 15:721-726.
24. Elleri D, Allen JM, Dunger DB, Hovorka R. Closed-loop in children with type 1 diabetes: specific challenges. Diabetes Research Clin Practice 2011; 93:S1310S135.
25. Bergenstal RM, Garg S, Weinzimer SA, Tamborlane WV. Safety of a hybrid closed loop insulin delivery system in patients with type 1 diabetes. JAMA 2016; 316:1407-1408.
26. Buckingham BA, Raghinaru D, Cameron F, Bequette BW, Chase HP, Maahs DM, Slover R, Wadwa RP, Wilson DM, Ly T, Tandy A, Hramiak I, Clarson C, Stein R, Gallego PH, Lum J, Sibayan J, Kollman C, Beck RW for the In Home Closed Loop Study Group. Predictive low-glucose insulin suspension reduces duration of nocturnal hypoglycemia in children without increasing ketosis. Diabetes Care 2015; 38:1197-1204.
27. Bolinder J, Antuna R, Geelhoed-Duijvestijn P, Kroger J, Weitgasser R. Lancet. 2016; http://dx.doi.org/10.1016/ S0140-6736(16)31535-5
28. Ly TT, Roy A, Grossman B, Shin J, Campbell A, Monirabbasi S, Liang B, von Eyben R, Shanmugham S, Clinton P, Buckingham BA. Day and night closed-loop control using the integrated Medtronic hybrid closed-loop system in type 1 diabetes at diabetes camp. Diabetes Care 2015; 38:1205-1211.
29. Garg S, Weinzimer SA, Tamborlane WV, Buckingham BA, Bode b, Bailey TS, Brazg RL, Ilany J, Slover RH, Anderson SM, Bergenstal, RM, Grossman B, Roy A, Cordero RL, Shin J, Lee SW, Kaufman FR. Glucose outcomes with the In-Home use of a hybrid Closed-loop insulin delivery system in adolescents and adults with type 1 diabetes. Diab Technol Ther January 2017, ahead of print.doi:10.1089/dia.2016.0421
30. Russell SJ, El-Khatib FH, Nathan DM, Damiano E. Blood glucose control in type 1 diabetes with a bihormonal bionic endocrine pancreas. Diabetes Care 2012;35:2148-2155.l
31. Russell SJ, El-Khatib FH, Sinha M, Damiano E, Nathan D. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. N Engl J Med 2014; 371:313-325.
32. Kowalski AJ. Can we really close the loop and how soon? Accelerating the availability of an artificial pancreas: a roadmap to better diabetes outcomes. Diabetes Technol Ther 2009; 11 (S1): S113-S119.
33. Hirsch IB. Realistic expectations and practical use of continuous glucose monitoring for the endocrinologist. J Clin Endocrinol Metab 2009; 94:2232-2238.

STUART J. BRINK, MD
Senior Endocrinologist, New England Diabetes and Endocrinology Center (NEDEC), Waltham MA, USA
Associate Clinical Professor of Pediatrics, Tufts University School of Medicine, Boston MA, USA
Address: New England Diabetes and Endocrinology Center (NEDEC), 40 Second Avenue, Suite #170, Waltham MA 02451-1136 USA.
E-mail: stuartbrink@gmail.com

February 2017

ENDOCRINOPATHIES IN PATIENTS TREATED FOR CHILDHOOD CANCER

Ciril Kržišnik

Introduction

According to European and North American data approximately 1 in 300 children will be diagnosed with cancer before the age 20 years, and 1 in 500 to 1000 young adults between the ages of 20 to 40 years is childhood cancer survivor (CCS). [1,2]

Cancer treatments can have many long-term consequences that depend on the drugs and doses used, radiation therapy protocols and irradiated organs, and age at the time of treatment. Cytotoxic drugs and radiation can cause endocrinopathy, cardiomyopathy, nephropathy, osteonecrosis and loss of bone density, cognitive impairment and structural abnormalities of brain and also second cancers of different histological type. [3,4,5,6,7]

Endocrine disorders are common in CCS appearing immediately, or months, many times years or even decades after surgery, chemotherapy (ChT) and/or radiotherapy (RT).

The prevalence of an endocrine disorder in adult CCS was reported to be aproximately 40 to 60 %. [8,9]

It is well known that endocrinopathies could be due to treatment exposure to alkylating agent based chemotherapy and radiotherapy but recent studies have proven that selective mitogen-activated kinase inhibitors and immune system modulators have been also associated with endocrine dysfunction.[10,11,12] Endocrine abnormalities detected in CCS include disorders of the

hypothalamic – pituitary axis, thyroid, puberty, gonads, bone, body composition, and glucose metabolism. Healthcare providers should be equipped to diagnose and manage acute and long-term endocrine complications that may arise in maturing CCS.[13]

DYSFUNCTION OF THE HYPOTHALAMUS AND PITUITARY

Hypothalamo – pituitary dysfunction is the most common endocrine complication in CCS, it was described in 88% of patients.[3] It can be due to tumor development, surgical intervention or radiotherapy in this region. Panhypopituitarism due to autoimmune hypophysitis provoked by immune system modulator Ipilimumab was also described.[12]

Growth hormone deficiency – short stature

Poor linear growth and short stature are the most common sequelae of childhood cancer therapy.[14] Growth failure could be due to growth hormone deficiency (GHD), exposures to spinal and total-body irradiation (TBI), pubertal disorders, chemotherapy treatments including glucocorticoids, hypothyroidism and renal disease.[15,16,17,18]

GHD in CCS is common in cranial radiotherapy doses of 12 to 64 Gy to hypothalamo – pituitary region,while the impact of chemotherapy alone on GH secretion is less frequent [19, 20] Imatinib, a tyrosine kinase inhibitor (TKI), has been associated with growth deceleration by impeding the kinase mediated release of GH.[21]

GH secretion should be investigated when linear growth velocity decelerates in children who survived cancer. The effect of GHD on growth may be masked by precocious puberty and by hyperinsulinemia in the context of rapid weight gain. Patients exposed to spinal radiotherapy are at risk of having skeletal disproportions [22, 18]

Biochemical evaluation for GHD requieres dynamic testing using arginine, levodopa, glucagon, clonidine and insulin. Plasma levels of IGF-1 and IGFBP3 are not reliable screening tools in CCS exposed to cranial radiotherapy and are associated with high rates of false-negative results.[23]

Treatment with recombinant GH replacement therapy is proposed one year after successfully completing cancer or brain tumor treatments. The mitogenic potential of GH stimulating tumor

growth is a safety concern in CCS. [24] Studies suggest that rGH in patients with brain tumors are not associated with primary disease recurrence while an increase in the development of second neoplasm, especially meningeoma was reported.[25,26] After longer follow up period, recent analysis found negligible differences regarding developing second neoplasm between the CNS survivors who were or were not treated with rGH. [27] Besides increased growth rate, improvements in bone mineral density, cardiovascular function, reduction in metabolic syndrome and sustained improvements in quality of life are important.[28]

The benefits and risk of rGH have to be carefully weighed in children and adult cancer survivors and further studies are needed to investigate the risk of developing second neoplasms.

Disorders of LH and FSH secretion

Central precocious puberty

Central precocious puberty (CPP) is due to early activation of the hypothalamic – pituitary – gonadal axis leading to the onset of puberty prior to the age of 8 years in girls and 9 years in boys.[29] CCP results in premature closure of growth plates and results in decreased adult height.

Clinical signs of puberty in young children, especially menarche can generate significant psycho – social problems. Pubertal development can be triggered prematurely by tumors located near hypothalamus or radiotherapy to the hypothalamo/pituitary region. Additional risk factors include hydrocephalus, female sex, exposure to radiotherapy before the age of 5 years and increased BMI.[30,31]

In children with CCP the mean growth velocity use to be 8-10cm/year (+2 to +4 SDS for chronological age) at diagnosis but the linear growth could be normal or poor due to occurence of GHD or spinal damage from radiotherapy.[32]

Measurement of testicular volume for diagnosis of puberty in boys exposed to gonadotoxic chemoptherapy and/or testicular irradiation could be misleading as treatment related germ cell injury can impair testicular growth without affecting the ability to produce testosterone. Laboratory testing should be performed in cases of rapid growth and penile enlargement and pubarche.

Laboratory investigations include basal levels of LH and FSH and estradiol or testosterone. Due to pulsatile nature of gonadotropins, stimulated values may be necessary to establish the diagnosis. GnRH agonists are used in stimulation testing and a pubertal LH value and LH to FSH ratio over 1 is consistent with CPP.[33] Radiographic evaluation includes assesement of bone age, while in females a pelvic ultrasound demonstrating pubertal sized uterus and ovaries maybe useful in confirming the diagnosis [33,34]

Precocious puberty can be treated with GnRH agonists in order to suppress the secretion of gonadotropins what could allow longer time for linear growth and improve final height. This treatment can act synergistically with rGH and improve final height of GH deficient CCS who also has CPP.[35] Final height can be improved also using aromatase inhibitors in combination with rGH in order to prolong the delay in closure of growth plates but this therapy has been considered at the moment experimental.[36]

Deficiency of gonadotropins

Hypogonadotropic hypogonadism due to deficiency of LH/FSH can result in delayed or arrested pubertal development during childhood. LH/FSH deficiency can occur after tumor and /or surgery related damage or after doses of radiotherapy to the hypothalamic-pituitary area exceeding 30 Gy.[37] Secondary hypogonadism may occur also in case of ipilimumab induced auto-immune hypophysitis. Replacement therapy is needed for the development and maintenance of secondary sex characteristics, optimal bone mass and adequate metabolism.

Central adrenal insufficiency-ACTH deficiency

Terciary or secondary adrenal hypocorticism can occur in CCS following tumor and/or surgery related damage or after the exposure of the hypothalamus/pituitary to radiotherapy doses ≥30 Gy. [38]

Disorders of the hypothalamo – pituitary – adrenal axis in children were reported in 14% to 40% of CCS exposed to cranial radiotherapy[3, 39]

The incidence of ACTH deficiency in patients receiving total body iradiation – TBI in the scope of bone marrow transplantation – BMT was 6% to 60%.[40,41] A large proportion of these patients

experienced transient forms of this condition as a result of their exposure to high-dose glucocorticoids.

ACTH deficiency has been associated with the use of tirosine kinase inhibitor Imatinib and immune system modulator Ipilimumab which can induce auto.immune hypophysitis and panhypopituitarism.[42, 43]

Symptoms of ACTH deficiency include fatigue, weakness, nausea, vomiting, anorexia and abdominal cramping. In case of severe illness, patients with ACTH deficiency may develop life threating complications including hypoglicaemia related seizures and hypotensive shock. Adrenal crisis should be treated with hydrocortisone.

The diagnosis can be confirmed with low morning levels of cortisol but also ACTH. The treatment of ACTH deficiency use to be replacement therapy with hydrocortion but patients and their families should be educated to increase the doses of hydrocortison during stress and times of illness.

Central hypothyroidism – TSH deficiency

Terciary or secondary hypothyroidism known also as central hypothyroidism is reported only in 6% CCS.[44] It can occur as a result of tumor and/or surgery related damage or after hypothalamic/pituitary exposure to radiotherapy doses ≥30 Gy.[45, 46] TSH deficiency may occur also in the context of ipilimumab-induced auto-immune hypophysitis as described before.

Clinical signs of hypothyroidism use to be poor linear growth, delayed bone age, fatigue, fluid retention, constipation, cold intolerance, proximal muscle weakness and depression.

Laboratory results reveal a serum free T4 below normal and low or normal TSH values. Levothyroxine should be used to treat central hypothyroidism.[47]

Hyperprolactinemia

Dopamin holds a predominant role in the regulation of prolactin secretion through a direct effect on anterior pituitary lactotrophs. Dopamin inhibits the basally high-secretory tone of the cell. Disruption of hypothalamic – pituitary connections due to tumor growth, surgery, or doses of radiotherapy > 30-50 Gy can

result in hyperprolactinemia because of the loss of hypothalamic inhibition on prolactin secretion[48, 49]

Patients with hyperprolactinemia may present with galactorrhea but uasually patients are asymptomatic. Elevated prolactin levels may suppress LH and FSH secretion and cause hypogonadism.

Central diabetes insipidus

Hypothalamic neurons are responsible of the production of anti-diuretic hormone (ADH) which is carried by axonal transport to the posterior pituitary from which it is secreted into the circulation. ADH regulates the body's retention of water by activating aquaporin CD water channels in the plasma membrane of renal collecting duct cells. It also increases peripheral vascular resistence and thus blood pressure.

The deficiency in ADH results in polyuria, polydipsia and dehydration when access to free water is compromised.

Central diabetes insipidus can be a mode of revelation of childhood brain malignancies such as dysgerminomas or hypophyseal non – Hodkin's lymphomas.[50, 51] In such cases it can initially be isolated and as the tumoral infiltration worsens additional pituitary functions become deficient.

More often diabetes insipidus occurs in the context of panhypopituitarism due to a tumor close to sellar region or a consequence of surgical intervention in this area. Central diabetes insipidus does not occur as a late effect of cranial radiation therapy.[52] The management of central diabetes insipidus consists of replacement therapy using desmopressin with close monitoring of fluid intake and urine output in order to avoid overtreatment and ensuing hyponatremia and seizures.

DISORDERS OF THE THYROID

As the thyroid is radiosensitive, thyroid dysfunction is common in CCS. It was reported in 66% of patients exposed to neck radiotherapy.

Thyroid disorders include hypothyroidism, hyperthyroidism, nodules and cancer.[3, 53]

Primary hypothyroidism

Primary hypothyroidism (PH) is common in CCS. It was reported in general in 17% survivors but in Hodgkin's lymphoma patients receiving > 45 Gy PH was detected in 50%.[54]

The risk of PH has been attributed to direct or scatter radiation of the neck including cranio – spinal radiotherapy as well as total body irradiation for cytoreduction before hematopoetic stem-cell transplant – HSCT.[55, 56]

Subclinical or compensated PH is more common than overt hypothyroidism. Chemotherapy alone has usually not been associated with PH but tirosine kinase inhibitors like sunitinib and imatinib can cause hypothiroidism in 7-85%.[57, 58] PH has been detected also in majority of patients treated for neuroblastoma with Metaidobenzylguanidine – MIBG. [59]

Laboratory analysis reveals elevated plasma TSH level with normal or low free T4. Therapy consists of levothyroxine at substitutive doses.

Hyperthyroidism

Hyperthyroidism was diagnosed in 5% of CCS.[54] It occured mostly in patients after hematopoetic stem-cell transplant. [60, 61]

Autoimmune induced thyroid disease

Elevated thyroid autoantibodies have been reported in cases of hypothyroidism and hyperthyroidism in CCS of allogenic HSCT.[62] The transfer of auto-immunity from the stem-cell donor to the HSCT recipient is suspected.

Autoimmune and non-autoimmune thyroiditis could be trigerred by newer anticancer agents such as the monoclonal antibodies and also interferon – a, human recombinant cytokine used in treatment of some tumors and hematologic malignancies.

Thyroid neoplasms

Second thyroid neoplasm may occur even two decades after the diagnosis of the primary cancer in CCS.[63]

The association between the development of thyroid cancer and direct or scatter radiation of the neck is well known, it can

appear in 20% of exposed patients.[64] The most common second primary cancer was papillary thyroid carcinoma and the risk was highest in patients who had received ≥ 20 Gy, were females and were very young, less than four years old at the time of diagnosis of primary cancer.[65,66]

Hodgkin's lymphoma is the primary cancer most commonly associated with thyroid cancer. Alkylating agents in conjunction with < 20 Gy of irradiation can increase the incidence of thyroid neoplasm.[63]

The diagnosis of second primary thyroid cancer in CCS relies on the presence of a positive result on a fine needle aspiration biopsy of a suspected nodule.

The treatment and prognosis are identical to thyroid cancer diagnosed in general population.[67]

DISORDERS OF THE GONADS

a. Males

The testes are components of both, the reproductive system and endocrine system. The reproductive compartment consists of germ cells and the Sertoli cells that support them. Leydig cells which produce testosteron are a part of the endocrine compartment. The two compartments are affected differently by cancer therapies.

Leydig cell dysfunction

Hypogonadism after chemotherapy is rare but high doses of alkylating agents such as cyclophosphamide can cause low levels of testosterone.[68] Leydig cell dysfunction as a result of testicular irradiation is dependent on the age of the exposure and the dose of irradiation. Pre-pubertal males receiving doses > 24 Gy are at high risk for hypogonadism, while pubertal males are at risk when exposed to >33 Gy.[69] Subclinical hypogonadism with elevated LH and low normal testosterone levels rarely requires testosterone replacement. But CCS at risk should be followed for signs and laboratory evidence of hypogonadism and if needed replacement therapy should be started during pubertal years and adulthood.[70]

Germ cell dysfunction

Germ cell dysfunction with subsequent azospermia was reported in 38% males exposed to chemotherapy and/or testicular irradiation.[7] Small testicular volume, elevated FSH, and low inhibin

B levels are associated with poor fertility prognosis in males. Germ cells are more sensitive to testicular irradiation and chemotherapy than Leydig cells. Chemotherapy agents associated with germ cell dysfunction are cyclophosphamide, procarbazine, ifosfamide, busulfan, melphalan and cisplatin. Sperm cryopreservation is recommended in cancer patients prior to therapy with gonadotoxic agents.[70, 72, 73]

b. Females

Ovarian follicles are responsible for estrogen production and oocyte maturation. Both functions could be affected by ovarian damage due to cancer treatment.

The extent of the damage depends on the cronological age of patients and the intensity of therapy and also on the number of viable follicles or » ovarian reserve« at the time of exposure.[74,75] Cancer treatments associated with ovarian toxicity include abdominopelvic irradiation and chemotherapy agents such as cyclophosphamide, procarbazine, busulfan, melphalan, and thiotepa.

Gonadal lesions are less extensive in females than in males.[76,77,78] Chemotherapy is less toxic to the ovaries of pre-pubertal females in comparison to pubertal and adult females. The ovarian toxicity of abdominopelvic irradiation is age and dose dependent.

The incidence of premature menopause was highest among CCS exposed to both alkylating agents and irradiation. Elevated gonadotropins, low anti-mullerian hormone levels and reduced ovarian volume are found in case of ovarian insufficiency.[79, 80, 81] Despite declining ovarian reserve in CCS, some survivors have succcessful pregnancies with live birth rates up to 73%.[82]

In young CCS with ovarian failure poor linear growth and bone mineralisation are common.

Older hypo-gonadal females can develop menopausal symptoms and are at risk for osteoporosis and cardiovascular disease.[83]

The use of mature oocyte cryopreservation represents a viable option for young pubertal females prior to gonadotoxic therapies what could improve fertility prospects in the CCS population.[84]

ENDOCRINE DYSFUNCTION IN PATIENTS AFTER TREATMENT FOR HODGKIN'S DISEASE AND SARCOMAS IN CHILDHOOD

The combination surgery, chemotherapy and radiotherapy has greatly improved the long-term survival of children with Hodgkin's disease (HD) and sarcoma (SA), but the intensive therapies can lead to different late sequelae including endocrine dysfunctions. Our analysis of endocrine function in some patients who were treated for HD and SA at University children's hospital Ljubljana is presented.

Patients and methods.

23 patients treated for childhood cancer were studied; there were 14 patients with HD (4 females, 10 males) and 9 with SA, 8 of the soft tissue and one osteogenic (2 females, 7 males). The selection was based on patient's consent, age over15 years at the time of follow-up (f/u) examination and the follow-up perod exceeding 5 years.

The mean age at diagnosis was 10.4 years (range 5.0-14.0) for the patients with HD, and 9.5 (range 5.0-13.2) for the patients with SA. At the time of f/u the mean age of the patients with HD was 22.2 years (range from 17.3 to 30.0), while that of patients with SA was 18.8 years (range from 18.0 to 22.0).

The post-treatment evaluation for the HD group was performed at of 11.7 years (range 5.0-21.0) and for the SA patients 9.3 years (6.0-12.0) after treatment.

Most patients with HD were treated with 6 cycles of MOPP (MOPP = mechlorethamine, vincristine, procarbazine, prednisone). In addition, radiation therapy (RT) ranging from 30 to 40 Gy was applied to one of several areas involved, depending on the extent of the HD. One patient treated with the combination of MOPP – ABVD received only 3 cycles RT (ABVD = doxorubicin, bleomycin, vinblastine, dacarbazine). Two patients had no chemotherapy.

The patients with SA were treated with surgery, chemotherapy and radiation. Chemotherapy for most of the patients included cyclophosphamide, actinomycin D, vincristine D, vincristine (VAC) +/- adriamycin following the so-called T_2 or T_6 or T_{10} protocols of Memorial Hospital, described by Memorial-Sloan Kettering Cancer Center, New York investigators. Bleomycin and methotrexate were added in some cases. The doses of radiation varied from 30 to 60 Gy (*Table VI.1*).

All patients underwent general physical examination. Height, weight and clinical abnormalities were recorded, as well as Tanner stages of pubic hair and genital development, and the onset and the course of puberty.

Endocrine assesement

Thyroid status was assesed by measurement of the serum concentration of T4, T3 and TSH in basal state and 30 minutes after stimulation with TRH. Growth hormone (GH) was stimulated with arginine and L-DOPA. Deficiency was defined as a GH level <10 mcg/l after stimulation. In some patients only IGF I values were determined. Testosterone and estradiol levels were measured in basal state. Concentrations of LH and FSH were determined before and after i. v. administration of gonadotropin realising hormone (GnRH). Basal and stimulated levels of cortisol after i.v. application of synthetic ACTH were determined in the morning. Measurement of hormones was performed at the central laboratory of University Medical Centre Ljubljana using standard laboratory procedures.

Results

The abnormalities found at physical examination in the course of puberty and the results of endocrine evaluation are presented in Tables VI.1 and VI.2.

Table VI.1 - Age at treatment and study, clinical abnormalities and endocrine dysfunction in patients trated for HD (Table VI.1.a) and SA (Table VI.1.b)

Table VI.1.a

Pts. no.	Sex	Age (years) at		Years F/U	Diagnosis	Therapy			Clinical abnormalities
		treat.	study			surgery	radiation (close)	Cht	
1	F	13	23	10	HD III	-	30 Gy, neck bil., mediastinum, paraaort., lgl, spleen	MOPP	obesity, spontaneous abortion at 22 years, sterility
2	M	12	26	14	HD III	-	30 Gy, TNI + spleen	MOPP	retard puberty, atrophy of soft tissue, hypothyrosis
3	M	13	23	10	HD III	laparatomy	30 Gy, TNI + spleen	MOPP	atrophy of neck
4	M	10	22	12	HD II	laparatomy	30 Gy, neck bil.	MOPP	astenia, azoospermia
5	F	13	22	9	HD II	laparatomy	30 Gy, mediastrinum, neck bil.	MOPP	obesity, one child
6	M	5	18	12	HD III	laparatomy	37,5 Gy, neck + inverted Y	MOPP	astenia, caries, short statue
7	M	12	24	12	HD I	-	40 Gy, inverted Y	-	bilateral gynecomastia, azoospermia
8	F	11	22	11	HD III	-	30 Gy, neck	MOPP	atrophy of neck
9	F	9	24	15	HD III	-	30 Gy, mediastinum, paraaort., lgl.	MOPP	none- pregnant
10	M	12	21	9	HD III	-	30 Gy, mediastinum, paraaort., lgl.	MOPP	none
11	M	14	19	5	HD II	-	-	MOPP, ABVD	none
12	M	5	8	13	HD III	-	30 Gy, TNI	MOPP	soft tissue atrophy, short statue
13	M	6	17	10	HD II	laparatomy	36 Gy, neck bil.	MOPP	none
14	M	9	30	21	HD II	-	40 Gy, inverted y	-	lwft fem. nerve paresis, muscle atrophy of the left leg

Table VI.1.b

Pts. no.	Sex	Age (years) at		Years F/U	Diagnosis	Therapy			Clinical abnormalities
		treat.	study			surgery	radiation (close)	Cht	
15	M	5	16	11	liposarcoma orbitae dex.	extenteration	50 Gy	-	asymetry of face and orbit, occurence of the third dention
16	M	10	22	12	ERMS auriculare sin. Lymph node metast.	Excicion, lymphadenectomy	60 Gy	VAC	atrophy of the left side of the face, epilepy (treated by Tegretol)
17	M	13	19	6	ERMS epipharyngis	-	46 Gy	$T_6 + T_2$	soft tissue and bone atrophy of the right side of face
18	M	9	19	10	ERMS epipharyngis lymph node metast.	-	50 Gy	VAC	blindness, bilateral atrophy of the face, eunochoid skeletal proportions, pituitary dwarfism
19	F	13	16	2	ERMS lymph node metast., cheek	extirpation	40 Gy	VAC	atrophy of the neck
20	M	11	21	10	ERMS paratesticular	orchiectomy lymphadenectomy	-	VAC	eunuchoid skeletal proportion
21	M	6	16	10	Synovioma right knee	excision (2XX)	-	T_2	none (champion in riding)
22	F	12	18	6	femur osteosarcoma	AK amputation	-	T_{10}	prothesis
23	M	6	17	11	ERMS left scapular region	excision	36 Gy	VAC	muscular atrophy in the left scapular region

Table VI. 2. Results of endocrine investigations in patients treated for HD (a) and SA (b)

Case	T4 (nmol/l)	THS (mU/l)		Cortisol (nmol/l)		Testosteron (nmol/l)	Estradiol (nmol/l)	LH (mU/l)		FHS (mU/l)		GH (mog/l)		IGFI (U/l)
		basal	maximal	basal	maximal			basal	maximal	basal	maximal	basal	maximal	
(a)														
1	111	2.70	19.20	534	1020	2.45	0.13	14.5	28.7	19.6	29.6	1.5	13.9	1490
2	81	4.40	25.90	348	959	17.90	0.03	15.6	26.1	9.2	8.9	1.1	43.1	1530
3	102	1.60	9.30	690	954	24.00	0.11	16.8	103.0	15.6	31.7	0.4	56.2	-
4	96	2.12	10.60	434	1443	23.50	0.09	6.9	64.3	17.9	39.9	1.0	1.7	-
5	120	1.31	18.39	172	630	2.20	0.36	3.1	15.4	8.2	12.4	0.9	5.1	2266
6	103	0.94	0.76	401	603	29.90	0.05	9.5	12.1	24.6	23.0	0.2	50.1	1979
7	135	4.14	33.84	534	949	17.30	0.10	14.7	80.2	32.2	67.1	0.8	1.7	990
8	109	1.60	11.50	282	756	1.72	0.43	17.2	58.5	31.3	15.3	0.7	13.0	840
9	104	1.54	15.40	316	660	2.50	0.06	6.2	12.2	4.6	5.1	0.3	3.8	1394
10	74	1.01	7.86	421	796	10.40	0.16	19.7	81.7	32.1	68.1	0.06	6.6	2864
11	134	2.32	14.00	636	1346	18.20	0.02	1.6	23.3	8.9	21.8	-	-	779
12	114	1.77	15.22	398	652	17.20	0.25	3.8	35.2	12.6	27.9	-	-	1501
13	89	4.62	53.20	441	1269	10.60	0.07	6.0	24.1	15.5	21.5	-	-	2732
14	105	1.60	11.80	626	946	9.20	0.10	4.9	22.0	21.0	35.2	-	-	870
(b)														
15	108	2.00	11.80	361	804	28.12	0.03	10.5	24.1	8.5	12.1	0.9	16.6	900
16	94	2.90	17.10	716	1083	19.63	0.14	31.3	105.0	67.7	91.1	2.1	23.2	480
17	123	3.38	23.18	263	478	15.00	0.08	5.8	34.5	9.3	17.6	-	-	970
18	134	2.90	9.30	453	741	13.81	0.08	10.6	36.2	13.0	25.0	0.6	1.8	280
19	114	5.84	37.66	767	1124	1.50	0.29	2.9	11.5	7.2	10.0	0.3	1.1	585
20	81	0.04	3.35	542	844	15.30	0.24	14.7	53.7	10.6	16.7	0.1	13.6	2986
21	94	2.90	15.30	180	387	14.40	0.04	8.3	-	9.1	-	0.7	54.7	430
22	95	2.43	14.90	1032	1280	4.40	0.97	6.9	64.4	11.5	27.6	1.1	16.9	5238
23	128	1.73	13.17	372	751	6.90	0.14	10.9	72.4	23.2	37.7	-	-	1266
Normal range	53-182	0.17-4.05	25.00	at 8 a.m. 200-690		after puberty M 13.7-35.4 F 1.0-3.0	M 0-0.20 F 0.13-0.78	M 1-8.4 F 1.6-9.3 (phollic. phase)		M 1-10.5 F2.6-9.3 (phollic. phase)		0.0-5.6	>10	423-3790

Growth hormone: GH was evaluated in 17 patients. Subnormal stimulated levels but normal IGF I values were found in 6 patients (HD: n=4, SA: n=2). Pituitary dwarfism with diminished levels of GH and IGFI was found in one patient (No.18).

Thyroid:

Thyroid dysfunctions were found in 7 patients. Though T4 and T3 levels were normal, primary latent hypothyroidism with either normal or elevated basal TSH levels and hyperresposivenes to TRH was found in 5 patients (Nos. 2, 7, 13, 16, 19) (HD: n=3, SA: n=2). Of the 7 patients with HD who had irradiation of the neck with incidental exposure of the thyroid, 3 (Nos. 13, 16, 19) had thyroid dysfunction. Of the 4 patients (Nos. 16, 17, 18, 19) with SA who had irradiation of the neck, 2 (Nos. 16, 19) had thyroid dysfunction. Two patients (Nos. 7 and 20) with thyroid dysfunction had no irradiation of the neck.

Hypothalamo – pituitary – adrenal axis:

Low levels of cortisol and exaggerated respond to ACTH indicating a hypothalamo-pituitary-adrenal dysfunction were found in one patient treated for HD (No. 5). In one patient treated for SA (No. 21) low basal and normal stimulated levels of cortisol were measured, while another one (No. 22) had high basal and stimulated levels of cortisol.

Hypothalamo – pituitary – gonadal axis:

Dysfunctions were found in 11 patients treated for HD (F=2, M=9) (76.6%). Three males had decreased basal levels of testosterone (Nos. 10, 13, 14); two of them were treated with MOPP and irradiation, one received only irradiation (inverted Y). One female (No. 9) had decresed values of estradiol and blunted response of FSH to stimulation. She was considered to be sterile after 3 years of childless marriage. Elevated values of LH in the basal state were foound in 7 patients (F=2, M=5) (Nos. 1, 2, 3, 6, 7, 8, 10), while an exaggerated response to Gn-RH was found in 8 (F=2, M=6) (Nos. 1, 2, 3, 4, 7, 8, 10, 12). Increased FSH values in basal and stimulated condition were discovered in 8 patients (F=1, M=7) (Nos. 1, 3, 4, 6, 7, 10, 12, 14) who had MOPP and RT above the diaphragm. In one female and in one male patient (Nos. 9, 13), only basal FSH values were elevated. In those with hypogonadism, infertile marriage was registered in 3 (Nos. 1, 4, 7) and azoospermia

was proven in two patients (Nos. 4, 7). Two of the patients were obese; one of the two also exhibited gynecomasty. Puberty occured late in one case (No. 2).

Out of the 9 patients with SA, primary gonadal dysfunction was found in 7 (F=1, M=6) (77.8%). In two males (Nos. 18, 20) eunuchoid proportions were registered. Increased levels of testosterone were found in one female (No. 22). Increased levels of LH in basal and stimulated conditions were found in 5 male patients (Nos. 15, 16, 18, 20, 23). Exaggerated response of normal basal LH values was found in one female (No. 22) and one male (No. 17). Elevated levels of FSH were observed in 5 patients (F=1, M=4) (Nos. 16, 18, 20, 22, 23).

Discussion

There was a high prevalence of endocrine dysfunction, exceeding 80%, in our patients treated for Hodgkin's disease and bone and soft tissue sarcomas. Clinically evident somatic abnormalities were caused by endocrinologic deficiency only on 5 cases (22%) (Nos. 1, 2, 7, 18, 20), while all others were due to tumor, radiation therapy or surgery. There was only one case of clinically evident pituitary dwarfism, in patient No.18 whose height and weight were bellow the 3rd percentile. Primary hypogonadism and eunuchoid proportions were also found in this patient. He was treated at the age of 9 for nasopharyngeal rhabdomyosarcoma; the value of radation included the base of the skull, consequently the pituitary gland received 50 Gy. In all cases of blunted GH response to provocative stimuli and normal IGF I values, the patients' height was in the normal range. In general, GH measurement would be of clinical value in cases of retarded growth or decreased growth velocity in still growing children and adolescents in order to start GH replacement therapy when necessary.

In the patients studied there were no clinically important adrenal abnormalities.We believe that there is no need to evaluate the adrenal function in patients treated for HD and SA, but on the other hand, the thyroid and especially hypohalamo – pituitary – gonadal axis should be examined exstensively due to high percentage of dysfunction (87%) found in our patients.

Thyroid dysfunction with decreased stimulated TSH levels and with high basal levels and/or exaggerated response to TRH was found in 30.4% of the patients who were clinically euthyreotic.

While the role of chemotherapy is uncertain, it is generally accepted that radiotherapy of the neck is an important cause of of thyroid dysfunction. Out of eleven of our patients who had the thyroid exposed to radiation, 5 had thyroid dysfunction. For the remaining two whose necks were not irradiated, the reason for pathological values could be chemotherapy at least in one case (No. 20).

It has been demonstrated that elevation of TSH levels in the presence of radiation-damaged thyroid tissue is carcinogenic and that administration of thyroid hormone depresses TSH levels and decreases the possibility of radiation-induced thyroid carcinoma (85). It is why the children who were exposed to irradiation of the neck should be followed for life. Check-ups of the structure and thyroid function should be part of follow-up examinations. Since elevated serum thyroglobulin levels are associated with an increased risk of developing thyroid cancer, thyroglobulin measurements could be be a useful adjunct to clinical examination[86]

Hypothalamo – pituitary – gonadal dysfunction: Only 2 of the 14 HD patients had sexual hormone levels within normal range; one female (No. 5) treated with MOPP and radiation above the diaphragm and one male who received MOPP in combination with ABVD and no radiation (No. 22). It is well known that chemotherapy of HD by the MOPP combination may cause a gonadal dysfunction. In our patients an additional cause for hypogonadism (azoospermia) was irradiation with the inverted Y technique. Patient No.7 had irradiation on a Co 60 unit without additional shielding of the testes. The doses to the testes by the same inverted Y on a linear accelerator and with additional shielding is significantly lower (1.5% of the applied tumor dose). Additional shielding of the testes is therefore recommended in all cases when radiation is applied to the lower abdomen. Because of the high percentage of hypogonadism in children treated for HD with MOPP, it would seem that, for some of them, radiation is the reasonable choice until less toxic chemotherapy regimens are available. ABVD has been proven to be less toxic than MOPP, but still not harmless.

Hypogonadism followed chemotherapy in all except one patient with SA.The girl received the VAC regimen when she was 13; the same regimen produced hypogonadism in all 4 male patients. The regimens T« and T6 seem to be less harmful to the gonads; possibly because the cumulative dose of cyclophosphamide was lower than in VAC.

All patients investigated were postpubertal. The shortest intervals between treatment and endocrine investigations were 5.0 and 6.0 years for HD and SA respectively.The endocrine sequelae discovered in our patients can be considered permanent.

The normal development of puberty in all patients investigated except one is in accordance with the known less toxic effects of alkylating agents on the Leydidg cells in boys and theca cells in girls. Additionally, irradiation of males is reported to damage, in a dose-dependent manner, first the germinal epithelium and the the Leydig cells. Due to the fact that testes are more vulnerable than the ovaries hypogonadism was observed also more often in male than female patients included in our study. There were several attempts to prevent gonadal lesions in males treated for malignancies. Gn-RH analogues and androgens before chemotherapy and irradiation have been found to protect partially male gonads but clinical trial have not been encouraging so far.[87]

CONCLUSION

Endocrinopathies are very common in patients who were treated for childhood cancer. The results of our study confirm the high prevalence of endocrine dysfunction also in patients treated for HD and SA. The function of the thyroid gland and of the hypohalamo-pituitary-gonadal axis should be assesed at frequent intervals in order to detect endocrinological abnormalities and to introduce replacement therapy when necessary. A high proportion of patients treated for cancer in childhood suffer from gonadal dysfunction. It is therefore, important to search for drugs or pretreatment that could prevent or deminish the late sequelae.

REFERENCES

1. Ward E, Desantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. Ca Cancer J Clin 2014; 64: 83-103.
2. Anon. Treatment of cildhood cancers: late effects. Prescrire Int 2015; 164: 236-9.
3. Hudson MM, Ness KK, Gurney JG et al. Clinical ascertainment of health outcomes among adults treated foe childhood cancer. JAMA 2013; 309: 2371-81.
4. de Fine Licht S, Winther JF, Gudmundsddottir et al. Hospital contacts for endocrine disorders in Adult life after Childhood Cancer in Scandinavia: a population-based cohort study. Lancet 2014; 383: 1981-9.
5. Velenšek V, Mazić U, Kržišnik C et al. Cardiac damage after treatment of childhood cancer: a long-term follow-up. BMC Cancer 2008; 8: 141-4.

6. Jones D, Spunt S, Green D et al. Renal Late Effects in Patients Treated for Cancer in Childhood: A Report from the Children¨s Oncology Group. Pediatr Blood Cancer 2008; 51: 724-731.
7. Karas-Kuželički N, Mencej-Bedrač S, Jazbec J et al: Risk factors for symptomatic osteonecrosis in childhood ALL: A retrospective study of a Slovenian pediatric ALL population between 1970 and 2004. Exp Ther Med 2016, 12: 840-846.
8. John T, Lomeli N, Bota DA. Systemic cisplatin exposure during infancy and adolescence causes impaired cognitive function in adulthood. Behav Brain Res 2017; 319: 200-206.
9. Erman N, Todorovski L, Jereb B. Late somatic sequelae after treatment of childhood cancer in Slovenia. BMC Res Notes 2012; 5: 254-257.
10. Brignardello E, Felicetti F, Castiglione A et al. Endocrine health conditions in adult survivors of childhood cancer: the need for specialised adult-focused follow-up clinics. Eur J Endocrinol 2013; 168: 465-72.
11. Lodish MB. Clinical review: kinase inhibitors: adverse effects related to the endocrine system. J Clin Endocrinol Metab 2013; 98: 1333-42.
12. Corsello SM, Barnabei A, Marchetti P et al. Endocrine side effects induced by immune checkpoint inhibitors. J Clin Endocrinol Metab 2013; 98: 1361-75.
13. Barnes N, Chemaitilly W. Endocrinopathies in survivors of childhood neoplasia. Front Pediatr 2014; 2: 1-12.
14. Chow EJ, Liu W, Srivastava K et al. Differential effects of radiotherapy on growth and endocrine function among acute leukemia survivors: a childhood cancer survivor study report. Pediatr Blood Cancer 2013; 60: 110-5.
15. Bayram C, Yarali N, Fettah A et al. Evaluation of endocrine late complications in childhood acute lymphoblastic leukemia survivors: a report of single-center experience and review of literature. Turk J Haematol 2017; 34: 40-5.
16. Wilson CL, Gawade PL, Ness KK. Impairments that influence physical function among survivors of childhood cancer. Children 2015; 2: 1-36.
17. Armstrong GT, Chow EJ, Sklar CA. Alterations in pubertal timing following therapy for childhood malignancies. Endocr Dev 2009; 15: 25-39.
18. Clayton PE, Shalet SM. The evolution of spinal growth after irradiation. Clin Oncol 1991; 3: 220-2.
19. Merchant TE, Rose SR, Bosley C et al. Growth hormone secretion after conformal radiation therapy in pediatric patients with localised brain tumors. J Clin Oncol 2011, 29: 4776-80.
20. Rose SR, Schreiber RE, Kearney NS et al. Hypothalamic dysfunction after chemotherapy. J Pediatr Endocrinol Metab 2004; 17: 55-66.
21. Rastogi MV, Stork L, Druker B et al. Imatinib mesylate causes growth deceleration in pediatric patients with chronic myelogenous leukemia. Pediatr Blood Cancer 2012; 59: 840-5.
22. Iwayama H, Kamijo T, Ueda N et al. Hyperinsulinemia may promote growth without GH in children after resection of suprasellar brain tumor. Endocrine 2011; 40: 130-3.
23. Blijdrop K, van den Heuvel-Eibrink M, Pieters R et al. The limited screening value of insulin-like growth factor –I as a marker for altwerations in body composition in very long-term adult survivors of childhood cancer. Pediatr Blood Cancer 2012; 59: 711-6.

24. Chemaitilly W, Robison LI. Safety of growth hormone treatment in patients previously treated for cancer. Endocrinol Metab Clin North Am 2012; 41: 785-92.
25. Woodmansee WW, Zimmermann AG, Child CJ et al. Incidence of second neoplasm in childhood cancer survivors treated with GH: an analysis of GeNeSIS and HypoCCS. Eur J Endocrinol 2013; 15: 168-71.
26. Child CJ, Zimmermann AG, Jia N et al. Assessment of primary cancer incidence in growth hormone-treated children: comparison of a multinational prospective observational study with population databases. Horm Res Paediatr 2016; 85: 198-206.
27. Patterson BC, Chen Y, Sclar CA et al. Growth hormone exposure as a risk factor for the development of subsequent neoplasms of the central nervous system: a report from the Childhood Cancer Survivor Study. J Clin Endocrinol Metab 2014; 99: 2030-7.
28. Follin C, Thilen U, Ahren B et al. Improvement in cardiac systolic function and reduced prevalence of metabolic syndrome after two years of growth hormone (GH) treatment in GH-deficient adult survivors of childhood-onset acute lymphoblastic leukemia. J Clin Endocrinol Metab 2006; 91: 1872-5.
29. Muir A. Precocious puberty. Pediatr Rev 2006; 27: 373-81.
30. Armstrong GT, Whitton JA, Gajjar A et al. Abnormal timing of menarche in survivors of central nervous system tumors: a report from the childhood cancer survivor study. Cancer 2009; 115: 2562-70.
31. Oberfield SE, Sorrano D, Nirenberg A et al. Age at onset of puberty following high-dose central nervous system radiation therapy. Arch Pediatr Adolesc Med 1996; 150: 589-92.
32. Carel JC, Lahlou N, Roger M et al. Precocious puberty and statural growth. Hum Reprod Update 2004; 10: 135-47.
33. Lee PA. Central precocious puberty. An overview of diagnosis, treatment, and outcome. Endocrinol Metab Clin North Am 1999; 28: 901-18.
34. Haber HP, Wollman HA, Ranke MB. Pelvic ultrasonography: early differentiation between isolated premature telarche and central precocious puberty. Eur J Pediatr 1995; 154: 182-6.
35. Gleeson HK, Stoeter R, Ogilvy-Stuart AL et al. Improvements in final height over 25 years in growth hormone (GH)-deficient childhood survivors of brain tumors receiving GH replacement. J Clin Endocrionol Metab 200388: 3682-9.
36. Stephen MD, Zage PE, Waguespack SG. Gonadotropin-dependent pecocious puberty: neoplastic causes and endocrine considerations. Int J Pediatr Endocrinol 2011; 2011: 1-6.
37. Relander T, Cavallin-Stahl E, Garwicz S et al. Gonadal and sexual function in men treated for childhood cancr. Med Pediatr Oncol 2000; 35: 52-63.
38. Rose SR, Danish RK, KearneyNS et al. ACTH deficiency in childhood cancer survivors. Pediatr Blood Cancer 2005; 45: 808-13.
39. Laughton SI, Merchant TE, Sklar CA et al. Endocrine outcomes for children with embryonal brain tumors after risk adapted craniospinal and conformal primary-site irradiation and high-dose chemotherapy with stem-cell rescue on the SJMB-96 trial. J Clin Oncol 2008; 26: 1112-8.
40. Gundgurthi A, Garg MK, Nair V et al. Endocrine complications after busulphan and cyclophosphamide based hematopoetic stem cell transplant: asingle tertiary center experience. Ind J Endocrinol Metab 2013; 17: 855-63.

41. Ogilvy-Stuart AL, Clark DJ, Wallace WH et al. Endocrine deficit after fractionated total body irradiation. Arch Dis Child 1992, 67: 1107-10.
42. Bilgir O, Kabapcilar l, Bilgir F et al. Is there any relationship between imatinib mesylate medication and hypothalamic-pituitary-adrenal axis dysfunction? Int J Clin Pract 2010; 64: 45-50.
43. Blansfield JA, Beck KE, Tran K et al. T-lymphocyte- associsted antigen-4, blockage can induce autoimmune hypophysitis in patients with metastatic melanoma and renal cancer. J Immunother 2006; 28: 593-8.
44. Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK et al. A population-based study of thyroid function afterradiotherapy and chemotherapy for a chidhood brain tumor. J Clin Endocrinol Metab 2003; 88: 136-40.
45. Fernandez A, Brada M, Zabuliene L et al. Radiation - induced hypopituitarism. Endocr Relat Cancer 2009; 16: 733-72.
46. Rose SR, Lustig RH, Pitukcheewanont P et al. Diagnosis of hidden central hypothyroidism in survivors of childhood cancer. J Clin Endocrinol Metab 1999; 84: 4472-9.
47. Slawik M, Klawitter B, Meiser E et al. Thyroid hormone replacement for central hypothyroidism: a randomised controlled trial comparing two doses of thyroxine (T4) with combination of T4 and triiodthyronine. J Clin Endocrinol Metab 2007; 92: 4115-22.
48. Fitzgerald P, Dinan TG. Prolactin and dopamine: what is the connection? A review article. J Psychopharmacol 2008; 22: 12-9.
49. Constantine LS, Woolf PD, Cann D et al. Hypothalamic-pituitary dysfunction after radiation for brain tumors. N Engl J Med 1993; 328: 87-94.
50. Ramelli GP, von der Weid N, Stanga Z et al. Suprasellar germonomas in childhood and adolescence: disagnostic pitfalls. J Pediatr Endocrinol Metab 1998; 11: 693-7.
51. Silfen ME, Garvin JH, Hays AP et al. Primary central nervous system lymphoma in childhood presenting as progressive panhypopituitarism. J Pediatr Hematol Oncol 2001; 23: 130-3.
52. Spoudeas HA. Growth and endocrine function after chemotherapy and radiotherapy in childhood. Eur J Cancer 2002; 38: 1748-59.
53. Chow EJ, Friedman DL, Stovall M et al. Risk of thyroid dysfunction and subsequent thyroid cancer among survivors of acute lymphoblastic leukemia: a report from the childhood cancer survivor Study. Pediatr Blood Cancer 2009; 53: 432-7.
54. Armstrong GT, Stovall M, Robinson LL et al. Long term effects of radiation exposure among adult survivors of childhood cancer: results from the childhood cancer survivor study.Radiat Res 2010; 174: 840-50.
55. Laugton SJ, Merchant TE, Sklar CA et al. Endocrine outcomes for children with embryonal brain tumors after risk-adapted craniospinal and conformal primary-site irradiation and high-dose chemotherapy with stem-cell rescue on the SJMB-96-trial. J Clin Oncol 2008; 26: 1112-8.
56. Chow EJ, Friedman DL, Stovall M et al. Risk of thyroid dysfunction and subsequent thyroid cancer among survivors of acute lymphoblastic leukemia: a report from the childhood cancer survivor study. Pediatr Blood Cancer 2009; 53: 432-7.
57. Illouz F, Braun D, Briet C et al. Endocrine-side effects o fanti-cancer drugs: thyroid effects of tyrosine kinase inhibitors. Eur J Endocrinol 2014; 171: 91-9.

58. Desai J, Yassa L, Marqusee E et al. Hypothyroidism after suntinib treatment for patients with gastrointestinal stromal tumors. Ann Intern Med 2006; 145: 660-4.
59. Haugen BR. Drugs that suppres TSH or cause central hypothyroidism. Best Pract Res Clin Endocrinol Metab 2009; 23: 793-800.
60. Sanders JE, Hoffmeister PA, Woolfrey AE et al. Thyroid function following hematopoietic cell transplantation in children: 30 nyears experience. Blood 2009; 113: 306-8.
61. Jung YJ, Jeon YJ, Cho WK et al. Risk factors for short term thyroid dysfunction after hematopoietic stem cell transplantation in children. Kor J Pediatr 2013; 56: 298-303.
62. Au WY, Lie AK, Kung AW et al. Autoimmune thyroid dysfunction after hematopoietic stem cell transplantation. Bone Marrow Transplant 2005; 35: 383-5.
63. Veiga LH, Bhatti P, Ronckers CM et al. Chemotherapy and thyroid cancer risk: a report from the childhood cancer survivor study. CancerEpidemiol Biomarkers Prev 2012; 21: 92-101.
64. Taylor AJ, Croft AP, Palace AM et al. Risk of thyroid cancer in survivors of childhood cancer: results from the British childhood cancer survivor study. Int J Cancer 2009; 125: 2400-5.
65. Bhatti P, Veiga LH, Ronckers CM et al. Risk of second primary thyroid cancer after radiotherapy for a childhood cancer in a large cohort study: an update from the childhood cancer survivor study. Radiat Res 2010; 174: 741-52.
66. Casagranda L, Oriol M, Freycon F et al. Second malignant neoplasm following childhood cancer: A nested case-control study of a recent cohort (1987-2004) from the Childhood Cancer Registry of the Rhone-Alpes region in France. Pediatr Hematol Oncol 2016; 6: 371-82.
67. Metzger ML, Howard SC, Hudson MM et al. Natural history of thyroid nodules in survivors of pediatrc Hodgkin lymphoma. Pediatr Blood Cancer 2006; 46: 314-9.
68. Kenney LB, Laufer MR, Grant FD et al. High risk infertility and long term gonadal damage in males treated with high dose syclophosphamide for sarcoma during childhood. Cancer 2001; 91: 613-21.
69. Izard MA. Leydig cell function and radiation: a review of the literature. Radiother Oncol 1995; 34: 1-8.
70. Kenney LB, Cohen LE, Shnorhavorian M et al. Male reproductive health after childhood, adolescent, and young adult cancers; a reportfrom the Children's Oncology Group. J Clin Oncol 2012; 30: 34008-16.
71. Green DM, Zhu L, Zhang N et al. Lack of specifity of plasma concentrations of inhibin B and follicle-stimulating hormone for identification of azoospermic survivors of childhood cancer: a report from the St Jude lifetime cohort study. J Clin Oncol 2013; 31: 1324-8.
72. Meistrich MI. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. Fertil Steril 2013; 100: 1180-6.
73. Goosens E, Van Saen D, Tournaye H. Spermatogonial stem cell preservation and transplantation: from research to clinic. Human Reprod 2013; 28: 897-907.
74. Chemaitilly W, Mertens AC, Mitby P et al. Acute ovarian failure in the childhoodood cancer survivor study. J Clin Endocrinol Metab 2006; 91: 1723-8.

75. Sklar CA, Mertens AC, Mitby P et al. Premature menopause in survivors of childhood cancer: a report from the childood cancer survivor study. J Natl Cancer Inst 2006; 98: 890-6.
76. Rivkees SA, Crawford JD. The relationship of gonadal activity and chemotherapy-induced gonadal damage. JAMA 1988; 259: 2123-5.
77. Kržišnik C, Jereb B, Bratanič N et al. Endocrinopathies in patients after tratment for Hodgkin´s disease and sarcomas in childhood. Zdrav Vest 1994; 63: 45-9.
78. Zadravec Zaletel L, Bratanič N, Jereb B. Gonadal function in patients treated for Hodgkin's disease in childhood. Radiol Oncol 2010; 44: 187-93.
79. Brydoy M, Fossa SD, Dahl O et al. Gonadal dysfunction and fertility problems in cancer survivors. Acta Oncol 2007; 46: 480-9.
80. El-Shalakany AH, Ali MS, Abdelmaksoud AA et al. Ovarian function in female survivors of childhood malignancies. Pediatr Hematol Oncol 2013; 30: 328-35.
81. Visser JA, de Jong FH, Laven JS et al. Anti-Mullerian hormone: a new marker for ovrian function. Reproduction 2006; 131: 1-9.
82. Reulen RC, Zeegers MP, Wallace WH et al. Pregnancy outcomes among adult survivors of childhood cancer in the British childhood cancer survivor study. Cancer Epidemiol Biomarkers Prev 2009; 18: 2239-47.
83. Aisenberg J, Hsieh K, Kalaitzoglou G et al. Bone mineral density in young adult survivors of childhood cancer. J Pediatr Hematol Oncol 1998; 20: 241-5.
84. Practice Committee of American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril 2013; 99: 37-43.
85. Lubin JH, Adams MJ, Shore R et al. Thyroid cancer following childhood low dose radiation exposure: a pooled analysis of nine cohorts. J Clin Endocrinol Metab 2017; doi: 10.1210/jc. 2016-3529.
86. Indrasena BS. Use of thyroglobulin as a tumor marker. World J Biol Chem 2017; 26: 81-85.
87. Meistrich ML, Shetty G. Hormonal suppression for fertility preservation in males and females. Reproduction 2008; 136: 691-701.

Ciril Kržišnik, MD, PhD
Pediatrician endocrinologist, senior councillor
Professor emeritus
University of Ljubljana, Faculty of Medicine
Vrazov trg 2, 1000 Ljubljana, Slovenia
E-mail: ciril.krzisnik@mf.uni-lj.si

February 2017

Tiparul executat la

IMPRIMERIA MIRTON
RO-Timişoara, str. Samuil Micu nr. 7
Tel.: 0256-225684, 272926; Fax: 0256-208924;
e-mail: mirton@mirton.ro; www.mirton.ro

ISBN 978-973-52-1727-3