PEDIATRIC ENDOCRINOLOGY AND DIABETES

2016 UPDATE

Editors:

Iulian P. VELEA
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Stuart J. BRINK

EDITURA MIRTON

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Preface

Starting with 2010, the first half of May represents a month when the doctors involved in Pediatric Endocrinology and Diabetes are meeting in Timisoara for exchanging experiences and new ideas.

Timisoara, capital of Banat County, also called the "Town of Flowers and Parks" or "The Little Wien" is becoming again this year, like every year before, the capital of the Romanian Pediatric Endocrinology and Diabetes.

According to its declared aim to increase the professional training level of the specialists in the field, The Romanian Society of Pediatric Diabetes, Nutrition and Endocrinology, is publishing the 6th volume of the Pediatric Endocrinology and Diabetes Update including the most important lectures presented during the Congress.

Because of the tradition to print this book yearly, the editors decided that, starting with this year, the title of the volume will be "Pediatric Endocrinology and Diabetes – 2016 Update", meaning that every year we will have a so-called "Yearbook of ENDOPED".

We kindly thank to all our speakers from abroad for their efforts, involvement and understanding without which it would not have been possible editing this book.

Editors.

Contents

ADRENAL INSUFFICIENCY IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS

Stuart J. Brink

Introduction

Adrenal insufficiency can be a primary, secondary or tertiary problem and it can be congenital or acquired. If the adrenal gland itself is hypoactive, this would be defined as a primary adrenal insufficiency (AI) whereas if the pituitary gland is the source of the problem, this would define secondary AI with deficiency or insufficiency of ACTH, adrenocorticotropic hormone. Tertiary AI would be defined, then, as hypothalamic insufficiency where CRH, corticotrophin releasing hormone, is deficient since the neuro-hypothalamic-pitutiary-adrenal axis can be interrupted at any of these anatomic sites.

The two adrenal glands sit atop the kidneys with first descriptions of these suprarenal tissues by the Italian anatomist, Bartolommeo Eustachio, in 1563. Their function was not understood until Thomas Addison's seminal clinical descriptions of AI in the 1855 [1] in London [2] with a good number of these original Addison AI patients' probably secondary to tuberculosis since that was one of the common severe manifestations of tuberculosis at the time.

Embryologically mesodermal cells give rise to the adrenal cortex whereas the adrenal medulla origins are ectodermal in origin. Earliest differentiation occurs about 5-6 weeks after conception and, by about 7-8 weeks in fetal development, sympathetic neural cells invade the primitive adrenocortical cells and then encapsulation of the adrenals glands take place. Thereafter, the enlarging fetal adrenal zone generates androgenic precursors for placental synthesis of estriol while the outer and the transitional zones are mainly responsible for mineralocorticoids and

glucocorticoids. At birth, the fetal adrenal glands are roughly twice the size of adult adrenals.

The mature adrenal cortex consists of three histologically distinct zones or regions:

- the glomerulosa just underneath the adrenal – or suprarenal – capsule,
- the fasciculata in the middle of the cortex and
- the innermost reticularis next to the adrenal medulla in the center of the triangular shaped adrenal glands atop the two kidneys.

The three zones plus a "transitional" zone between the glomerulosa and fasciculata can be classified morphologically but with more recent classifications based on specific enzymes and hormone production that is now known.

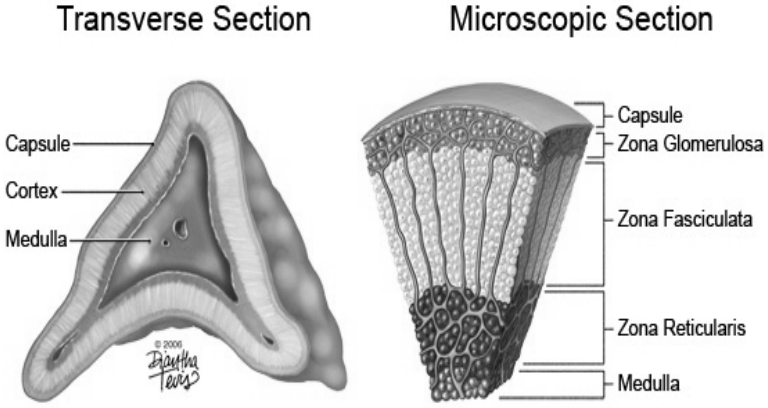
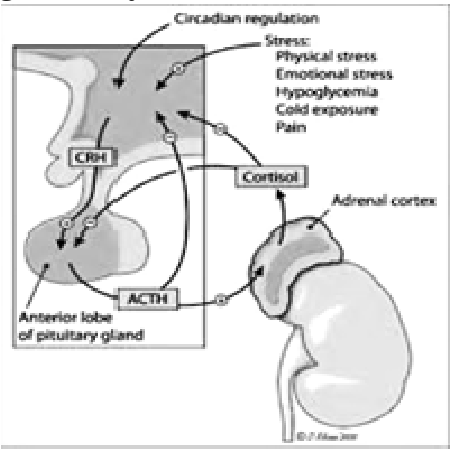


Fig. 1.1. – Structure of adrenal gland

Different genes also seem to be expressed in these different parts of the adrenals that also highlight the work that takes place in each of these regions with the outermost zona glomerulosa predominantly producing mineralocorticoids, the middle zona fasciculata predominantly producing glucocorticoids and the inside zona reticularis predominantly producing androgens (with some estrogens) while the adrenal medulla is mainly responsible for catecholamine production with control modulated by pituitary ACTH responding to hypothalamic CRH for the cortex and through the renin-angiotensin system, although with some influence particularly in high ACTH states, for mineralocorticoids, predominantly aldosterone.

Soon after birth, the relatively large adrenal gland, particularly the large fetal zone begins to involute while the other zones expand over the first three years of life. The zona reticularis doesn't reach "full maturity" until adolescence. The three zones show lots of overlap functionality.

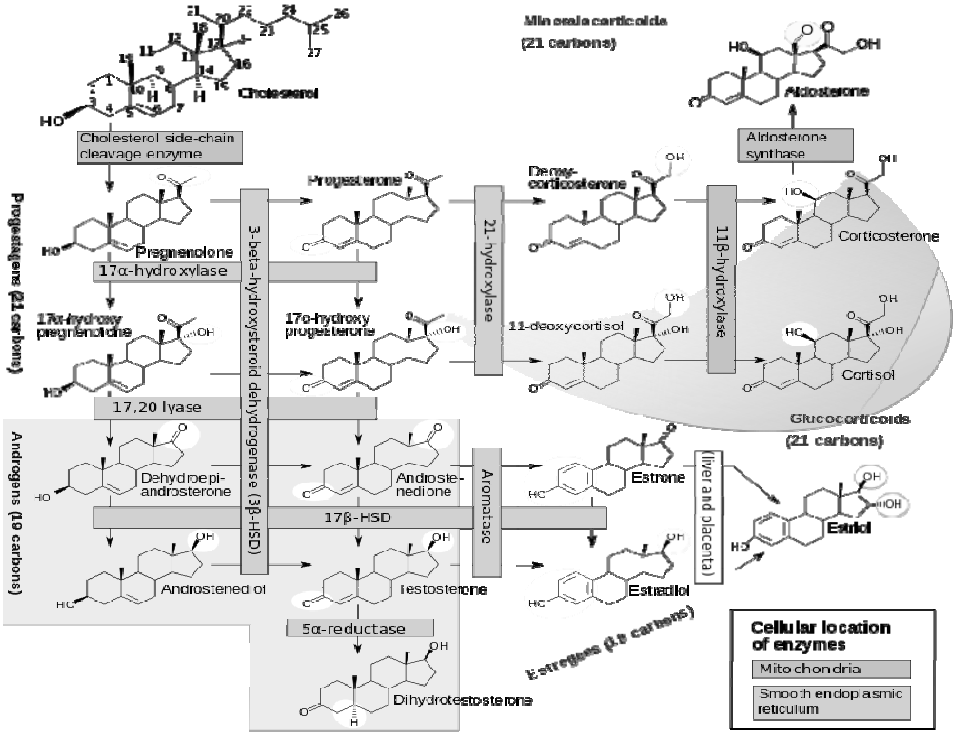
The brain-hypothalamic-pituitary-adrenal axis, like the thyroid and gonadal systems, communicate to control the hormonal end-products in their respective "trees." The steroid biosynthetic pathways are now well known with specific enzymes also characterized and involved with specific known deficiency or insufficiency conditions controlled by a variety of genetic markers. The complex interaction of genes, enzymes, cholesterol as precursor to subsequent steroids and the interaction with the vasculature and the neurologic systems gives us the final capacity of the adrenal glands in addition to interactions with other members of the endocrine system (ie. IGF-1 and fibroblast and epidermal growth factors). With knowledge of the steroid pathways beginning with cholesterol and the enzymes existing at key steps in these pathways, descriptions of clinical expected "consequences" of variations, mutations, excess or deficient responses, coupled with improved genetic and metabolic measurements allows for not only better diagnoses and understanding of these complex situations but also consideration for specific treatment options.



Cholesterol is transferred into the mitochondria of the steroidogenic cells via the steroid acute regulatory protein (StAR) with conversion to steroid hormones via activity of a series of P450 enzymes. Normal progression to the end products, aldosterone, cortisol, testosterone (or estrogen) and the various steps along the pathways then produce either normal steroid functions or myriad abnormalities more or less common according to excess or deficiency conditions that exist or come along. Conditions of glucocorticoid deficiency occur as a result of impaired function of one or several links of the hypothalamic-pituitary-adrenal (HPA) axis, enzyme deficiencies caused by a variety of now known genetic mutations or certain diseases or disorders or events which interfere with such hormone production.

The steroid biosynthetic tree starts with cholesterol and then various enzymatic processes produces different steroids downstream. When there is an enzyme absent or diminished in its function, then the subsequent steroid intermediary is not produced and the effects of these products absence becomes clinically apparent. At the same time, when there is a blocked or absent enzymatic step, the precursor products still are stimulated to be produced and therefore increase relatively or absolutely and their own effects may also become clinically apparent as well as biochemically measurable with modern laboratory techniques. Primary AI can occur because of congenital abnormalities (genetic/enzymatic) and

several relatively rare situations are known to exist. In addition to the obvious problems of lack of production of the end-hormone (ie. cortisol, androgens, mineralocorticoids) with expected clinical consequences, if there is a genetic or enzymatic block, the steroid precursors before the block become excessive and sometimes produce their own clinical signs and symptoms. Acquired AI can occur secondary to cancer, trauma, hemorrhage, infection, surgery, radiation as well as autoimmune adrenalitis.



Secondary AI and tertiary AI also can occur in congenital or acquired format as well, sometimes but not always with other hormone systems also involved depending upon which other hormones are increased and/or decreased. Trauma to or around the brain, hypothalamus and pituitary regions as well as to the adrenal glands themselves, tumors and cysts, surgery, hemorrhage, hydrocephalus or autoimmune hypophysitis. Congenital abnormalities of CRH and ACTH also exist in which case adrenal insufficiency would be expected without some of the effects associated with primary glucocorticoid deficiency, ie. no hyperpigmentation because the hyperactivity response of the "normal" hypothalamus-pituitary cannot occur.

Under normal circumstances, ACTH and cortisol usually peak around 6-8 am with trough levels around midnight to 2 am. 80-90% of

cortisol is transported by CBG, corticosteroid binding globulin. Normal levels of cortisol measured at the same time as ACTH levels will provide good information about baseline (morning) function. Salivary and urinary cortisol levels can also provide information about sufficiency or insufficiency states.

Primary Adrenal Insufficiency

Primary AI [3] can occur with absolute or partial insufficiency. There can be destruction or dysfunction of the adrenal cortex to produce such insufficiency and genetic etiologies may be more common than expected[4] although somewhat expensive and difficult to diagnose.[5] Most of the time there is also concomitant preservation of adrenal medullary function. Prevalence of primary AI is estimated to be about 1:8,000-10,000. Primary AI can also be classified as either congenital or acquired. See *Table 1.1.*

Table 1.1

Primary Adrenal Insufficiency

A. Primary Congenital AI

1. CAH: Congenital Adrenal Hyperplasia
2. Adrenoleukodystrophy
3. Neonatal adrenoleukodystrophy
4. Steroid pathway mutations: DAX-1, SF-1, StAR
5. Allgrove Syndrome: Triple A Syndrome
6. Congenital Adrenal Aplasia/Hypoplasia: IMAGe Syndrome
7. Familial glucocorticoid deficiency
   - Type 1
   - Type 2
8. Kearns-Sayre Syndrome
9. Smith-Lemli-Opitz Syndrome
10. Wolman Disease

B. Primary Acquired AI: Addison's Disease

11. Adrenal trauma, surgery
12. Adrenal hemorrhage: Trauma, Surgery, Hemorrhagic diathesis, Antiphospholipid antibody
13. Infection: Waterhouse-Friderichsen Syndrome (associated with adrenal hemorrhage): Tuberculosis, HIV/AIDS, Meningococcus, Histoplasmosis, Coccidiomycosis, Blastomycosis, Cytomegalovirus, Other bacterial septicemias including pneumococcus, streptococcus and pseudomonas
14. Infiltration: Sarcoidosis, Amyloidosis, Scleroderma
15. Medication: Ketoconazole, fluconazole, Etomidate anesthesia, Mitotane
16. Autoimmune AI
    - Isolated autoimmune adrenalitis (Addison's)
    - AIRE 1 Autoimmune Polyglandular Syndrome 1
    - Autoimmune Polyglandular Syndrome 2
    - Autoimmune Polyglandular Syndrome 4
17. Glucocorticoid Resistance

Secondary Adrenal Insufficiency

Secondary AI is a category of adrenal insufficiency where there is lower than usual adrenal cortical production reflecting hypopituitarism and particularly lower ACTH levels and/or hypothalamic insufficiency with lower corticotropin releasing factor (CRF). With less CRF and/or less ACTH, the adrenal gland has lower stimulation and therefore lower levels of cortisol, as well as other adrenal steroids. There can be isolated ACTH or CRF deficient or insufficient states or there can be combinations with other hypopitutiary or hypoactive hypothalamic problems. Some endocrinologists would subcategorize hypothalamic abnormalities as Tertiary AI to distinguish it from pituitary problems,

Secondary and Tertiary AI categorization is presented in *Table 1.2.*

Table 1.2.

Secondary and Tertiary Adrenal Insufficiency

- Congenital malformation or other genetic conditions affecting the midline brain, hypothalamus and/or pituitary gland (including anencephaly, microcephaly, septo-optic dysplasia)
- Hypothalamic abnormalities associated with localized tumors or cysts, hemorrhage, trauma, infiltration/infection, radiation and/or surgery of the brain, hypothalamic or pituitary regions
- Pituitary disease, tumors, cysts, hemorrhage, trauma, infiltration/infection, autoimmunity, radiation and/or surgery
- Suppressed CRH/ACTH from exogenous glucocorticoids/withdrawal
- Isolated ACTH (? also CRH) disease or insufficiency
- Genetic mutations with variable hypopituitarism
    - Pit-1
    - Prop-1
    - HESX-1
    - LHX-4
    - POMC

Presentation of Adrenal Insufficiency [6]

Symptoms and signs of AI [7] can be variable depending upon age of presentation, severity of adrenal hormonal insufficiency, duration of AI and concomitant co-morbidities (ie. growth hormone deficiency or insufficiency, thyroid hormone deficiency or insufficiency, diabetes insipidus and/or adrenal medullary deficiency or insufficiency. These can include mild or severe fatigue; weight loss including emaciation, failure to thrive in infants and young children; gastrointestinal symptoms such as nausea, vomiting, diarrhea; hypovolemia with salt craving, hyponatremia, acidosis,

hypotension; anemia, pallor; hyperpigmentation associated with ACTH excess; hypoglycemia.

Table 1.3 presents an overview of common signs and symptoms associated with AI in its myriad manifestations.

Table 1.3.

Signs and Symptoms of Adrenal Insufficiency

Glucocorticoid deficiency	**Mineralo-corticoid deficiency**	**Adrenal Androgen deficiency**	**Increased MSH**
Fasting hypoglycemia	Salt craving	Decreased pubic, axillary and body hair	Hyperpigmenta-tion
Increased insulin sensitivity	Hypotension	Decreased libido	
Decreased gastric acidity	Hyponatremia, hyperkalemia, metabolic acidosis	Delayed or absent adrenarche, puberty	
Nausea, vomiting, mild diarrhea	Nausea, vomiting		
Fatigue, malaise	Fatigue, malaise		
Muscle weakness	Muscle cramping, weakness		
Weight loss, failure to thrive	Weight loss		
Overt growth failure			

Sometimes adrenal insufficiency will be much more subtle or asymptomatic and presentation occurs only with high index of suspicion[4] because of associated diagnoses such as hypothalamic, pituitary diseases, autoimmune thyroid disease, type 1 diabetes, pernicious anemia, hypogonadism or several concomitant endocrinopathies that would make a knowledgeable clinician check adrenal status particular those of an autoimmune nature. In the rare syndromes associated with adrenal aplasia, hypoplasia or other forms of genetic abnormalities, being aware of the possibility of a syndrome and the clinical manifestations that would raise concern for adrenal status, causes the astute clinician to consider adrenal disease as part of the differential diagnosis (ie. progressive or degenerative neurologic conditions, achalasia, visual or tear problems).

Condition of an initial presentation that spares one category of adrenal steroids does not mean that it will be spared over longer term follow-up and changes in production of each of the categories of adrenal steroids should be reassessed periodically especially if symptoms are checked and changing.

In primary chronic adrenal insufficiency associated with low cortisol levels, there is hypersecretion of ACTH and other POMC peptides. This includes various forms of MSH, melanocyte stimulating hormone, which is

reflected in hyperpigmentation of the skin and mucous membranes since both α-MSH and ACTH are secreted concomitantly from the anterior pituitary gland as both are cleavage products of POMC. Such hyperpigmentation is most prominent in sun-exposed skin areas exposure and extensor surfaces of the knuckles, axillae, palmar creases, knees and elbows as well as gingival borders of the mouth and vaginal mucosa.

Associated nonspecific clinical findings of AI also may include small heart on chest x-ray, nonspecific anemia, azotemia, eosinophilia, lymphocytosis and hypoglycemia. Conditions with AI that are part of specific syndromes demand attention to other clinical manifestations in their varying presentation patterns and often collaborative clinical experience of multiple subspecialists. Conditions of AI associated with autoimmunity demand clinical attention to other autoimmune disorders that may or may not be associated in subclinical form (positive antibodies but no symptoms) at presentation and diagnosis but which will change over time (months or years later) demanding ongoing surveillance and detection in an effort to avoid severe symptoms or signs of such co-morbidities (ie. ovarian and testicular failure, other forms of hypopituitarism manifestation, thyroid, diabetes, celiac disease).

A. Primary Congenital Adrenal Insufficiency

1. CAH: congenital adrenal hyperplasia

Congenital adrenal hyperplasia can be caused by a variety of enzyme deficiencies in the adrenal cortex. There are six major forms of CAH each felt to be caused by a mutation of one of the six enzymes required for the biosynthesis of cortisol. The enzymatic processes each are involved with very specific clinical signs and symptoms that depend on whether the specific enzyme absence is full or partial and whether the enzyme deficiency results in high or low production of the various steroid products in the mineralocorticoid, androgen or glucocorticoid systems.

The decrease in cortisol secretion results in a decrease in negative feedback at the level of the hypothalamus-pituitary such that the resulting increase in CRH and ACTH attempts to produce more cortisol – if the mutations allows some degree of enzymatic activity. At the same time, such increases in ACTH produce elevated production of the usual cortisol precursors before the blocked pathway site and this can add to different clinical manifestations and difficulties.

More than 80-90% of CAH is thought to be caused by a deficiency of 21 hydroxylase with an incidence of approximately 1:10,000-1:15,000. It is also thought to be quite similar all around the world but where there is increased consanguinity as in some more isolated tribal communities the incidence of rarer forms of CAH increases.

Classical 21 hydroxylase deficiency (21OHD CAH), is characterized by too low cortisol levels associated with increased androgens so that testosterone, dihydrotestosterone and androstenedione levels are increased with virilization. The virilization in females can be rather severe and the more so because 21OHD CAH in its most severe manifestations not only decreases cortisol secretion but also because the enzymatic block allows exposes of the very early female fetus to masculinize the genital precursor tissues. There are also salt losing varieties but with nonspecific demarcation between the mineralocorticoid deficient varieties and those who can more or less maintain sodium and potassium balance unless there are severe condition of stress that intervene. Someone with a simple virilizing form of 21OHD CAH may only show mineralocorticoid imbalance under such extreme conditions, ie. severe illness, surgery.

Non-classical 21OHD CAH may escape detection at birth particularly in males where the virilization can be more subtle compared to females who appear to be males with underdeveloped penises (in fact, virilized clitoral bodies), labioscrotal sacs devoid of testes since they are, in reality, females with ovaries overwhelmed by the androgenic hormones. Usually such females can be easier to identify clinically in the nursery compared to the males and so early mineralocorticoid deficiency crises can be detected earlier in females than males. The virilization of newborn girls with 21OHD CAH may be severe enough that the baby is thought to be a boy – but without palpable testes.

CAH due to 21OHD is the most common cause of ambiguous genitalia. Classical hyponatremic crises typically occur in males with 21OHD CAH about 1-3 weeks after birth, out of the hospital. In circumstances around the world where there is no available, knowledgeable medical care, such infants usually die in coma from severe hypoglycemia, hyponatremia and hypovolemia but this also can occur throughout the more developed nations.

Over the past decades in most Western countries, newborn screening has been expanded to include not only congenital hypothyroidism but also screening for elevated levels of 17 hydroxyprogesterone on the second or third day of life to detect 21OHD CAH before serious salt loss appears. The test is not available in most countries of the world or in parts of the world where most births do not take place in hospitals but at home. After the first few days to a week or so after birth, both boys and girls with salt losing forms of 21OHD CAH will have progressive hyponatremia, hyperkalemia, dehydration, failure to thrive, vomiting, hypotension, hypovolemic shock, coma and death if not diagnosed and treated not only with salt and fluids but with glucocorticoids and mineralocorticoids.

The finding of genital ambiguity at birth is a medical and social emergency with family distress, shock, embarrassment, anger made especially difficult by social conventions where the first questions after is the baby healthy involve whether or not the baby is a boy or a girl. So,

under such circumstances, sometimes there can be no apparent answer at first. Medical personnel must be trained and equipped to deal with the social manifestations of this angst in addition to the best ways to biochemically and genetically figure out the appropriate strategies for diagnosis and treatment.

CAH is inherited in an autosomal recessive fashion where parents are usually asymptomatic carriers of the gene mutations responsible. The carrier incidence of 21OHD is thought to be about 1:50 and usually parents are healthy carriers of the mutated CYP21A genes.

Presence of a uterus on ultrasonography in a virilized newborn without palpable testes should raise the diagnostic possibility of virilizing 21OHD CAH and emergency sodium and potassium levels should be checked as should blood pressure monitoring.

Blood urea nitrogen and/or serum creatinine should be checked to rule out renal disease.

Ultrasonography can also potentially identify ovaries as well as inguinal testes.

A buccal smear looking for X chromatin Barr bodies can be done rather quickly but needs an experienced examiner and may be unreliable in neonates. Formal karyotypes would be diagnostic but also take some time for results to return to help established XX or XY situations. Results of measurements of 17 hydroxyprogesterone that are elevated would be diagnostic. Test for mineralocorticoids are expensive and likely will be difficult to interpret or not available with quick turn around times from laboratories under most circumstances.

Those with simple virilizing but not salt losing forms of CAH have intermediate forms of 21 hydroxylase deficiencies affecting more of the glucocorticoid pathways than the mineralocorticoid pathways. Some of these patients are even milder and only present for diagnostic consideration after a severe illness, trauma event, anesthesia or surgical event where the demands are increased. Some do not come to medical attention until later in childhood or adolescence because of premature adrenarche or puberty, tall stature otherwise unexplained by family patterns or accelerated height velocity associated with androgen excess. Such early adrenarche or full pubarche does not typically occur in very rapid fashion but in an early but ongoing fashion so that detailed history may be important to elucidate from the child, teen or parents.

Other tests to be considered would include assays of other components of the steroid pathways, ACTH concomitant with cortisol levels, urinary 17 ketosteroid and urinary cortisol levels and sometimes ACTH stimulation testing particularly looking at 17 hydroxyprogesterone and other intermediary metabolic products ongoing of the steroid pathways especially the androgens. Adrenal imaging to rule out adrenal tumors both benign and malignant - but hormone producing - should be considered as appropriate in older children and adolescents.

Surgery to change the appearance of the external genitalia of female infants with the salt-losing and non-slat-losing but virilizing forms of CAH is available but experienced surgeons with support staff that include social workers and psychologists are deemed very important to assist with such complex decisions. In girls who are more completely masculinized, there many be more significant internal anomalies required multiple step surgical procedures. In some patients, more virilization occurs that would otherwise be expected presumably because there is more intermittent androgenization taking place even with ongoing glucocorticoid and mineralocorticoid therapy. The reasons for such "refractory androgen suppression" are not well understood. Therapeutic approaches have considered bilateral adrenalectomy as a means to reduce the androgen excess, peripheral androgen and blockade accompanied by provision of estrogen but optimal treatment is not known with a great deal of individual variability. As such youngsters grow and begin to better understand their CAH and such complex situations, discussion of details of their diagnosis, treatment options, self-care involvement with age and maturity often respond well to psychosocial supportive intervention in addition to nurturing relationships with the physician and nurse clinical experts. There is also some discussion of the prenatal exposure to these excess androgens and whether this "masculinizes" the fetal brain influencing postnatal, adolescent and adult sexuality and behavior but there are no definitive studies and there is much variability in such outcomes to date.

Those with non-classical 21OHD CAH are thought to have milder mutations of the CYP21A alleles on chromosome 6 with signs of androgen excess at varying ages and into adolescence or even adulthood. Anyone with increased linear height velocity otherwise unexplained and associated with even mild androgen excess or irregular periods as well as poor breast development in females should also be considered as having some variant of CAH. Sometimes there is positive family history of such premature adrenarche. Girls are not necessarily virilized at birth and in both sexes, the variety of clinical presentation is extremely variable. Differential diagnosis of older children and adolescents as well as adults include virilizing carcinomas of the gonads and adrenal glands, polycystic ovarian syndrome and varying forms of precocious puberty as well as exogenous steroid exposure. Unilateral testicular enlargement as result of testicular adrenal rest tumors have been reported and routine examination of testes in males with CAH should be taught for self-examination by patients as reach adolescence and move into adulthood – as well as for health care providers. Some studies have suggested specific high risk ethnic populations where such late androgen excess conditions may be as common as 1 in 25-30, ie. Ashkenazi Jews or as common 1:1000 individuals (PCOS-like, menstrual irregularities, hirsutism, clitoral enlargement, severe forms of acne, etc) [8]

Presence of hypertension rather than hypotension should prompt consideration of alternative types of CAH such as classical forms of 11β-

hydroxylase deficiency, 17,20 lyase deficiency, 17α-hydroxylase deficiency with excessive mineralocorticoids (hypertension and hypokalemia) at the same time that there is deficient glucocorticoid production contrasts with other variants where some of these CAH forms may only present with sexual infantilism or other forms of gonadal abnormalities (incomplete male differentiation sometimes to the extent of incorrect female gender assignment) depending exactly in which tissues and to what extent such abnormal enzyme aberrations exist.

Earlier enzymatic deficiencies such as cholesterol desmolase deficiency (lipoid CAH) have low levels of all steroid hormones with decreased or absent ACTH responses and high levels of ACTH and the absence of this very early enzyme causes failure of conversion of cholesterol to pregnenolone very early in the steroid pathway in the adrenals and gonads. Very early presentation is associated with cardiovascular collapse following increasing severity of adrenal insufficiency and as with many other of these enzyme disorders, milder variant are also reported.

3β-OH steroid dehydrogenase deficiencies felt to reside in mutations in chromosome 1 in classical salt wasting forms (with decreases in both mineralocorticoids and glucocorticoids as well as androgens) and in non-classical forms (affecting more as abnormalities of puberty, menses with some virilization) also need to be considered under such circumstances.

Treatment then would depend upon the exact deficiency and its severity. Mutations in nuclear receptors such as DAX-1 and steroidogenic factor-1, SF-1, as well as mutations in StAR protein interfere with steroidogenesis reflecting types of congenital adrenal lipoid hypoplastic states with hypoadrenalism (and often hypogonadism as well if those tissues are involved). In the IMAGe Syndrome there is a reported association of Intrauterine growth retardation, Metaphyseal dysplasia, Adrenal hypoplasia and Genital abnormalities such as micropenis, cryptorchidism and occasionally hypospadius and the IMAGe Syndrome appears to be familial with autosomal recessive inheritance and the adrenal insufficiency requires adrenal hormone replacement treatment.

In any or all of these variants of CAH, if there is mineralocorticoid and or glucocorticoid abnormalities, then sodium chloride, mineralocorticoid replacement, intravenous fluid blood pressure management and/or glucocorticoids all are needed as emergency or urgent treatment measures with appropriate monitoring of all such parameters. Once the emergency situation is under control and parenteral administration is no longer required, then maintenance oral hormone replacement treatment (often with some extra sodium chloride for awhile) will be needed forever as well as education for dealing with intercurrent illness, surgery and other stress situations where extra hormone replacement is warranted.

Diagnostic approach to the infant with suspected CAH is presented in Figure 4 adapted from Henwood and Levitt Katz [9]:

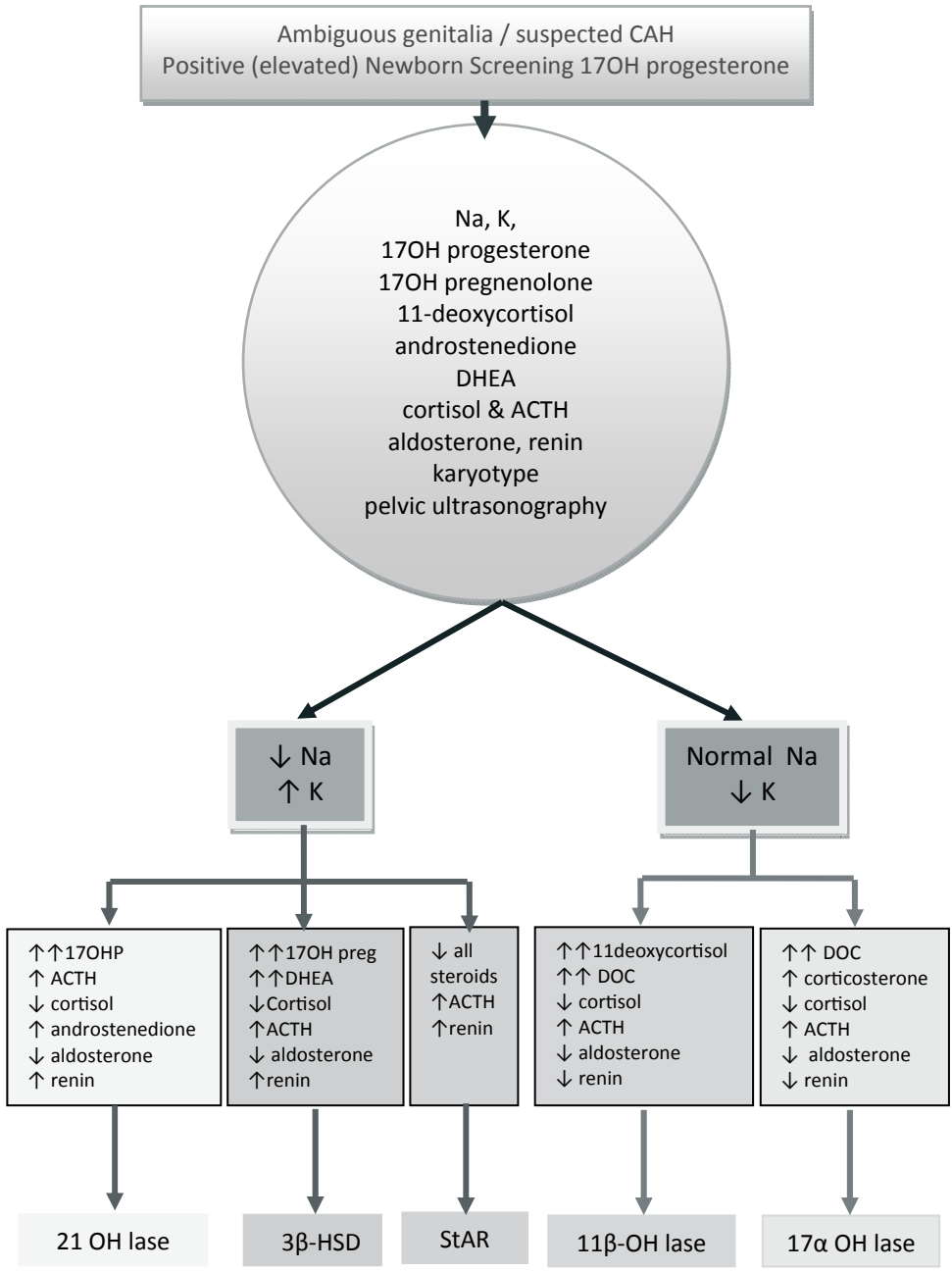


Figure 1.4. - Suspected CAH - Diagnostic Approach

Treatment of CAH depends upon the age of the patient, the severity of presentation, whether or not there is need for only glucocorticoid replacement or also mineralocorticoid treatment. This would be directly dependent on the site of the enzyme abnormality and it severity and be reflected in clinical presentation as well as lab parameters.

Glucocorticoids can be replaced with cortisone acetate, hydrocortisone, prednisone, prednisolone or dexamethasone alone or in combination depending upon availability, cost and individual therapeutic considerations and response. Overtreatment with glucocorticoids in children can compromise height but this is no longer a concern in adults and so longer acting glucocorticoids often are used in adolescents and adults compared to children. The lowest doses of glucocorticoid to produce the desired beneficial improvement in day-to-day quality of life, optimizing energy and growth while at the same time avoiding the common virilization from underdosing of glucocorticoids (purposeful or accidental) is the therapeutic goal. Similarly, paying attention to *mineralocorticoid* (and salt) needs particularly in younger babies, infants and children is important and often there is a need for fludrocortisone in addition to glucocorticoids replacement to treat CAH. Here, too, there is great individual variation in need and efficacy and compliance factors with some patients and families are a great barrier.

Specific *salt supplementation* is often used in neonates, infants and very young children especially before and up to about 1-2 years of age depending upon such individual idiosyncrasies of that child's type of CAH.

Sex hormone replacement at or around puberty aimed to mimic normal pubertal progression and optimize final height also is required in those types of CAH where sex steroid abnormality co-exists.

Surgical correction of severe ambiguous genitalia (ie. virilization of genetic females) depends upon surgical experience, degree of abnormalities and ability to predict and optimize adult sexual functioning. There remains much debate about what should be offered and done, when it should be done and the entire decision making process under such circumstances in addition to the debates about sexual identify, subtle effects of excess or deficiencies of androgens and estrogens on the fetal and developing brain as well as societal restrictions, religious and cultural norms.

Some experiments have shown positive effects using combinations of growth hormone, gonadotropin blockade, aromatase inhibitors in specific circumstances where there is significant height compromise, precocious puberty with demonstrated improvement in final height and pubertal effects accordingly.

2. Adrenoleukodystrophy, X-linked

Adenoleukodystrophy (ALD) is also known as **Siemerling-Creutzfeldt's Disease or Schilder's Disease** and is a biochemical abnormality associated with elevated plasma and tissue concentrations of

very long chain C24, C25 and C26 fatty acids (VLCFAs). The VLCFAs accumulate because of peroxisome cellular organelles, fill the adrenal cells with abnormal cholesterol esters and ultimately the adrenal cells atrophy and die. There appears to be two forms of adrenoleukodystrophy with some variable expression and time to death. One form is the childhood *inflammatory cerebral ALD* and the other is a *noninflammatory ALD.*

Cerebral ALD is a progressive brain dysfunction which starts with milder symptoms such as decreased muscle strength, decreased visual acuity, slurred speech, peripheral neuropathies, sphincter problems and unusual behaviors. Over years, the symptoms progress to dementia, blindness, paralysis, coma and death and, with the neurologic degenerative stages, there is often but not universally adrenal insufficiency of glucocorticoids as well as mineralocorticoids.

The *adrenomyeloneuropathic form*, called AMN, involves distal axonal dysfunction as well as adrenal insufficiency. For both ALD and AMN, clinical expression is variable and both involve ABCD-1 gene mutations which code for the peroxismal membrane protein ALDP that regulates transport of VLCFA into peroxisomes. Because these are x-linked mutations, classical adrenal insufficiency and the neurologic dysfunctions associated with these rare adrenoleukodystrophies show up in young males. Some patients first show up with learning problems and ADHD but there is progressive neurologic involvement that is very different than most types of learning difficulties or attention deficit disorders.

The adrenal insufficiency generally shows up later than the neurologic symptoms and signs and there may also be gonadal failure in adolescence or young adult patients including impotence.

From an adrenal treatment perspective, hormonal replacement with glucocorticoids and mineralocorticoids is fairly standard but the key is recognizing and making the diagnosis when the predominant complaints are subtle but progressive neurologic abnormalities so that awareness of the associated adrenal insufficiency condition is very important. Neurological treatment is palliative and symptomatic but generally unsatisfactory.

Treatment experimentally has been with stem cell transplantation as well as with Lorenzo's oil to decrease the VLCFA levels with some documented improvement in brain MRI offering some hope but this has not been replicated in several other study attempts.

Adrenoleukodystrophy, neonatal autosomal recessive, PEX-1 mutation, Zellweger Syndrome (severe form) and **Infantile Refsum's Disease** (milder form)

The neonatal autosomal recessive form of ALD is associated with a PEX-1 (and other variants) mutation rather than the ABCD-1 gene mutation but also with variable presentation and severity.

Zellweger Syndrome has been used for the more severe form and Infantile Refsum's Disease for the milder variations.

In neonatal ALD, there can be not only adrenal atrophy but also neurologic abnormalities with associated convulsions, hypotonia, craniofacial abnormalities, progressive hearing and visual loss as well as liver disease.

Leopard spot retinal pigmentation may also be present in neonatal ALD and so raise the possibility of a PEX-1 mutation diagnosis.

VLCFA levels are high and generally the prognosis is poor with progressive neurologic deficiencies in addition to adrenal insufficiency and ultimately coma and death in childhood before 7 years of age.

Allgrove Syndrome (Triple A; Quadruple A)

The association of the triumvirate of **adrenal insufficiency** occurs with **achalasia** and **alacrymia** (thus the **Triple A Syndrome**) described by Allgrove [10] and sometimes also autonomic dysfunction such as postural hypotension and abnormal papillary reflexes as well as ataxia.

Allgrove syndrome has been attributed to mutations in the AAAS chromosome region on the 12th chromosome whose product aladin, also has given rise to the name ALADIN Syndrome. The proteins are expressed in the central nervous and gastrointestinal systems.

In this very rare syndrome with fewer than 100 cases described to date, Allgrove Syndrome, clinical presentation is similar to congenital adrenal hypoplasia with hypoglycemia, hypovolemia, hyponatremia but with variable presentations over the first decade of life.

Glucocorticoid insufficiency is more common than mineralocorticoid deficiency. There are increased ACTH levels concomitant with cortisol deficiency and no response to ACTH stimulation testing since the adrenal gland is resistant to ACTH biochemically.

Genetic testing for the AAAS chromosome region is available. Achalasia sometimes precedes other clinical features by several years and the hypoglycemia as well as hyperpigmentation can be significant.

Treatment. Cortisol replacement therapy is very effective in relieving symptoms of Allgrove Syndrome but consultation with gastroenterology and ophthalmology as well as neurology for management issues is important as well as genetic counseling because of the familial nature of Allgrove Syndrome.

Familial Glucocorticoid Deficiency, autosomal recessive

Another extremely rare genetic condition exists where the adrenal gland is insensitive or resistant to ACTH. There are fewer than 100 cases reported in the literature.

Such patients can present with congenital adrenal insufficiency or later in life and the genetic defect seems to cause inability of the adrenal gland to respond to ACTH so that cortisol levels are low while ACTH levels are extremely high.

Glucose levels also are usually low while electrolytes are normal because mineralocorticoid function as opposed to glucocorticoid cell response is normal. There can also be failure to thrive and slow growth.

Such familial glucocorticoid deficiency is an autosomal recessive condition involving the ACTHR/MC2R genes.

Treatment is with glucocorticoid replacement. In some affected individuals, tall stature and increased linear growth are apparent without obvious growth hormone or androgen excess; in others, there is hypoandrogenemia marked by less pubic hair especially in affected adolescent females.

Glucocorticoid Resistance to ACTH

Resistance to the effects of ACTH results in AI from partial (or complete) end-organ insensitivity.

When this rare syndrome occurs, there is decreased cortisol production since the adrenal cortex “cannot” receive signals from ACTH to “produce” cortisol. At the same time, without such resistance or insensitivity to ACTH in the mineralocorticoid or and, pathways in the adrenal glands, the elevated ACTH levels “sensing not enough cortisol” increase as well as androgens so that associated with low cortisol effects are electrolyte, blood pressure and virilizing androgen consequences: hypokalemic alkalosis, hypertension, acne, hirsutism, clitoromegaly and virilization, precocious puberty particularly precocious adrenarche, ambiguous genitalia, oligomenorrhea or amenorrhea, hypofertility and male pattern baldness depending upon severity of the defect and the variable effects on the fetus, newborn, child, adolescent or adult. Inheritance can be autosomal receive or autosomal dominant with mutations in the glucocorticoid receptor or ligand.

Laboratory testing include measurement of electrolytes, renal function, mineralocorticoid, glucocorticoid and androgenic steroids and their precursors as well as ACTH in addition to appropriate imaging studies to better define the anatomy. Karyotyping may also be needed.

Treatment involves high doses of glucocorticoids titrated to bring morning cortisol to normal range with lowering of ACTH. Treatment of the mineralocorticoid induced hypertension and hypokalemic alkalosis may also be needed.

Anti-androgenic treatment may also be needed until the lowered ACTH ceases to produce excess androgens.

Similarly, if the virilization causes ambiguous genitalia, surgical correction may also be needed.

Kearns-Sayre Syndrome

This syndrome is one of the mitochondrial myopathies where adrenal insufficiency occurs but is not a prominent common part of the typical syndrome complex, just more common than might be happenstance.

It is thought to be caused by mitochondrial deletion abnormalities and diagnosis requires 2 of the following conditions: onset less than 20 years of age, pigmentary retinopathy, progressive external ophthalmoplegia or ptosis plus 1 of the following: cardiac conduction abnormalities, elevated cerebrospinal protein levels or cerebral ataxia. Deafness is also an associated finding. Muscle biopsy shows "ragged red fibers." Associated multiple endocrinopathies include adrenal insufficiency, hypoparathyroidism with hypocalcemia, growth hormone deficiency, hypogonadism, diabetes mellitus and thyroid dysfunction so all these complex possibilities need lab confirmation and surveillance accordingly.

Treatment is supportive and includes standard glucocorticoid and mineralocorticoid replacement.

Smith-Lemli-Opitz Syndrome

Smith-Lemli-Opitz Syndrome is an autosomal recessively transmitted syndrome complex related to loss of function gene mutation in chromosome 11 encoding 3β-hydroxysterol reductase and a complex of congenital anomalies including microcephaly with global developmental delay and psychomotor retardation, prominence of the metopic suture, ptosis, upturned nose, micrognathia, cleft palate, limb abnormalities such as syndactyly, polydactyly or short thumbs, congenital heart defects, hypospadius or more severe genital abnormalities with failure of masculinization, lung and kidney abnormalities, failure to thrive and poor growth and rarely, adrenal insufficiency.

The enzyme is necessary for the synthesis of cholesterol, a precursor for androgen and other steroid products. Elevated 7-dehydrocholestrol, the precursor to cholesterol, or the ratio of this precursor molecule to cholesterol is confirmatory in addition to special genetic testing.

Treatment of the glucocorticoid and mineralocorticoid deficiencies per standard treatment protocols with extensive specialty involvement based on the specific individual patient needs.

Wolman Disease

Wolman Disease, one of the lipid storage diseases, is thought to have a prevalence of ~1:100,000 and is an autosomal dominant gene mutation on chromosome 10 (LIPA gene) for cholesterol esterase so that cholesterol entry into the steroidogenic pathways is limited by the lysosomal acid

lipase deficiency. This causes cholesterol and triglyceride accumulation in the liver, GI track and adrenal glands.

Two forms are described: *Wolman Disease* which is the severe manifestation in infants and *Cholesteryl Ester Storage Disease* with a later onset and milder presentation.

Clinical findings include hepatosplenomegaly and fibrosis, vomiting, diarrhea, steatorrhea and gastrointestinal malabsorption as well as adrenal calcifications and adrenal insufficiency.

Diagnosis is confirmed with lysosomal acid lipase enzyme testing ass well as finding of disseminated organ foam cell infiltration, elevated cholesterol levels, xanthomas of the liver, adrenal, spleen, lymph nodes, bone marrow and small intestines, lungs and thymus. Standard endocrine treatment involves glucocorticoid and mineralocorticoid replacement.

B. Acquired Adrenal Insufficiency: Addison's Disease[11]

1. Primary Acquired Adrenal Insufficiency

Primary acquired AI can occur because of infection or infiltrative processes as well as secondary to immunologic attack of the adrenal glands. In past years as well as in financially challenged parts of the world, tuberculosis was the primary infection causing acquired AI.

Throughout the world, HIV/AIDS, meningococcemia, other overwhelming bacterial septicemias like pneumococcus and streptococcus as well as cytomegalovirus, histoplasmosis, coccidiomycosis and blastomycosis all have caused AI. All such overwhelming infections hitting the adrenal glands produce what is called Waterhouse-Friderickson Syndrome where predominantly glucocorticoid insufficiency causes all the classical symptoms and signs of AI and treatment is directed not only at recognizing the state of AI and maintaining high dose glucocorticoid replacement but also maintaining circulation with appropriate electrolyte and fluid management and being aware of the potential for severe adrenal hemorrhage when there is a sudden catastrophic circulatory collapse.

Infiltration by sarcoidosis, amyloidosis and scleroderma also can be associated with AI.

In all such circumstances treatment of the underlying condition is necessary at the same time that high dose glucocorticoids, fluid and electrolyte restoration and maintenance are necessary. If the septic state is severe enough, or if there is subsequent adrenal hemorrhage adding to the AI situation, death is a high likelihood.

Hemorrhagic conditions can cause severe, adrenal hemorrhage as can adrenal trauma, per se, or surgery involving the adrenal glands and kidneys; as a consequence of such damage, AI may occur temporarily or permanently. Antiphospholipid antibodies also can be associated with adrenal hemorrhage and AI. Several specific medications, ketoconazole,

fluconazole, etomidate anesthesia and mitotane also can damage the adrenal glands and produce AI. These conditions generally are known by thorough past history but sometimes can be subtle manifestations where AI may be the primary presenting situation. As with infections and other infiltrative processes causing AI, the treatment of the specific cause will remain important to know about and to address therapeutically, as much as possible, in addition to the consequences of the AI state itself.

2. Autoimmune Adrenalitis

Autoimmune adrenalitis [12] is the second most common cause of adrenal insufficiency following CAH in many parts of the world (where diseases such as tuberculosis, malaria and other infectious disease may take precedence) with a prevalence estimate of 8% of AI patients. Pathologically, this can represent isolated autoimmune adrenalitis with destructive infiltration of the adrenal glands or such autoimmune adrenalitis can be part of four subsets of autoimmune polyglandular syndromes: APS-1 with mucocutaneous candidiasis, hypoparathyroidism and AI (less common than APS-2); APS-2 with type 1 diabetes mellitus and/or autoimmune thyroid disease, either euthyroid, hypothyroid or hyperthyroid with or without goiters and AI; APS-4 with AI and any other autoimmune disease not otherwise listed such as alopecia, vitiligo, Sjogren syndrome, Crohn's disease, chronic active hepatitis and some types of hypergonadotropic hypogonadism. (APS-3 includes autoimmune thyroid disease plus other autoimmunopathies such as atrophic gastritis, pernicious anemia with positive gastroparietal antibodies, vitamin B12 and folic acid as well as iron deficiencies and celiac disease but NOT adrenal insufficiency).

In general, it is thought that about 40% of autoimmune adrenalitis is associated with some other type of autoimmunopathy.

Isolated adrenalitis, primary AI, in many situations around the world, particularly in richer or middle-income nations, may be the most common acquired cause of AI in recent decades since tuberculosis and bacterial sepsis are not only less common but also responsive to available infectious disease treatments as well as improved supportive care.

Adrenalitis is associated with increased risk with certain HLA phenotypes (DR3-DQ2 and DR4-DQ8) as well as polymorphism in the CTLA-4 gene particularly in APS-2 conditions. Awareness of such genotypic risks makes it possible to screen other family members with earlier identification of potential AI and increased awareness of symptoms so that earlier and less severe presentations is possible.

Positive 21α-hydroxylase antibodies as well as 17α-hydroxylase antibodies, P450 side chain cleavage (P450scc) enzyme antibodies and adrenal cortical antibodies (ACAs) all have been associated with not only risk of future adrenalitis but also helpful in making the diagnosis without

adrenal tissue availability. In some reports, about 90% of patients with other autoimmune diseases but who screen and remain persistently positive for adrenal antibodies develop AI within 3 years. [13]

While AI adrenalitis can occur in children and adolescents, the mean age of presentation is 30 years old.

Clinical presentation can be with any of the classical symptoms and signs of AI but with variable expression depending upon precipitating circumstances. Unexplained fatigue, unexplained weight loss, sleep disturbance, hyperpigmentation from ACTH excess trying unsuccessfully to boost the low glucocorticoid production, syncope, hypotension, unexplained gastrointestinal illness, hyponatremia and dehydration in any combination may occur. In someone with known type 1 diabetes, relatively sudden unexplained severe hypoglycemia or need for reduced insulin requirements may be indicative of AI.

Sometimes hypoglycemic seizures even without concomitant diabetes are the first presentation of AI secondary to adrenalitis and sometimes hypovolemic shock and coma especially if precipitated by an unrelated infectious disease or other illness. Although the immune disorder of adrenalitis is congenital, the formation of antibodies and the progressive destruction of the adrenal glands usually appear later in life although there can be exceptions in children with some increases already in the teenage years. More typically, such AI presents in 20-40 year olds and isolated autoimmune adrenalitis is unusual in children and teens. It is thought, in adaptation of the Eisenbarth model for type 1 autoimmune-mediated diabetes mellitus, development of AI is a process that progresses from a stage of potential development because of genetic predisposition, to subclinical stages detected with positive antibodies against adrenal cortex components but only minor compromise of laboratory adrenal levels then further progression toward clinical abnormalities and more severe failure of the adrenocortical functions. Progressive worsening of cortisol levels, increasing ACTH levels, worsening cortisol secretory response to exogenous ACTH stimulation and clinical AI early only with severe precipitants while in later stages, more symptomatic AI with potential for hypovolemia, shock, coma, seizures and death if treatment is not successful.

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.

The Eisenbarth Adrenalitis Natural History Model is presented in *figure 1.5.*

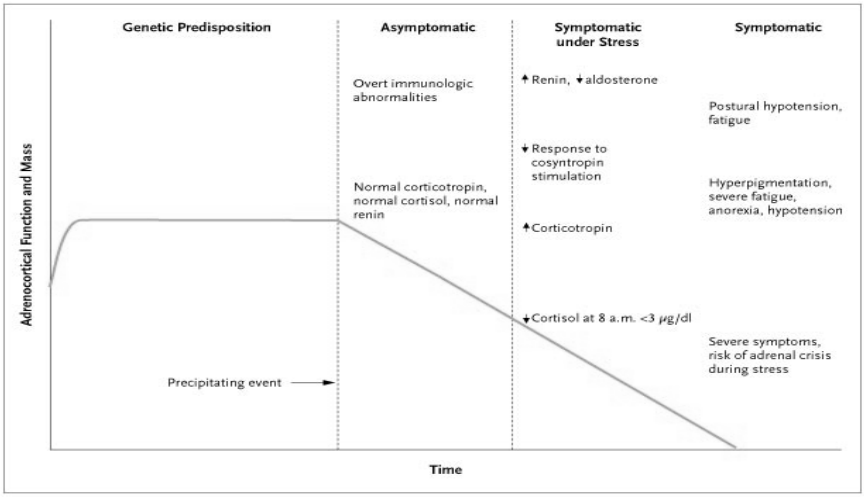


Figure 1.5. - Eisenbarth Adrenalitis Natural History Model

Diagnosis involves a high index of suspicion based on history and physical exam paying particular attention to other autoimmune diseases that may co-exist. If available and affordable, adrenal antibodies should be checked and morning cortisol with a concomitant ACTH level should confirm low cortisol levels, high ACTH levels and there should be relatively prompt response not only to fluid and electrolytes to restore volume depletion and glucose to correct hypoglycemia.

If not definitive, more subtle AI may require ACTH stimulation testing[4] as described in the lab testing section to follow. Similar confirmation can occur with genetic HLA typing.

If adrenal tissue is available for pathologic study, lymphocytic infiltration of the cortex can be seen. Following such infiltration, there is scarring and destruction of the cortical cells producing insufficient adrenocortical glucocorticoids, sometimes insufficient mineralocorticoids as well despite compensatory hypersecretion of CRF and ACTH in an (futile) attempt to stimulate increase adrenal steroid production.

Autoimmune Polyglandular Syndromes[14,15]

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. [16]

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
  - Grave's Disease
  - Goiter
  - Vitiligo
  - Adrenal insufficiency or adrenalitis
  - 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

Premature ovarian failure occurs in up to 20% of women with APS [17] with some implication of oophoritis as a possible explanation.

Secondary Acquired Adrenal Insufficiency

Secondary acquired adrenal insufficiency [18] involves abnormalities of CRF in the hypothalamus and/or ACTH in the pituitary gland. Primary hypothalamic and/or pituitary congenital anomalies, tumors, cysts, trauma, hemorrhage, infection, hypophysitis with lymphocytic infiltration of these areas or surgery and radiation that involves the hypothalamus or pituitary area can all be associated with AI.

Patients treated with glucocorticoids for legitimate but prolonged reasons, also show suppression of CRF and/or ACTH as a direct result of the amount and duration of such glucocorticoid treatment. The higher the dose and the longer the treatment duration, the more suppression that occurs. Cushing's Syndrome that occurs from any such excess of CRF or ACTH as well as excess corticoids from a unilateral tumor, is associated with suppression of one's own adrenal glands; after treatment and with absence of increased CRF or ACTH, glucocorticoid levels fall dramatically and before the remaining normal adrenal tissue can recover, there can also be AI. Cortisol administered during pregnancy can also cross the placenta. It is thought that this produces fetal cortisol concentrations about 10% of maternal levels but this may be associated with hypoglycemia and partial AI in the newborn. In contrast, dexamethasone, which can cross the placenta more readily than cortisol, if used during pregnancy, can be expected to produce AI. Anencephaly can be associated with absence or inadequacy of normal hypothalamic/pituitary function and in such infants, adrenal insufficiency may also occur.

Exogenous chronic glucocorticoid treatment and/or withdrawal

In those patients where prolonged administration of glucocorticoids, usually for anti-inflammatory or anti-cancer therapy, has taken place, CRF and ACTH are suppressed resulting in endogenous adrenal cortex atrophy.[19] Doses used, particularly high doses and duration of treatment with dexamethasone, hydrocortisone, prednisone and other varieties of steroids can all be associated with more serious adrenal atrophy and AI but with great variability in response. As patients are weaned and their steroid doses decreased, AI may occur and slower weaning may be necessary. Oral, dermal, intranasal or pulmonary glucocorticoids can all produce systemic levels of glucocorticoids that affect the hypothalamic-pituitary-adrenal axis with full (or partial) suppression of endogenous cortisol production (as well as interference with growth). Since DHEA-sulfate is produced concomitantly, this can be used with ACTH measurements as a relative marker of endogenous adrenal suppression in such patients in place of cortisol measurements. The vast majority of such patients weaned of high dose and/or prolonged glucocorticoid therapy

recover adrenocortical function within six to twelve months of withdrawal; before that time, stress doses of steroids may be needed.

Hypothalamic or pituitary disease or its treatment

Congenital anomalies of the hypothalamic or pituitary region, infection, infiltration, surgery, hemorrhage, trauma, radiation, chemotherapy and various types of genetically determined hypopituitarism can all involve CRH or ACTH insufficiency. With such positive stimulation, the adrenal cortex can be insufficient in its production of glucocorticoids as well as adrenal androgens. Depending upon timing of such abnormalities in fetal life or in the neonate, child, adolescent or adult, AI may then be present in a full or partial state. Genetic conditions such as septo-optic dysplasia, holoprosencephaly and conditions associated with microcephaly or anencephaly may have hypothalamic or pituitary hypofunctioning and thus tertiary (CRH) or secondary (ACTH) deficiencies may be present. This is especially true for the congenital malformation of the brain with midline defects. Conditions with cleft lip and cleft palate fall into this group as well. If there is associated hypogonadotropic hypogonadism during fetal life, micropenis and incomplete virilization may be present.

Lymphocytic infiltration of the pituitary gland is a rare cause of hypopituitarism but usually present in adults and not children or adolescents.

Infiltrative processes such as hemochromatosis, sarcoidosis, histiocytosis and amyloidosis also is quite rare in younger patients.

Various infections including encephalitis and meningitis may also involve the hypothalamic/pituitary axis. Any overwhelming sepsis and any conditions associated with hemorrhagic disorders either as part of infections or secondary to bleeding diathesis can also involve the hypothalamic/pituitary region.

Craniopharyngioma and other tumors or malignancies with invasion of the hypothalamic/pituitary region, surgical response and/or radiation effects in these areas all can be associated with various degrees of hypopituitarism including CRH/ACTH insufficiency leading to secondary AI.

Panhypopituitarism (anterior and/or posterior pituitary involvement) or multiple anterior pituitary hormone deficiencies can co-exist. Not infrequently, initial pituitary deficiencies involve growth hormone and gonadotropin deficits, then later on ACTH and TSH insufficiencies/deficiencies become more apparent. While growth hormone deficiency commonly shows up relatively soon after such evens, secondary AI can occur immediately or many years later so that ongoing surveillance should continue indefinitely and vigilance particularly during intervening

stress, infection and/or anesthesia and surgery are especially vulnerable periods for due diagnostic consideration.

Similarly, post radiation effects can be immediate or delayed.

Isolated ACTH (? CRH) disease/insufficiency

Isolated ACTH (? also CRH) disease or insufficiency is another unusual disorder with clinical and genetic heterogeneity. It may present in infancy with hypoglycemia, seizures or show up later with growth failure and delayed puberty since what seems to be isolated ACTH deficiency or insufficiency may only be the forerunner of other hypothalamic or pituitary factors. Frequently, the first manifestation of hypopitutiary or hypothalamic insufficiency may be growth hormone axis abnormalities while thyroid and adrenal functions are relatively spared only to show up later, months or years later. Gonadal axis abnormalities also are variable and also may show up later as well.

CRH gene abnormalities as well as receptor abnormalities are possible as are inactivating mutations, loss-of-function mutations. Mutations associated with ACTH deficiency include hose of T-box 19 and POMC. Clinical finding are variable with presentation in neonates as well as children. With POMC abnormalities, hyperphagia in the newborn may change to later obesity associated with red hair, hepatic failure and neonatal cholestasis. The appetite effects are thought to be related to lack of appetite suppressing effects of MSH within the CNS and the pigment changes reflecting loss of melanin synthesis by melanocytes.

Genetic mutational disease with variable hypopituitarism

Several different molecular pathways that regulate the development of the hypothalamus and pituitary have been identified beginning in the 1990s. [20] These include Pit-1, PROP-1, HESX-1, LHX-4 and POMC among others and can implicate panhypopituitarism or isolated thyroid, gonadal, adrenal and growth axis abnormalities in isolated or multiple combinations depending upon specific mutations and variable penetration/clinical effects. Transcription factors as well as posttranslational protein modification may also have implications in multiple hormone functions and specifically related to the brain-hypothalamic-pituitary-adrenal axis and its feedback loops. These research efforts are continuing and the benefits of genetic, molecular pathway elucidation will permit more accurate diagnoses as well as provide potential novel targets for pharmacologic endocrine treatments in the coming years.

The Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php) is an ongoing catalog of disease-related mutation. Online Mendelian Inheritance in Man (OMIM) at

www.nchi.nlm.nih.gov/sites/enrez?db=omim) is another resource about known genetic diseases, causative genes, symptoms, signs and literature references.

Hypothalamic and pituitary tumors such as craniopharyngioma can be associated with ACTH deficiency in about 25% of patients. [21] AI is rarely the presenting complaint but may contribute to the clinical picture. After surgery or radiotherapy, a large number of such patients will have ACTH deficiency either temporarily or permanently and some will eventually evolve to develop this months or years later with complaints of otherwise unexplained fatigue, weight loss or other nonspecific complaints. Treatment with glucocorticoids can sometimes unmask a previously mild degree of diabetes insipidus. Similarly, combined thyroid and adrenal insufficiency under such circumstances, when the hypothyroidism is treated, sometimes unmasks a more significant adrenal insufficiency so that careful assessment and follow-up of potential AI is critically important to avoid precipitating an adrenal crisis/emergency. If in doubt, it is important to provide glucocorticoid replacement before thyroid hormone replacement and to follow-up up clinical parameters and lab parameters closely to optimally balance needs.

AI Diagnostic Lab Tests to Consider

Clinical suspicion raises the possibility of AI in addition to awareness of known association with certain genetic, syndromic conditions.

General testing would include complete blood count including differential analysis, electrolytes especially sodium, and potassium, morning cortisol with concomitant ACTH. Afternoon and evening cortisol levels are usually lower than early morning blood samples and may be difficult to differentiate between abnormally low cortisol values and AI values.

Urinary free cortisol measurements can be useful as well since unbound or free plasma cortisol is excreted by the kidneys and if the free plasma cortisol levels are low, as would be expected in AI, then low urinary free cortisol values would indicate AI. Laboratories that measure urinary free cortisol would have normal values for adults, lower values correlating with general weight and body size in children and growing adolescents.

Salivary cortisol measurements can be collected at home without the accompanying angst of doctor's offices, laboratories, emergency rooms and hospital environs. Some believe that this provides a less stressful situation in which to assess adrenal status since the blood cortisol patterns are reflected with similar circadian rhythms even with salivary steroid levels usually about 90% lower than plasma cortisol levels. Because absence of nighttime cortisol decreases in Cushing's and other glucocorticoid excess conditions is so important, and this is relatively difficult to measure in an ambulatory patient, salivary cortisol measurements often play a more

important role for hypercortisol states rather than AI. Key with all such laboratory assessments is affordability and availability of reliable laboratories with quality control provisions to ensure appropriate results.

DHEA-sulfate sometimes is also helpful as an adjunct to identifying adrenal functional status.

If autoimmunity is considered, then *adrenal antibodies*, most specially, 21 hydroxylase and 17 hydroxylase antibodies can be diagnostic I since the diurnal variations of f positive.

Plasma renin and *aldosterone* levels while difficult to standardize and obtain, also may be helpful although expensive.

Stimulation testing with ACTH with measurement of cortisol pre-dose and post-dose can also be diagnostic. ACTH can be given in low (0.1-1 μgm) or higher dosage (250 μgm) with normal values available for comparison with those of the patient in question. Baseline, 30 minute and 60 minute cortisol assessment allow for such assessment with expectation of having results that peak at or above 20 ugm/dl post-intravenous ACTH administration. Primary AI patients would be expected to have little or no response to either the low or higher dose ACTH stimulation situation whereas secondary or tertiary AI patients may have some response dependent upon degree and duration of ACTH and/or CRF insufficiency/deficiency and ability of the otherwise unstimulated adrenal cortex to still respond to the testing dose.

Recent primary AI guidelines[4] reviewed the available literature comparing low dose and high dose ACTH stimulation testing without definitive conclusions except to continue to recommend the standard 250 ugm dose as having the higher sensitivity in detection of AI for children and adults until more definitive comparative data are available.

Insulin tolerance testing, mostly done for assessment of growth hormone secretion and *glucagon tolerance testing*, also mostly done for assessment of growth hormone secretion are both potent stimuli of ACTH secretion. Both can be done in ambulatory facilities by trained laboratory personnel aware of the side effects of insulin and glucagon used under such specific conditions. Baseline glucose (as well as growth hormone) levels are measured in addition to being measured at 15-30 minute intervals for the first hour and then at 30 minute intervals for the second and third hours with such stimulation testing done before 10 am. Expectations would be that normal glucose levels on the first fasting sample would decrease following insulin administration and then return to normal as the insulin intravenously absorbed effects wear off over time. As the body responds to this relative or absolute hypoglycemia, endogenous glucagon and adrenocorticoid responses help raise glucose levels. Growth hormone assists in this “recovery” and so measurement of GH allows determination of growth hormone sufficiency, insufficient y or deficiency. After glucagon administration, glucose levels rise acutely and then fall and it is believed that the falling glucose also stimulates an ACTH

and cortisol response so glucagon stimulation testing can also be utilized to assess hypothalamic-pituitary-adrenal axis responsivity.

The down side to insulin tolerance testing is the need for intravenous access and multiple samples plus the obvious risks of hypoglycemia producing a hypoglycemic seizure or loss of consciousness.

The down side to glucagon tolerance testing is the common side effect of nausea and vomiting as well as significant headache that follows intramuscular glucagon administration. Under both testing circumstances, trained personnel must be aware of how to monitor the patient, how to emergently treat severe hypoglycemia should it occur and physician presence should be available to assist as needed when such diagnostic procedures are considered.

Radiographic imaging with plain xrays, computerized tomography (CT) and magnetic resonance imaging (MRI) can be invaluable in identifying mass lesions, cysts and tumors, congenital anomalies, calcification and hemorrhage of the adrenal glands themselves as well as the hypothalamic and pituitary regions.

Specific genetic testing is more and more available and more affordable, although often very expensive. Some specialty academic centers and laboratories can provide such testing at no cost if the senior staff are consulted and samples can be obtained locally and transported under special arrangements to make more definitive diagnoses for many of the rare causes of primary, secondary or tertiary AI. Some specialty societies also have made special committees and programs available to assist in such cooperative diagnostic endeavors for financial distressed parts of the world where these expensive laboratory tests are not otherwise available.

Emergency AI Treatment and Adrenal Crisis

In emergency conditions, drawing appropriate samples of blood (serum, plasma) for analysis and then starting emergency electrolyte and fluid management, maintaining airway, breathing and circulation, stress doses of hydrocortisone or other glucocorticoids as well as mineralocorticoids can be life-saving. Unnecessary delay can result in prolonged seizures, coma and death.

Partial impairment of the hypothalamic-pituitary-adrenal axis in any or all of its components must frequently be entertained as a diagnostic consideration in critically ill patients or in those patients who have an unexplained, severe clinical decline (ie. worsening blood pressure, inability to maintain hydration and cardiac output, prolonged coma or coma following admission etc). Relative hypoadrenal function is critically ill patients in the emergency room or intensive care unit is not uncommon and so sometimes the better part of valor is to draw the “critical” lab blood sample, administer the appropriate fluid, electrolyte solutions for such emergency situations and also administer stress doses of glucocorticoid while awaiting clinical response and lab results.

In those with chronic conditions or in those already diagnosed with AI, changes in clinical status and intercurrent events (surgery, anesthesia, infections, for example) demand that appropriate initial education of patient and family be mandatory. As in most chronic medical situations, however, repetitive and follow-up education is also mandatory and should be so documented in the medical records at least annually. Written handouts and teaching tools are available on numerous websites to facilitate and support such educational efforts. Age-appropriate education, family education and support, friends and spouses should all be involved with such educational efforts and invited to periodic follow-up appointments. Medic-alert identification, bracelets or necklaces, save lives and in AI, if the patient cannot identify their diagnosis and medications, can be invaluable. Wallet-card identification and, more recently, mobile phone identification protocols (adding a note and/or phone listing entitled "ICE" [in case of emergency]) also can serve the same function to notify emergency care responders on the scene of an accident, in an emergency room or clinical/office/hospital evaluation center that AI has been diagnosed – and thus allow consideration for specific fluid, electrolyte, blood pressure and cardiorespiratory support as well as glucocorticoid and mineralocorticoid emergency dosage.

In a crisis, intravenous hydrocortisone up to 100 mg may be needed as an initial bolus with dose titration according to age of patient, size of patient and clinical condition as well as precipitating event.

Emergency 10-20 cc/kg normal saline with 5-10% glucose solution to maintain blood pressure and optimize circulatory efforts is standard and not much different than other similar emergency situation except for the needed glucocorticoids parenterally. Higher doses of glucose can be provided if hypoglycemia does not respond on follow-up and sequential blood glucose testing.

Hydrocortisone usually is then provided as a continuous infusion up to 100 mg every 8 hours for the first 24 hours and may be continued for longer periods of time according to clinical response, clinical status and other clinical parameters that must be tracked, recorded and communicated to care providers.

After the initial crisis, tapering of glucocorticoids from these very high initial stress doses should be done over the subsequent few days back to maintenance oral dosage with decisions about mineralocorticoids similarly adapted to the situation.

Smaller infants and children as well as adolescents need the same treatment strategies but with smaller doses per protocol: approximately 20-25 mg for the infant and toddler, approximately 50 mg for the young child and approximately 100 mg for the adolescent with close clinical monitoring for need for dose adjustment. The more obese the patients, in general, the higher the doses needed for emergency as well as maintenance treatment.

If intravenous hydrocortisone or other glucocorticoid is not available, then intramuscular preparations can be utilized in appropriately increased emergency dosage and then sustained with follow-up tapering similar to intravenous coverage. Clysis or gastric tube placement may be needed for saline and fluid support if intravenous access is unavailable for any reason.

It should be noted that in some neurosurgical procedures, dexamethasone is utilized to help prevent cerebral edema. While a potent glucocorticoid, dexamethasone has minimal or no mineralocorticoid effect and so attention to this detail will be important. Dexamethasone has the added benefit, in emergency conditions for AI, of not interfering with blood cortisol lab measurements so that an ACTH stimulation test can be done the net morning even while dexamethasone treatment continues with reasonable assurance that the cortisol response to the injected ACTH will give some reliable diagnostic information.

Home treatment of intercurrent illness should include education and follow-up reminder education at least annually. Dose increases of usual maintenance glucocorticoids should be discussed and known and instructions on when to contact the endocrine team or other providers of ongoing health care should also be reviewed. Frequently, glucocorticoid doses are doubled and may also be given every 4-6 hours instead of 2-3 times a day for usual maintenance until the intercurrent event concludes. Vomiting and diarrhea may be significant enough as to require intravenous fluid replacement for several hours or a few days according to the precipitating illness. Home weight assessment can serve as an excellent guide to clinical dehydration particularly if stable and no weight change is occurring vs. ongoing acute weight loss that persists.

In planned surgical or anesthesia procedures in an otherwise well and well-controlled AI patient, this author recommends intravenous hydrocortisone for adults in stress dosage of 100 mg every 6-8 hours (with adapted doses for younger children/ smaller sized adolescents/adults) and tapering doses thereafter according to duration and complexity of procedures and then switching back to oral maintenance doses according to clinical condition and response. Some would recommend 100 mg/m^2 as an initial stress glucocorticoid dose.

Acute situations usually do not require additional mineralocorticoid dosage, only glucocorticoids, since higher dose hydrocortisone has some mineralocorticoid activity. Injectable glucocorticoid in a syringe that combines powdered medication and diluent is also available when oral medications cannot be taken for any reason. No danger occurs in giving such medications in one dose inappropriately but not giving “stress dosage” early enough can be serious or fatal. [22]

In patients who routinely do not require mineralocorticoid replacement, under conditions of stress, mineralocorticoid support may also be required if the higher hydrocortisone doses do not prove efficacious by fluid, sodium, potassium and blood pressure monitoring results.

Home availability of parenteral emergency glucocorticoids is also a strong consideration for all patients with AI and especially for those who have a history of adrenal crisis. This includes provision of drug, syringes or pens and needles or needle-tips for safe administration, written and verbal education and follow-up review for proper use as well as when to contact the health care team for emergency support, questions and possible transport to a facility where further assistance can be provided.

Adrenal Maintenance Treatment

Glucocorticoid and mineralocorticoid replacement treatment is complicated by frequent side effects related to undertreatment as well as overtreatment plus the usual problems of a chronic condition that requires two or three times/day dosing with noncompliance a big issue to address prospectively and in an ongoing fashion – particularly if clinical and/or laboratory testing provides evidence for this possibility.

Overtreatment can cause signs and symptoms of Cushing syndrome with weight excess, hypertension and impaired linear growth as well as hyperglycemia. Undertreatment allows AI signs and symptoms to persist and continues the added risks of adrenal crisis, coma and death particularly during periods of stress, anesthesia, surgery, infection etc.

Maintenance treatment attempts to mimic the normal physiologic circadian rhythm of glucocorticoid production with higher early morning levels and then decreasing levels throughout the day and into the night. Mineralocorticoid circadian rhythms are not so dramatic, however. This leads to higher morning dosage when compared to afternoon and/or evening glucocorticoid prescribed whereas mineralocorticoids may be only needed in the morning or, if not lasting long enough, in equal doses twice-a-day.

Usual glucocorticoid doses are approximately 10-15 mg/m^2 /day in twice-a-day or three-times-day split dosage. This may be as low as 5-6 mg/m^2 /day in younger children but there is considerable variation in normal secretory rates so this must be individualized. As with most medications, smaller initial doses are generally provided and then titrated according to either laboratory parameters or clinical findings (ie. fatigue). Exactly which diagnosis caused the AI may also influence therapeutic replacement needs vis-à-vis severe, moderate or minimal insufficiency and whether this remains stable or changes over time with longer duration of AI. One therapeutic goal is to optimize growth and development while keeping ACTH levels at relatively normal values at the same time there are no clinical symptoms of AI persisting. Fatigue, malaise and weakness are important questions to ask on follow-up to help assess individual dose requirements as titration of dose occurs. With CAH, the adrenal may require more aggressive suppression than with other forms of AI in an attempt to minimize virilizing secondary affects from even intermittent

ACTH nonsuppression. This also decreases epiphyseal growth plate advancement thought related to inadequate glucocorticoid replacement and this continued androgen excess state.

In some children and adults, mild mineralocorticoid deficiency may not be clinically evident but this may contribute to continued elevated ACTH level. This is then brought under more optimal control by addition of a small dose of mineralocorticoid once or twice-a-day in addition to glucocorticoid replacement. Treatment with mineralocorticoid thus may allow relative reduction in the glucocorticoid previously provided and so the combination of both adrenal hormones may be superior to just glucocorticoids alone and this minimized the potential for height reduction from virilizing effects not fully suppressed.

Long acting glucocorticoids, prednisone and dexamethasone, are more preferred in adults with AI because they are often needed only once or twice-a-day and thus may promote better compliance compared to twice or three-times-a-day shorter hydrocortisone replacement preferred in children and adolescents. Smaller incremental dosage adjustments are more easily carried out with the weaker, shorter acting glucocorticoids as well. It is not clear from various research attempts that the goal of mimicking the circadian rhythm is borne out by outcome variables and exact dosage recommendations are difficult to be supported by prospective research endeavors.

Typically recommendations are to start with a morning plus evening dose of hydrocortisone for a twice-a-day regimen. Usually this entails a higher morning dose and somewhat lower bedtime dosage. This dose is then titrated according to diagnosis, appropriate ACTH levels accordingly and clinical history of fatigue, weakness and other parameters of normal adrenal status. Whether or not one can titrate to optimize ACTH levels without overtreatment remains debatable; some authorities[4] continue to recognize clinical parameters over ACTH titration in deciding exact replacement and maintenance dosing since definitive studies have not been conclusive. With growth, it is expected that the dose would increase through puberty and thereafter stabilize except if obesity occurs – then the dose increases further. Exact needs and changes with pregnancy are not well established but clinical parameters and laboratory parameters followed.

Some do better with hydrocortisone on a three-times-a-day regimen, ie. prebreakfast, mid-afternoon and then bedtime. Others use the same three-times-a-day regimen but with prebreakfast, lunchtime and then bedtime. Usually hydrocortisone effects last about 6-8 hours but with significant individual variability and detailed interval history may be the best guide as to exact timing of doses needed for any individual patient (ie. is there fatigue and when does this occur during the day?).

If mineralocorticoid is needed because of unexpected higher glucocorticoid doses on a weight or body surface area basis, then fludrocortisone is stared first with a once-each-morning dose of 0.05 mg

and then increased to 0.1 mg each morning with twice-a-day dosing based on clinical history and lab parameters sometime using 0.05 mg twice-a-day, morning and bedtime and other times up to 0.1 mg twice-a-day. Sometimes there is a higher dose at one or another time needed compared to the second dosage. Mineralocorticoid doses do not change very much by patient size or age. Newborns and infants require relatively higher doses of mineralocorticoids than may otherwise be expected. Mineralocorticoid dose excess can result in hypertension and hypokalemia so blood pressure, sodium and potassium levels should be monitored periodically to help with dose adjustments and needs.

Sodium chloride supplementation is usually needed in neonates, infants and children with primary AI. The typical dosage is about 2-4 grams/day (4 gram = 1 teaspoon = 5 cc). Older children, adolescents and adults can usually titrate their own salt requirements on their own and typically do not require specific NaCl supplements.

As a general guideline, hydrocortisone lasts roughly 8-12 hours, prednisone or prednisolone lasts approximately 12-18 hours and dexamethasone lasts approximately 24-36 hours. General dose equivalence is approximately: 30 mg hydrocortisone ~ 6 mg prednisone or prednisolone ~ 0.1-0.5 mg dexamethasone) [23]

In middle-school and high-school aged patients and in many adults, three-times-a-day dosing prescriptions are associated with significant missed doses and noncompliance, particularly for the mid-day dose.

Since hydrocortisone in some patients does not last 8-12 hours but closer to 6 hours (after which time fatigue or malaise is reported), this dilemma can be “solved” by switching to prednisone on a twice-a-day schedule because prednisone has a longer half-life than cortisol and is about six times more potent than cortisol. A somewhat higher morning prednisone plus a somewhat lower bedtime prednisone dose can have quite satisfactory results under such circumstances treating AI just as changing timing of doses may be helpful.

Prednisolone is also available in some countries as a liquid preparation and special pharmacies can make liquid preparation of hydrocortisone or even dexamethasone if consistent compounding procedures are followed.

As with all chronic conditions in childhood and adolescence (and adulthood), periodic follow-up is key with knowledgeable health are providers who can establish rapport, regularly follow-up height and weight with plotted growth charts to make sure that there is no slowed or accelerated growth occurring and can monitoring blood presure, steroid lab results, ACTH, electrolyte and mineralocorticoid results and androgenic and pubertal processes. These need to be documented systematically with appropriate ongoing surveillance.

Education needs to be ongoing and periodically repeated (ie. at least annually).

Our routine procedure is to make sure that this happens prior to the winter increase in expected infections, to make sure supplies are kept current and that emergency procedures known, understood and being followed including specific sick-day guidelines and when/how to contact us.

We routinely follow-up AI patients at least every 3 months through the end of the high school years and then 2-3x/year thereafter into the college and adult years.

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ADRENOGENITAL SYNDROME: CONGENITAL ADRENAL HYPERPLASIA - ADRENAL TUMORS

Ciril Kržišnik

Introduction

The adrenogenital syndrome is a type of hyperadrenocorticism characterized by excess production of adrenal androgens. When it occurs congenitally it is due to hyperplasia of adrenal glands and is manifested in girls with masculinization of genitalia which became ambiguous, later they get low voice, acne, amenorrhea and masculine hair distribution.

Symptoms in boys include also enlarged penis and small testes. In patients precocious sexual development is observed which is isosexual in boys and heterosexual in girls. Children with this condition are usually taller than average but develop into short adults.

A postnatal variety of adrenogenital syndrome is caused usually by adrenal tumors.

1. Congenital adrenal hyperplasia (CAH)

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders of cortisol biosynthesis due to deficiency of specific enzymes in zone fasciculate of the adrenal cortex.

Impaired cortisol synthesis leads to chronic elevation of ACTH via the negative feedback system, causing overstimulation of the adrenal cortex and resulting in hyperplasia and oversecretion of the precursors to the enzymatic defect. Impaired enzyme function at each step of adrenal

cortisol biosynthesis leads to combination of elevated precursors and deficient products.[1,2,3]

CAH due to 21-hydroxylase deficiency

The most common enzyme deficiency that accounts for more than 90% of all cases is 21-hydroxylase deficiency (21OHD). This P450 enzyme (CYP 21, P450c21) hydroxylates progesterone and 17-hydroxyprogesterone (17OHP) to yield 11-deoxycorticosterone (DOC) and 11-deoxycortisol. (*Figure 2.1*) [4,5]

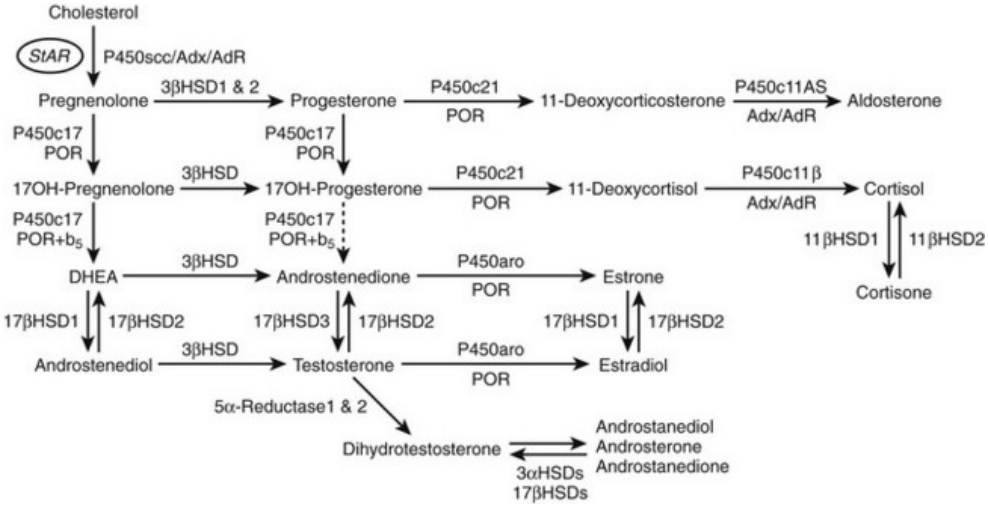


Figure 2.1. - Major steroidogenic pathway in the adrenal cortex[4]

These conversions are necessary for synthesis of aldosterone and cortisol. In the severe, 'salt-wasting' form of the disease both hormones are deficient while in 'simple virilising disease' secretion of aldosteron is sufficient to prevent salt loss.

In both forms termed **'Classical 21-hydroxylase deficiency'** levels of adrenal androgens have been elevated.[6]

Patients with more severe forms are at risk of a salt-wasting crisis in infancy and an adrenal crisis at any age, especially during physiological stress situations. Classical CAH is common, it occurs in about 1 in 15 000-20 000 births in most populations, it is estimated that 75% of patients have salt-wasting phenotype and the rest simple-virilizing phenotype.

CAH is caused by mutations in the CYP 21 gene. There are 2 steroid 21-hydroxylase genes-CYP21P (CYP 21A1P, CYP21A) and CYP21 (CYP21A2, CYP21B) which are located close to HLA complex on the short arm of chromosome 6 (6p21.3) between the HLA-B and HLA-DR loci. CYP21 is the active gen while CYP 21P is pseudogene due to different

mutations. Majority of mutations causing 21-hydroxylase deficiency are recombinations between CYP 21 and CYP21P what prevent normal activity of enzymes involved in steroidogenesis. Disease severity correlates well with different mutations. [7,8]

The primary clinical manifestation is the virilisation of the external genitalia of the affected female fetus, in which the development of the uterus, ovaries, and fallopian tubes remains unaffected by the androgens. The degree of virilisation varies, ranging from mild clitoromegaly to complete fusion labioscrotal folds, with severe clitoromegaly simulating a phallus. (*Figure 2.2*)

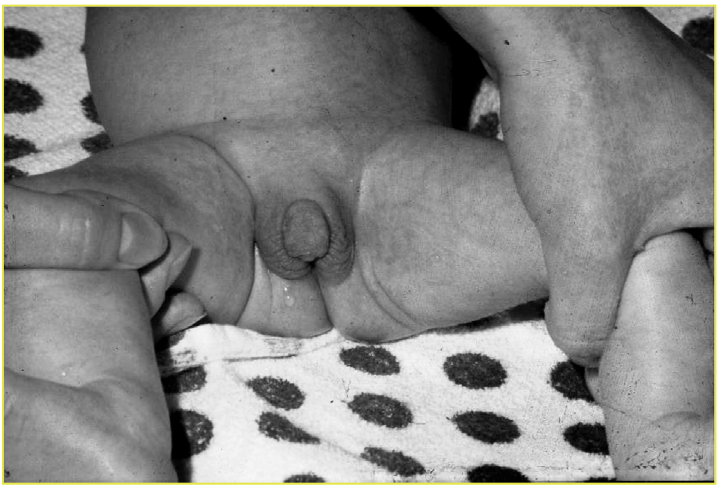


Figure 2.2. - Virilised genitalia of a newborn girl suffering of CAH

A male infant with this defect appears normal at birth, although sometimes penile enlargement may be apparent. Many of these patients are admitted to hospital in case of salt wasting one to two weeks after birth due to progressive weight loss, anorexia, vomiting, dehydration, hypotension, hypoglycemia, hyponatremia and hyperkalemia (*Figure 2.3*). In these patients levels of 17-hydroxyprogesteron (17 OHP) are increased, diagnosis is based on measurement of 17 OHP in basal condition and after stimulation with ACTH.

Treatment of CAH involves glucocorticoid administration to suppress ACTH stimulation and hypersecretion of adrenal androgens and fludrocortisone to treat aldosterone deficiency in case of salt-wasting forms. Hydrocortison, 15-20 mg /m2/24h, administered orally in 3 divided doses is recommended while the dose of fludrocortisone 0.05-0.2mg daily is usually sufficient. Therapy must be adjusted throughout childhood at regular intervals. Overtreatment will cause growth stunting and weight gain, while undertreatment will cause excessive height gain, skeletal

advance, an early appearance puberty, ultimately resulting in relative short stature.[9]

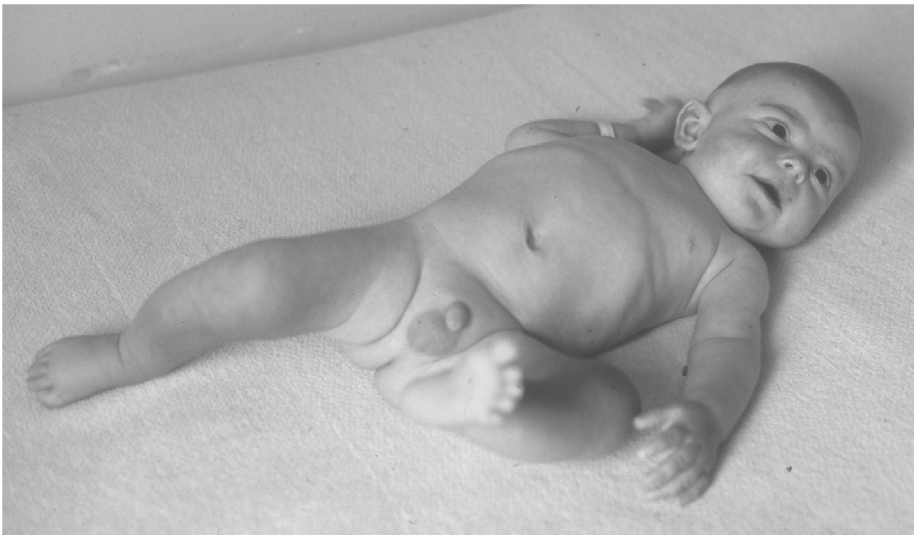


Figure 2.3. - A 3 weeks old boy with salt-wasting form of CAH was admitted to hospital due to shock and dehydration. No other clinical signs of CAH were registered except hyperpigmentation of mammillae and scrotum due to ACTH-MSH excess

Virilised females undergo surgery in the first or second year of life when partial resection of the clitoris is performed. Vaginoplasty and correction of the urogenital sinus could be done at the same time or could be postponed to the time of puberty to prevent frequent revisions.[10]

The adequacy of glucocorticoid replacement therapy is monitored by determining serum concentrations of adrenal precursors 17-OHP and androstenedione. Besides, the assessment of linear growth and skeletal age, by bone age determination, is required as a reflection of appropriate therapy.

To avoid adrenal insufficiency, two to threefold higher dose of glucocorticoids should be given during stress situations, such as febrile illnesses and surgery, and subcutaneous glucocorticoid is used in severe emergencies. Mineralocorticoid therapy is monitored with serum sodium and potassium and plasma rennin activity levels.

'None classical or Late-onset form' of CAH is due to the same affected genes but patients have milder manifestations of the disease without ambiguous genitalia but they do have acne, hirsutism, and in girls, irregular menstrual cycles or amenorrhea are common. Late onset CAH in girls may be confused with polycystic ovary disease.[11]

CAH due to 11-beta – hydroxylase deficiency

The second most common cause of CAH 11-beta-hydroxylase deficiency occurs only in 5-8% of all cases of CAH. It is due to a mutation in the CYP11B1 gene located on the long arm of chromosome 8 (8q24). Patients have clinical signs of glucocorticoid deficiency and excess of androgens and deoxycorticosteron. Genitalia in females are ambiguous, there is postnatal virilisation in males and females. Hypertension and hypokalemia are a result of excessive mineralocorticoid production.

Therapy includes suppression with glucocorticoids and vaginoplasty and clitoral recession if needed.[12]

CAH due to 3-beta-hydroxysteroid dehidrogenasse deficiency

The rare form of the disease which occurs in less than 2% of patients with CAH is due to deficiency of 3-beta-hydroxysteroid dehydrogenase. This enzyme expressed in the adrenal cortex and gonad is encoded by the HSD3B2 gene located on the short arm of chromosome 1 (1p13.1).

Affected subjects have clinical signs of glucocorticoid and mineralocorticoid deficiency with salt-wasting crisis. There are ambiguous genitalia in females and males, precocious adrenarche and disorders of puberty are common.

Glucocorticoid and mineralocorticoid replacement are necessary. Besides surgical correction of genitals and sex hormone replacement consonant with sex of rearing are necessary.[13]

CAH due to 17-hydroxylase deficiency

Less than 1% of CAH are caused by 17-hydroxylase deficiency. The enzyme is expressed in both the adrenal cortex and the gonad and is encoded by a gene on chromosome 10 (10q24.3). Patients cannot synthesize cortisol but an active glucocorticoid corticosterone is synthesized in excess. This can cause hypertension and hypokalemia. As patients are unable to synthesize sex hormones affected males are incompletely virilised and present as phenotypic females.

Female patients usually present with failure of sexual development at the expected time of puberty.

Therapy includes administration of glucocorticoids, antihypertensive drugs and sex hormone replacement.[14]

Disturbed steroidogenesis with adrenal hyperplasia presenting with glucocorticoid deficiency and ambiguous genitalia could be due also to mutations on the short arm of chromosome 8 (8p11.2-Congenital lipoid adrenal hyperplasia) and the long arm of chromosome 7 (7q11.3-Deficiency of P450 oxidoreductase) but they are extremely rare.

Prenatal diagnosis and treatment of CAH

In majority of patients CAH is due to 21-hydroxylase deficiency. Possibilities of prenatal diagnosis and treatment have been investigated for a long time. In families where the parents have an affected child there is a possibility to analyze in the 1st trimester of a new pregnancy DNA obtained by chorionic villus sampling or during the 2nd trimester by amniocentesis. CYP21 gene mutations analysis enables prenatal diagnosis and treatment of affected females.

Mothers at risk could be given dexamethasone to suppress hypersecretion of adrenal androgens by the fetal adrenal in case of CAH. If started by 6 weeks of gestation, it ameliorates virilisation of the external genitalia in affected females.

Chorionic villus biopsy is performed to determine the sex and genotype of the fetus. Therapy is continued only if the fetus is an affected female.

Recent studies have shown that prenatal dexamethasone treatment is associated with orofacial clefts, decreased birth weight, poorer verbal working memory, and poorer self-perception of scholastic and social competence. Many medical societies have cautioned that prenatal treatment of CAH with dexamethasone is not appropriate for routine clinical practice as this treatment is inconsistent with the classic medical ethical maxim to “first do no harm”.[15]

Newborn screening

Screening of CAH is important especially for affected males as they are usually undiagnosed until they have severe adrenal insufficiency in the first weeks of life. Analysis of 17-hydroxyprogesterone has been done in dried blood obtained by heel-stick and absorbed on filter paper cards at the same time as screening of congenital hypothyroidism and phenylketonuria are performed. Affected infants are recalled for additional testing and immediate therapy usually in ten days could be started what is important in preventing many cases of adrenal crisis in affected males.[16, 17]

Novel treatment strategies in CAH

Glucocorticoids have been the mainstay of CAH treatment. The delivery of glucocorticoid replacement therapy trying to mimic the physiologic secretion pattern has been introduced. Examples include modified release oral glucocorticoids and continuous subcutaneous hydrocortisone pumps. Non glucocorticoid approaches to address the androgen excess have emerged, such as inhibition of key androgenic enzymes and adrenocorticotropin secretion blockade by corticotrophin-releasing hormone receptor antagonists. [18]

2. Adrenogenital syndrome due to adrenal tumors

Adrenocortical tumors are very rare in childhood.

Epidemiolgical and molecular evidence suggests that in most cases they are derived from the fetal adrenal due to germline mutations of the tumor suppressor gene TP 53 with loss of heterosigosity in the tumor.[19]

Tumors can occur in all age groups but most commonly in children younger than 6 years of age and are more frequent in girls. Virilization is the most common presenting symptom but symptoms of glucocorticoid excess are also possible. In *males* clinical picture is similar to that of simple virilizing CAH: accelerated growth velocity and muscle development, acne, penile enlargement and the precocious development of pubic and axillary hair. In *girls* masculinization of previously normal female with clitoral enlargement, growth acceleration, acne, deeping of the voice, and premature pubic and axillary hair are observed. In untreated patients mortality is 100%.

The treatment of choice is surgery alone for stage I and II and surgery followed by intensive chemotherapy (cisplatin, doxorubicin, etoposide and mitotane) for advanced stage (III and IV) disease. For patients with tumors smaller than 5 cm and with no signs of lymph node or distal metastases, survival is favorable with a median exceeding 10 years. However the overall 5-year survival rate for all patients in adrenocortical cancer is only 30 per cent. [20, 21, 22]

We present our three patients.

The *first* was a girl who was admitted at twelve months as parents have observed accelerated growth, acne, pubic hair and enlarged clitoris since age 8 months (*Figure 2.4*).

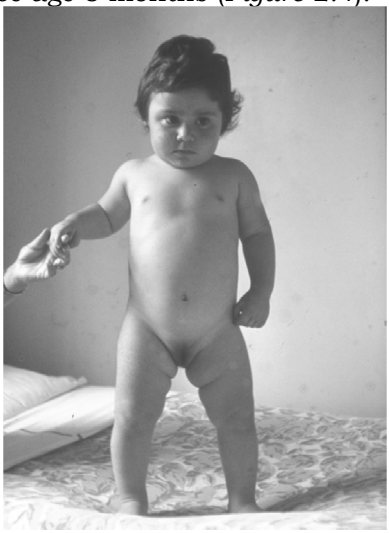


Figure 2.4.a

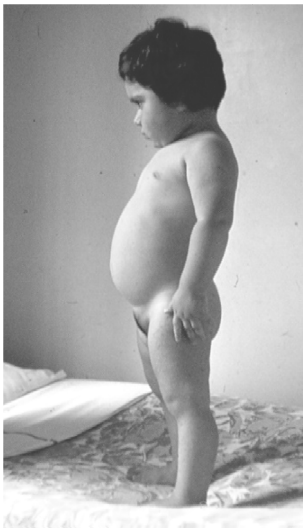


Figure 2.4.b

Radiological examination revealed unilateral tumor in suprarenal region which was extirpated by surgeon. Encapsulated tumor was adrenal carcinoma. (*Figure 2.5.*)

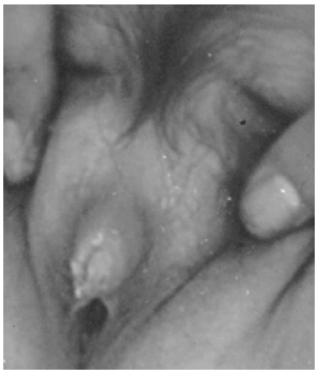


Figure 2.4.c. - 8 months old girl with signs of androgen excess: rapid growth, premature pubic hair and clitoris enlargement

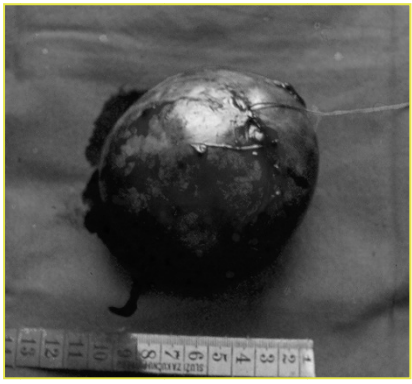
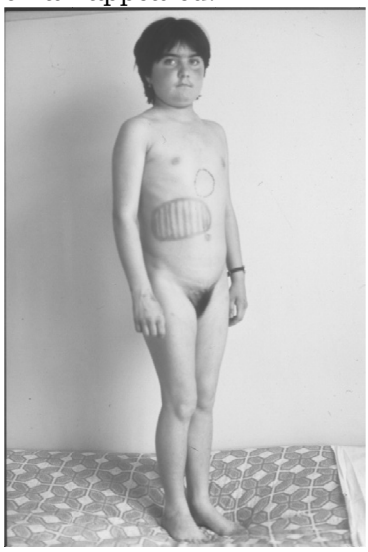


Figure 2.5. - Encapsulated adenocarcinoma which was extirpated by surgeon

In one year majority of symptoms of androgen excess disappeared and her further development was normal.

The second patient (*figure 2.6.*) was 9 years old girl who was complaining of physical changes which started one year before. She got acne, her voice deepened, her face became round and flushed, intensive pubic hair appeared.[5]



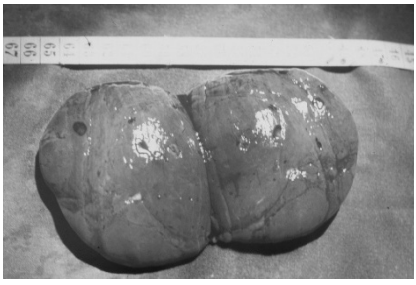


Figure 2.6. - 9 years old patient affected by adrenocortical carcinoma

At physical exam tumor was palpated in the right abdominal region, clitoris was enlarged; pubertal stage was P4, A 2-3, B 1. Radiological findings revealed tumor in the right adrenal gland and metastases in liver, lungs and brain. Adrenocortical carcinoma of the right adrenal gland and kidney were removed and chemotherapy with mitotane was performed. Treatment was not successful and the patient died three months after operation.

The third patient was a 14 years old girl who was admitted because of a typical Cushing syndrome with hypertension, progressive acne vulgaris, generalized obesity with rounded face, flushed cheeks, and numerous striae. (*Figure 2.7.*)

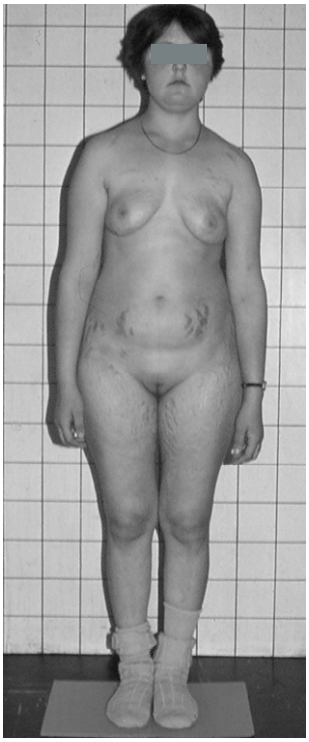


Figure 2.7. - 14 years old girl with clinical signs of Cushing syndrome due to cortical adrenal carcinoma

At clinical exam hypertrophy of clitoris and large tumor mass in the left abdomen was stated. CT presented a large tumor in the region of the left adrenal gland with necrotic center and numerous metastases in both lungs. Plasma levels of cortisol, testosterone, aldosterone, and estradiol were markedly elevated.

The high plasma cortisol levels did not show normal diurnal variation, hyperglycemia was also found.

The tumor of the left adrenal gland was completely removed along with the left kidney. Histology showed cortical adrenal carcinoma which was poorly differentiated.

The patient received high doses of hydrocortisone during the operation which was continued in the physiological replacement dose later on. Postoperatively the levels of hormones normalized but pulmonary metastases progressed. After 12 days mitotane therapy was started in low dose and increased to 10 g daily after one week. Two days later high fever with signs and symptoms of adrenal crisis appeared which was controlled with high doses of hydrocortisone and parenteral hydration. A dose of mitotane was reduced to 7 g per day and she tolerated this dose of cytostatic well. The pulmonary metastases regressed continuously during this treatment and disappeared after 5 months. The initial dose of mitotane caused severe hyperthermia and Addison's crisis, which appeared presumably because of cytolitic effect of mitotane on malignant and also normal adrenocortical tissue. The patient had to receive permanent substitution therapy as with our follow up studies we found practically no hormone activity of the remaining cortex of the adrenal gland. The patient has been in excellent condition without

signs of tumor later on, but she could be followed only for ten years. It has been proven that aggressive treatment with radical surgery combined with cytostatics could be effective even in metastatic tumors. [23]

Conclusion

Adrenogenital syndrome due to adrenocortical tumor could mimic simple virilizing CAH in boys but in girls enlargement of clitoris which was normal at birth and progressive masculinisation use to be signs and symptoms of malignancy and need immediate diagnostic procedure and treatment.

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SOME CLINICAL ASPECTS OF HORMONE INSENSITIVITY

Ciril Kržišnik

Introduction

It has been generally accepted that hormones regulate cellular activities and homeostasis regulating also cellular differentiation and genetic expression. Endocrine glands release hormones into the blood stream where they are carried through the body and reversibly bind to their target receptors.

Hormones as the first messengers, binding to specific cell membrane receptors transmit the signal information across the membrane and produce a response in the cytosolic side of the cell. The response results in the change of concentrations of so called the second messengers inside the cells which include cAMP, cGMP, Ca ions, inositol 1,4,5 triphosphate and diacylglycerol. One common response to a second messenger is the activation of protein kinaze which use ATP to phosphorylate serine, threonine, and tyrosine residues to target enzymes what lead finally to specific biological effect.[1]

Defects in the hormone receptor or post-receptor pathways lead to hormone resistance or insensitivity. Some clinical aspects of growth hormone and androgen insensitivity are presented.

GROWTH HORMONE INSENSITIVITY (GHIS) – LARON SYNDROME (LS)

GHIS is characterized by clinical features of growth hormone (GH) deficiency and biochemical findings of GH resistance. The syndrome was first described in Israel by Zvi Laron in 1959 in three siblings with severe

short stature and very high levels of serum GH. Many more cases have been reported from the Mediterranean region, different parts of Europe, Ecuador and also Asia. The autosomal recessive disease is seen commonly in consanguineous families. [2,3]

GHIS is due to defects in GH receptor or post-receptor pathways. GH receptor (GHR) is a protein encoded by the GHR gene which is located on the short arm (p) of chromosome 5 between position 13 and 12 (*Figure 3.1.*).

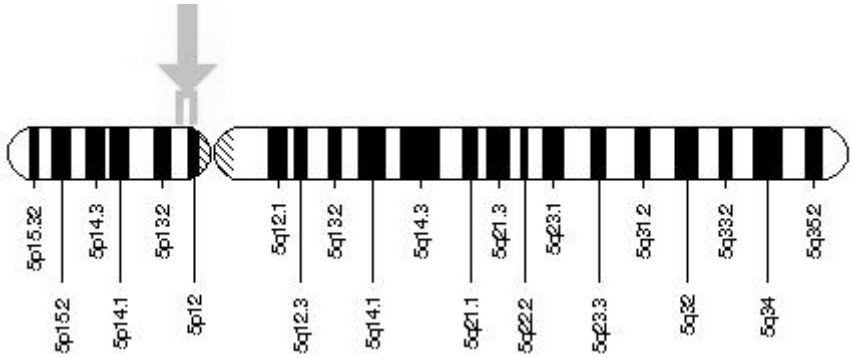


Figure 3.1. - Location of GHR gene on chromosome 5 (httpps://ghr.nlm.nih.gov/gene/GHR)

GHR are embedded in the outer membrane of cells throughout the body and are most abundant in liver cells. The GHR has three parts: an extracellular region, a transmembrane region that anchors the receptor to the cell membrane and intracellular region which transmits signals to the interior of the cell (*Figure 3.2.*).

The binding of GH to GHR triggers signaling via the intracellular region of the receptor that stimulates the growth and division of cells. This signaling leads to the production, primarily by liver cells, of another important growth promoting hormone called insulin-like growth factor (IGF-1). Thus both, GH and IGF-1 stimulate growth and influence metabolism.

It has been established that at least 70 mutations in the GHR gene can be involved in GH insensitivity. The most common defect has been mutation in the extracellular domain of the GH receptor associated with low levels of GH-binding protein, which is the circulating form of this extracellular domain. Defects in the post-receptor pathway (JAK, sTAT), IGF-1 gene and IGF receptor have also been described.[4,5,6]

Children suffering of GHIS have clinical signs of GH deficiency.

Many of them are short at birth but apparent short stature is usually observed in infancy as well as protruding forehead, relative macrocrania, saddle nose and mid-facial hypoplasia.

Hairs are thin, sparse, nails grow slowly, and teething is delayed and defective with overcrowding being common feature due to small mandible.

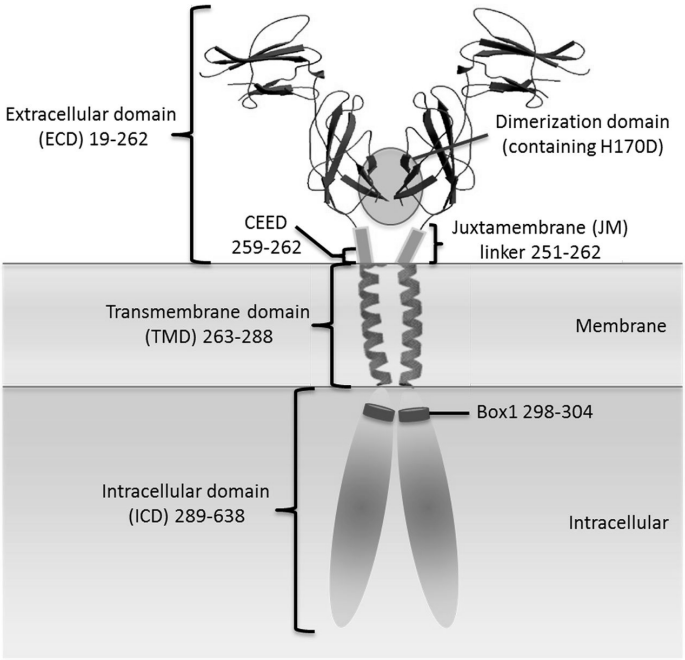


Figure 3.2. - GH receptor [4]

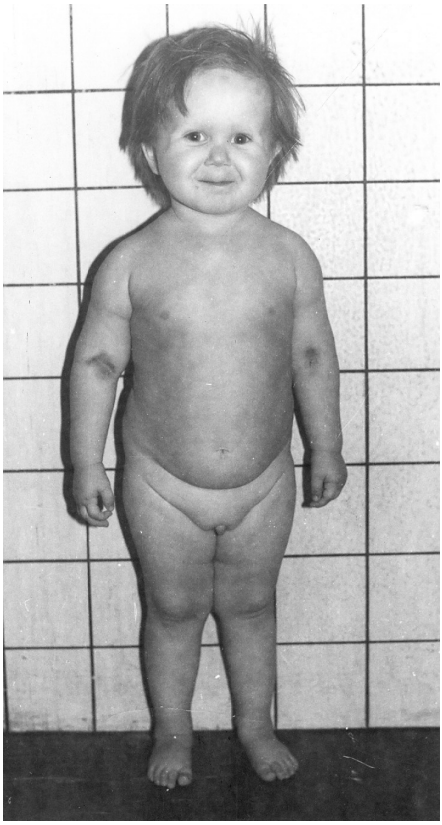
Voice is high-pitched, small hands and feet-acromicria is common, boys often have hypogenitalism, many patients are obese. They grow at a lower speed during childhood resulting in very short adult stature. Final height has been 111-142 cm in males and 108-136 cm in females.[7]

In patients the levels of circulating GH use to be high, while IGF-1 concentrations are very low and do not rise on administration of exogenous GH. The only treatment for this syndrome is subcutaneous administration of IGF-1. [8]

We present our patient J.G. from Slovenia who has had all clinical and laboratory characteristics of Laron syndrome (LS) described before. Birth weight was 3200 gr, length 49 cm. After delivery, hypoglycemia, tremor, hypotonicity, relative obesity and prolonged jaundice were noted. He was referred to us at 16 months of age when he measured 63 cm and weighed 7450 grams. He was hypotonic, had a doll-like chubby face, saddle nose, receding chin, accumulation of abdominal fat, and micropenis. Basal serum GH was elevated 39,5mcg/l (normal basal level 0,0-5,6 mcg/l) and increased further after glucagon stimulation. At age 4

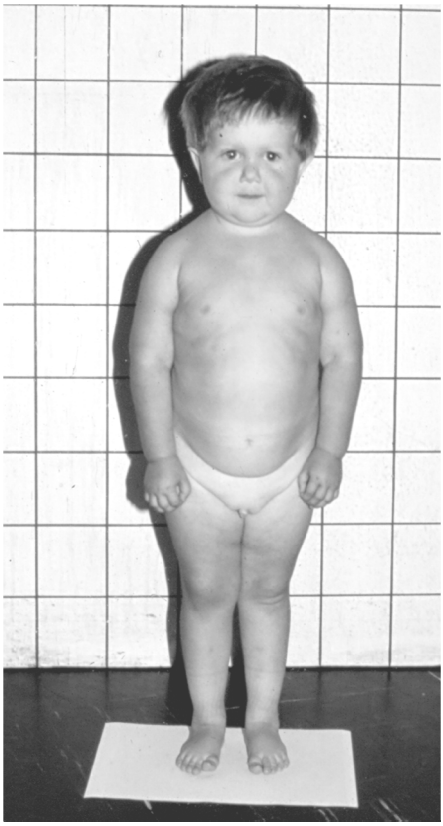
years he measured 74 cm, weighed 10 kg, he resembled a pituitary dwarf (*Figure 3.3.a*).

At 6 years when his length was 87 cm and weight 14,30 kg (*Figure 3.3.b*), an IGF-1 generation test was performed. The patient received 4 units of GH s.c. for one week. The levels of IGF-1 were undetectable before and after stimulation what confirmed GHIS. Besides low IGF binding protein -3 was detected. Gene analysis revealed an abnormality in the extracellular domain of the GH receptor gene: mutation of exon 4: del C.



3a

Figure 3.3.a: Patient G.J. at 4 years, showing typical appearance of Laron syndrome: dwarfism, obesity, large forehead, saddle nose, acromicria, hypogenitalism



3b

Figure 3.3.b. L.S. patient at 6 years

The patient started treatment with recombinant IGF-1 in the framework of multicenter study (Kabi Pharmacia Upjohn) at age 9,3 years when he measured 106 cm (-4.5 SDS) and weighed 27 kg. The IGF-1 was

administered s.c. in a dose of 120 mcg/kg twice daily into the thigs, forearms and abdominal region in a rotating manner. The patients pretreatment growth velocity of 4cm /yr increased to 10 cm in the first, 8,5 cm in the second, 7,5cm in the third, 8 cm in the fourth and 6 cm in the fifth year of therapy. A significant increase in body weight was found (*Table 3.1*).

Table 3.1.

Anthropometric and clinical changes in GHIS patient during five years of IGF-1 treatment

	Duration of IGF-1 Treatment						
	0	1 yr	2 yr	3 yr	3,5 yr	4 yr	5 yr
Age (yr)	9,3	10,4	11,4	12,4	13	13,4	14,5
Bone Age (yrs)	8,5	9	10	11,2	-	13,0	13,0
Body Length (cm)	106	116	124,5	132	136,5	140	146
Height SDS	-4,5	-3,5	-2,8	-2,4	-2,1	-2,0	-2,1
Body Weight (kg)	27,0	31,8	43,3	54	60,6	64,3	77
Arm Span (cm)	109	118	126	133	136,5	141,5	148
Sitting Height (cm)	60,0	64,0	69,0	72,5	74,2	75,5	76,5
Head Circumference (cm)	49,5	51,5	53	55	56	56,5	57
Growth Velocity (cm/yr)	4,0	10,0	8,5	7,5	-	8,0	6,0
Penile Length (cm)	2,0	2,0	2,0	2,5	3	3	3,5
Penile Circumference (cm)	4	4	4,5	4,5	5	5	5
Testicular Volume (ccm)							
left	1	1	1	1	3	8	15
right	1	1	1	1	2	6	12
Stage of puberty (Tanner) P	1	1	1	1	1	2	2-3

During treatment striking maturation of the facial appearance was noted, the hair became stronger and more abundant and nails became thicker and harder. Enlargement of the nose and voice change from high-pitched to normal for age were observed. Though some values of blood glucose were below the normal range, no clinically evident signs and symptoms of hypoglycaemia were observed during IGF-1 therapy. Hypertrophy of subcutaneous tissue at the injection sites of IGF-1 in both thigs and forearms was observed after two years of therapy. At the age of 13 years initial enlargement of testes and appearance of pubic hair were registered. [9,10,11]

We could state that IGF-1 therapy was successful in our patient as in five years his linear growth increased 40 cm but he became very obese. He continued to grow during puberty, at 17 years he measured 164 cm. Lipohyperthrophy at the site of IGF-1 injections could be due to insulin -

like effect of IGF-1 at the level of adipocytes, which is also mediated via insulin receptors.

As parenteral application of IGF-1 decreases secretion of patient s own GH, during treatment of GHIS combined therapy with IGF-1 and GH is recommended. The latest analysis of LS patients had shown they are protected from cancer. [12, 13, 14]

ANDROGEN INSENSITIVITY SYNDROME (AIS)

Androgen insensitivity syndrome (AIS), previously described as testicular feminization is due to partial or complete unresponsiveness of the target cells to the action of androgenic hormones in genetically male individuals[15, 16]

AIS is broken down into three classes based on phenotype:

- Complete Androgen Insensitivity Syndrome (CAIS),
- Partial Androgen Insensitivity Syndrome (PAIS),
- Mild Androgen Insensitivity Syndrome (MAIS).

AIS is an X-linked disease which is due to mutation in the androgen receptor (AR) gene which is located on the long arm of the X chromosome (Xq 11-12) (*Figure 3.4.*). AR protein is encoded by 8 exons. Over 400 AR mutations have been reported. [17, 18, 19, 20]

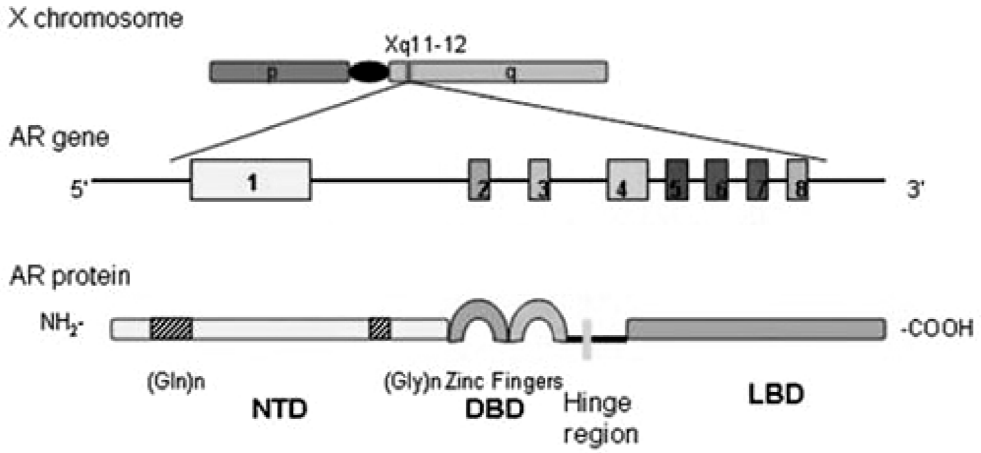


Figure 3.4. - Human androgen receptor gene is located on the long arm of the X-chromosome[20]

Androgens exert their effects by mediating the differentiation and development of the male phenotype via AR. It is expressed in fetal tissues at 8 weeks of gestation, before the onset of androgen action and is activated to coordinate expression of suitable responsive genes.

In the human male embryo, testes begin to secrete androgens at 9 weeks of gestation. Testosterone (T) and dihydrotestosterone (DHT) form a complex with AR and produce different biological messages. T which peaks between 11 and 18 weeks of gestation stimulates differentiation of the Wolffian duct into epididymis, vas deferens and seminal vesicles. Development of the prostate from urogenital sinus and masculinization of the primordial external genital into penis and scrotum require more potent androgen DHT. DHT is derived from testosterone by enzyme 5 alfa-reductase which is expressed in target tissues. The presence of AR in tissues is needed to express biological effect of T and DHT. [20, 21]

Patients suffering of AIS usually present with 46 XY karyotype, incompletely descended testes and female or partially masculinized external genitalia. There have been three subgroups based on genital phenotype.

1. Complete Androgen Insensitivity Syndrome (CAIS)

Patients suffering of CAIS present normal female external genitalia with short blind ending vagina, absence of Wolffian duct derived structures like epididymides, vas deferens and seminal vesicles, absence of prostate. At puberty breast development is observed but axillary and pubic hair is absent or sparse, and menarche does not occur. Many patients are recognized when they present inguinal hernia.

2. Partial Androgen Insensitivity Syndrome (PAIS)

PAIS comprises a wide spectrum of clinical phenotypes. Individuals with predominantly female external genitalia present mild clitoromegaly, some fusion of the labia and pubic hair at puberty.

Patients of predominantly male appearance of external genitalia exhibit micropenis, perineal hypospadias and cryptorchidism. In some individuals, Wolffian duct derived structures can be partially or fully developed while testes are usually found undescended.

At puberty affected subjects usually develop gynecomastia with no increase in the size of phallus. In the testes reduced number of germ cells with azoospermia can be observed and later on at puberty carcinoma in situ is common.

3. Mild Androgen Insensitivity Syndrome (MAIS)

The phenotype is usually male, but genitalia may be underdeveloped for a male or only simple coronal hypospadias or prominent midline raphe of the scrotum could be found.

At puberty MAIS takes two forms with various degrees of gynecomastia, high-pitched voice, sparse sexual hair and impotence. In

one form spermatogenesis and fertility are impaired, while in another they are sufficient to preserve fertility.[22]

We present two patients suffering of AIS treated at University Children's Hospital Ljubljana, Slovenia.

A 15-year old girl was admitted due to epileptic status. Convulsions could not be blocked with conventional therapy, the patient died few hours after admission. The height of the girl was 170 cm, weight 60 kg, pubertal stage B 4, A 1, P 1-2. Autopsy revealed tumors in brain and lungs which were metastases of seminoma. Primary tumors – seminomas were found in degenerated undescended testes in the intra-abdominal area. The lack of uterus and ovaries and hypoplastic vagina were also stated. The karyotype 46 XY confirmed the CAIS (*Figure 5*).

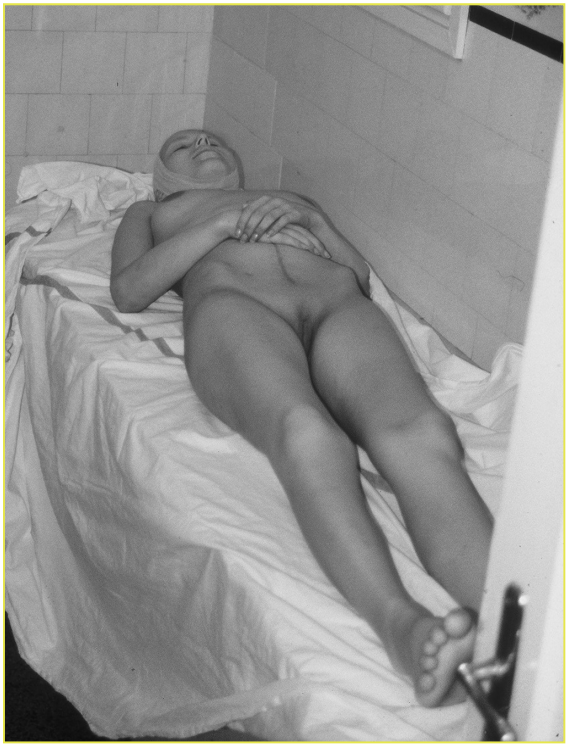


Figure 3.5. - 15 year old girl with CAIS who died due to metastases of seminomas

The second child was 8 months old girl who was referred to our department due to tumor in the left inguinal region. Biopsy revealed testicular tissue, while the testis on the right side was located in the intra-abdominal region. Short, blind vagina was stated, ultrasound examination

confirmed there was no uterus or ovaries. CAIS was confirmed by 46 XY karyotype (*Figure 3.6.*).

There are some controversies in management of patients suffering of AIS.[23]

XY individuals with CAIS in whom external genitalia are normal female are raised as females.

Usually the testes are removed after puberty when feminization of the affected individual is complete since feminization occurs partly by testicular estrogens and partly by peripheral conversion of androgens to estrogens.

The reason for the postpubertal gonadectomy is the risk of testicular malignancy which seldom occurs before puberty but it can occur. It is why some clinicians propose gonadectomy also before puberty.

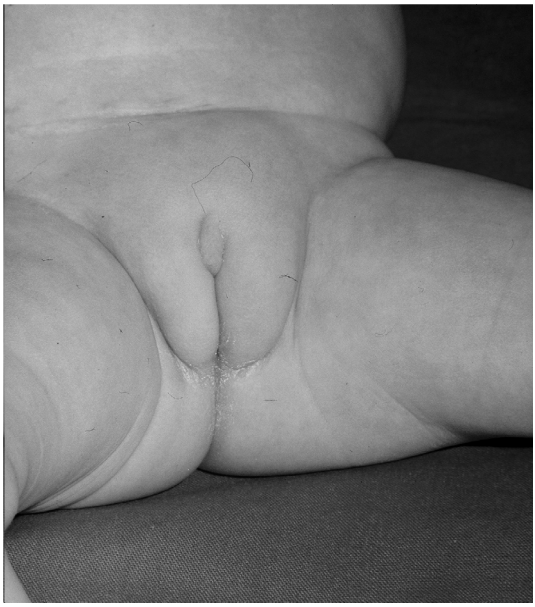


Figure 3.6. - 8 months old girl with CAIS.
Biopsy of tumor in the left inguinal region revealed testicular tissue.

In PAIS with predominantly female genitalia prepubertal gonadectomy is preferred to avoid increasing clitoromegaly and the possibility of malignant tumor. [24]

In infertile subjects with MAIS therapeutic trial with testosterone in an effort to predict potential androgen responsiveness is recommended. [25]

But in general in AIS the main problem has been the possibility of malignancy of degenerated testis and gonadectomy is recommended.

The optimal time of this operation has not been agreed yet.

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RICKETS AND VITAMIN D

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Aims

The common denominator of all rickets is hypophosphatemia. Hypophosphatemia prevents apoptosis in the hypertrophic cells in the growth plate. In the absence of apoptosis the hypertrophic cells accumulate in the growth plate and form the rachitic bone.

It follows that diagnosis of rickets should be based on the etiologies of hypophosphatemia.

The three major entities that can lead to hypophosphatemia are high PTH activity, high FGF-23 activity, and renal defects that lead to Pi wasting.

Hallmarks of high PTH activity are: hypophosphatemia, phosphaturia, disturbance in vitamin D metabolism, and low calcium. Hallmarks for high FGF-23 activity are hypophosphatemia and phosphaturia with inappropriately low 1,25-(OH)2-D.

Hallmarks for renal rickets are hypophosphatemia and phosphaturia with high 1,25-(OH)2-D that causes hypercalcuria.

Introduction

The first complete clinical description of rickets was published 350 years ago by Francis Glisson[1]. He also coined the term rachitis, derived from the Greek word for spine, which he thought was one of the first organs affected. The word also sounded similar to the Old English “wrickken”, meaning to twist.

Two centuries later, the cause for the most common etiology of rickets was discovered when cod liver oil and sunlight were found to prevent rickets. In 1928 the Nobel Prize for chemistry was awarded to Adolf Windaus "for his studies on the constitution of the sterols and their connection with vitamins". During the following decade the structure of

vitamin D was established. The use of cod-liver oil for the treatment and prevention of rickets and fortification of milk with vitamin D became commonplace, and led to eradication of the most common cause of rickets[3]. That is, until the more recent surge in rickets incidence.

The discovery that vitamin D deficiency results in poor overall mineralization of the skeleton and is the most frequent cause for rickets led to the assumption that rickets develops due to abnormal mineral ion homeostasis. However the mineral involved is not calcium, as was believed, but rather phosphorus. Here we review the evidence for phosphorus deficiency as the common denominator for all rickets.

Rickets is a disease of the growth plate, and hence only children are affected. The growth plate hypertrophic layer is divided into upper (early) and lower (late) hypertrophic zones. The terminal cell of the lower calcified hypertrophic zone undergoes apoptosis *(Figure 4.1).*

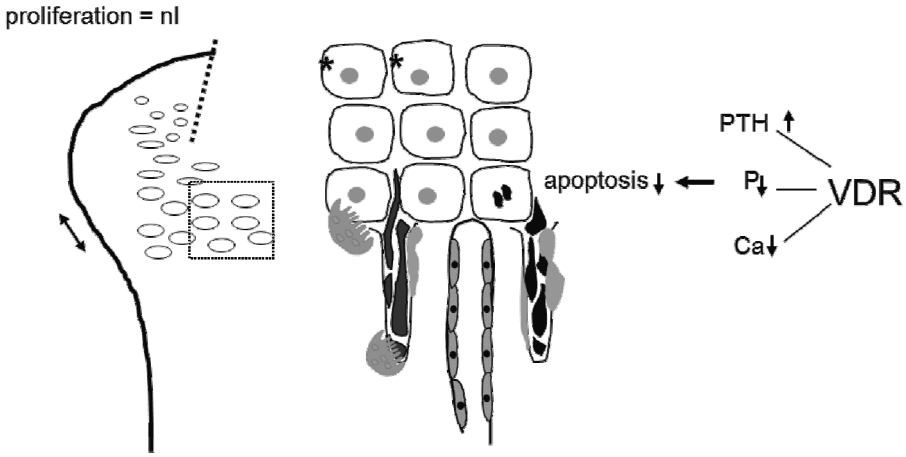

Figure 4.1. Severe rachitic bone changes in a child with vitamin D receptor defect (vitamin D dependent rickets type 2). The wide calcified hypertrophic zone of the growth plate is clearly evident. This is due to the defective apoptosis of the terminal hypertrophic chondrocyte due to hypophosphatemia.

It is the process of apoptosis that is defective in rickets, and the availability of phosphorus is essential for proper apoptosis of the terminal hypertrophic cell. Clinical evidence of the role of phosphate in the process of new bone formation was underscored over 50 years ago by Fraser et al [4]. They showed the *in vivo* response of rachitic bones to phosphate supplementation in two children with untreated vitamin D resistant rickets who received continuous intravenous infusion of isotonic phosphate. Normalizing serum inorganic phosphorus concentration induced an

immediate decrease in serum calcium. Radiographic evidence confirmed recovery from rickets within five days.

Prevention of rickets in patients with vitamin D receptor (VDR) mutations[5] and in VDR knock-out mice administered high amounts of dietary calcium[6] demonstrates that neither vitamin D nor the VDR are required for normal growth plate maturation, and that rickets develops due to impaired mineral ion homeostasis.

Analysis of growth plate morphology in VDR null mice reveal normal resting and proliferating chondrocyte layers; however the hypertrophic calcified chondrocyte layer is expanded. Proliferation studies with BrdU labeling have failed to demonstrate a change in chondrocyte proliferation in the VDR null mice.

On the other hand, apoptosis was markedly diminished in the late hypertrophic chondrocytes, suggesting that impairment in the programmed cell death of these cells leads to the characteristic findings of rickets. Surprisingly, diminished apoptosis can be rescued by restoring phosphorus serum levels only, without correction of serum calcium levels.[7]

Similarly, studies on Hyp mice, a model of X-linked hypophosphatemia, and mice that were weaned onto a phosphorus-restricted/high-calcium diet, revealed the same findings observed in VDR null mice.[8] Development of hypophosphatemia was associated with a decrease in the number of apoptotic hypertrophic chondrocytes and expansion of the growth plate. This indicated that the histological findings observed are not unique to VDR null mice, but rather a general phenomenon coincident with hypophosphatemia.

The role of inorganic phosphorous (Pi) in hypertrophic cell apoptosis

Apoptosis of epiphyseal chondrocytes provides a mechanism for the removal of terminally differentiated cells from cartilage columns, and promotes the invasion of vascular elements of bone marrow, including osteoblasts and osteoclasts, for generation of new bone[9] (*Figure 4.1.*).

Rickets is a disease of insufficient apoptosis of the terminal hypertrophic chondrocyte. Apoptosis is controlled by ECF phosphorus, which, in turn, is controlled by PTH and FGF23.

Treatment of embryonic tibial chondrocytes with Pi induced cell death in a dose and time dependent manner, and that Pi is a stage-specific inducer of apoptosis in mature chondrocytes. Cells that were at an earlier developmental stage than the terminally differentiated hypertrophic-tibial cells remained viable despite an increase in the medium in Pi content[10, 11]

Apoptosis is multifactorial.

In one of the apoptotic mechanisms in mammals mitochondrial cytochrome c, is released, leading to cytosolic assembly of the apoptosome, a caspase activation complex involving apoptotic protease-activating factor

1 (APAF1) and caspase-9, these being the hallmarks of apoptosis. There are, however, mitochondrial regulated cell death pathways that are independent of APAF1/caspase-9. Like cytochrome c, apoptosis-inducing factor (AIF) is localized in mitochondria and released in response to death stimuli. AIF-dependent cell death displays structural features of apoptosis, and can be genetically uncoupled from APAF1 and caspase-9 expression[12].

In vitro analyses in primary chondrocytes demonstrate that phosphate mediates hypertrophic chondrocyte apoptosis by activating the caspase-9-dependent mitochondrial pathway.[8] Studies in mice treated with a caspase-9 inhibitor recapitulated the growth-plate phenotype observed in the rachitic models, thus confirming the importance of the mitochondrial apoptosis pathway in growth plate maturation and the critical role of phosphorus in hypertrophic chondrocyte apoptosis.

We previously showed that patients with type 2 vitamin D-dependent rickets show spontaneous healing of the rachitic bone during puberty, even when the primary underling cause of rickets or treatment compliance does not change.[13] We now believe that this might be caused by the activation by estrogens of a non Pi-dependent apoptotic cascade in the hypertrophic cells. Estrogen affects skeletal growth and promotes growth plate fusion. This fusion was addressed by Chagin and et al who showed that the selective estrogen receptor modulator Tamoxifen enhances apoptosis of chondrocytes within the resting and hypertrophic zones.[14] Fas Ligand (FasL) secretion was stimulated by Tamoxifen and blocking either FasL or Fas decreased Tamoxifen-induced apoptosis of chondrocyte.

Hypophosphatemia and Osteomalacia

While rickets is a disease of the hypertrophic chondrocytes in the growth plate, osteomalacia is the general term for the softening of the bones due to defective bone mineralization.

During growth and until epiphyseal fusion at puberty both rickets and osteomalacia co-exist in the presence of hypophosphatemia. The clinical signs, craniotabes and bowing are due to the osteomalacia of infancy and childhood.

During adulthood only osteomalacia presents in patients with hypophosphatemia. One of the early biochemical signs in patients with rickets and osteomalacia is elevated alkaline phosphatase (ALP). The role of Pi in osteomalacia is summarized later in this review.

Phosphorus transporters

Cells obtain phosphorus in the form of negatively charged inorganic phosphate (Pi) from the extracellular environment by means of secondary-active transport. Three classes of sodium-phosphate cotransporters are expressed in the kidney. They consist of the type I cotransporter, NaPi-I (SLC17A1), type II co-transporters, NaPi-IIa (SLC34A1) and NaPi-IIc (SLC34A3), and type III co-transporters, PiT1 (Glvr-1(SLC20A1) and PiT2

(Ram-1 (SLC20A2).[15] Type III co-transporters account for less than 1% of the mRNAs of the renal sodium-phosphate co-transporters.

Phosphorous metabolism *(Figure 2).*

Plasma phosphorous (Pi) levels are maintained in a very narrow range. They are higher during infancy (4.5 to 8.3 mg/dl or 1.5-2.65 mmol /L (Factor X 0.322) and childhood (3.7 to 5.6mg/dl or 1.5-2.65 mmol /L), than during puberty and adulthood (2.5 to 4.5mg/dl or 0.9-1.5 mmol /L)[16]

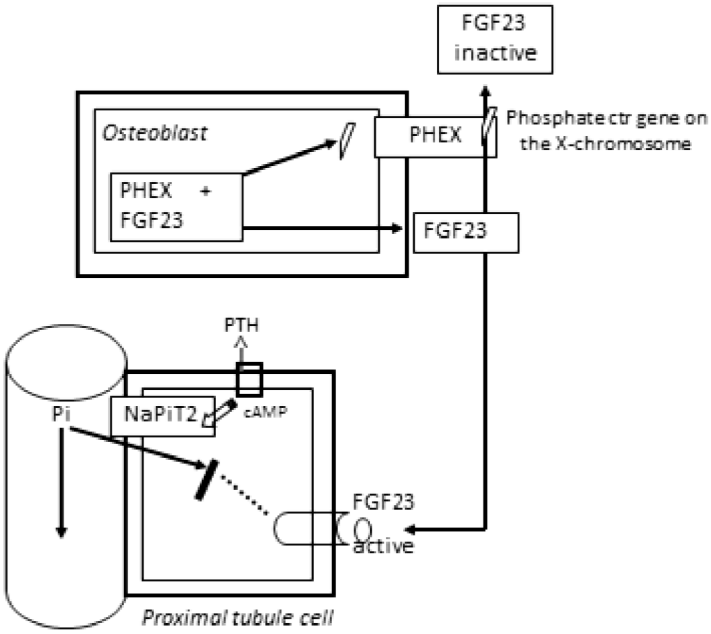

Figure 4.2. - In the presence of hypophosphatemia, two major processes occur in the bone. In the growth plate hypophosphatemia causes arrest of apoptosis in the hypertrophic chondrocytes leading to rickets while in the rest of bone, hypophosphatemia inhibits osteoblasts maturation and mineralization leading to osteomalacia. In the presence of high phosphorous, FGF23 is secreted in order to promote phosphaturia and normalize phosphorous. (the precise relationship between DMP1 and PHEX remains to be established)*

Phosphorus metabolism is tightly controlled and can be divided into two levels. PTH leads to phosphaturia by enhancing the endocytosis of the sodium-phosphorus transporters from the luminal epithelium in the kidney. FGF-23 is expressed and released from the osteoblasts.

The osteoblast secretes not only the FGF-23 but also the protease that inactivates FGF-23, the PHEX protein. In cases where there is disturbance in the inactivation of FGF-23, as demonstrated in x linked

hypophosphotemic rickets due to a mutation in the PHEX protease, the high amounts of FGF-23 will cause severe phosphaturia due to suppression of NAPi transporter expression.

The skeleton and muscles, the principal Pi reservoir in the body, comprise approximately 80% of the total body Pi, the remainder being in soft tissues and extracellular fluids. Of Pi filtered in the kidneys, 80 to 90% is re-adsorbed in the proximal tubule.

In adults, the average amount excreted is almost equivalent to that of absorbed Pi in the intestines.

During periods of rapid growth and development at infancy and puberty a positive phosphate balanced is established. The complex homeostasis of Pi is controlled by the Pi itself, and by calcitriol, PTH, and FGF23, through coordinated function of several organ systems.[17] Calcitriol is responsible for the absorption of Pi in the intestines mainly by the type II co-transporter NaPi-IIb that is not expressed in the kidney.[18] In the osteoblast, calcitriol and phosphorus have a positive effect on the generation and secretion of FGF23, while PTH is responsible for the release of Pi from the bone.[19]

The three main factors responsible for Pi re-adsorption by the sodium/Pi co-transporter in the proximal tubule are; urinary Pi, PTH, and FGF-23. Renal handling of Pi is regulated by hormonal and non-hormonal factors. Changes in urinary excretion of Pi are almost invariably mirrored by changes in the apical expression of NaPi-IIa and NaPi-IIc in proximal tubules. Phosphate deprivation increases NaPi-IIa and NaPi-IIc expression in the proximal tubule. Both NaPi-IIa and NaPi-IIc, the main sodium /Pi cotransporters, are regulated in a similar fashion by PTH, FGF23, and dietary phosphate. PTH and FGF23 accelerate the cotransporter endocytosis while FGF-23 also accelerates cotransporters degradation in the lysosome.[15]

FGF23 as the osteoblast Pi threshold keeper

While ALP and STC1 secretion protect cells from low Pi levels, FGF23 appears to protect cells from hyperphosphatemia. Hyperphosphatemia negatively impacts osteoblasts; overloading of Pi causes cell death. NaPi transport stimulation above a critical threshold, as occurs with extracellular Pi above 5 mM, is evident in Fgf23-null mice.[20,21, 22] For them accumulation of unmineralized osteoids and elevated ALP is marked.

Studies in Hyp mice provide indirect evidence of the relation between high Pi levels and FGF 23 secretion. In Hyp mice FGF23 is 5 to 25 fold higher than in normal mice. However, when dietary Pi decreases, FGF23 concentration decreases by more than 3-fold, with levels correlating directly to those of serum Pi.[19] In concordance with these findings post - operative to partial thyroidectomy serum FGF23 levels are elevated while hyperphosphatemia presents due to a reduction in PTH secretion. FGF23 levels normalize after recovery of parathyroid function and normalization of serum Pi levels. The peak level of serum phosphorus always precedes that

of FGF23 by several days, suggesting that elevated Pi is a primary stimulus for release of FGF23.[23] The significant effect of dietary phosphorus on serum FGF23 concentrations was demonstrated by the measure of considerably higher levels of FGF23 following high-normal Pi dietary intake (2300 mg/d) compared with those following low-normal Pi dietary intake (625 mg/d).[24] In patients with elevated serum phosphorous due to renal function decline, the circulating concentration of FGF23 increases.[25] This indirect evidence ascribes FGF23 with the role of maintaining a phosphorus threshold that protects osteoblasts from high phosphorus levels.

Mechanistic classification of rickets

From the supposition of phosphorus as the common denominator of all rickets, based on the defective apoptotic cascade in the hypertrophic cells, we propose a new classification for the differential diagnosis of rickets, focused on the mechanism leading to hypophosphatemia - high PTH activity, high FGF23 activity, or renal phosphaturia (*Table 4.1.*).

Rickets due to high PTH

Insufficient intake or poor absorption of dietary calcium decreases serum-ionized calcium levels. Immediate recognition of such by the calcium sensing receptor in the parathyroid glands leads to increased expression, synthesis, and secretion of PTH.

In a vitamin D–deficient state, absorption of only 10–15% of dietary calcium and 50–60% of dietary phosphorus results in hyperparathyroidism. PTH, like 1,25-(OH)2-D, enhances expression of RANKL in osteoblasts, which increases production of mature osteoclasts that mobilize calcium stores from the skeleton[26]. Simultaneously, PTH 1decreases renal phosphorus reabsorption, leading to hypophosphatemia and rickets. Thus, in infants with vitamin D–deficient rickets serum calcium levels are normal, serum phosphorus levels low, and 1,25-(OH)2-D, levels normal or high.[27]

Other defects or alterations in vitamin D metabolism lead to rickets due to hyperparathyroidism and consequent hypophosphatemia.

Rickets resulting from sunlight deprivation and nutritional vitamin D deficiency, (the most common cause of rickets), or the rare mutation in Cyp2R1 all share the hallmark of low 25(OH)D3 levels[27,28]. Mutations in 1-alpha-hydroxylaze, VDR, and post receptor mutations share the common finding of normal 25(OH)D3 levels.

Dietary calcium deficiency per se induces secondary hyperparathyroidism, which leads to rickets. Such may develop in newborns of mothers whose calcium intake during the 3rd trimester of pregnancy was inadequate.[29]

Table 4.1. - A mechanistic classification of rickets
From: Tiosano D, Hochberg Z. Hypophosphatemia: the common denominator of all rickets. J Bone Mineral Metab 27:392-401, 2009

		25(OH)D	1,25(OH)D	Ca	Alk. Phos.	PTH	FGF23	Associated Clinical and biochemical characteristics.
PTH dependent	Vitamin D def/ Sunlight dep. / Nutritional	↓	↑	↓	↑	↑	N / ↓	
	25 OHase mutation/ Cyp2R1	↓	↓	↓	↑	↑	N / ↓	
	Vitamin D dependent /CYP27B1 mutations in 1alpha-OHase	N	↓	↓	↑	↑	N / ↓	
	VDR mutations	N	↑	↓	↑	↑	N / ↓	Alopecia
	Post Receptor mutations	N	↑	↓	↑	↑	N / ↓	Alopecia
	Dietary Calcium def.	N / ↓	↑	↓	↑	↑	N / ↓	
	Hyperparathyroidism	N	↑	↓	↑	↑	N / ↓	
FGF 23 dependent	XLH/ PHEX mutation. Increased FGF23 production.			N	↑	N / ↓	↑	
	ADHR/Decreased FGF23 degradation.			N	↑	N / ↓	↑	
	ARHR/ DMP1 mutation. Increased FGF23 production.			N	↑	N / ↓	↑	
	HRH/Klotho excess/Gain of function.			N	↑	↑	↑	
	TIO/*MEPE and sFRP4?* Fibrous dysplasia /*GNAS1*activating mutation. Increased FGF23 production			N	↑	N / ↓	↑	
	Epidermal nevus syndrome /FGFR3 End organ gain of function			N		N / ↓	↑	Epidermal nevus
Renal	HHRH/ SLC34A3			↑		↓	N /↓	Heterozygotes- Renal stones without rickets.
	Fanconi Syndrome			↑		↓	N / ↓	aminoaciduria, glucosuria, bicarbonaturia

In a prospective study conducted in the Middle East the majority of patients in Turkey who suffered from rickets had vitamin D deficiency, compared to those in Egypt who suffered from calcium insufficiency combined with vitamin D insufficiency. Strict vegetarian diet or on a diet high in phytate, which binds calcium, may be calcium deficient.[30, 31]

Primary hyperparathyroidism is very rare in the prepubertal age, among those with an active growth plate. There have been a number of reports of the association of hypophosphatemic rickets and primary hyperthyroidism.[32]

Rickets due to high FGF23

FGF23 functions principally as a phosphaturic factor.[33] It is secreted by osteocytes and osteoblasts in response to high serum phosphate levels and 1,25-(OH)2-D.[18,34] Impaired FGF23 synthesis or action, due to FGF23 gene mutations; mutations in GALNT 3, which is involved in FGF23 post-translation; or mutations in Klotho, which is required for the conversion of FGFR1(IIIc) into the FGF23 receptor, lead to severe hyprphosphatemia and tumoral calcinosis.[35-37]

During secretion, FGF23 is cleaved at the C-terminus between amino acids 179 and 180.

Mutations near this site in the RXXR furin-like cleavage domain of FGF23 (R176Q and R179W) impair proteolytic inactivation of FGF23, resulting in high FGF23 levels and leading to autosomal dominant hypophosphatemic rickets (ADHR).[38] Though an initial study suggested that Phex processes FGF23[39], subsequent studies have failed to establish Phex-dependent cleavage of FGF23. The role of FGF23 in the kidney and its role as a phosphaturic hormone have been reviewed repeatedly.[17]

The hallmark of all clinical entities that share high FGF23 activity is rickets/osteomalacia with hypophosphatemia due to renal phosphate wasting and inappropriately low 1,25-(OH)2-D levels. Causes for high FGF23 include overproduction by osteocytes, bone tumor or bone fibrous dysplasia, degradation defect of FGF23 through increased Klotho production, and end organ gain of function.

Increased FGF23 production by osteocytes

X-linked hypophosphatemic rickets (XLHR) is a dominant disorder characterized by impaired phosphate uptake in the kidney.

XLHR is caused by inactivating mutations in PHEX that lead to increased circulating FGF23 levels.[40]

Autosomal recessive hypophosphatemia rickets (ARHR) is caused by inactivating mutations in *DMP1*, a member of the small integrin-binding ligand N-linked glycoprotein family of extracellular matrix proteins that augment mineralization.

Loss of function of DMP1 results in increased transcription of FGF23 by osteocytes.[41]

Decreased FGF23 degradation

Autosomal dominant hypophosphatemic rickets (ADHR), caused by mutations (R176Q and R179W) in the RXXR furin-like cleavage domain of FGF23, impairs proteolytic inactivation of FGF23.[38]

Increased FGF23 production by tumors and fibrous lesions

Polyostotic fibrous dysplasia (PFD), also called McCune-Albright syndrome, is caused by an activating mutation in the guanine nucleotide binding protein, alpha stimulating gene (*GNAS1*), and results in fibrodysplastic tissue. In some patients hypophosphatemia results from elevated circulating FGF23 levels [42].

Tumor-induced osteomalacia (TIO), or oncogenic osteomalacia, is a paraneoplastic syndrome of renal phosphate wasting, aberrant vitamin D metabolism, and osteomalacia that is associated with elevated FGF23 levels[43]. Both, PFD and (TIO) disorders are associated with increased levels of MEPE and sFRP4, which regulate PHEX and DMP1 metabolism.[44]

Increased FGF23 due to increased KLOTHO production

Hypophosphatemic rickets and hyperparathyroidism

The association of hyperparathyroidism and rickets has been reported, but remains controversial. In the reported cases some patients had parathyroid gland adenoma while others had hyperplasia[32]. Brownstein et al investigated a patient with hypophosphatemic rickets and hyperparathyroidism due to parathyroid hyperplasia as well as to other skeletal abnormalities. The disease was due to a de novo translocation with a breakpoint adjacent to alpha-Klotho, which encodes a beta-glucuronidase. Plasma alpha-Klotho levels, beta-glucuronidase activity, and circulating FGF23 levels were markedly elevated[45] .

FGFR1, FGFR2, and FGFR3 mutations

In vitro studies indicate that the N-terminal region of FGF23 binds to and activates FGFR1,-3, and -4. In cases where mutations occur in these receptors, FGF23 is elevated and may lead to rickets.

Linear sebaceous or epidermal nevus syndrome (ENS)

There have been a number of reports of the association of hypophosphatemic rickets and epidermal nevus caused by a mosaicism of activating FGFR3 mutations in the human epidermis. Some of these patients presented with ipsilateral focal bone disease associated with hypophosphatemic rickets, elevated circulating FGF23 levels, and aberrant 1,25-(OH)2-D, levels, similar to other syndromes caused by elevated FGF23.

Osteoglophonic dysplasia (OD) is a rare disorder with a skeletal phenotype associated with FGFR1, FGFR2, and FGFR3 mutations that may regulate FGF23 expression in bone or the renal handling of phosphate.[46] Patients with OD present with

Renal diseases affecting phosphate transporters

Mutations in NaPi-IIc (SLC34A3)

Hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is caused by inactivating mutations in the SLC34A3 gene encoding NaPi-IIc.[47] This rare autosomal recessive disorder was first described in a large consanguineous Bedouin kindred.[48]

Hypophosphatemia is secondary to renal phosphate wasting. The disease has varying degrees of expression.

In the *heterozygote state*, there is a slight loss of Pi that leads to a low Pi level in the kidney, enhanced1α–hydroxylase (CYP27B1) expression, and increased intestinal calcium absorption with hypercalciuria.

In the *homozygote state*, or in compound heterozygotes, the urinary loss of phosphorus is more significant, resulting in low serum phosphorus and severe rickets.

Fanconi syndrome

Fanconi syndrome is characterized by generalized proximal tubular dysfunction that is manifested by phosphaturia, generalized aminoaciduria, glucosuria and bicarbonaturia. Genetic defects or toxins may be the cause. Cystinosis is the most common genetic cause for Fanconi syndrome in childhood. Radiologic evaluation often reveals florid rickets. The pathophysiologic mechanism(s) involved in Fanconi syndrome is not completely understood. However, in some diseases such as Dent's disease and Lowe oculocerebrorenal syndrome the mechanism for phosphaturia has been explored.

Dent's disease is an X-linked renal tubular disorder, a form of Fanconi syndrome that is characterized by proteinuria, hypercalciuria, nephrocalcinosis, kidney stones and renal failure.

The disease is caused by mutations in the chloride channel 5 (CLCN5) gene. Phosphaturia results from defective endocytosis and redistribution of NaPi IIa (SLC34A1) and NaPi IIc (SLC34A3) from the plasma membrane to intracellular vesicles, and is associated with low–molecular weight proteinuria and hypercalciuria.[49]

Lowe oculocerebrorenal syndrome is caused by mutations in the gene *OCRL1*, which encodes a phosphatidylinositol 4,5-bisphosphate 5-phosphatase that is also involved in endocytosis. Other features of LOS include elevated lactate dehydrogenase and creatine kinase levels.[50]

Conclusions

The common denominator of all rickets is hypophosphatemia. Hypophosphatemia prevents apoptosis in the hypertrophic cells in the growth plate. In the absence of apoptosis the hypertrophic cells

accumulate in the growth plate and form the rachitic bone. It follows that diagnosis of rickets should be based on the etiologies of hypophosphatemia.

The three major entities that can lead to hypophosphatemia are high PTH activity, high FGF-23 activity, and renal defects that lead to Pi wasting. Hallmarks of high PTH activity are: hypophosphatemia, phosphaturia, disturbance in vitamin D metabolism, and low calcium. Hallmarks for high FGF-23 activity are hypophosphatemia and phosphaturia with inappropriately low 1,25-(OH)2-D.

Hallmarks for renal rickets are hypophosphatemia and phosphaturia with high 1,25-(OH)2-D that causes hypercalcuria.

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BONE AGE AND SKELETAL MATURITY

Ze'ev Hochberg

Aims

This chapter reviews the anatomical and histological maturation of long bones and cuboid bones as they are represented in the hand and wrist radiogram.

It summarizes briefly the endocrine regulation of these maturational processes and attempt to uncover endocrine function and malfunctions, as they unfold in the radiogram.

It further reiterates the presently used methods of skeletal maturity assessment and raises some doubts about the entire paradigm and its uncertainties. It suggests that beyond the reading of a "bone age", the hand and wrist films may be used in understanding skeletal maturity in a more meaningful way**.**

Introduction

In the dialectics of human anthropology and auxology "bone age" is felt to be an expression of the biological maturity of a child. Inferring from bone maturity, a clinician would contemplate diagnostic considerations and evaluate height prediction, but also recommend sport activity and dancing or the timing for orthodontic procedures and orthopedic surgery. Moreover, pediatricians and endocrinologists feel that the assessment of bone maturity by means of a hand and wrist radiogram reflects on the child's biological age. This is accomplished in a variety of methods but all of them compare a given film to various standards, averaging or summarizing the maturity of several bones, followed by designation of a "bone age".

Whereas the aim is noble, the means are naive.

A radiogram of the hand and wrist can at best reflect the maturity of the bones that are depicted on that film. Even in terms of bone maturation, the recognition of bones' shapes and changes of configuration provide relative little insight into maturational processes, and more so as one utilizes the method of averaging or summarizing several bones to give a unified reading for the entire film.

More importantly, the hand and wrist film may provide information that is not being evaluated by reading of a "bone age". Although the radiogram reveals only calcified elements, it provides a glimpse at a whole variety of processes in bone and cartilage growth, differentiation and calcification which, in turn, are regulated by control mechanisms. Thyroid hormones, sex steroids, calcium regulating hormones and growth hormone (GH) are but a few of these control mechanisms, and their effect may become evident when hand and wrist radiograms are carefully examined. Comprehension of these mechanisms may infer on an underlying endocrine action or derangement and lead to a more meaningful reading of the radiogram.

This chapter reviews the anatomical and histological maturation of long bones and cuboid bones as they are represented in the hand and wrist radiogram. It summarizes briefly the endocrine regulation of these maturational processes and attempt to uncover endocrine function and malfunctions, as they unfold in the radiogram.

It further reiterates the presently used methods of skeletal maturity assessment and raise some doubts about the entire paradigm and its uncertainties. It suggests that beyond the reading of a "bone age", the hand and wrist films may be used in understanding skeletal maturity in a more meaningful way (*figure 5.1.*). *The 'bone maturity' radiogram (A) provides a glimpse at the mechanism of bone and cartilage growth, differentiation, ossification and calcification (B).*

Following early reflections and the pioneering work of Greulich and Pyle[1], other methods have been developed for the assessment of skeletal maturity. They all average or summarize several bones of the hand and wrist, to define a "bone age". The very act of averaging entails a loss in distinction of individual segments. The following pages will attempt to show the importance of individual segments in evaluating the mechanisms and pathophysiology of bone growth and maturation.

The terminology of 'bone age' and the assignment of "years" as its units are insensible. Age, expressed in year units, is a precise measures with no variance and in our age and aging – conscious civilization it entails the meaning of a calendar age.

On the other hand, 'bone age' is neither; its reading is imprecise, children at a certain chronological age have often a wide variation in their skeletal maturity and children with different calendar age have similar 'bone ages'.

This chapter will reason against that abuse of terms. The units of a skeletal maturity have accordingly been 'years' in inverted commas. This

term often misleads children and parents who perceive a retarded or an advanced "bone age" as a physical impairment. It also misleads the medical professionals to apprehend it as a mean to evaluate the child's biological age. The entry in Medline does not even read "bone age" and is erroneously labeled "*Age determination by skeleton*". This misconception rises to its peak when a court reassigns a child's calendar age according to a "bone age" film. Would it not make more sense to use for that purpose the child's mental age or his psychomotor or developmental age? I underscore this misconception and suggest that we omit the "age" in this term and speak of skeletal maturity or skeletal development and that skeletal maturity be assigned units of "**maturity index"** rather then "years". We can still use the available figures from the standards of references. I recommend saying that "*This child, at the age of 10 years, has a skeletal maturity index of 9*".

The progression (tempo) of skeletal maturity correlates weakly with puberty, with chronological age or with growth velocity. These poor correlations reach their extreme in cases of complete dissociation of skeletal maturity and growth, which would ultimately bring about the **eunuch** habitus.

The tempo of bone maturity does correlate better, however, with the degree of ponderosity. This implies that the unique metabolic and endocrine milieu of obesity regulates this independent index, and as such it needs to be viewed as a measure of this milieu.

It is noteworthy that in the insightful earliest attempt to develop skeletal maturity standards of reference, Todd called as early as 1937 an Atlas of Skeletal Maturation[2], emphasizing the maturation process and not the age equivalence.

So many years ago, he sensibly made the comment that "... differences in age equivalents are not the result of *variations of the normal* but are sequel to sub-clinical modifications of time relationships of the several successive features of the maturation process".

The methods developed by **Tanner and Whitehouse** start similarly with maturation scores, but then, alter direction into the "bone age" paradigm[3].

Bone maturation and growth

Primary and secondary ossification centers

In the young embryo the skeleton develops out of the primordial hyaline cartilage.

The cartoon in *figure 5.1.* shows the succession of events from a hyaline cartilage, as it transforms into a primary-then a secondary ossification centers. Nutrient vessels deliver pluripotent stem cells, which give rise to the bone marrow, as a source of building blocks for the ossification centers. Only after the primary- and secondary ossification

centers grow concentrically and verge upon each other, a growth plate ensues to become the site of enchondral ossification.

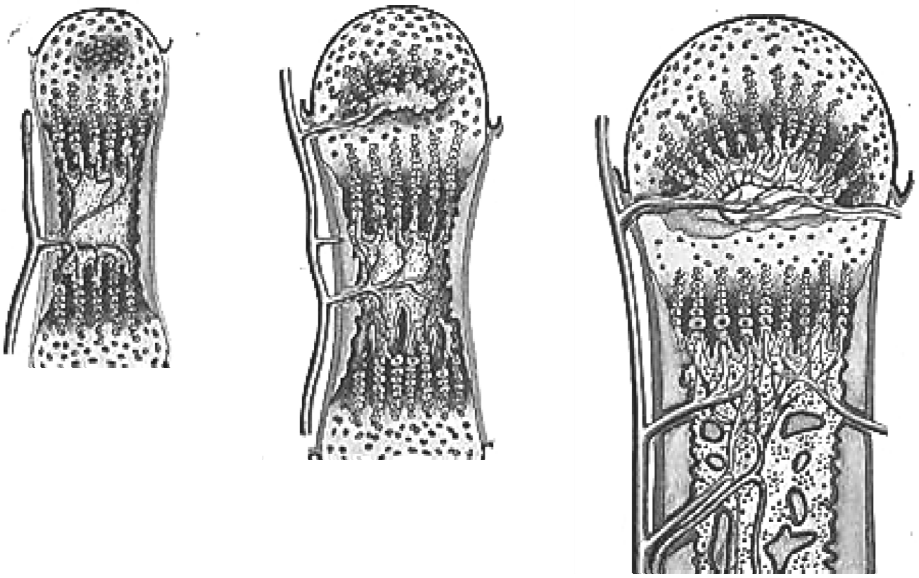

Figure 1. – Bone develops from its mesenchymal anlage.

In the case of long bones, the central part of the shaft (diaphysis) is the embryonic site of the primary ossification center (*figure 5.2.*). Later in the course of maturation, secondary ossification centers would develop at either ends of the bone (epiphysis). As primary and secondary ossification centers approach each other, a growth plate ensues.

In the case of cuboid bones, such as carpal bones of the hand or vertebrae, primary ossification occurs later in infancy or childhood in the bone center. As hyaline cartilage transforms to become a nodule of growth cartilage, groups of young chondroblasts proliferate several times before the cell cycle rests. These cells then hypertrophy at the expense of their matrix in the center of the cartilage nodule. Hypertrophic cells generate a proteoglycan-rich matrix that allows on the one hand for calcium deposits to surround the cells and on the other hand, for blood vessels and later on for bone marrow to penetrate the matrix, mobilizing osteoprogenitor cells to invade the site of hypertrophy and calcification. Now the stage is ripe in the calcified cartilage for a programmed death of the hypertrophic cartilage cells and an invasion of the remaining lacunae by bone resorbing and bone forming cells. Bone is deposited on residues of calcified cartilage.

Secondary ossification centers develop at one or both epiphyseal ends. The process is similar to that of the primary ossification center. As two centers of a long bone expand and approach each other, a growth plate

evolves between them. Chondrocytes at either ends rearrange in cartilaginous nodules proliferate and the same sequence of events of proliferation, central hypertrophy, calcification and degeneration, that established the first primary ossification center, is repeated.

Long bones of the hand represent for the reader the long bones of the legs, which contribute more than 50% of height. Cuboid bones of the hand represent the cuboid bones of the vertebrae.

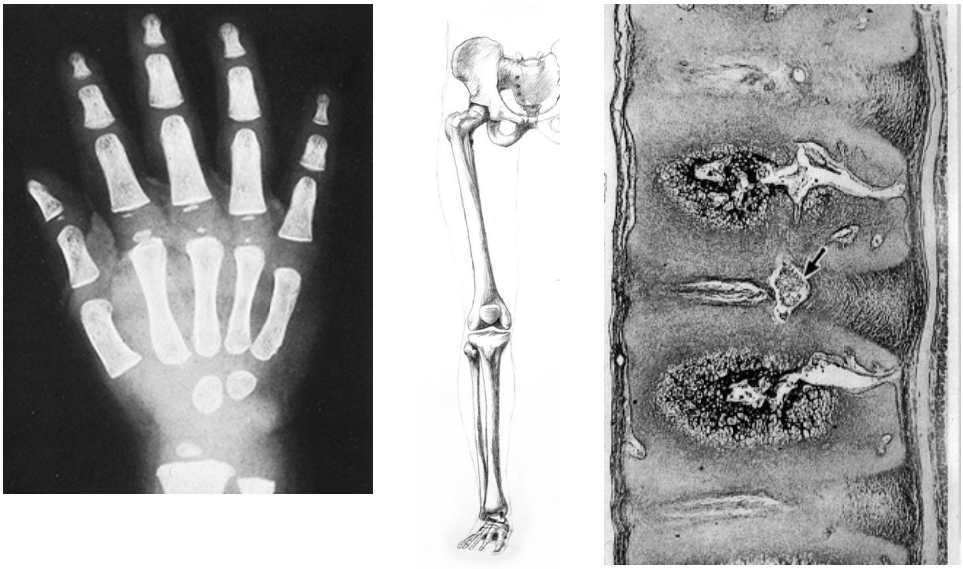

Figure 5.2. - Long and Cuboid bones.

In the short bones of the hand, metacarpals and phalanges, two nodules of chondro-epiphyses initially form, but only one end forms a secondary ossification center and only this one end would contribute to longitudinal growth of the bone. At the other end, cartilage would progressively be replaced by bone continuously from the primary ossification center and retains only a thin articular cartilage.

Fusion of primary and secondary ossification centers

The Fusion of an epiphysis with a metaphysis occurs in most long bones during the last year of puberty and implies the cessation of the bone's growth. Fusion occurs at different ages for different bones. Fusion starts with fine bridges between the now mature primary and secondary ossification centers.

Histologically, it is characterized by extension of osteogenesis towards the reserve zone and the gradual elimination of initially the proliferative- and later the hypertrophic zone. Matrix calcification proceeds from metaphysis to epiphysis, to form osseous bridges that eventually would replace cartilage cell columns. Fusion progresses from the marginal

regions centrally, and the cartilage tube would gradually shrink in size until it disappears. Growth of a given bone culminates with the initial bridging, and it may take a whole year before the entire cartilage tube ossifies and calcifies.

The cuboid bones (carpals and vertebrae)

Whereas primary ossification centers of long bones are well ossified at birth, the carpal bones of the hands portray a unique opportunity to observe primary ossification centers in their make. Conceptually, understanding and evaluating carpal bones maturation reflect on vertebral maturation.

Cuboid bones develop through a pure process of osteogenesis. Centripetal ossification of the hyaline cartilage occurs through the osteogenetic phases of cartilage hypertrophy and calcium deposition, followed by resorption and formation of bone. Osteoclasts, osteoblasts and growth factors are initially supplied by nutrient vessels, then by the later developed bone marrow.

Implications for skeletal maturity readings

In all the methods that are utilized for clinical appraisal of skeletal maturity, the user is expected to read each bone separately. This would enable a meaningful evaluation of the underlying mechanisms. Yet, as soon as individual bones are read, the user is then expected to average the scores in some of methods or sum them up in others. As the TW2 method for evaluation of bone maturity was developed, Tanner et al realized that the "long-" and "short bone" readings (RUS, for Radius Ulna and Short bones) are often in a disparity with the reading of the carpal bones[4]. They indicated that the mean difference between carpals and RUS indices in normal girls is 0 over the range of maturity index 2-11, with a SD of 0.7-1.0. But even within the RUS bones, maturity of the short bones of the metacarpals and phalanges often differ from maturity of the radius and ulna. The reason for those differences lay with the two processes of chondroplasia and osteogenesis that reveal themselves in the hand and wrist film. The appearance in an X-ray film of primary ossification centers of the cuboid bones and of secondary ossification centers in the epiphyses of long- and of short bones during early childhood reflect the maturational process of osteogenesis.

The appearance of the secondary ossification centers of long- and short bones during later childhood and adolescence reflects a combination of osteogenesis and chondroplasia[6]. For an insight into the mechanisms behind the film, the minimal effort required is to group the individually read bones to RU - radius and ulna, S - short bones and C - the cuboid carpal bones. This simple tool allows for some insight of possible aberrance of the basic processes of osteogenesis and chondroplasia.

The maturity scores of carpal bones resemble often the RU score and the RUC score is more homogeneous then the RUS score. Understanding

the control of each of these two processes allow for inferences on underlying pathology from the X-ray film.

Mostly, the clinician is not interested in the maturation of bones of the hand and wrist. Bone maturity films are expected to reflect on those distant bones that contribute to the child growth. It is commonly felt that maturity of cuboid carpal bones may stand for maturity of the cuboid vertebrae and that maturity of short bones of the hand may stand for maturity of the femur and tibia. Yet, these logical assumptions still require a scientific confirmation. The advantage of the hand – wrist area is that a single film covers 30 different ossification centers, which represent the various growth patterns.

The radiographic perception

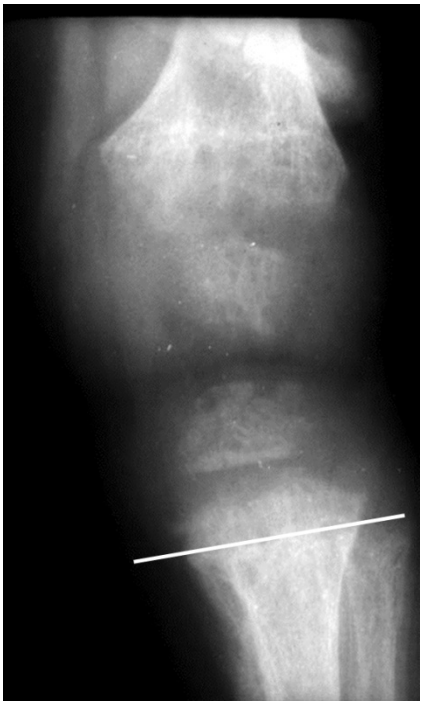

Figure 5.3. – The demarcation line. (This film of a four-year old girl with hereditary vitamin D-resistant rickets due to a mutated vitamin D receptor demonstrates the demarcation between the calcified matrix and the bone (while line).

Unless a special action is taken, the x-ray film shows mostly calcified tissues. These include in the normal hand and wrist the bone, but also the calcified cartilage.

The demarcation line

Whereas hyaline cartilage of the bone anlage and the proliferating zone are translucent to X-rays, calcified matrix and bones are visible (*Figure 5.3.*). Thus, the boundaries of a tissue that would be visible on a radiogram of a growth plate are calcified bone trabecula at the epiphyseal end but also the calcified matrix of the hypertrophic chondrocytes at the metaphyseal end.

Such a view through the growth plate provides a unique opportunity to assess conditions of disturbed calcification. *Figure 3* shows the typical X-ray of a child with rickets. The thick radio-opaque layer of calcified cartilage is obvious. It reflects on increased matrix calcification by rachitic hypertrophic chondrocytes. The ends of the metaphyses are splayed because of an increase in the appositional growth at the marginal regions. These changes occur in response to retarded or absent enchondral bone growth.

Thus, the demarcation line of a radiogram separates chondroplasia, with its non-calcified cartilage from osteogenesis, with its calcified cartilage and resorption front.

Normal variations

The density of phalangeal epiphyses may vary among bones of the same film. Hyperdensity of a single epiphysis indicates local sclerosis, mostly with no pathological significance, or may result from current or a history of local arthritis or chondritis.

Some cuboid bones may show two ossification centers that would ultimately fuse. Adjacent carpal bones may fuse into a single atypical larger bone. Carpal bones may show unusual disfiguring bony processes or irregular mineralization. All these are more common in girls then they are in boys[1].

Maturational sequence

Bones mature in an orderly fashion. The sequence of bone ossification and calcification allows for unique assignment of an index to the radiographic appearance of each bone. A deviation from these orderly events can be used clinically to identify retrospectively an insult to bone maturity. A severe systemic disease, malnutrition or an endocrine malfunction may prevent the ossification of a primary or a secondary ossification center. Thus, the age at onset of malnutrition or maternal deprivation can be read after years of recovery from missing ossification centers that should have appeared on that age.

Assessment of bone maturation

From the very beginning of this discipline, human anthropologists have appreciated the importance of longitudinal follow up of skeletal maturity. The earliest attempt to develop a standard of reference was reported in 1937, by Todd's Atlas of Skeletal Maturation[3]. Dental age has been advocated, mostly in Scandinavia, and the methodology is well developed, but the variation is so great that it provides little help in clinical practice.

Greulich and Pyle atlas method

Developed from films taken in the 1930s and 40s, this method is probably still the most frequently used[1].

The atlas calls for a comprehensive analysis of bones of the hand and wrist, compared to a standard set from the median of a range evaluated for each chronological age. The technique of maturity reading varies among raters. Greulich and Pyle themselves suggested that raters should be adapted to individual needs or preferences.

Although instructions are given for individual assessment of each bone and epiphysis, the user tends to be influenced by the rest of the hand. Despite the clear instructions, it has been implied that the original concept of the atlas method was that skeletal maturation is even.

The series used in the original atlas is till in use today and it is obvious to experienced raters that they do not represent an ideal set of radiographs and that in many of the original radiographs various bone maturity were uneven. Even though, a recent reevaluation of each individual bone in a cohort of normal individuals, showed the reliability of the system to be accurate with deviations not greater than six months.

TW2 individual bones method[3]

Scoring bone by bone, this system is potentially superior to the atlas method. The longitudinal nature of the series of 113 radiograph and the scoring system accommodates for the fact that the data were collected over a period of 20 years as of the 1950's. Moreover, the score of individual bones allows for a more meaningful statistical analysis in search of the biological meaning of each bone's maturation. Yet, as soon as each bone is assessed, the assessor is asked to desert all this useful information from individual bones and to give a combined score that gives more weight to the long bones, the radius and the ulna, then to the short bones.

The scoring system even accounts for sex differences and eliminates thereby the very clear and unique effects of sex steroids. Then the user is to abandon the rational of the scoring system to give a unified "bone age".

The excessive weight given to the long bones entails a potential for apparent discontinuous maturation. Thus, the progression of the radius and ulna scores from stage F to G, with no intermediate stage, increases the maturation index from 11 to 12.

The TW2 method intermingled measures of bone maturity with size. The artificial attempt to grade each bone into 8 or 9 maturity stages gave extra weight to minor insignificant changes. Poor positioning of the hand when the radiograph is taken alters the radiographic appearance of the epiphyses and makes interpretation difficult and inconsistent.

The use of computed image analysis of bone maturation might have increased the precision and reproducibility of reading. In its newest version[5] it allows for interpolation between stages, to produce a continuous score, that is, however, still an average of several bones, losing their singular identity.

Adult height prediction

Skeletal maturity index correlates in normal individuals closely with the remaining growth potential, and as such has been used to predict the fusion of growth plates.

This tool has been used in predicting adult height in normal children, in short or tall children, but also in patients with growth disorders.

It is essential to perceive that delayed bone maturity does not necessarily reflect increased growth potential, but can rather resonate a chronic disease with compromised potential. Thus, children with hypothyroidism or growth hormone deficiency have delayed bone

maturation but they do not have a better potential for later growth. On the other hand, patients with aromatase deficiency or defective estrogen receptors keep growing despite their retarded bone age.

In studies on the accuracy of predictions the mean values were acceptable at ± 1 cm, but the distribution of predictions is as wide and unacceptable as ± 6 cm (95% confidence limits). The reasons lay with the precision of assessments and with the unified "bone age", but also with our limited knowledge of the bones that need to be emphasized.

We do not know which of the long or the short bone maturity represent best that of the legs' bones.

Skeletal maturity as an argument in functions

The terminology of "*bone age*" and its assignment of "*years*" as its units are problematic.

Age, and its units years, are precise measures with no variance, and make an ideal argument in a function.

The use of bone age "years" (or index) as an argument in a function, such as in growth charts, brings in a great deal of imprecision. It has become a common practice to plot auxological data or even laboratory results against bone age "years". This is entirely unjustified. First of all, no normative data were ever collected against an argument of skeletal maturation. The assumption that a bone maturity index can replace the chronological age on the X-axis has not been tested in a scientific way. In fact, in many clinical conditions it does not, as skeletal maturation lags many months behind abrupt endocrine changes, such as onset of normal or abnormal puberty or the outset of a disease. The "age" in "bone age" insinuates a concept of precision and unity, which it does not provide. This is due to individual variability, imprecise quasi-quantification and the pattern of individual bones maturation. As mentioned above, both inter- and intra-observers variability may be extremely high. This is created by the long experience it takes to master the art of hand and wrist radiology, but also by the disposition towards various bones in the film and the decision to use average, sum scores or specific bones only, such as the RUS method.

Deviations of the skeletal maturity of children from their chronological ages are as common as deviations of any biological parameters (height, serum thyroxine, etc) from their mean values.

The distribution is narrow in infancy, increase gradually during childhood to maximal deviation at near-puberty.

In the study of growth we have accepted the practice of expressing stature as "*standard deviation score*" (SDS). This has freed us from the misconception of "height age" or "weight age", entailing an optimal growth at the 50th percentile. It would be sensible to use SDS values in expressing skeletal maturity; yet, such normative values are yet to be developed.

PRACTICAL GUIDELINES

There is more than one way to read bone maturity films. The following are personal steps I take in reading them.

I use the Greulich and Pyle Atlas.

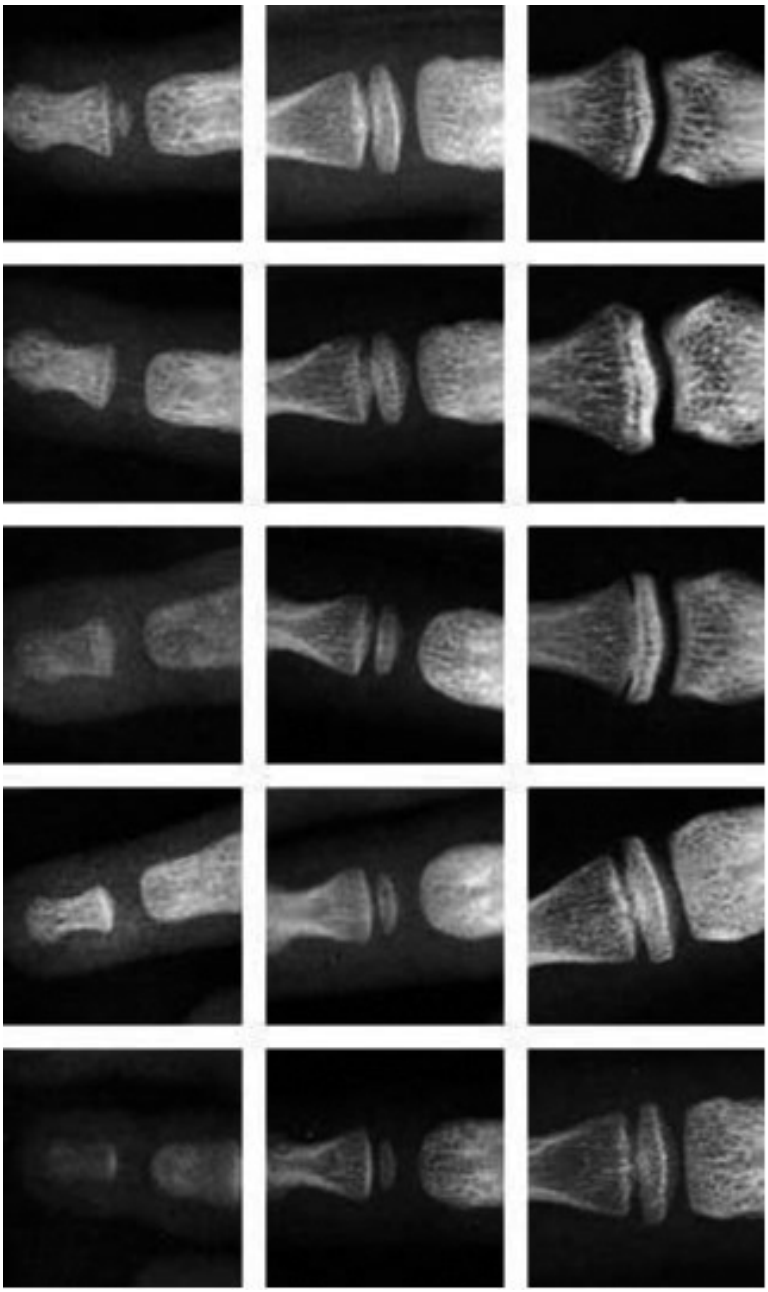

Figure 5.4. - Maturation of the ring finger. (images from the Greulich and Pyle atlas[1])

To refrain from a bias by the child's age, I do not look at the plates' title up to the very end. I start by a rough estimate of the film.

In young children I use for that purpose primary and secondary ossification centers.

In late adolescence, I use epiphyseal fusion and in between I use the maturity indicators of the Atlas.

I read bone groups following a regular order. I read long bones (radius and ulna) first, then the carpal bones and finally the short bones (metacarpals and phalanges).

Each bone group gets its own maturity index. Missing ossification centers are labeled *"Less than …"* It is important to realize that unified maturity is uncommon. Do not average the scores as you lose the perception of bone biology in the clinical setting. Each bone group is then compared to next and previous plates. If you feel experienced, you can give intermediate scores. I then try to identify the outlined most retarded bones. I scrutinize for the ossification age of the respective bones and discuss with patient and family possible insults during that age.

The film is also viewed as diagnostic radiography. Look for disease-specific pathology, growth arrest lines, irregular epiphyses, etc. The film is compared to previous bone maturity films of the same subject. The change (Δ) in bone maturity is related to the Δ chronological age for each of the bone groups ($\frac{\Delta \text{ maturity score}}{\Delta \text{ chronological age}}$). This ratio is then related to the growth velocity.

Now that all this information is available, one can contemplate bone biology and mechanism of disease.

Conclusions

The principal message of this text is that the analysis of a bone maturation film provides the clinician with a lot more than only a "bone age" reading.

It provides a unique opportunity to glimpse into the biology of bone, to contemplate mechanisms of growth and to consider the hormones and growth factors that contribute to bone growth and development.

As such, these x-ray films are a powerful research tools in the hand of the clinician. Comparing the hand radiogram in a given diagnosis to the known histology, human or experimental pathology of bones in that same condition renders a strong relationship between x-ray and mechanism. This can then be used to analyze unknown clinical conditions. To take advantage of the entire information provided by a hand and wrist film, one must keep in mind that different bones undergo different developmental processes. In the simplest way, long bones grow differently than do cuboid or flat bones. Epiphyses grow in the bone's longitudinal axis (in both directions) but also sideways. Developmentally, each axis has often its unique growth factors and critical time periods.

The center of a growth plate has a different growth mechanism than does the marginal region of that same plate. Finally, miscellaneous bones

have different mechanisms. In the most elemental way, the radius and the metacarpals, being both long bones progress often in a different tempo. Because they so do, I call the *metacarpals* and *phalanges "short bones*". The message is to read each bone separately, to document the development of each bone separately and to not average those readings.

Even if the maturity index designation takes time to be adopted, the labeling of bone maturity units as "years" has to remain within apostrophes. Whereas the calendar unit year is a precise measure bone maturity reading is subjective. The use of "years" units often deludes patients to apprehend bone age "years" as a mean to evaluate the child's biological age. I prefer the use of the same measures as index units.

Whereas this monograph is meant to broaden the utilization of the hand and wrist X-ray films, the bone biologist in the clinical setting has other bed-side tools. Body proportions give important information on the growth of unique body parts. Basic research enhances our understanding of the molecular and structural mechanisms which pertain to bone growth and maturation. The clinical aspects of these developments are in the hands of the bone biologist in the clinical setting.

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THERAPEUTIC NUTRITION IN THE CASE OF TYPE 1 DIABETES IN CHILDREN

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Introduction

The term nutrition derives from the Latin "*nutritio*" and refers to all the processes by which the body assures the nutrient intake to obtain sufficient energy for the metabolic processes in both health and disease.

In case of children this notion becomes particularly important as we are talking about ensuring the intake of energy and nutrients necessary for normal growth and development of the body as individual genetic "pattern".

Based on these records, in the therapeutic strategy of diabetes mellitus (T1DM) in children, nutrition has gained importance that is equal with the substitution of insulin, diet being an essential part of care for type 1 diabetes.

Even before the discovery of insulin, diabetic children were kept alive by a severely restricted diet concerning the consumption of carbohydrates and energy. These measures had led to unbalanced diets for years, with the emergence of nutritional complications, complications that now are history since the introduction of new types of insulin regimens and hence therapeutic schemes trying to achieve insulin substitution similar to the physiological insulin secretion.

In children with T1DM, the body found in continuous growth and development, the achievement of "nutritional therapy" raise a number of issues for both the physician and the medical team (required to identify the specifics of the initial nutrition), and especially for the child and his family having to apply rules of "therapeutic nutrition" imposed by the new statute, namely the one of "diabetic". Under these conditions the physician must know and have practical experience in the nutrition of the healthy

child, to understand the eating habits of the family, to remove diet mistakes, instil in the child and his family respect for the nutritional recommendations in conformity with the insulin therapy. Since the onset of diabetes, the child and his family should be informed, educated and trained in order to understand nutrition objectives in both the short (making up the menus, meal planning, etc.) and long-term.

The current management of therapeutic nutrition in diabetes in children focuses on a healthy, balanced diet with the correct intake of carbohydrates, protein and fibre and low-fat just like for healthy children.[1,2]

Objectives

If insulin substitution could imitate the insulin secretion to the pancreas, the diabetic child should not worry about his diet more than a healthy child. Due to this inconvenient, the participants to the T1DM in children (medical team, on one hand, and his family, on the other hand) must follow the achievement of the therapeutic objectives. Diet objectives in T1DM aim to achieve the general objectives of the T1DM treatment, while it also takes into account the achievement of nutritional "targets" of the children without diabetes.[3,4,5,6] These objectives aim at:

- Obtaining normal and durative glycemic level by reducing postprandial hyperglycaemia as much as possible, avoiding the "accidental" hyperglycaemias connected to additional food ingestion between meals, preventing the appearance of excessive hypoglycaemias immediately after or later after the meal;[7,8,9,10]
- Assuring the proper energy intake in order to obtain normal growth and development, as well as physical activity similar to that of the children of the same age and without diabetes;
- Satisfying the appetite without exceeding the normal limits,
- Ensuring social and professional integration;
- Creating a feeling of greater degree of independence.

If the latter objectives are easier to achieve, in fact, the first one represents the "key" of therapeutic nutrition in DM and the most efficient means of prevention of the degenerative chronic complications.

Nutrition principles of the children with T1DM

As stated by the American Diabetes Association (ADA), the principles of therapeutic nutrition in T1DM, as currently and unanimously accepted are the following:[4,5 6,11,12,13]

a. *The nutrition of the child with DM must be as similar as possible to the one of the children without diabetes* in order to ensure a stature-weight growth and a normal sexual maturation.

The menus shall take into account the permitted caloric value, the food restrictions and permissions, the culinary preferences of the child with diabetes (in the case where there are no nutritional mistakes), as well as the material possibilities of their families.[3]

From a theoretic point of view, the paediatric diabetologist shall take into account the Recommended Diet Ratio (RDR), which offers the standards of an efficient nutrition [14] for a healthy population regarding the energy intake, the carbohydrates, proteins, lipids, vitamins, minerals etc. Fortunately, the RDR is continuously changing, according to the newest research, so that to assure the quality and duration of life.

b. *the number and composition of meals* (quantity of carbohydrates for a meal at the same hour), must be respected on a daily basis, even by those who benefit from a therapy by continuous insulin infusion.[13]

The daily modification of the glucidic intake for the meal at the same hour is only accepted in diseases where the reduced digestive tolerance limits the intake (ingestion).

Otherwise, the variations of the ingestion from one day to the other lead to an improper adaptation of the insulin doses, that is, to making wrong therapeutic decisions.

c. *meal planning* in correlation with the practiced insulin-therapy scheme and the type of insulin injected, but with regard to the meal schedule and the snacks in order to prevent significant variations of the glycemia. (hypo- / hyperglycemia).

d. *individualization of diet means* preparing a diet for each child [3] taking into account: age, sex and physical activity, socio-economic level of the family, school timetable, associated pathologies etc. Once the meal schedule is established, it has to be observed 7 days a week.

A problem that has to be mentioned is the term of "free diet", given by situations, personal food preferences etc. Although its advantage is that it psychologically "comforts" the patient, its greatest disadvantage is that, as it is free of any criteria, not even the orienting ones, it encourages the patient's lack of discipline, so that he does not know how far his freedom extends. This is the reason why some of the followers of this diet (once supported by illustrious names) are subject to the most severe complications.

e. *satisfaction of the appetite and taste* of the child is achieved by gastro-technical variation. This principle must be analyzed with each meeting between the physician and the patient during the entire childhood, by monitoring the somatometric landmarks of the child (height, weight) and reference to age and gender nomograms.

f. *optimal proportion of the nutritive principles* (*Table 6.1.*), with a high content of food fibre and reserved attitude towards "sugar", which is occasionally permitted, under the form of sweets in low quantities, consumed at lunchtime. Limiting the lipid and protides intake in the Recommended Diet Ratio and avoiding salt excess.

The composition of the diet

Establishing the energetic needs

Once the T1DM has been diagnosed, the daily energetic need is established depending on the age and sex using the standards for non-diabetic children (*Tables 6.1. and 6.2.*) [15,16,17,18] or following the formula:

Kcal/day = 1000 + (A x 100) where A = years of age.[4]

Table 6.1.

Proportion of calories provided by the nutritive factors depending on age

Age (years old)	Energetic need (Kcal/day)	% caloric percentage resulted from each nutritive principle		
		Carbohydrates	Proteins	Lipids
1-3 years old	1 300	53-55	13-15	30-33
4-6 years old	1700	54-55	13-14	30-33
6-14 years old	2 400-2 700	55	13	32
15-18 years old (B)	2700	55-56	13	31-32
15-18 years old (G)	2200	55-56	13	31-32

Table 6.2.

Caloric need depending on age, sex, weight, height (*)

Category	Age (years old)	Weight	Height (cm)	Kcal/day
Infants	0 – 6 months	6 kg	60 cm	320
	7 – 12 months	9 kg	71 cm	500
Children	1 – 3 years old	13 kg	90 cm	740
	4 – 6 years old	20 kg	112 cm	950
	7 – 10 years old	28 kg	132 cm	1130
Boys	11 – 14 years old	45 kg	157 cm	1440
	15 – 18 years old	66 kg	176 cm	1760
Girls	11 – 14 years old	46 kg	157 cm	1310
	15 – 18 years old	55 kg	163 cm	1370

(*) adapted after Frary C.D., Johnson R.K. – Energy in: Mahan L.K., Escott-Stump S.,"Krause`s Food, Nutrition & Diet Therapy",10th Ed.,W.B. Saunders Co., 2004)

The energy need varies depending on sex (*Table 6.2.*), but only after puberty period.[4,18]

Establishing the caloric intake will not be rigid, instead, it will take into account the temperament of each child (some children are more sedentary and may become overweight, others are more active, they have

an increased energy consumption, so they need energy supplement according to the effort made).

Is it necessary to observe all diet recommendations or only some of them? There is only one correct answer: YES all of them! In support of this statement, the USA Department of Agriculture has developed the so-called "food guide pyramid" It has been introduced in the guidelines to support adults and elder children, to make them consume more fruits, vegetables and cereals.

Figure 6.1. – Food guide pyramid

New food guides include in the diet all types of food, the main principle being *balance and moderation.* They recommend the increase of vegetable, fruit and cereal intake, as they represent an important source of vitamins, minerals, carbohydrates, fibres, and they are generally low fat.[19]

The medical team aims at convincing both the parents and their children that therapeutic nutrition in T1DM is not a "dogma" but is flexible and can be changed depending on the momentary increase or decrease of the energetic needs, on the change of tastes, on the situations that might reduce appetite (undercurrent diseases). These changes (adjustments) shall be made only by the physician under the conditions of correct adaptation of the insulin doses. Trying to completely *"free"* the energetic intake depending on the appetite is risky and may lead to *"anarchy",* to the impossibility to correctly adapt insulin doses and, eventually, to the premature installation of the chronic nutritional complications (met in the case of the child with T1DM, as well as to the degenerative ones.

It is important to mention that timely obtaining and maintaining the ideal glycemic balance shall not be based on decreasing caloric and carbohydrate intake, but on correct division of the carbohydrates by meals and adapting the insulin doses to the different events (period of growth, infectious stress, hormonal stress - puberty, intense physical effort etc)

Carbohydrates

Carbohydrates are organic compounds that are structurally different from simple carbohydrates (which contain 3-7 carbon atoms) to complex polymers (polysaccharides).

Carbohydrates come from various types of food, where they can be found under different forms: polysaccharides (amidines, glycogen), disaccharides (sucrose, maltose, lactose) and monosaccharides (glucose, fructose, maltose). The latter ones represent the only form in which absorption takes place. Taking into account the recommendations regarding rational diet of the non diabetic child (*Table 6.1.*), and the results of the several research works performed in the case of diabetic adults, most authors claim that in the diet of the child with T1DM, carbohydrates must represent 50-55% of the daily caloric need, out of which 90% must be polysaccharides, and the remaining 10% monosaccharides.

Increasing the carbohydrates ratio up to 50-55% from the daily caloric intake initially led to controversies that made several groups of researchers to assess their effects on the glycemic values.

Initially, the different food with similar carbohydrate content was thought to give similar glycemic responses. Therefore, the American Associations for Dietetics and Diabetes presented for the first time in 1950 *"The Exchange Lists for Meal Planning".*[20] Although the initial simplistic idea, according to which the same ratio of carbohydrates from different types of food gives similar glycemic responses, was not agreed upon for a long period, the term of equivalence is not disrespected.

After the year 1970, solid reasons appeared to prove the inequality of the glycemic response caused by various types of food with similar carbohydrate content. This inequality of the glycemic response produced by different types of food has been studied by Jenkins et al. that launched the concept of *"glycemic index".*

The glycemic index (GI) proposed by Jenkins et al[21] is the ratio (expressed in percentage) between the area under the glycemic response curve of the food under test (S1) and the area under the glycemic response of the reference food (S2) to the same amount of carbohydrates over the course of 3 hours.

IG = S1 : S2 x 100 where:

S1 = surface of the glycemic curve 3 hours after the ingestion of 50 g carbohydrates in the food tested
S2 = surface of the glycemic curve 3 hours after the ingestion of 50 g glucose.

The reference food originally used was glucose, which was then replaced with bread (*Table 6.3.*).

Although the concept of glycemic index brought novelties in dietetics and classification of food by the glycemic index (*Table 6.3.*) can facilitate the application of this information in day to day nutrition,[22] the utility of its application is still questionable,[23] two unknowns still remaining under discussion[21]: the value of the glycemic index of the food when it is in the composition of mixed meals and the variation of the individual factor.

Besides individual factors, other factors may influence the amount of glycemic response: hydration state, particle size (amidines or other

polysaccharides), presentation of food,[22] the technique of cooking, food temperature, amount and type of associated dietary fibre, the presence phytates or natural enzyme inhibitors, fat and protein content from the mixed meal, the state of insulin treatment, the insulin therapy scheme used and, not least, the degree of mechanical food processing by chewing.

Table 6.3.

Glycemic index of some foods as compared to the white bread reference calculation (by Bantle)

FOOD GROUP	FOOD	GLYCEMIC INEX
BREAD	White bread	100
	Wholemeal bread	99
	Rye bread	58
CEREALS	white rice	83
	Spaghetti and other pasta	66
VEGETABLES	Dried potatoes	116
	New potatoes, boiled	81
	French fries	135
LEGUMINOUS	Beans	60
	Green pea	56
	Frozen peas	74
	Green lentils	43
FRUIT	Apples	53
	Bananas	79
	Grapefruit	36
	Grapes	62
	Oranges	66
	Pears	47
SUGARS	Glucose	138
	Sugar	86
	Fructose	30
DAIRY PRODUCTS	Milk écrémé	46
	Yogurt	52
	Ice cream	52

Years, sugar was not allowed in diet of the diabetic patients, because the simple sugars are rapidly absorbed and produce quick rise of glycemic sugar, while complex carbohydrates are absorbed more slowly and cause therefore slower increase of glycaemia. Small quantities of sugar consumed at the end of lunches, does not produce in the case of well-balanced diabetics, than the equivalent amounts of sugars from other foods.[4, 21, 22] In the diet of patients with type 1 diabetes small amounts of glucose can be consumed within a "healthy" diet,[22] if ingested during the meals or after them [24] without exceeding 30 grams of sugar per day.

We accept that sucrose should not comprise more than 10% of daily caloric intake.[25] Particular attention will be given to the consumption of beverages sweetened with sucrose because they often lead to weight gain[26] on the one hand, and on the other hand, these beverages containing sugar

are difficult to “cover” with insulin and can cause hyperglycemia. The current recommendation is to use glucose sweetened beverages only to prevent or treat hypoglycemia.

Some authors recommend increasing the intake of carbohydrates, up to 60% of energy requirements[27], but most children do not touch any necessary carbohydrates 50% of daily caloric calculated.

In one patient well-balanced proportion of carbohydrates should not be less than 45%, otherwise, it can lead to an increased intake of fat and protein.

In the case of children with T1DM, establishing the necessary carbohydrates in absolute value depending on the age group (e.g. 1-3 years old, 4-6 years old, 7-12 years old, etc., as shown in Table I for non-diabetic children) is a serious error. Using this method will rarely ensure an optimum daily percentage of 50-55% carbohydrates of the energy needs, the difference being compensated by excess lipids and/ or protein.

As currently practiced in our clinic, from the beginning, we recommend that the carbohydrate intake represent at least 50% of the daily caloric needs as calculated above, and in time we adapt (to increase or decrease) the caloric needs to the somatic indices (height, weight).

When we have the certainty that the correct dosage of insulin is adapted, we proceed to the next stage where the patients receiving a scheme of multiple injections or insulin pump are taught to calculate "how many grams of carbohydrates are metabolized by one unit of insulin for each meal” offering the alternative of liberalization of the carbohydrate quantities consumed in a meal with the condition of avoiding weight curve fluctuations. This allows children and young people with DM to adjust prandial insulin dose according the carbohydrate consumption.

Proteins

The human body continuously synthesizes proteins with structural and functional role as required. It is estimated that the daily amount of protein renewed is of 3%. Although there is this constant process of renewal (turn-over) in the human body, proteins are given less attention in the dietary recommendations in children with DM.

In the period of low carbohydrate consumption, proteins were classified as “free” foods and the children were encouraged to eat “all they can” which brought to overcoming the recommended protein intake at that time.[24]

Children with DM have a higher protein need as compared to the non-diabetic ones. In order to ensure normal growth and development, the child, taking into account the age, is recommended 0.9 to 1.7 g protein/ body kg / day intake, amount which represents 13-15% of the total daily energy. Today it is considered that a high protein diet speeds up the installation of diabetic nephropathy and the decrease of food proteins for the diabetics with incipient nephropathy decreases microalbuminuria.[22]

In conclusion, diabetics should avoid consuming proteins above average.[24]

Regardless of the age group, the optimal ratio of animal protein/ vegetable protein = 1, a ratio of 2: 1 being considered excessive. [22] Foods rich in protein, especially those of animal origin, have a high content of saturated fat; instead foods containing vegetable protein (ex. beans, vegetables) have the advantage of becoming associated with dietary fibre and complex carbohydrates favouring the installation of satiety. [24] On this scale some authors tempted even a strict vegetarian or semi-vegetarian (lacto-ovo-vegetarian) diet, considering that a lasting vegan diet may explain the decreased incidence of chronic complications.[28] These recommendations are not commonly used in children because there is a permanent balance between the processes of degradation and synthesis, without fluctuations, which maintains a relatively constant amount of protein in the body.

In conclusion *proteins must be weighed as well as carbohydrates.*

Lipids

Lipids are essential constituents of the body (they maintain the position of organs and nerves, protect bones from mechanical pressure, maintain constant body temperature).

There are three categories of lipids that are important for nutrition: simple lipids, compound lipids, sterols (cholesterol, bile salts) and fat-soluble vitamins.

Due to the hydrophobic character, lipids can only be transported into the aqueous environment of the plasma in the form of complexes with the proteins called lipoproteins.

Normally, insulin prevents lipid mobilization by two mechanisms: it dephosphorylates the hormonsensitive lipase, impairing lipolysis, and on the other hand, favours the reesterification of glycerol-3-phosphate with AG. Thus, it explains why the lack of insulin in DM promotes lipolysis and adipose tissue melting.[30] Thus, unlike the rest of the population, patients with T1DM are prone to early atherosclerosis. Abnormal plasma levels of the specific lipoproteins influence the development of atheromatous plaques and vascular lesions in T1DM.

An unsatisfactory glycemic control as a result either of insufficient insulin treatment or an excessive intake of saturated fatty acids and cholesterol in the diet worsens dyslipidemia. [22] Under these conditions, diet "manipulation" may influence the relationship between the diabetes mellitus – hyperlipidemia.[1] The diet established must be aimed at maintaining LDL, HDL, VLDL levels as normal as possible for the corresponding age and sex groups.[24] In order to achieve this, in the nutrition of the child with T1DM lipids should not exceed 30-33% of daily caloric intake, a ratio in which the saturated fatty acids (concentrated mainly in animal products) will represent 10% monounsaturated fatty acids (the most common being the oleic acid) = 12 to 14% of

polyunsaturated fatty acids (represented mainly by linoleic acid) = 6-8% and cholesterol of less than 100 mg/1000 calories,[6.22] without exceeding 250 mg/day. [31]

From the quality point of view the main measure is aimed at reducing saturated fatty acids.

According to current recommendations amount of saturated fatty acids must be reduced (in order to reduce total cholesterol and LDL), while the intake of monounsaturated and polyunsaturated fatty acids must be increased. This recommendation has epidemiological support.

The special attention should be given to food lipid sources. Lipids are found in products of animal origin, oils, margarine, milk, meat, sauces. Combinations like cakes, creams, mixed cereals, snacks have increased lipid content while fruits, vegetables and grains have low lipid content.

The association of hypercholesterolemia - cardiovascular risk has led to dietary restrictions on high-cholesterol foods. Although cholesterol is homeostatically controlled and each individual responds differently to ingested food cholesterol, the intake should be limited.

Table 6.4.

Lipids content of foods[32]

g. of lipids / 100 g product	Food
0 g lipids	Fruit, vegetables, jams, jellies, juices
1-3 g lipids	Popcorn, soup, bread, light cheese
4-6 g lipids	Milk, yogurt,
> 7 g lipids	Cheese, meat, chocolate, mayonnaise, sauces, cakes, creams.

Simple measures like: replacing butter with margarine, avoiding fried foods, using vegetable oils instead of animal ones, fish meat instead of fatty meat or ham as well as limiting the number of eggs consumed reduce cholesterol intake and hence LDLc serum levels, one of the predisposing factors for atherosclerotic diseases.[33]

Food fibres

In the early 1970s, many researchers struggled to use high fibre diets with and without high carbohydrate content. Fibre diet is used both in the prevention and in the treatment of chronic diseases such as diabetes, colon cancer, hypercholesterolemia, diverticulitis, etc. Fibre is a complex component in the diet, through the high content of organic components.[34]

James Anderson (cited by 21) in a study in which the amount of carbohydrates represented 55-70% of the daily caloric intake, prescribed a fibre content of 20-25 g/ 100 kcal. The results obtained (good compliance, reduced postprandial glycaemia, reduced insulin requirement and cholesterolaemia) confirm data from the literature showing that diets rich in fibre/ carbohydrates have a beneficial effect on glycaemia,

cholesterolaemia and LDL[13] compared to diets that were only supplemented with substances rich in fibre.

Regarding the fibre supplements, Arky[21] shows that they are not as well tolerated as high-fibre foods, causing: abdominal discomfort, bloating and nausea in most of the patients, many of whom are discouraged on the one hand by the large quantities of fibres needed to cause a decrease in glycaemia and cholesterol, and on the other hand of the bad taste of the meals.

The fibres are not subject to enzymatic digestion, they transit the stomach and the small intestine, forming a so-called "matrix" with the capacity to absorb water, iron and other nutrients.[35] Hydrosoluble fibres increase the viscosity of gastric and intestinal gastric contents, delay the evacuation of the gastric content and decrease the absorption rate of nutrients in the small intestine. In other words, due to increased hydrophily, they create a feeling of satiety, and on the other hand they form a protecting "tape" along the intestine that reduces the absorption of carbohydrates, lipids and cholesterol.[22]

The consumption of *soluble fibres* (oats, beans, vegetables, citrus, etc.) seems effective in modulating the level of glycaemia and lipids. It is recommended that fibre shall be gradually introduced, starting with 1 g each day, focusing on the soluble fibres.

According to other authors *insoluble fibres* decrease the absorption of zinc in contrast with the soluble fibres that increase its absorption as well as that of the other divalent cations especially Ca^{++} and Mg^{++} in the colon [36]

Although not establish an optimal amount of fiber to be consumed, it is clear that both adults and children consume insufficient amounts [37, 38]

In elder children, natural complex food fibre consumption should be encouraged compared to the refined ones, with emphasis on fruits, wholemeal bread, whole wheat cereal for breakfast, beans and vegetables at other meals (especially at lunch).

Increased consumption of food fibre in children younger than 5 years of age will be made with even greater caution, gradually starting after the age of 2 [39], because they cannot tolerate large amounts [6] having a smaller physical capacity of alimentation, therefore requiring a more "dense" diet in terms of energy compared to the elder children and adolescents. Their excessive growth can induce the decrease of energy demand, the reduction of nutrients and of mineral absorption, and if an unconventional diet is administered (with too much fibre) the children may refuse the food.

So increasing fibre intake in children of this age group should be gradual, to levels considered practical for each child because:

- diet rich in fiber may compromise energy intake (decreases the absorption of proteins, fats, carbohydrates) and can lead to retardation of growth - especially at young ages.
- the fibers decrease the bioavailability of vitamins and minerals (Fe, Ca, Zn).[40]

In the first year of life is not necessary fiber diet. By age 2, child's diet should contain at least 5 g fiber / day it receives from fruits, vegetables and grains. These are foods that should be part of child's diet at this age.

There is a consensus regarding fibre intake in relation to age. It is calculated using the formula: **fibre intake (g)/ day = "age + 5"** the level being considered sufficient and safe at the same time.

In conclusion, dietary fiber has many advantages over the health of the child, especially the bowel. While it is almost impossible to determine daily intake of fiber per child with diabetes, consider that you have reached the target amount of 20 g / 1000 kcal / day. [31] This amount has advantages only when the diet comprises at least 50% carbohydrate.[6]

Minerals

The minerals required by the body are divided into two categories: microelements (calcium, magnesium, phosphorus, sodium, potassium, sulfur and chlorine) and trace elements (iron, iodine, zinc, manganese, chromium, cobalt, selenium, molybdenum, fluoride).

Calcium is the most important minerals in the human body. 99% of calcium is found in the skeleton and teeth, and the remaining 1% is found in soft tissues, blood and only 0.1% is in the extracellular fluid.[41] Standing in the body, there is an equilibrium constant between calcium intake from food and calcium deposits from the skeleton. Optimal calcium intake in children about 1300 mg/day. The most important sources of calcium are milk and dairy products (yogurt, cheese). Nowadays, there are also calcium-fortified foods (soy milk, fruit juices, cereals). However dietary intake of calcium has been shown to be suboptimal low compared with other micronutrients.[42]

Sodium

Sodium is rapidly absorbed in the small intestine and is eliminated from the body through the kidneys (mostly), some sweat and only 2% in the faeces. The kidneys remove sodium is controlled by aldosterone.

5-40% of the sodium in the body is found in the skeleton.[43]

Average intake of sodium always adapts to the body's needs. So in terms of a low intake loss through urine and sweat diminish, while in terms of an increased intake, urinary excretion equals intake. [44] Sodium is brought into the body through food, such as table salt, milk, cheese, bread, carrots, spinach, radishes. It has been calculated that the salt of the organism comes from three sources: 3 g of food Rovin, 3 g originate on average, in the preparation of foodstuffs and 4 g is supplemented with meals.[44] The association of diabetes with hypertension led recomanmdarea limiting salt intake. In this regard, Lestradet[45] calculated that, in children with diabetes, salt intake should not exceed 2 mEq / kg / day of sodium (3 g for a child 20 kg or 4.80 g a child 30 kg). So as not to exceed the "daily dose of table salt per child with diabetes should not be any salt shaker."

Sweeteners (artificial sweeteners)

Chemicals used instead of sugar for sweetening foods, sweeteners represent an alternative determined by the need of patients to satisfy their sweet sensation (*Table 6.5.*).

Adults generally do not exceed the recommended doses of sweeteners, while children with diabetes have this tendency. For these reasons and due to the small volume of information on the intake of sweeteners in children, it is recommended to use a wide range in order to reduce possible side effects as when using only a single exclusive one.

A special place among sweeteners is occupied by fructose. Unlike glucose, fructose is absorbed more slowly into the intestinal lumen, being circulated by a distinct system. The slower rate of fructose absorption is probably due to the partial transformation the level of intestinal cells in glucose.

Table 6.5.

Types of sweeteners

Type	Name	Provenience	Maximum intake
Non-calorigenic	saccharin cyclamate	Synthetic synthetic	4 mg/ kg body / day 2,5 mg/ kg body / day
Calorigenic	Fructose Aspartam	Natural Synthetic	0,5 g / kg body / day 50 mg / kg / day
Sugar substitutes (polyols)	Xylitol Sorbitol	Natural Natural	90 g / day 30-50 g/kg body/day

Sugar substitutes (polyols) can be found in fruits and vegetables. Due to reduced energy intake they are used in the food industry in the manufacture of chewing gum.

Stevia or "sweet grass" is a perennial plant whose leaves are used as a sweetener and a "medicine" to lower glycaemia. Stevia extract is a natural sweetener without calories but having a sweetness of 100-300 times higher than the sugar. Its use has proved beneficial in diabetic children who have a greater "need" for sweet than adults.

Nutritional therapy planning (meal planning)

Meal planning pursues three main objectives [46]:

- obtaining and maintaining euglycemia by reducing postprandial hyperglycemia as much as possible as well as the "accidental" ones related to additional food intake between meals, and preventing hypoglycaemia later after the meals;
- appropriate energy intake to ensure normal growth and development but also for physical activity identical to that of non-diabetic children of the same age;
- satisfying the appetite but without exceeding the limits.

The first objective is "the key" of nutritional therapy in DM and the most effective method of preventing degenerative complications.

The composition of menus must take into account the admitted calorific value, the food restrictions and permissions, the food preferences of the children with DM (when there are no food mistakes, and not least the material possibilities of his family.[3]

A problem that has to be mentioned is the term of "free diet", given by the situations, personal food preferences etc. Although its advantage is that it psychologically "comforts" the patient, its greatest disadvantage is that, as it is free of any criteria, not even the orienting ones, it encourages the patient's lack of discipline, so that he does not know how far his freedom extends. This is the reason why some of the followers of this diet (once supported by illustrious names) are subject to the most severe complications.

Factors involved in achieving the objectives

a. Regularity of alimentation

In the absence of particular "events", the daily caloric intake must be the same [47] respecting the proportion between the nutrients, its division by meals and the meal schedule.[46]

Although there are differences related to geographic area, ethnicity, cultural and religious level[3] currently the idea of compliance with the quantity of carbohydrates consumed from day to day (as well as that of having the meals at the same time) is more and more promoted as well as having a meal schedule every day of the week. If the carbohydrate intake varies from day to day, the insulin dose adjustment based on the results of self-control is difficult, if not impossible.[4]

b. Food fractionation.

In order to avoid hyperglycemia and hypoglycemia the general principle recommends fractionation of food (three main meals and three snacks) and observance of the proportion of nutrients and dietary fibre in each meal. This initial recommendation will not only adapt to the insulin therapy scheme used (multiple injections, insulin pump) but it also depends on each child's metabolism. Snacks between meals are essential as they contribute to reducing the amount of carbohydrates eaten at meals and help prevent postprandial hyperglycemia and hypoglycemia in-between meals.

c. The distribution percentage of the caloric and carbohydrate intake per meal.

The distribution percentage at which nutrition therapy is "started" involves three main meals: breakfast = 20% lunch = 30%, dinner = 20% and 3 snacks 10% at the middle of the insulin action time.

It should not be forgotten that this distribution covers both carbohydrates and proteins and lipids, ensuring a balance between the different foods that make up each meal.

c. Synchronization with insulin regime.

"Meal planning", through the two relatively constant elements (mealtimes and quantity of carbohydrates consumed at each meal and snack) aims to achieve a parallelism between insulinemia obtained from insulin injections and the glycemic value (as a result of absorption of nutritive principles, and on the other hand of the "blocking" of the endogenous glucose production by the insulin concentration). Thus, in the case of the analogues, the snacks will be 2 hours after the prandial injection: at 9:00, at 15:00 (if lunch was at 13:00) and in the evening before going to bed at 21:00. In the case of human insulin, snacks will be 3 hours after injection, i.e. at 9:00, 16:00 and 22:00 respectively.

As a result of this simultaneous increase of the glycaemia and insulinemia, there is a significant increase in glucose uptake and hence its use by the tissues.[46]

Nutritional intervention

Nutritional dietary recommendations require individualization for each child, depending on several factors. Young child nutrition recommendations compel adaptation periods due to child rearing. Caloric needs assessment will be made at least every 6 months. Special problems are found in the diet of infants with diabetes. At this age will encourage breastmilk especially at night, thereby reducing the risk of hypoglycemia. After 3 months of age, the child will receive the same diet as a healthy child, and the pace of meals will be as regular as possible to maintain blood glucose levels in limits as well. [6] Regarding the daily variation in calories and carbohydrates, in a study of 168 children with type 1 diabetes compared with non-diabetics 405 children, Hackett et al.[48] concluded that the slightest variation in young children is due to the influence and greater parental involvement in observing diet.

Insulin regimen

Adjusting nutritional recommendations – in the sense of changing the percentage distribution by meals - will be performed only by a physician after the analysis of the evolution of the glycemic profile.

In children and adolescents treated with multiple injections, the diet can be more flexible if the metabolic and glucidic balance (objectified by HbA1c value) is good, and patients were educated in advance how to handle the type and quantity of food. This permissive attitude can create two problems: increased incidence of hypoglycemia that can be prevented by observing the interval between meals and especially by eating whole rations of carbohydrates[3] and weight increase with the emergence of overweight or obesity (in which case caloric intake and insulin doses must decrease, following carefully the degree of metabolic control) [3,4,13]

Physical activity

Physical activity is an integral part of the therapeutic measures in T1DM with immediate and long term effects.

Importance of physical activity is considerable. Practiced regularly produce decreased insulin sensitivity, reduce cardiovascular risks, help maintain body weight and induce a feeling of well-being.

In the case of healthy people, during exercise, despite increasing glucose uptake by the muscles, the glycemic values do not change significantly, situations which do not occur in patients with T1DM. Therefore when analyzing the results of glycemic self-monitoring for nutrition adjustments, we will take into account the type of physical activity carried out by each child following its inclusion in one of three situations: physical activity, physical exercise and fitness[49] that are defined as follows:

- physical activity is produced by the body movements through skeletal muscle contraction and it consumes additional energy as compared to the energy use while resting;
- physical exercise involves repetitive, planned and structured body movements to improve or maintain one or more components of fitness.
- physical condition (physical training, fitness) actually involves cardio-respiratory training, muscle training and flexibility.

It is generally accepted that an additional intake of 10-20 g carbohydrate (depending on age) is sufficient for practicing 45 minutes of exercise or swimming for 30 minutes. If physical activity is prolonged, it consumes the same amount every 60 minutes.[3] In preschoolers and school children, this additional intake varies between 5-10 g.

During intense exercise (cycling, football, tennis etc.) some of the carbohydrates can be taken in the form of sweetened beverages. [3]

Table 6.6.

Guidelines for Carbohydrate intake when Exercising to prevent low blood glucose (from the American Diabetic Association) [50]

		Grams of carbohydrate needed prior to exercise		
Duration of exercise	Exercise Intensity	Blood glucose < 90 mg/dl	Blood glucose 90-150 mg/dl	Blood glucose 150-250 mg/dl
15-30 min	Mild Moderate Hard	15 15 15	0 -15 15 15	0 0 -15 0 -15
30-60 min	Mild Moderate Hard	15-30 15-45 30-45	15-30 15-30 15-30	0 -15 15 15-30
60-90 min	Mild Moderate Hard	15-45 30-45 30-60	15-45 30-45 30-45	15-30 30-45 30-45
> 90 min	Mild Moderate Hard	Follow guidelines for 60 – 90 min of activity. Check blood glucose and consume 15 g of carbohydrate for every 30 min of exercise		

These estimates, however, have an approximate character, there being significant inter-individual variability and even an intra-individual one, that makes it mandatory to monitor glycaemia levels before, during and after the exercise practiced, to establish more precisely the additional carbohydrate intake and/ or the insulin dose to be administered in order to avoid metabolic imbalances (hypo- or hyperglycaemia).

Acute diseases often attract adjustment of diet, selection of food (sometimes) and insulin dose adjustments. In the case of febrile diseases the use of soups, juices, milk, soft grain, vegetable purées are recommended, while maintaining the carbohydrate intake. The glycaemic control should be intensified because ketonemia and hyperglycemia can be installed quickly and the patient will be instructed to reduce or suppress ketogenic food intake (proteins and lipids in particular).[50]

If the clinical picture is dominated by nausea and vomiting the patients should be advised to immediately consult the physician who will resort to food interruption and application of the perfusion.

Hypoglycemia.

The desire to obtain and maintain normal glycemia increases the risk of hypoglycaemia. The intensification of glycemic self-control has led to the concept of "biochemical hypoglycemia" in which glycemia <50 mg/ dl is not accompanied (in well balanced children and adolescents) by a clinical correspondent. In these cases and when there are minor signs of hypoglycemia, hypoglycemia treatment measures must be established. If hypoglycemia is repeated for two consecutive days, at the same hour, under the conditions of respecting the amount of carbohydrates ingested/ meal and in the absence of physical exercise responsible for the emergence of hypoglycemia, it is mandatory to lower the dose of insulin that led to hypoglycemia.

Recommendations on treatment targets will therefore take into account the child's age.

Gastrotechnic principles

Preparing the diet in T1DM in children and adolescents requires knowledge of food nutrients content[51] by the medical team (physician, dietician, nurse) but also by the child and his family.

Weighing the food will be required before consumption and not before cooking as through the preparing methods the carbohydrate concentration is modified (e.g. boiled potatoes contain 22g carbohydrates/100 g, while the french fries get to 44 g carbohydrate/100 g). There are some exceptions: bread, if toasted it will always be weighed before roasting, since dehydration increases the concentration of carbohydrates. Fruits and vegetables (> 10% carbohydrates) baked or cooked must also be weighed prior to the heat treatment.

Food savor is a major motivation for the selection, acceptance and consumption of food. In case of children with T1DM in order to remove the feeling of frustration caused by quantitative restrictions and meal timetables, it should be considered the satisfaction of pleasant sensations triggered by taste, smell, texture and flavor of the food consumed. The more the food consumption triggers pleasant sensations the bigger its nutritional impact and the more it attenuates the sometimes imperious need to consume the so-called "forbidden" foods.

Gastrotechnic methods is best to keep vitamins and minerals contained in food. It is recommended consumption of fruit and vegetables in large pieces, anti steam (in covered vessels that rush boiling / baking). We recommend reducing the consumption of fatty meat and use lean meat, fish, cheese and milk with low fat (yogurt instead of sour cream), cooking without added fat, use vessels that require small quantities of oil, avoid sauces and mayonnaise in supermarkets.[30] The sauces are prepared without flour and flour when it will be taken into account when calculating carbohydrates.[3]

Dietary education

Type 1 DM in children is a complex disease from both an etiopathogenetic viewpoint and in terms of prognosis and therefore it requires strict control on the long-term.[52]

In order to accumulate the necessary knowledge for a correct diet it is necessary to educate the child and his family.

If the medical team neglect the dietary education that should be initiated since its onset, they will trigger a similar attitude from the patient whose participation will be insufficient both due to lack of information and especially because of the subjective state of "wellbeing" that he has been in (over a long or short period of time), which will make him believe that respecting the diet is unnecessary.

If "the patient" (both the child and his family) understand the role of nutrition in the treatment, the risk of complications is distant in time. Maryniuk (cited by 53) believes that involving children in food preparation habits encourages them to comply with the new food habits (restrictions).

Diet is an alimentary symbol closely related to dietary habits and traditions of the population, family, religious prohibitions, etc. All these create certain food preferences, often damaging and inconsistent with rational nutrition.[17]

Nutritional therapy in DM often causes major changes in family lifestyles. Although the diet should be adjusted according to individual and family preferences, family nutrition will be adapted to the new situation of DM in the family (each family member must consume at the meals the same food without preparing special meals for the child, thus avoiding the "isolation" of the diabetic child) and the new gastrotechnic methods must

often be adopted.[12] But any change should be made after proper preparation; otherwise changing dietary habits will not be accepted.

Dietetic education will be continuous and the diet and nutrition of children and adolescents will be reviewed every 3-6 months.

The diet reviewing frequency seems to influence children's attitude towards the importance of the diet. Thus Vinik et al.[13] found that 41% of the patients whose diet was checked after three months feel that their diet is very important.

Conclusions

As an integral part of the DM treatment, nutrition should be similar to that of the non-diabetic children, thus ensuring a normal growth and development of the body.

Elaborating a "meal planning" and ensuring its observance are essential objectives of the alimentation of the patient with DM.

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PREVENTION OF MICROVASCULAR AND MACROVASCULAR COMPLICATIONS IN CHILDREN AND ADOLESCENTS WITH TYPE 1 DIABETES

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Introduction

Type 1 diabetes (Type 1 DM) is the most common chronic disease seen in childhood, and the occurrence of chronic complications represents a really concerning health problem, in one way by affecting the quality of life, and also by influencing the life expectancy in these patients.

Although microvascular and macrovascular complications are rare in pediatric patients with Type 1 DM, still early manifestations can appear very prematurely, immediately after diagnosis.

Microvascular complications can be seen after 5 years since the disease has started, but rarely before puberty.[1]

Vascular complications of Type 1 DM can be classified in:

- microvascular complications: retinopathy, nephropathy and neuropathy;
- macrovascular complications: affecting coronary and cerebral arteries.

In children with Type 1 DM it is very important that the family knows well the risk of the occurrence of these complications.

Identifying the predictive factors of complications will allow for the establishment of an early interventional plan, which might change the evolutional course of these complications for patients with diabetes.[2]

Preventing microvascular complications

In order to prevent vascular complications in diabetes, the most efficient measures are the following: maintaining a good glycemic control and medical education.

Preventing strategies in Type 1 DM can be applied before the onset of the disease by acting on the risk factors and in-depth knowledge of imunopathogenesis. In early stages of type 1 diabetes, the therapeutic strategies will be oriented towards the immune specific modulation on the pancreatic beta cells.[3,4]

It would be ideal if we could succeed in preventing the macrovascular and microvascular complications and not only treat them after they have appeared. Recent studies show that in Type 1 DM the risk of microvascular and macrovascular complications is present when the level of HbA1c is over 7%.

The guides that relied on the results of DCCT (Diabetes Control and Complications Trial) recommended the following target values for the prevention of microvascular complications: HbA1c lower than 8.5% for children under 6 years old, lower than 8% for children between 6 and 12 years old and lower than 7.5% for teenagers with ages between 12 and 19 years old. These guides were recently revised by ADA (American Diabetes Association), which recommends a target value lower than 7% for all group ages, in order to prevent complications.[5,6]

Intensive insulin treatment and maintaining a good glycemic control determine a significant decrease of the risk for vascular complications, as it has been demonstrated in DCCT and EDIC (Epidemiology of Diabetes Interventions and Complications). In a group of teenagers treated intensively with insulin, as opposed to conventional treatment, the risk for complications such as diabetic retinopathy has been reduced by 53%, for microalbuminuria by 54% and for neuropathy by 60%, and HbA1c dropped from 9.8% to 8.1%. More importantly, the benefits of intensive insulin treatment have been sustained in the following four years.[6,7]

Studies like DCCT have demonstrated the importance of intensive treatment with insulin in preventing the progression of microvascular complications. Based on these studies, clinical guides recommended treatment schemes with multiple insulin injections and lower HbA1c as targets, in children and also in adults.[8,9]

Other studies have shown a significantly reduced incidence of retinopathy in the last years (from 49% to 24%) and also a reduced incidence of microalbuminuria (from 7% to 3%), with an increase of BMI, besides some insignificant changes of HbA1c values in teenagers with Type 1 DM. Therefore, it can be interpreted that treatment with multiple injections with insulin can contribute to reducing complications, even if HbA1c values remain unchanged.[10]

Although maintaining a good glycemic control represents the gold standard for preventing the appearance of long-term complications on persons with diabetes, the importance of early identifying genetic markers and environmental factors that contribute to the evolution of the disease has been recently highlighted. Because early interventional methods can change the evolution of these complications, it is very important that families with diabetic children know these factors, and understand the importance of periodic screening of these complications.

Risk factors

The ***genetic factors*** have a complex role in producing microvascular complications, multiple genes being involved, which act on multiple pathways:

- genes involved in the renin-angiotensin system;
- genes involved in the metabolism of folates;
- genes involved in the oxidation of lipids;
- genes that codify the aldose-reductase enzyme and other enzymes. [11,12]

Environmental factors have a favoring role in the appearance of vascular complications, and the most important are:

- puberty;
- high BMI;
- hypertension;
- lipid metabolism disturbances;
- smoking. [11,12]

Puberty. Several studies have shown that puberty in children with diabetes represents an important risk factor for the appearance of microvascular complications. These aspects are also connected with high values of HbA1c, frequently seen in this period.

The risk of complications in puberty in a child with type 1 diabetes was studied in connection with:

- changes in corporal mass composition;
- the role of GH1/IGF1 axis;
- the role of androgenic hormones.

It has been seen that *changes in corporal mass*, such as adipogenesis represent an important factor in producing complications.[12,13]

A raise of the BMI by 2 SD (standard deviations) determines a double risk for microalbuminuria and a significant risk for the appearance of retinopathy. The risk of gaining weight in puberty is higher in girls with diabetes, which will lead to an exacerbation of insulin resistance and the appearance of complications.[12]

Insulin resistance also represents an early marker for cardiovascular complications in teenagers with diabetes.[14,15]

Insulin resistance in puberty is present in all teenagers, regardless that they suffer from diabetes or not. Euglycemic clamp tests in children

that are not obese and not diabetic showed a reduction in the sensitivity to insulin with 30-36% in children in Tanner stages 2 up to 4, comparing with children in Tanner stage 5, with adults or children in prepuberty.[16] Also we can say that girls, comparing to boys, presented a higher insulin resistance, in all stages of puberty, which can be correlated with the degree of adiposity - measured by the thickness of the skin fold.

The *role of the GH/IGF 1 axis* in favoring complications in teenagers with diabetes was suggested by: the increase of the GH plasma level in diabetic patients which already developed retinopathy, comparing to those without this complication and a low prevalence of diabetic retinopathy in patients with GH deficiency. However, clinical studies with selective inhibitors of the GH/IGF1 pathway did not show concluding results in preventing or treating microvascular complications.[17,18]

The release of GH in puberty is done in a pulsatile manner, in Tanner stages 2 and 3 for girls, and 4-5 for boys, corresponding to a growth spurt period. A similar model of GH release is present in teenagers, but there has been observed an exaggerated GH secretion followed by low levels of IGF1 in all puberty stages. These modifications of the GH/IGF1 axis, with permanent increased levels of basal GH, might contribute to the accentuation of the insulin resistance in adolescents with type 1 diabetes.[19]

Moreover, insulin is necessary to adjust hepatic receptors for GH; periods with insulin deficiency on a hepatic level will lead to an insufficient production of IGF1, which by negative feedback will lead to an increase of the GH level.[20]

Although growth deficiencies are rarely seen in children with diabetes, these abnormalities at the level of the GH/IGF1 axis are constantly found. The GH secretion is increased and the plasma levels of IGF1 and IGF-BP III (binding protein) tend to go down.

Puberty delay and insufficient physical development only occur in children with diabetes that present an unsatisfying glycemic imbalance on a long term basis.

Mauriac Syndrome, characterized by short stature, delayed puberty, Cushing-like distribution of fat, hepatic steatosis, osteopenia, limiting joint movement, is present in diabetic children with chronic underinsulinization. Clinically manifests with ,,full moon" face, prominent abdomen, diminished proximal musculature and the presence of hepatomegaly due to hepatic infiltration with glycogen and fat.[1]

Increased *androgenic hormones* and a higher free androgen index (FAI) were seen only in girls with microalbuminuria and diabetes in puberty.[21]

Teenage girls with type 1 DM are predisposed to hyperandrogenism and to polycystic ovary syndrome, with increased levels of testosterone and menstrual disturbances. Sex Hormone Binding Globulin (SHBG), a transporter protein for sexual steroid hormones, was found in lower levels in teenagers with diabetes, compared to control subjects. In teenage girls

with type 1 DM, the ovarian function can also be affected, especially the synthesis of androgenic ovarian hormones. However, the estrogen production was not observed to be affected.[22,23]

Therapeutic interventions in puberty.

When microalbuminuria is present, it is recommended to start early a treatment with *angiotensin converting enzyme (ACE) inhibitors*, which raise the sensitivity to insulin, just like it has been proved by studies done on adults.[24] It has been showed that *statins* have an important effect on the vascular function, independently from the effects on the lipid profile.[25]

Metformin is used as a first line therapy in improving sensitivity to insulin, in teenagers with diabetes type 2. In adults with type 1 DM, it has been proved that Metformin brings noticeable benefits over the vascular function. That is why it has been suggested that it can also be used in teenagers with insulin resistance and type 1 DM in puberty.[26,27]

The role of *physical exercise* is well known in improving the sensitivity to insulin in teenagers with type 1 DM, along with insulin treatment and diet. Because the exercise capacity and the cardiac function can be affected in teenagers with type 1 DM, especially in the ones with a higher BMI, it is imperative to carefully adapt the exercising program, in order to avoid aggravating the complications.[28]

Smoking, hypertension and dyslipidemia considerably raise the risk of complications and enhance the progression of retinopathy, nephropathy and macrovascular disease. It has been proved that *smoking* is associated with a high risk of persistent microalbuminuria and also macroalbuminuria; smoking effects are still not very clear concerning retinopathy, but early retinal microvascular modifications were indeed associated with smoking.[29] Smoking will lead to increased morbidity and mortality in patients with type 1 DM by favoring and aggravating the cardiovascular disease.[30]

Arterial hypertension, both systolic and diastolic, is encountered ten times more frequently in younger diabetic patients with type 1 DM compared to non-diabetics, especially in persons with renal impairment. Hypertension determines a predisposition to stroke, heart attack, heart failure and limb amputations. There is few data about hypertension prevalence in young people with diabetes, which prevents the establishment of an early treatment.

Dyslipidemia includes: increased triglycerides level, total cholesterol, LDL cholesterol and it is associated with both microalbuminuria and retinopathy; moreover, it represents an important risk factor for cardiovascular disease.

Long-term screening of complications

Prolonging life expectancy in children with type 1 diabetes is heavily correlated with the occurrence and also the evolution of microvascular and microvascular complications. The appearance of complications in diabetes

is insidious, but their detection can be achieved, several years prior to the development of clinical signs or the damage of organs. Systematic screening can detect anomalies in a very early stage, in which, through adequate interventions, complications could be reversible, stopped, or their progression might even be avoided (*Table 7.1.*).

Table 7.1.

Screening and intervention methods in vascular complications[31,32,33]

	When to commence screening	Frequency	Preferred method of screening	Other screening methods	Potetntial intervention
Retinopathy	After 5 yr duration in prepubertal children, after 2 yr in pubertal children	Annually	Fundal photography	Fluorescein angiography, mydriatic ophthalmoscopy	Improved glycemic control, laser therapy
Nephropathy	After 5 yr duration in prepubertal children, after 2 yr in pubertal children	Annually	Overnight timed urine excretion of albumin	24-hr excretion of albumin, urinary albumin/creatinine ratio	Improved glycemic control, blood pressure control, ACE inhibitors
Neuropathy	Unclear	Unclear	Physical examination	Nerve conduction, thermal and vibration threshold, pupillometry, cardiovascular reflexes	Improved glycemic control
Macrovascular disease	After age 2 yr	Every 5 yr	Lipids	Blood pressure	Statins for hyperlipidemia Blood pressure control
Thyroid disease	At diagnosis	Every 2-3 yr	TSH	Thyroid peroxidase antibody	Thyroxine
Celiac disease	At diagnosis	Every 2-3 yr	Tissue transglutaminase, endomysial antibody	Antigliadin antibodies	Gluten-free diet

Nephropathy

International Society for Pediatric and Adolescent Diabetes (ISPAD) and the International Diabetes Foundation (IDF) recommends that the annual screening of microalbuminuria should start at the age of 11 and 2 years after the onset of the disease.

Microalbuminuria is defined by a rate of albumin excretion of 20-200 µg/min or 30-300 mg/day and the albumin/creatinine report should be 2.5-25 mg/mmoL for boys and 3.5-25 mg/mmoL for girls.

Retinopathy. ISPAD recommends that the screening for retinopathy should start before patients turn 11 and after a period of 2 years of disease, through an ophthalmoscopy with dilated pupils. The screening

should be done annually or even more frequently, if several other risk factors for affecting the eye sight are involved.

Neuropathy. Diabetic neuropathy, peripheral and autonomic, will have to be evaluated annually, according to ISPAD, also at the age of 11, and in children that have the disease for longer than 2 years. Strict glycemic control could prevent the development of diabetic neuropathy and also can reduce the risk of progression of this complication. If the neuropathic signs are present, before beginning the treatment, it is necessary that other etiological factors be excluded, such as chronic alcohol consumption, B_{12} deficiency and uremia.

Blood pressure will be monitored at least annually. Arterial hypertension is defined as an average between arterial systolic and diastolic pressure which exceeds the 90th percentile for age and sex, in 3 successive determinations. In a teenager, blood pressure shouldn't exceed 130/90 mmHg. The confirmation of hypertension will be made by measuring blood pressure for 24 hours.

Dyslipidemia will be immediately evaluated after type 1 diabetes onset, in all children over 10 years old; then, the screening will be made every 2 years if there is a history of familial hypercholesterolemia or cardiovascular disease, and every 5 years for people with normal values and no medical history.

The target values for some parameters which have an important role in reducing the risk of microvascular and cardiovascular complications in children with type 1 DM are presented in *Table 7.2*.

Table 7.2.

Target values of the parameters which trigger vascular complications[31]

Parameter	Target level
LDL cholesterol	< 100 mg/dL (<2.6 mmol/L)
HDL cholesterol	> 40 mg/dL (>1.1 mmol/L)
Triglycerides	< 150 mg/dL (<1.7 mmol/L)
Albumin/creatinine ratio	< 2.5–25 mg/mmol in males < 3.5–25-mg/mmol in females
Blood pressure	< 130/90 for adolescents
HbA1c	< 7.5%, without severe hypoglycemia
BMI	< 95th percentile

Diabetic retinopathy

Even if by maintaining a glycemic control very close to normal the appearance of retinopathy, or it's progression, can be delayed, almost every diabetic person though, in time, will develop non-proliferative retinopathy, initially mild, which can progress to moderate or severe forms.

It is essential to detect the disease as soon as possible, through screening methods, and the application of the laser treatment with photocoagulation should be done before the eyesight is severely affected.

The risk of developing diabetic retinopathy can go up to 98% of all cases of type 1 diabetes, after 15 years of disease progression.[31]

Diabetic retinopathy is characterized by multiple lesions of the retina: modifications of the vascular permeability, capillary microaneurysms, capillary degeneration, excessive formation of new blood vessels (neovascularization). Also, changes are made at the level of the neural retina, with retinal electrophysiological alterations, clinically expressed by the incapacity of distinguishing colors.

Non-proliferative retinopathy is defined by retinal microvascular abnormalities of the blood flow. This type of retinopathy can be classified as follows: mild, which presents only microaneurysms; moderate, which besides the microaneurysms also presents microhemorrhages; severe, which presents more than 20 retinal hemorrhages in the 4 quadrants. Severe retinopathy is characterized by progressive ischemia and retinal infarctions, which cause cotton wool spots.[31]

Proliferative retinopathy is characterized by the proliferation of new vessels, with a high risk of losing the eyesight through the appearance of retinal hemorrhages and retinal detachment.

Macular edema can occur in any stage of the retinopathy and it can affect the eyesight. Diabetic macular edema or diabetic maculopathy is classified separately from the retinopathy stages and is defined by microaneurysms, which determine increased exudation and swelling of the central retina. It has a severe prognostic, but it is very rarely encountered in children and adolescents with type 1 diabetes.[31]

In the non-proliferative stage, hyperglycemia can lead to the destruction of intramural pericytes, the thickening of the basal membranes and the alteration of the vascular permeability. In this stage, most patients don't have visual disturbances. As non-proliferative retinopathy evolves, the degeneration of the retinal capillary will be produced, with the appearance of capillary occlusions and the presence of hypoxia. Secondary to hypoxia, ischemic lesions can be found, followed by the release of angiogenic factors as the disease progresses to the proliferative phase. In this phase, secondary to neovascularization and fluid accumulation at the retina level – macular edema, visual disturbances appear. In severe cases, retinal hemorrhages are associated with architectural distortions, with the development of fibro-vascular membranes, and finally retinal detachment.[34]

Cataract is very rare in childhood and in a diabetic child has a prevalence of <1%, although in adulthood it is particularly common. The risk factors for cataract occurrence in a diabetic child are: adolescence, long term hyperglycemia, diabetic ketoacidosis at its onset; much higher HbA1c values at the onset of diabetes and possibly also genetic factors.[35]

Bilateral acute cataract in young patients recently diagnosed with type 1 DM can occur in several weeks or months after establishing the diagnosis. Visual symptoms are due to secondary myopia, hyperosmotic modifications of the lens, caused by prolonged hyperglycemia. The severity

of the cataract varies from mild forms to complete eyesight loss in just a few days.

The pathogenesis of cataract in a diabetic child is still not very clear. Recent studies underline the importance of the polyol pathway, in which glucose is converted to sorbitol; sorbitol being excessively produced, it will create a hyperosmotic effect, which will lead to cataract formation. Other authors showed that increased glucose levels in the aqueous humor induces glycation of the lens proteins, generating free radicals and forming advanced glycosylation products. Along with an increased level of free radicals, the antioxidant capacity of the lens decreases and the susceptibility to oxidative stress goes up.[36]

Although metabolic control has a major impact over the development of this complication, genetic factors also have an important role, because it has been shown that only 50% of these cases also develop proliferative retinopathy. It is very important that every diabetic patient that presents or not ophthalmologic symptoms be sent to an ophthalmologic evaluation, both at the onset and during the evolution of the disease.

The best method for early detecting retinopathy is the fundus eye examination, indirect ophthalmoscopy, angiography that show functional and structural anomalies of the blood vessels, and also optic coherence tomography that determines only structural anomalies and macular edema.

Besides maintaining a good glycemic balance, the treatment of retinopathy includes: laser photocoagulation, local treatment with steroids, vitrectomy and recently, the intraocular administration of vascular endothelial growth factor (VEGF) antagonists. Unfortunately, there is no treatment that could slow down the progression of the disease before using these invasive treatment methods.

Laser therapy can reduce by 50% the progression of visual disturbances in patients with proliferative retinopathy. This therapy is not recommended in patients with non-proliferative retinopathy, in milder forms especially. After therapy, side effects can appear: reduced peripheral and nocturnal vision, color perception disturbances, choroid and vitreous hemorrhages, visual sequelae.

Diabetic nephropathy

Diabetic nephropathy is one of the most common vascular complications, present at 15-40% of patients diagnosed with type 1 DM, with a peak of incidence situated between 15 and 20 years since the onset of diabetes. Diabetes is the most frequent cause of renal terminal disease and it can occur in 30-40% of the patients with type 1 diabetes, in adult period. The risk of nephropathy increases along with the duration of diabetes, the degree of metabolic control and genetic predisposition to arterial hypertension.[37,38]

The appearance and the progression of nephropathic lesions is due to a complex process, in which various cell populations from the kidney

level are involved. In diabetes, besides the impairment of the filtration function and excretion of the kidney, there are also other functions involved, such as: the release of erythropoietin, the renal activation of vitamin D, the capacity of maintaining fluid balance and arterial pressure.

Hyperglycemia induces specific modifications at the level of renal tissue, with the impairment of endothelial cells, smooth muscle, mesangial cells, podocytes, collector tubes cells, myofibroblasts and inflammatory cells. Initially, hemodynamic changes and the raise of arterial pressure, which were reported early in the evolution of diabetes, have been considered to be caused by glomerular hyperinfiltration. The role of glomerular hyperinfiltration remains controverted, because it has been found that other factors also interfere: metabolic factors, the release of vasoactive factors, the alteration of the transduction signals, intrinsic defects at the glomerular arterioles level. Proteinuria reflects precisely these renal hemodynamic changes, with modifications at the level of the glomerular filtration barrier, by affecting glomerular epithelial cells and podocytes.[39]

Renal hypertrophy represents an early sign of renal impairment, sometimes observed even at the onset of diabetes. The hypertrophy seen at the glomerular level is associated with mesangial expansion and thickening of the glomerular basal membrane. Renal hypertrophy of the proximal tubules, which represent 90% of the renal cortex, is also seen, with an important increase of the glomerular filtration rate. It will be observed an increase in the glomerular filtration of glucose, fatty acids, proteins and aminoacids, growth factors, cytokines, with the triggering of pathological processes such as energetic imbalances, fibrosis and inflammation. Finally, the tubulointerstitial fibrosis represents the major determining factor for the progression of renal lesions in diabetes.[40,41]

The earliest clinical stage of nephropathy is microalbuminuria, which is defined by the presence of albumin in urine more than 30 mg/day or more than 20 μg/min. Sustained microalbuminuria is a predictive factor for progressing towards nephropathy, manifested by clinical albuminuria or macroalbuminuria, defined as the presence of albumin in urine in values greater than 300 mg/24 h or more than 200 μg/min. When albuminuria is associated with systemic arterial hypertension and a progressive decrease of renal filtration, these represent predictive factors for terminal renal disease onset in approximately 10 years.

The recommended screening methods for nephropathy are: determination of microalbuminuria from the morning urine, in order to avoid the confusion created by the increased urinary albumin excretion due to prolonged vertical posture; transitory excretion of urine albumin, which can also be precipitated by hyperglycemia, febrile diseases and physical exercises. Because of the variability of albumin excretion, in order to define microalbuminuria, it is necessary to be confirmed in at least 3 probes, during a 3 to 6 months period, before prescribing the treatment. It

is also recommended to determine the albumin/creatinine rapport in urine.[42]

Microalbuminuria represents the earliest stage of diabetic nephropathy, and its persistence represents an important predictive factor for the progression of nephropathy and the occurrence of cardiovascular disease.

In subjects with diabetes onset in childhood it was reported a prevalence of microalbuminuria of 4-20% during puberty.[43,44]

Other authors reported in the Oxford Prospective Study (ORPS) a prevalence of microalbuminuria in 25.7% of the subjects with an onset of diabetes in childhood in 10 years after the onset and 50.7% in disease duration of 19 years. A higher prevalence in subjects with the onset of the disease in childhood, compared to the subjects with the onset of the disease in adulthood could be explained by the effects of puberty over the renal function and albumin secretion.[45]

Microalbuminuria occurs rarely before puberty. Although previously it has been thought the duration of diabetes before puberty would not contribute to the risk of developing complications, now it's very clear that both the duration of diabetes and glycemic control have a significant effect over the occurrence of complications, even if this risk becomes obvious in puberty. Children with an early onset of diabetes usually present a quiet period before puberty, with an acceleration of albumin excretion in puberty. However, children with diabetes onset before the age of 5 can develop microalbuminuria even before puberty.

In children with diabetes lasting over 15 years, the risk of microalbuminuria is similar to those subjects who had diabetes onset before the age of 5, before 5 and 11 years old or after puberty. This suggests that the onset age doesn't influence the general risk of microalbuminuria, but it influences the age at which microalbuminuria is detected for the first time. The main risk factor for microalbuminuria and progression towards nephropathy is represented by inadequate glycemic control.[46]

Puberty also represents an important risk factor for microalbuminuria, both through associating insulin resistance with an increase of HbA1c, and through the hormonal changes that happen in this period. It also has been noticed that girls in puberty have a double risk of developing microalbuminuria, compared to boys. It has also been noticed an increased level of testosterone in teenage girls with microalbuminuria, compared to control subjects. The effect of puberty on the risk of microalbuminuria can be also explained by a rapid renal growth and increased glomerular filtration rate.[46,47]

Other risk factors are:

- arterial hypertension and circadian rhythm changes of arterial pressure, which were found in teenagers with type 1 diabetes;

- lipid anomalies that are frequently seen in teenagers with type 1 diabetes might be connected with renal impairment;

- smoking in teenagers is associated with a higher risk for microalbuminuria, while quitting will lead to a significant improvement of albumin excretion.

All these factors influence microalbuminuria, but extensive, further studies are necessary in order to establish a direct correlation between microalbuminuria in teenagers and type 1 diabetes.[48,49]

There is some data that highlights also the existence of a partial genetic risk for microalbuminuria and diabetic nephropathy, and during puberty the interaction between genetic factors, metabolic factors, hormonal factors and other environmental factors, all bring their contribution to the appearance of microalbuminuria. Longitudinal studies show that after puberty, microalbuminuria persists only in 50% of the cases, the rest of them having a favorable evolution, towards normoalbuminuria.[46]

However, even though microalbuminuria regresses, the morphological modifications caused by microalbuminuria can persist, increasing the risk of microalbuminuria occurrence and progression towards renal disease. Renal biopsy revealed glomerular basal membrane thickening and mesangial expansion. These modifications can be present both in subjects with microalbuminuria and in subjects with normal rates of albumin excretion, which represents a negative prognosis factor for the occurrence of nephropathy.[50]

In teenagers with type 1 diabetes, microalbuminuria doesn't represent just a predictive factor for diabetic nephropathy, but also for cardiovascular disease. Studies show that microalbuminuria represents a marker for vascular damage in general; endothelial dysfunction and a low degree of chronic vascular inflammation have been associated with microalbuminuria, with diabetic nephropathy and cardiovascular disease. Microalbuminuria is also associated with other risk factors, such as: hypertension, dyslipidemia, subclinical inflammation, glomerular hyperfiltration, renal function decline, but also with markers of subclinical atherosclerosis, such as endothelial dysfunction and increased carotid intima media.[51,52]

Along with the appearance of microalbuminuria, some measurea are required in order to attenuate the hyperfiltration effect on the kidneys:

- strict control of hyperglycemia;
- rigorous control of blood pressure;
- selective control of arterial dilation, with decreased glomerular capillary pressure by using angiotensin converting enzyme inhibitors;
- restricted proteins in diet, because increased ingestion of proteins raises the rate of renal perfusion.

Current therapies utilized in diabetic nephropathy, for systemic and intraglomerular hypertension, include: angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor antagonists (ARBs). All the international guidelines emphasize the importance of this therapy as a first

intention in diabetic nephropathy, alongside maintaining a good glycemic control.

In adults, it has been proved that using ACE inhibitors and ARBs reduces the progression from microalbuminuria to macroalbuminuria and increases regression rate to normoalbuminuria. Besides the obvious facts, in young adults controversies still exist regarding the use of ACEI long term to protect renal functions in patients that don't have hypertension. Side effects in long term use could be: cough, hyperpotasemia, headaches and impotence. What's more, there is also the risk of congenital malformations, if this medication is used during pregnancy.[31,52]

Diabetic neuropathy

Diabetic neuropathy can affect both autonomic and peripheral nervous system and can be diagnosed early in teenagers, through adequate screening methods. This complication can be due to the metabolic effects of hyperglycemia, and also due to the effects of insulin deficiency over the peripheral nerves.

In teenagers with type 1 diabetes, with early diabetic neuropathy, asymptomatic, the impaired cutaneous thermal sensation of the upper and lower limbs is often a common sign and is usually related with the duration of diabetes, but also with the degree of the metabolic control.

The damaging of the peripheral nerves, by reducing the velocity of the nerve conduction and action potential amplitude, can be detected even from puberty or immediately postpuberty, in 10% of the patients with type 1 diabetes.

In producing diabetic neuropathy, multiple mechanisms are involved:

- polyol pathway changes;
- non-enzymatic glycation;
- myoinositol metabolism disorders;
- deficiencies in the neurotrophic effect of insulin;
- growth factors related to insulin;
- nitric oxide and other factors.[38]

In diabetic neuropathy several vascular anomalies are produced, characterized by: thickening of the capillary basal membrane and endothelial hyperplasia, with a secondary decrease of the oxygen tension and hypoxia. In advanced neuropathic stages nerve fibers are affected, thermal and vibration sensation changes appear which evolve up to loosing sensorial perception. Clinical signs, such as hyperalgesia, allodynia and paresthesia, are present in 40-50% of patients with diabetic neuropathy and can seriously affect life quality.[54]

In general, the mechanism of development of diabetic neuropathy is not well known. Recently, there are controversies if the neuropathy is determined by the neuronal and glial damage or by the vascular changes. Through corneal microscopy, the degeneration of the optic nerve was found, with the loss of neuronal fibers, changes that significantly relate to

peripheral nerve damage and altered cutaneous, thermal and vibration sensation.[55]

It seems that in the beginning of neuropathy, longer nerve fibers are affected, with the early decrease of the nerve conduction speed at the level of the terminal nerves from the extremities; it's about the "glove and sock" distribution which manifests with paresthesia, dysesthesia (stings), sensation loss and nocturnal pain. In time, a progressive motor dysfunction develops characterized by dorsiflexion of the fingers and toes, which is a common manifestation in diabetic neuropathy.

Somatic neuropathy associated with diabetes, can be classified in two major categories: mononeuropathy (carpal tunnel syndrome, peroneal nerve paralysis, third pair of cranial nerves paralysis) and polyneuropathy, in which all peripheral nerve fibers are affected, motor, sensitive and from the autonomic nervous system.[56]

Clinical manifestations in diabetic polyneuropathy:

- loss of distal sensation, in fingers and toes;
- distal paresthesia;
- pain in the extremities;
- muscle weakness;
- lack of sweating in the extremities;
- postprandial gurgling, constipation, diarrhea;
- asymptomatic hypoglycemia;
- loss of deep tendon reflexes;
- leg ulcers.

Diabetic polyneuropathy diagnosis:

- patient history: paresthesia, loss of sensation, leg pain;
- clinical exam: loss of thermal sensation, vibration sensation, touch, stinging, at distal extremities; loss of deep tendon reflexes;
- laboratory tests: nerve conduction velocity, quantitative tests for determining thermal, pain, vibration sensitivity; tests for the autonomic nervous system function, nerve biopsy.[57]

Diabetic neuropathy is an important chronic complication in diabetics, but in adolescents and children with diabetes it's difficult to establish the incidence, prevalence, diagnosis and prognosis, due to a low number of studies in pediatric population.

Autonomic neuropathy is an underdiagnosed chronic complication of diabetes, although it affects multiple organs and systems and has various clinical manifestations: [58]

- cardiovascular: tachycardia at rest, orthostatic hypotension, exercise intolerance;
- digestive: gastroparesis, esophageal dysmotility, diarrhea and constipation;
- genitourinary: Urinary incontinence, neurogenic bladder, sexual dysfunction;
- metabolic: Asymptomatic hypoglycemia;

- sweating disorders: anhidrosis, heat intolerance, excessive salivation, dried skin;
- pupillary: impaired motor pupillary function;

However, the most severe consequences are represented by asymptomatic hypoglycemia and cardiovascular dysfunction and even sudden death. Although autonomic neuropathy is rarely manifested in childhood and adolescence, the subclinical signs of autonomic dysfunction, can be early diagnosed, after the onset of diabetes.

Risk factors for autonomic neuropathy appearance in youngsters with diabetes are represented by: duration of diabetes, low glycemic control, puberty, genetic predisposition, aldose reductase gene polymorphisms being present.

Tests for diagnosing subclinical autonomic neuropathy include: cardiovascular reflex tests, heart rate response to deep breathing, to standing up, to Valsalva maneuver, arterial pressure response from supine to standing up, pupillary response to light and adjustment to darkness. Orthostatic hypotension is due to inability of maintaining cerebral blood flow by adequate adaptation of the cardiac frequency and vascular tone.

More recent detection methods of autonomic cardiovascular dysfunction include: baroreflex sensitivity (BRS) and power spectral analysis of heart rate variability (HRV). Power spectral analysis of HRV is related to the measurement of the high frequency and low frequency spectral bands of HRV. These represent a more refined approach to the traditional method of simply measuring RR intervals in the time domain.[59,60,61]

Pupillometry is another method used for diagnosing autonomic neuropathy; the size of pupils at rest is mostly under the influence of the sympathetic nervous system, while the modification of the pupillary diameter as a response to a light stimulus is mediated by the parasympathetic nervous system.[62]

Usually, damaging the autonomic nervous system will coexist with other microvascular complications or it can represent predictive factors for these.

Autonomic neuropathy is one of the least studied complications in young people with diabetes, even if it's known that it is usually associated with a high morbidity and mortality in adulthood. Further, longitudinal studies are necessary, that can clarify the connection between autonomic neuropathy and microvascular complications. Extensive research will also be required, in order to establish "gold standard" tests to detect autonomic neuropathy in young patients and to clarify if subclinical autonomic neuropathy detected by cardiovascular test and pupillometry have a clinical significance for autonomic neuropathy in adults.

Therapeutic strategies for neuropathy include:

- improving metabolic control;
- using aldose reductase inhibitors to reduce the final products of the polyol pathway;

- use of lipoic acid, an antioxidant which will improve cellular nitric oxide and it's metabolites;
- use of anticonvulsants (lorazepam, valproic acid, carbamazepine, tiagabine and topiramate).

Actually, in diabetic neuropathy, besides maintaining a good glycemic control and pain management, there are no other therapies that can stop the progression of the disease.

Macrovascular complications

Even if the risk of macrovascular complications is virtually low under the age of 30, these complications represent the most common cause of death in adults with type 1 diabetes, especially in the presence of other risk factors, such as: a history of cardiovascular disease under the age of 55, a history of diabetes type 2, hypertension, dyslipidemia and smoking.

Macrovascular complications are often a cause of mortality in young adult. Mortality and morbidity by cardiovascular disease are obviously increased in diabetic patients as compared to non-diabetic patients.[63]

In medical literature there is little information available concerning the prevalence of predictive factors in developing vascular complications in young adults with type 1 DM.

In type 1 diabetes, the evolution towards vascular damage, is usually preceded by renal impairment. Cardiovascular damage in diabetes includes atherosclerosis with an early onset, myocardial infarction, stroke, heart failure, predominantly diastolic.[64]

Atherosclerosis is a complex process, which involves numerous mechanisms which precede the atherosclerotic plaque formation. One of the main roles is being assigned to endothelial dysfunction. Early diagnosing of atherosclerosis can be determined by ultrasound measuring the thickness of the medial intima at the aortic and carotidal level.

Dyslipidemia is frequently associated with poor glycemic control, and has an important role in initiating an atherosclerotic lesion progression. Treatment with statins in adults with diabetes has been proved efficient in primary and secondary prevention of the main cardiovascular damages.[65]

Clinical studies showed that Simvastin, Lovastatin and Pravastatin are safe and efficient in children and adolescents. These can be used in children over 10 years old. No side effects have been seen concerning growth, prepubertary stages, menarche, testicular volume, endocrine function, muscular and hepatic enzymes, though an increased risk for rhabdomyolysis is mentioned. However, in children, the safety of this treatment still has to be tested through clinical studies.[66]

Peripheral and cardiac vascular function, similarly to adults, is also affected in children and adolescents with type 1 DM. Endothelial dysfunction appears early in the evolution of diabetes, favoring the appearance of atherosclerosis. In adults with diabetes, the vascular function is affected independently from hypertension and coronary

disease. Diastolic dysfunction is characterized by a decrease in diastolic relaxation and ventricular filling.

Recent studies have confirmed the presence of diastolic dysfunction and reduced exercise capacity in teenagers with type 1 DM. Diastolic dysfunction has been correlated with increased levels of HbA1c, but not the duration of diabetes, which suggests reversibility of the dysfunction by imporving glycemic control.[67]

Prevention and treatment of macrovascular complications

The strategies for reducing the risk of developing macrovascular complications in children and adolescents with diabetes include:

- intensive glycemic control;
- trying to avoid smoking;
- early and constant treatment of arterial hypertension;
- treatment of dyslipidemia;
- a healthy life-style, with healthy eating and exercise.[31]

Diabetic patients which have a high risk of cardiovascular disease will be treated with: insulin intensively in order to maintain a better glycemic control, hypotensive agents, statins or fibrates for dyslipidemia and antiplatelet agents.[68]

Prognosis of complications

Type 1 DM is a chronic disease, with a reserved prognosis, given by the presence of chronic complications; it has been estimated that the life expectancy in diabetic patients is 10 years less than in non-diabetic patients. Nevertheless, in the past decades, the use of insulin pumps and obtaining a good metabolic control significantly reduced the incidence and the severity of complications. It is also proven that patients with microalbuminuria, through a good metabolic control, can obtain normoalbuminuria.

It is obvious that improving glycemic control on a long term can very much improve the prognosis of the disease, by reducing the incidence of chronic complications.

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MATURITY-ONSET DIABETES OF THE YOUNG (MODY): CLINICAL CHARACTERISTICS, DIAGNOSTIC AND THERAPEUTIC APPROACHES

Victoria Cret

Maturity onset diabetes of the young (MODY) is a heterogenous group of monogenic form of diabetes caused by a mutation in at least one of the genes known to affect insulin production or secretion. By now, there are described 13 genes whose mutations have been associated with specific subtypes of MODY. The most frequent MODY subtypes are caused by mutations in glucokinase (*GCK*) (MODY2) and in hepatocyte nuclear factor 1A (*HNF1A*) (MODY3),[1,2] account for up to 80% of all MODY cases. Approximately 10% of MODY cases are from mutations in hepatocyte nuclear factor 4A (*HNF4A*) (MODY1) and hepatocyte nuclear factor 1B (*HNF1B*) (MODY5). An additional 10% of MODY cases are because of newly discovered or as yet unknown gene mutations for which testing is not yet commercially available. [3]

Prevalence

An accurate prevalence of MODY is difficult to obtain because of overlapping clinical features with more common types of diabetes (type 1 and type 2), clinicians unawerness and high cost of genetic testing.

Althrough MODY has been well characterised in European and North American populations, the prevalence in Romania is not known. The only one study (Predatorr study, 2014) refears to age 20-79 years and established a prevalence of 11,6% (~ 2 milion people).

The frequency of the different genetic subtypes is variable and greatly depends on the clinical recruitment (either paediatric or adult) and on the geographic origin of tested patients.

The minimum population prevalence of MODY in the United Kingdom is estimated at 108 cases per million. Studies of population based childhood diabetes registries in Norway, Poland and Germany estimated the minimum prevalence of monogenic diabetes as 3.1/100 000 in Norwegian children,[4] 4.2–4.6/100 000 in Polish children,[5] 2.39/100000 in German children[6] and 2.1/100 000 in American children.[7] Un update from SWEET data base – The European Reference Network for Rare Diabetes (ERN Diabetes), in March 2016, has counted a total of 847 european patients with rare diabetes.

Diagnostic features

The diagnostis features of MODY are based upon the following criteria: strong family history of diabetes, age of onset before 25 years, sustained endogenous insulin production and absence of the beta-cell autoimmunity or features of metabolic syndrome. However, there can be significant clinical overlap of the MODY subtypes with both type 1 and type 2 diabetes mellitus. [1,2]

As a stable, asymptomatic, mild hyperglycemia is typically presents clinically in GCK-MODY while HNF1A-MODY and HNF4A-MODY present with hyperglycemia and the typical symptoms of diabetes such as polyuria/polydipsia, weight loss, and rarely diabetic ketoacidosis (DKA). In addition to hyperglycemia, patients with HNF1B-MODY may have renal abnormalities.

MODY cannot be diagnosed using non-genetic tests alone. As genetic testing for MODY can be cost prohibitive for many patients, it is necessary to determine which clinical and biochemical characteristics would be more predictive of a positive MODY diagnosis in a given patient. [8] A number of inexpensive and widely **available biomarkers** can help to select patients for genetic testing:

- pancreatic autoantibodies are present in up to 90–95% of newly diagnosed type 1 diabetes patients, [9] but are present in only 1% of patients with MODY.[10] Testing for glutamate-decarboxylase (GAD) and protein tyrosine phosphatase-like protein (IA2) auto-antibodies is recommended even long after the onset of diabetes. [11]
- urinary and serum C-peptide levels persist in patients with MODY, but are significantly reduced or undetectable in both adult and paediatric type 1 diabetes cases of long duration.[12,13]

The absence of pancreatic auto-antibodies and the detection of C-peptide outside the honeymoon period should therefore raise the suspicion of MODY.

- Serum high-sensitivity C-reactive protein levels are reduced in HNF1A MODY compared with type 1 and type 2 diabetes,[14] and can

be helpful in discriminating from type 2 diabetes when used in combination with traditional MODY selection criteria. [15]

- Features of insulin resistance such as high BMI, hypertension and dyslipidaemia are less prevalent in patients with HNF1A MODY compared with patients with early-onset type 2 diabetes.[16]

Two **prediction models** have been developed that estimate the prior probability of making a genetic diagnosis of MODY, based on simple clinical parameters: the first model differentiates MODY from type 1 and type 2 diabetes18 (online version available at http://www.diabetesgenes.org/content/mody-probability-calculator) and the second model differentiates HNF1A MODY from early-onset type 2 diabetes.[16]

Mutations in the HNF1B gene (previously named MODY5) are identified in 25% of patients with young-onset diabetes and renal cysts (RCAD) and/or variable structural abnormalities of the kidney. In about 1% of cases, HNF1B mutations could also be identified in patients with young-onset diabetes who met the criteria of two generations affected with diabetes and at least one family member diagnosed under 25 years and with no known renal disease. [17]

Rarer genetic causes of MODY because of mutations in other genes have been reported, but these additional subtypes are very rare and together they account for only ~ 1% of MODY.

CLASSIFICATION OF MODY AND PHENOTYPIC CHARACTERISTICS

Genes that are known to cause MODY are:

- hepatocyte nuclear factor 4 α (HNF4A; MODY1),
- glucokinase (GCK; MODY2),
- hepatocyte nuclear factor 1 α (HNF1A; MODY3),
- pancreatic and duodenal homeobox 1 (PDX1; MODY4),
- transcription factor 2 (TCF2) or HNF1B (MODY5),
- neurogenic differentiation 1 (NEUROD1; MODY6),
- Kruppel-like factor 11 (KLF11; MODY 7),
- carboxyl ester lipase (CEL; MODY8),
- paired-box-containing gene 4 (PAX4; MODY9),
- insulin (INS; MODY10),
- B-lymphocyte kinase (BLK; MODY11),
- adenosine triphosphate (ATP)-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8; MODY12),
- and potassium channel, inwardly rectifying subfamily J, member 11 (KCNJ 11; MODY13).
- additional genes responsible for MODY exist and remain to be identified.

Mutations in the GCK, HNF1A, HNF4A, and HNF1B genes are the most common causes of MODY, and they respectively account for 32%, 52%, 10%, and 6% of cases in the UK.[18]

Althrough MODY is described as a dominantly inherited disorders, de novo mutations in GCK, HNF1A and HNF4A are possible but rare, likely to be underestimated as patients are usually selected for mutation analysis on the basis of their positive family history.[19]

Genes causing MODY and their clinical and molecular characteristics are summarized in *Table 8.1.* [20]

Table 8.1.

Clinical and molecular characteristics of MODY subtypes

MODY gene	Chromosomal location	Frequency (% from MODYs)	Pathophysiology	Other features	Treatment
HNF4A	20q13	5	β-Cell dysfunction	Neonatal hyperinsulinemia, low triglycerides	Sensitive to sulfonylurea
GCK	7p13	15-20	β-Cell dysfunction (glucose sensing defect)	Fasting hyperglycemia from newborn	Diet
HNF1A	12q24	30-50	β-Cell dysfunction	Glycosuria	Sensitive to sulfonylurea
PDX1/IPF1	13q12	<1	β-Cell dysfunction	Homozygote: pancreatic agenesis	Diet or OAD or insulin
HNF1B	17q12	5	β-Cell dysfunction	Renal anomalies, genital anomalies, pancreatic hypoplasia	insulin
NEUROD1	2q31	<1	β-Cell dysfunction	Adult onset diabetes	OAD or insulin
KLF11	2p25	<1	β-Cell dysfunction	Similar to type 2 diabetes mellitus	OAD or insulin
CEL	9q34	<1	Pancreas endocrine and exocrine dysfunction	Exocrine insufficiency, lipomatosis	OAD or insulin
PAX4	7q32	<1	β-Cell	Possible	Diet or OAD

MODY gene	Chromosomal location	Frequency (% from MODYs)	Pathophysiology	Other features	Treatment
			dysfunction	ketoacidosis	or insulin
INS	11p15	<1	Insulin gene mutation	Can also present PNDM	OAD or insulin
BLK	8p23	<1	Insulin secretion defect	Overweight, relative insulin secretion defect	Diet or OAD or insulin
ABCC8	11p15	<1	ATP-sensitive potassium channel dysfunction	Homozygote: permanent neonatal diabetes; heterozygote: transient neonatal diabetes	OAD (sulfonylurea)
KCNJ11	11p15	<1	ATP-sensitive potassium channel dysfunction	Homozygote: neonatal diabetes	Diet or OAD or insulin

GCK-MODY (MODY 2)

Glucokinase (GCK) is a glycolytic enzyme that catalyzes the conversion of glucose to glucose-6-phosphate and acts as a β-cell glucose sensor controling glucose-mediated insulin release.

Heterozygous inactivating mutations in GCK cause GCK-MODY, also known as MODY2, which was first recognized in 1992.[21]

More than 620 mutations of GCK have been identified so far to cause hypoglycemia and hyperglycemia.[22]

The phenotype associated with GCK mutations is similar for all mutations. No genotype–phenotype correlation exists for GCK mutations.

The clinical disease manifests as mild fasting hyperglycemia from birth (99 to 144 mg/dl, glycosylated hemoglobin range 5.8% to 7.6%), but many patients are first discovered during routine screening such as during pregnancy that demonstrates slight deterioration with age. Patients are usually asymptomatic. Severe hyperglycemia and the risk of developing diabetic complications is very low in GCK diabetes. [23,24]

Patients with GCK-MODY do not require treatment outside pregnancy because glucose-lowering therapy is ineffective and there is a lack of long-term complications. [25]

In pregnancy, insulin may be required to prevent fetal overgrowth. The fetal growth in pregnancy is dependent on whether the mutation is inherited. [26] Women with GCK MODY may have babies that are large for gestational age if the baby has not inherited the GCK mutation. Management of pregnant women with GCK-MODY is based on fetal growth scans which are surrogates for fetal genotype.

HNF1A-MODY (MODY 3)

Hepatocyte nuclear factor 1 α (HNF1A) is a homeodomain-containing transcription factor, which is expressed in the liver, kidney, intestine, and pancreatic β-cells. HNF1A-MODY patients developed diabetes because of impaired glucose-induced insulin secretion.[26]

Mutations in HNF1A are the most common cause of MODY in Europe, North America, and Asia. More than 400 different HNF1A mutations have been discovered. [27, 28]

The HNF1A genes are highly polymorphic and very rare sequence variants of uncertain clinical significance are frequently identified (a total of 186 polymorphisms /variants of uncertain clinical significance have been published).

Heterozygous HNF1A mutations result in progressive β-cell dysfunction that leads to diabetes in early adult life. These mutations demonstrate high penetrance; 63% of carriers develop diabetes by 25 years of age, and almost all carriers develop diabetes by the age of 55. [29]

Patients heterozygous for *HNF1A* mutations have the onset in adolescence or early adult life, with progressive β-cell failure and increasing hyperglycemia. In fact, β-cell dysfunction occur before the onset of diabetes in *HNF1A* mutation carriers.[30] Even when blood glucose is in the normal range, *HNF1A* mutation carriers are seen to have a lower insulinogenic index and a lower early insulin response when compared to non-mutation carriers in the same family. [31]

The glucose raising at 2-hour on oral glucose tolerance test (OGTT) frequently is 5 mmol/L (80 mg/dl) higher than the fasting level, even when the fasting glucose level is less than 5 mmol/L.

Another feature of this group of patients is a low renal threshold for glucose. Glycosuria has been observed in young nondiabetic *HNF1A* mutation carriers[29] This is thought to be due to a reduced expression of the sodium-glucose transporter-2, reducing glucose reabsorption through the proximal tubule.

The risks of microvascular and macrovascular complications are similar to type 1 diabetes mellitus (T1DM) and T2DM, because hyperglycemia may be severe and worsens over time. Therefore, tight glycemic control and close monitoring for diabetic complications are required in these patients.[32]

A genotype–phenotype correlation exists for HNF1A mutations, the position and the type of HNF1A mutations within the gene influences the age at diagnosis of diabetes in the patient.

Patients with missense mutations in the first 6 exons of the HNF1A gene (predicted to affect all three isoforms) are diagnosed on average 12 years earlier (median of 18 years) than those patients with missense mutations located either in exons 8–10 (affecting isoform A only) or in the transactivation domain (median of 30 years). In contrast, patients with HNF1A truncating mutations are diagnosed at a median age of 20 years independently of the location of the mutation within HNF1A. [29,33]

High-sensitivity C-reactive protein (hs-CRP) is a novel non-genetic biomarker useful in distinction HNF1A patients from the others diabetes patients. The CRP gene has binding sites in the promoter of HNF1A gene, so that circulating hs-CRP has reduced levels in HNF1A-MODY patients compared with type 1 diabetes, type 2 diabetes, glucokinase (GCK) MODY and normal control subjects.[18]

Patients with HNF1A-MODY are sensitive to sulfonylurea therapy, which is recommended as first line treatment. An observational study suggests that patients with HNF1A-MODY can be switched safely from insulin to a sulfonylurea [34] with good control for many years, but most patients progress to insulin treatment. The initial dose should be low (one-quarter of the normal starting dose in adults: e.g., 20–40mg gliclazide daily) to avoid hypoglycemia.[1]

Glucagon-like peptide receptor agonist (GLP-1 RA) treatment is a new option for treatment in patients with HNF1A MODY with low risk of hypoglycemia, because of its insulinotropic effect in a strictly glucose-dependent manner, with no efect at plasma glucose concentrations. [1, 35]

Due to its progressive nature and early age at diagnosis, those with HNF1A and HNF4A MODY are at similar risk of microvascular and macrovascular complications, as patients with type 1 and type 2 diabetes, which is strongly influenced by the degree of glycaemic control. HNF1A MODY patients are at an increased risk of developing cardiovascular disease, so statin therapy by the age of 40 years, regardless of lipid status, has been recommended. [36] HNF1A mutations may predispose to familial liver adenomatosis through somatic inactivation of the remaining wild-type allele. In a cohort of 137 HNF1A MODY patients, 9 cases (6.5%) of liver adenomatosis were identified by liver ultrasonography. Patients with liver adenomatosis are at risk of haemorrhage, and some hepatologists advocate systematic liver ultrasonography in HNF1A MODY patients.

HNF4A-MODY (MODY 1)

HNF4A mutations represent less than 10% of MODY cases in Europe, and more than 103 mutations in 173 families have been identified so far. [37] It was the first MODY to be described.

Hepatocyte nuclear factor 1 α (HNF4A is a transcription factor involved in the regulation of genes that are required for glucose transport and metabolism.

The clinical profile of heterozygous HNF4A mutations is similar to HNF1A MODY. It is estimated that 10% to 29% of HNF1A-negative patients

actually have HNF4A mutations.[38,39] Patients with HNF4A diabetes are seldom diagnosed before adolescence.

Glycosuria does not present in HNF4A MODY, and low apolipoproteins (apoA11, apoCIII, and apoB) can be a diagnostic clue. *HNF4A* mutation carriers may also have a lower serum HDL-C, possibly due to reduced ApoA2 transcription, resulting in lipid profiles similar to those with type 2 diabetes. [39]

A similar response to sulfonylureas as in HNF1A patients has been observed in patients with HNF4A-MODY.

Finding an HNF4A mutation has important implications for the management of pregnancy.

Pregnancies where one parent (mother or father) has an HNF4A mutation are at risk of complications during delivery because of macrosomia and ultrasound monitoring of fetal growth is recommended.[40] Neonates who have inherited the HNF4A mutation are also at risk of neonatal hyperinsulinaemic hypoglycaemia and blood glucose should be checked from birth.

Invasive prenatal testing to predict the future risk of diabetes (or congenital hyperinsulinism for HNF4A mutations) is not indicated.

If the mother has GCK MODY or either parent has HNF4A MODY, testing of a fetal DNA sample obtained for other purposes (eg, high risk for Down syndrome) is useful for determining the fetal genotype. This predicts fetal size and hence risk of complications during delivery because of macrosomia.[41]

Furthermore, fetal genotyping using cell-free fetal DNA from maternal plasma sampling during early pregnancy is currently being developed to guide management of the pregnancy in mothers with risk of fetal macrosomia because of GCK or HNF4A mutations.

PDX1-MODY (MODY 4)

PDX1 (also known as insulin promoter factor 1 - IPF1) is a homeodomain-containing transcription factor that acts in pancreas development and insulin gene expression. PDX1-MODY is a very rare cause of MODY and was first described in 1997. [42]

Homozygous mutations can cause permanent neonatal diabetes due to pancreas agenesis. Heterozygous PDX1 mutations lead to β-cell dysfunction and MODY.

HNF1B-MODY (MODY 5)

This form of diabetes is caused by heterozygous mutations in hepatocyte-nuclear factor 4B (HNFIB). HNF1B is encoded by the transcription factor 2 (TCF2) gene, which is expressed in the liver, kidney, intestine, stomach, lung, ovary, and pancreatic islets and influences their embryonic development. More than 65 mutations have been detected to date. Exon or complete gene deletions account for approximately half of cases. [43]

HNF1B-MODY phenotypes are different from HNF1A-MODY because diabetes develops due to both insulin resistance and defective insulin secretion. It was initially described as a rare subtype of familial diabetes, but patients with heterozygous mutations in *HNF1B* rarely present with isolated diabetes.[1,44] It is characterized by progressive nondiabetic renal dysfunction of variable severity, pancreatic atrophy and genital abnormalities. Diabetes develops later, typically during adolescence or early adulthood. It is also called RCAD (renal cysts and diabetes syndrome).

Renal developmental disorders (especially renal cysts and renal dysplasia) are present in almost all patients with *HNF1B* mutations even in the absence of diabetes. Genital-tract malformations (particularly uterine abnormalities), hyperuricemia and gout can also occur, as well as abnormal liver function tests.

In addition to insulin deficiency related to pancreatic hypoplasia, patients also show some degree of hepatic insulin resistance which explains why they do not respond adequately to sulfonylurea treatment and require early insulin therapy.

Mutation carriers have lower exocrine pancreatic function, fecal elastase is always reduced abnormal in patients with HNF1B-MODY.[45]

The phenotype of renal cysts and diabetes (RCAD) patients is highly variable even within families sharing the same *HNF1B* mutation and therefore this diagnosis should be considered not only in the diabetes clinic but also in other clinics (nephrology, urology, gynecology, etc.). In patients found to have renal cysts, imaging of the pancreas is indicated, as the absence of the pancreatic body and/or tail is highly indicative of HNF1B-MODY.[1,46] Half of carriers develop diabetes.

A positive family history of renal disease or diabetes is not essential to prompt genetic testing, as spontaneous de novo mutations and deletions of this gene are common (one third to two thirds of cases).

Patients with HNF1B-MODY do not respond well to sulfonylureas and usually require early insulin therapy.[47]

NEUROD1-MODY (MODY 6)

Neurogenic differentiation 1 (NEUROD1) is a basic-loop-helix transcription factor that is involved in pancreatic and neuronal development. This is a very rare form of MODY.

Heterozygous NEUROD1 mutations lead to diabetes as children or adults, frequent known as type 2 DM.[48, 49] while mutations in both alleles result in neonatal diabetes with neurological abnormalities and learning disabilities. [50]

KLF11-MODY (MODY 7)

Kruppel-like factor 11 (KLF11) is a zinc-finger transcription factor that is expressed in pancreatic islet cells. KLF11 binds to and activates the insulin promoter in mouse insulinoma cell lines in a high-glucose

condition, which indicates that KLF11 is a glucose-inducible regulator of the insulin gene. [51] Two rare variants of KLF11 gene were identified in three families with early onset T2DM. [52]

CEL-MODY (MODY 8)

Carboxyl ester lipase (CEL), also called bile salt-stimulated lipase, is expressed in mammary glands and pancreatic acinar cells. CEL is a major component of pancreatic juice and is responsible for the hydrolysis of cholesterol esters as well as a variety of other dietary esters. CEL-MODY was first identified by Raeder et al.[53] in 2 Norwegian kindreds with autosomal dominant diabetes.

Heterozygous mutations in the CEL gene result in pancreatic atrophy, fibrosis, and lipomatosis together with exocrine insufficiency and later endocrine dysfunction and diabetes.[54]

PAX4-MODY (MODY 9)

Paired-box-containing gene 4 (PAX4) is a transcription factor that is essential for differentiation of insulin-producing β-cells in the mammalian pancreas. PAX4 gene mutations have been identified in Thai probands with MODY who did not have mutations in known MODY genes.

It has also been associated with ketosis-prone diabetes.[55]

INS-MODY (MODY 10)

Insulin (INS) gene mutations are rare causes of diabetes in childhood or adulthood, being more common cause of neonatal diabetes.[56]

Heterozygous INS gene mutations decrease proinuslin molecule folding or cause β-cell apoptosis in the endoplasmic reticulum.

The treatment is generally insulin, although some patients manage with oral antidiabetic drugs.

BLK-MODY (MODY 11)

B-lymfocyte kinase (BLK) acts as a stimulator of insulin synthesis and secretion in pancreatic β-cells via the transcription factors Pdx1 and Nkx6.1. BLK is a non-receptor tyrosine-kinase of the src family of proto-oncogenes.[57]

There is a higher prevalence of obesity in individuals with diabetes that was linked to 8p23 than in diabetic individuals with MODY linked to other loci.[58]

ABCC8-MODY (MODY 12)

The adenosine triphosphate (ATP)-binding cassette, sub-family C (CFTR/MRP), number 8 (ABCC8) gene encodes the sulfonylurea receptor 1 (SUR1) subunit of the pancreatic β-cell ATP-sensitive potassium (K-ATP) channel. Its activating homo- and heterozygous mutations cause neonatal diabetes, but heterozygous mutations can also cause MODY in patients whose clinical features are similar to those with HNF1A/4A MODY.[59]

The correct molecular diagnosis is important, as these patients can be treated with sulfonylureas.

KCNJ11-MODY (MODY 13)

The potassium channel, inwardly rectifying subfamily J, member 11 (KCNJ11) gene encodes Kir6.2, a part of the K-ATP channel. Its activating homozygous mutations cause neonatal diabetes, but heterozygous mutations have been associated with a large spectrum of diabetes phenotypes in a French family. [60]

The age at diagnosis varied from childhood to adulthood (13 to 59 years), and the treatment varied from diet to OAD or insulin.

CLINICAL CRITERIA FOR GENETIC TESTING (Sian Ellard)[2]:

1. Mild hyperglycaemia: testing for GCK mutations

The following features suggest a diagnosis of a GCK mutation:

- the fasting hyperglycaemia is ≥5.5 mmol/l (98% patients), persistent (at least three separate occasions) and stable over a period of months or years;
- HbA1c is typically just above the upper limit of normal and rarely exceeds 7.5%;
- in an OGTT the increment [(2 h glucose) − (fasting glucose)] is small (71% of patients in the large European study reported by Stride et al. [27] had an increment <3 mmol/l). An increment of 4.6 mmol/l is often used to prioritise testing and corresponds to the 90th centile (*S. Ellard and A. T. Hattersley, unpublished data*);
- parents may have 'type 2 diabetes' with no complications or may not be diabetic. On testing, one parent will usually have a mildly raised fasting blood glucose (range of 5.5–8 mmol/l) unless the mutation has arisen de novo. Testing of apparently unaffected parents'fasting glucose is important when considering a diagnosis of a glucokinase mutation.

2. Gestational diabetes: testing for GCK mutations

- persistently raised fasting blood glucose in the range of 5.5–8 mmol/l (99 – 108 mg/dl) before, during and after pregnancy;
- an increment of <4.6 mmol/l (82 mg/dl) on at least one OGTT (either during or after pregnancy);
- parent may have mild type 2 diabetes but often this has not been detected and so the absence of family history should not exclude the diagnosis.

3. Children and young adults with diabetes and a strong family history of diabetes: testing for HNF1A mutations

- young-onset diabetes (typically before 25 years old in at least one family member);

- non-insulin-dependent outside the normal honeymoon period (3 years), e.g. not developing ketoacidosis in the absence of insulin, good glycaemic control on less than the usual replacement dose of insulin, or detectable C-peptide measured when on insulin with glucose >8 mmol/l;
- family history of diabetes (at least two generations).

This may be insulin treated and considered to be 'type 1' diabetes or 'type 2' diabetes. At least two individuals within the family would typically be diagnosed in their 20s or 30s. There may also be an affected grandparent, although often these are diagnosed after 45 years.

OGTTs in early stages tend to show a very large glucose increment, usually >5 mmol/l (90 mg/dl). Some individuals may have a normal fasting level but a value within the diabetic range at 2 h.

- absence of pancreatic islet autoantibodies;
- glycosuria at blood glucose levels <10 mmol/l (180 mg/dl) is often seen, as these patients have a low renal threshold;
- marked sensitivity to sulfonylureas resulting in hypoglycaemia despite poor glycaemic control before starting sulfonylureas;
- several features suggesting monogenic diabetes rather than a diagnosis of young-onset type 2 diabetes should be considered: no marked obesity or evidence of insulin resistance in diabetic family members, absence of acanthosis nigricans and whether the family is from an ethnic background with a low prevalence of type 2 diabetes (e.g. of European descent).

4. ***Children and young adults with diabetes and a strong family history of diabetes: testing for HNF4A mutations:***

- the clinical characteristics are similar to HNF1A, except there is not a low renal threshold and the age of diagnosis may be later;
- HNF4A mutations should be considered when HNF1A analysis does not detect a mutation but the clinical features are strongly suggestive of HNF1A;
- patients are often sensitive to sulfonylureas;
- HNF4A mutations are associated with macrosomia (approximately 56% of mutation carriers) and transient neonatal hypoglycaemia (approximately 15% of mutation carriers).

The possibility of HNF4A mutations should be considered when diabetic family members have marked macrosomia (>4.4 kg at term) or if diazoxide-responsive neonatal hyperinsulinism has been diagnosed in the context of familial diabetes.

5. ***Babies with diazoxide-responsive neonatal hyperinsulinaemic hypoglycaemia and a strong family history of diabetes: testing for HNF4A mutations:***

- mutations of HNF4A are a cause of neonatal hypoglycaemia that remits during infancy or early childhood, with diabetes developing later in life;

- macrosomic babies with diazoxide-responsive hyperinsulinism and a strong family history of diabetes should be considered for HNF4A mutation screening.

Current genetic tests for diagnosing monogenic diabetes rely on selection of the appropriate gene for analysis according to the patient's phenotype.

Next-generation sequencing technology provides the potential for the simultaneous analysis of multiple genes in a single test to detect mutations in all known MODY and neonatal diabetes genes, assay at a similar cost to testing a few genes by Sanger sequencing. [61]

Conclusions

MODY is a genetically and clinically heterogeneous group of monogenic diabetes, underdiagnosed because of significant clinical overlap of the MODY subtypes with both type 1 and type 2 diabetes mellitus.

The diagnostis features of MODY are based upon the following criteria: strong family history of diabetes, age of onset before 25 years, sustained endogenous insulin production and absence of the beta-cell autoimmunity or features of metabolic syndrome.

There are described 13 genes whose mutations have been associated with specific subtypes of MODY, but the most frequent MODY subtypes are caused by mutations in glucokinase (*GCK*) (MODY2) and in hepatocyte nuclear factor 1A (*HNF1A*) (MODY3).

Knowledge of these rare causes of diabetes, the use of additional clinical clues, and other biomarkers will improve pozitive genetic detection rates, allowing appropriate treatment and genetic to the patients and their family members.

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PEDIATRIC DYSLIPIDEMIA. CLINICAL APPROACH

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Summary

In the last years, it has become more and more evident that atherosclerosis originates in childhood. The cardiovascular risk factors, starting in childhood, progress into adult life and have been associated with a moderate to high risk of future cardiovascular disease (CVD).

In order to prevent the development of risk factors and future CVD events, with effective management of both genetic and acquired factors, it is important to identify those patients who are vulnerable to this pathology. A variety of techniques has been used to identify children and adolescents with early atherosclerotic vascular changes due to genetic and/or acquired CVD risk factors.

Recently, the expert groups in Europe, as well as in the US have established guidelines for screening of dyslipidemia in the pediatric age group. These guidelines, aiming to support the cardiovascular health and to reduce the CVD risk in children and adolescents included the recommendations for screening, treatment and follow-up of the children and adolescents at risk for premature CVDs (e.g. myocardial infarction and stroke). The screening addressed to all children with family history of hypercholesterolemia and/or premature CVD, but also all the children between 9 and 11 years old, and, then again between 17 and 21 years of age. The later recommendation (including all children) is still controversial, but prevention and early effective therapeutic intervention are the keys to decreasing the incidence of CVD in the adult life.

The paper presents the actual recommendations for screening, therapeutic means and follow-up of all children and adolescents with dyslipidemias and/or risk for premature CVDs.

Introduction

The cardiovascular diseases are the leading cause of death in many developed countries[1,2], including Romania.

Although, the clinical symptoms of the atherosclerotic disease are not evident until adulthood, the vascular lesions are known to begin forming earlier during adolescence or, even childhood.[3,4]

The fact that atherosclerosis originates in childhood became quite evident in the last decades, [5-8] with the help of various techniques used to identify the children and adolescents with early atherosclerotic vascular changes. Many imagistic studies (coronary intima-media thickness, brachial artery distensibility and carotid artery calcium scores) revealed the subclinical disease in children.[3,4,9,10]

The atherosclerotic vascular lesions are determined by genetic or/and acquired CVD risk factors. When CVD risk factors are present at a young age, these do progress into adulthood and have been associated with a moderate to high risk of CVD in the adult life.[11] In order to prevent the development of the risk factors and CVD events, it is of great importance to identify the patients at risk as early as possible.

The primary prevention will aim for extending the interval between the identification of the risk factors and the expression of the CV disease. Therapy will depend on age, but the initial approach will always include diet and changes in lifestyle. When needed, drug therapy will be used according to the approval for different age groups. Drug therapy should be closely monitored with lipid profile, vascular imaging, hematologic and metabolic parameters (keeping in mind the potential adverse effects of the drugs) (12).

Screening

The first guidelines for lipid screening in children and adolescents, published 25 years ago, were revised in the last years and completed with other risk factors and associated conditions that could accelerate the risk for CVD.[11,13,14]

The screening is now recommended for two categories of children and adolescents[15]:

- the first, includes all children older than 2 years, with family history of hypercholesterolemia (one or both parents known with hypercholesterolemia or treated with lipid-lowering medications, or who have a family history of premature CVD (men younger than 55 years, women younger than 65 years), or children with unknown family history

(like for example adopted children) who have a moderate to high risk for premature CVD (*Table 9.1.*) [11];

- the second category (universal screening) referrs to all children aged between 9 and 11 years, regardless of general health or the presence/absence of CVD risk factors; in this category, it is recommended to repeat the screening between 17 and 21 years of age.

Tabel 9.1.

Criteria for moderate and high CVD risk factors and conditions [11]

Criteria	Moderate risk	High risk
BMI	≥ 95thp but < p97th	≥ 97th p
Hypertension	HBPwithout treatment	HBP with treatment
HDL-c	< 40 mg/dl	-
Cigarette smoking	-	Current smoker
Predisposing medical conditions	Kawasaki disease with regressed coronary aneurysms	Kawasaki disease with current coronary aneurysms
	Chronic inflammatory diseases (SLE, JRA)	Diabetes mellitus (type 1 and type 2)
	Nephrotic syndrome	Chronic renal disease/ end-stage renal disease/ postrenal transplant
	HIV infection	cardiac transplant

Legend: BMI= body mass index; CVD = cardiovascular disease; HBP = high blood pressure; HIV = human immunodeficiency virus; HDLc= high-density-lipoprotein cholesterol; SLE= systemic lupus erythematosus; JRA= juvenile rheumatoid arthritis.

Investigations included in the screening

The lipid panel

The fasting lipid panel should be the main tool used for screening. The standard lipid panel includes serum total cholesterol (TC), triglycerides (TG), high-density-lipoprotein cholesterol (HDLc), low-density-lipoprotein cholesterol (LDLc). If the patient is not fasting, a non HDLc panel will be more reliable (TC-HDLc), because it is not affected by beverages and food, unlike TG and calculated LDLc, which are affected by eating or drinking beverages (most of the commercial laboratories are using the Friedwald equation TC - TG/5 + HDLc for the calculation of LDLc). On the other hand, directly measured LDLc and the calculated non- HDLc, are very little influenced by food and beverages. If non-HDLc is high (> 145 mg/dl), than two fasting lipid profiles should be obtained within 3 months, and with a minimum of two weeks between the evaluations.[15]

The interpretation of the results should keep in mind the normal serum lipid levels, accepted for children and adolescents, which differ from those in adults (*Table 9.2.*)[16,17] Worth mentioning that, the lipid profile is changing during growth, so that in the first 2 years of life, TC, HDLc and LDLc increase, followed by a plateau, until the onset of puberty. During puberty, TC and LDLc decrease (with a mean of 10-20 %) and increase

after puberty (>15-16 years of age). Therefore, the first screening, around 10 years of age, should be reliable. If the lipid profile is not significantly modified, it should be repeated between 17-21 years.[15] The results of the lipid screening should be interpreted as significant if LDL ≥ 130 mg/dl, non-HDLc ≥ 145 mg/dl, HDLc < 40 mg/dl, TG ≥ 100 mg/dl (for children < 10 years of age) and TG ≥ 130 mg/dl (for children >10 years of age). The lipid profile should be repeated, as mentioned before, after 2 weeks.[15]

Table 9.2.

Lipid cutpoints (mg/dl) for children, adolescents and young adults

Category	**Children & adolescents < 17 ys**			**Young adults > 17 years**		
	A	**B (*)**	**H (**)**	**A(x)**	**B(x)**	**H(x)**
TC	< 170	170 - 199	≥ 200	< 190	190 - 225	≥ 200
LDLc	< 110	110 - 129	≥ 130	< 120	120 -160	≥ 130
Non-HDLc	< 120	120 - 144	≥ 145	< 150	150 - 190	≥ 145
TG (0-9 y)	< 75	75 - 99	≥ 100			
TG(10-19y)	< 90	90 - 129	≥ 130	< 115	115 -150	≥ 150
Category	**A**	**B**	**Low*** **	**A**	**B**	**Low**
HDLc	> 45	45 - 40	< 40	> 45	45 - 40	< 40

Legend:
A = acceptable, B = Borderline, H = high
* and ** represent the 75th (p75) and the 95th percentile (p95) for each lipid
*** represent the 10thpercentile (p10) for HDLc
x the cutpoints for TC, HDLc and non- HDLc represent the 95th percentile (p 95) for 20-24 y old subjects; acceptable values are at < p75;
For HDLc: low is < p25, acceptable values are > p50;

Taking into consideration other causes of dyslipidemia, the evaluation should be completed with a comprehensive metabolic panel (CMP) including liver function, renal function, electrolytes) and, other specific tests for common secondary causes of dyslipidemia, which are listed below (*Table 9.3.*)[11]

Table 9.3.

Common secondary causes of dyslipidemia[11]

Condition	Screening tests
Liver disease	CMP
Renal disease	CMP, Urine analysis
Hypothyroidism	TSH, FT4
Diabetes mellitus	CMP, UA, fasting glucose, HbA1c
Obesity/insulin resistance	CMP, fasting glucose, Insulin
Medication	Steroids, oral contraceptives, retinoids, protease inhibitors

Legend: CMP = comprehensive metabolic panel (liver function, renal function, electrolytes); UA= urine analysis; HbA1c = glycated haemoglobin A1c, TSH = Thyroid stimulating hormone, FT4 = Free thyroxin T4;

Cardiovascular health begins in childhood.

Evaluation of Carotid Intimal Media Thickness

Carotid intimal media thickness (CimT) is an important indicator for clinical coronary artery disease in adults. Increases in CimT seem to be directly associated with an increased risk of myocardial infarction and cerebrovascular accident, later, in asymptomatic adults. [18]

A large prospective cohort study found that increased CimT in adulthood is significantly associated with elevated childhood serum LDLc levels, along with elevated systolic BP, elevated BMI and smoking. Also, the study revealed that LDLc levels above the 80th percentile, for patients aged 12 to 18 years, are directly related to an increased CimT measured in young adults. The same authors found a correlation between the number of risk factors present in childhood and adult CimT, concluding that exposure to multiple cardiovascular risk factors during childhood and adolescence may initiate the endothelial damage and contribute to the development of atherosclerosis. [4]

Another large study, analyzing the same association between traditional cardiovascular risk factors and carotid intimal media thickness in young adults found that an increased BMI and elevated LDL- c during childhood significantly predicted increased carotid intimal media thickness in adulthood (with childhood LDLc levels showing the highest correlation).[19]

In a follow-up study, other researchers showed the association between ideal cardiovascular health during childhood and optimal cardiometabolic outcomes in adulthood. The authors concluded that ideal childhood cardiovascular health was inversely associated with carotid intimal media thickness levels. [20] The limitation of this study came from the fact that diet and physical activity were not standardized. These results sustain the same ideea, showing that low levels of cardiometabolic risk markers in childhood are associated with thinner carotid intimal media thickness, which alos means a lower risk of future atherosclerotic disease.[21,22]

Early diagnosis of dyslipidemia in children is essential to prevent the formation of atherosclerotic plaques and all the long-term sequelae that follow later, in adulthood.[23] Previous research has shown that cardiovascular risk factors, not only persist over time, but usually worsens with age. [23]

Therapeutic intervention

The early intervention in childhood, through diet and lifestyle modifications, has proven to be effective in reducing the cardiovascular risk factors later in adulthood. To prevent the early formation of the

vascular atherosclerotic lesions, the innapropriate serum lipid levels should be corrected. [11]

Also, the healthcare providers should encourage protection from tobacco smoke as well as regular physical activity in all children and adolescents. Lipid–lowering medication is rarely needed for the treatment of dyslipidemia in children, usually in those with genetic forms (e.g. familial hypercholesterolemia).

Nutrition

The boards of the most important societies in the field reviewed the evidence in the nutrition area and made appropriate recommendations for diet and nutrition in children (2 years and older). These recommendations are intended for pediatric care providers to use with their patients, in order to reduce the CV risk in the future adult.[11]

It is also wellknown that good nutrition begins at birth and has many benefits, including the potential to reduce the risk of cardiovascular disease later in adulthood. That is why, nutrititon would be the first tool used for the management of dyslipidemia in children. When the parents of a child with dyslipidemia would be informed that his lipid levels are innapropriate and this fact may expose him to a future CVD risk, the information might represent a wake-up call for the family, helping them to implement the nutritional recommendations, aiming to reduce the CVD risk.

In order to optimize the cardiovascular health, nutrition should start with breast feeding (for the first 6 to 12 months). After 6 months of age, solids should be added gradually, and if reducing breast-feeding, the transition to iron- fortified formula (until 12 months) should be recommended. Fat intake, in infants less than 1 year of age, should not be restricted without medical indication.

In children older than 1 year, a diet low in saturated fat is recommended. The milk recommended should be with reduced-fat content (2%) or even fat-free depending on the child's growth and appetite, the intake of other nutrient dense foods, intake of other sources of fat and potential risk for obesity and CVD. At this age (1-2 y) sweetened beverage intake should be avoided, pure fruit juice limited to 100 ml/day, Parents should encourage the child to drink water and get him used with a diet rich in fruits, vegetables, whole grains and limit sodium intake. This is the period of transition to table food, when the fat content of daily caloric intake should not exceed 30 % (with saturated fat 8 - 10% of daily kcal, monounsaturated and polyunsaturated fat up to 20% of daily kcal) Cholesterol less than 300 mg/day, avoiding trans fat as much as possible.

In children older than 2 years, energy intake needs to match growth demands and physical activity.

The general recommendations for the fat content of the daily energetic requirements (in healthy children) are: 30-40% of calories from fat aged 1-3 years, 25-35% for aged 4-18 years. Of the daily amount of fat,

the saturated fat should be less than 10 %, cholesterol < 300 mg/day, reduced trans- fat content.

The remaining 70% of calories in the diet will include 15-20% from protein and 50-55% from carbohydrate sources. The meal portions should be based on the energetic requirements for age and sex. High dietary fiber content should be encouraged. The dietary fiber from foods should be calculated as a minimum of (age (years) +5) grams/day for young children; in adolescents, the required amount is 14 g/1000 kcal of energy requirements. [11]

Fruits and vegetables are important low calorie sources of nutrients including vitamins and fiber, so a minimum of 3 - 4 portions daily would be appropriate. Naturally sweetened juice should be limited to 150 ml/day, a limited sodium intake would be associated with lower blood pressure.

A portion of 500 ml fat-free milk daily is recommended in all children and adolescents, as milk provides essential nutrients, for growing children, including protein, calcium, magnesium, and vitamin D, that are not readily available elsewhere in the diet. Consumption of fat - free milk, in children older than 2 years and adolescents, optimizes these benefits, without compromising nutrient quality, while avoiding excess saturated fat and calorie intake.[11]

In children with identified hypercholesterolemia and elevated LDLc, the diet would be more restrictive in fat content with saturated fat less than 7% of calories and dietary cholesterol limited to 200 mg/d, trans fat < 1 %. These restrictions have been shown to be safe and modestly effective in lowering the LDLc level.[12]

In children with elevated TG, the reduction of simple carbohydrate intake and weight loss will be followed by a decrease in TG levels. The reduction of simple carbohydrate intake should be associated with a higher intake of complex carbohydrates and, also, a reduced saturated fat intake. If high serum TG is associated in obese children, a reduced caloric intake and an increased physical activity are recommended.

Physical activity and lifestyle

There is strong evidence for the beneficial effects of physical activity and unfavorable effects of a sedentary lifestyle on the overall health of children and adolescents.

Like dietary choices, the physical activity patterns are established in childhood and carried forward into adulthood. That is why, family is the first responsible to encourage children to learn good dietary habbits and to be active. School should also sustain physical activity in children and adolescents. Health care providers should support recommendations for a minimum of 3 days/week to even 1 hour daily physical activity in schools.

There is strong evidence that an increased moderate to vigorous physical activity is associated with lower blood pressure, reduced body fat, decreased BMI, lower total cholesterol, LDLc and triglycerides, higher HDLc and decreased insulin resistance in childhood and adolescence. [11]

It is recommended that children and adolescents should have daily moderate activity and a minimum of 1 hour/day, 3 days weekly, of vigorous activity. For example, jogging or playing football in the yard could be considered moderate to vigorous activity, while running, playing tennis or training for football, can be considered vigorous physical activity

Reducing sedentary time is strongly associated with a favorable CV profile. It is recommended that leisure screen time should not exceed two hours daily. In children 1-5 years old, television viewing altogether, with the family, will be discouraged, total media time should be limited to a maximum of 1- 2 hours daily. Family activity should be encouraged at least once a week, especially with children aged 3-10 years.

Drug Therapy

Medication is rarely needed to treat dyslipidemia in children. The decisions regarding the need for medication therapy should be based on the average of results from two fasting lipid profiles (at least) obtained at two weeks interval (minimum).[11]

Elevated LDLc

Patients with LDLc levels ≥ 250 mg/dl and /or TG ≥500 mg/dl should be referred to a lipid specialist, in an academic medical center in order to receive proper therapy.

In children with increased LDLc levels, the therapeutic approach should be different depending on the associated factors (Table 9.4.).[15]

Table 9.4.

LDLc 130- 189 mg/dl	Negative family history; No risk factors	Diet + Therapeutic lifestyle counselling
LDLc 130- 159 mg/dl	2 high risk factors **or** 1 high risk factor+≥2 Moderate risk factors **or** CVD	Diet + Drug (statin)
LDLc 160- 189 mg/dl	1 high risk factor **or** + ≥2 moderate risk factors **or** positive family history	Diet + Drug (statin)
LDLc ≥ 190 mg/dl	-	Diet + Drug (statin)

If diet and lifestyle modifications do not succeed in 6 months, than drug therapy is needed. The drugs of choice recommended to lower serum LDLc, in children older than 10 years, are the statins (a 3hidroxi 3methyl-glutaryl coenzyme A reductase inhibitor). In general statin therapy is not recommended before the age of 10 years, unless there is a significant family history of high-risk CVD conditions and/or premature events, the child has one or more risk conditions or multiple risk factors.[11,15] The treatment goal is to decrease LDLc <130 mg/dl (ideally <110 mg/dl).[11]

Statin therapy, when needed, should be started with the lowest dose available, once daily. If the goal is not achieved, the dose could be increased, gradually. The liver function and muscular enzymes should be

checked monthly because adverse effects of statins, even if rare at standard doses, include myopathy and hepatic enzyme elevation.

Table 9.5.

The recommended doses for statins in children[11,14]

STATIN	Age	Dosage
Atorvastatin	10 -17 y	10-20 mg
Fluvastatin	10 -16 y	20-80 mg
Lovastatin	10 -17 y	10-40 mg
Pravastatin	8 -13 y 14 -18 y	20 mg 40 mg
Rosuvastatin	10 -17 y	5-20 mg
Simvastatin	10 -17 y	10-40 G

If lifestyle modifications combined with statin does not succeed in reducing serum LDLc, other lipid lowering medications should be considered. The therapeutic alternatives are: ezetimibe (a cholesterol absorbtion inhibitor) or a bile acid sequestrant. The later, binds bile salts in the intestinal lumen, preventing their enterohepatic re-uptake in the terminal ileum; that results in a depletion of bile salts in the liver and a signal for increased production. Unfortunately, these have frequent gastrointestinal side effects, although are safe and moderately effective.

Elevated TG

Drug therapy is rarely needed for children with elevated TG. When TG levels are extremely high (exceeding 500 mg/dl), the patients are at risk for pancreatitis and require to be referred to a lipid specialist.

Usually, TG serum levels are increased in children who are also obese, and in these patients, TG serum levels will normalize along with weight loss and lifestyle changes (physical activity).

The addition of omega-3 fatty acids (from fish oil) has been shown to modestly lower TG in adults, so might be efficient, but the effective dose has not been established for children.[11]

Mixed dyslipidemia

In clinical practice, the most frequently encountered dyslipidemia is a combination of high TG with low HDLc [11] commonly seen in obese children, with insulin resistance. This pattern (called "atherogenic dyslipidemia") may also be genetic.

The treatment of this type of dyslipidemia includes lifestyle modification (healthy eating habits, daily physical moderate/strenuous activity) aiming to decrease body weight.

Low HDLc

An isolated low HDLc also increases the CVD risk (24). Low HDLc is, usually, encountered in obese children with insulin resistance and is associated, almost always, with increased TG levels. Genetic forms might

occur. Therapy consists in lifestyle modifications aiming to reduce the CVD risk factors

Drug therapy follow-up

Statin treatment should be closely monitored: monthly in the first 3 months of therapy, every 3 months in the first year, two times per year, in the following years. Investigations should include: the lipid profile, liver function (ASAT, ALAT), creatinkinase (CK). The clinical exam will focus on growth and pubertal development (height, weight, BMI, Taner stage), BP and possible adverse effects.

If clinical side effects or laboratory abnormalities are noted, the medication should be withhold for at least two weeks, investigations rechecked, and, if abnormalities resolved medication may be restarted, with close monitoring.

Concerning side effects of statins, there were some concerns that statins may affect brain development by decreasing the cholesterol synthesis but the trials on treated children with familial hypercholesterolemia, which usually start treatment early (at age 2) infirmed this assumption (12). The risk of type 2 diabetes is dose related and, seems to be associated with other pre-existing diabetes risk factors.[25]

Conclusions

Atherosclerosis originates in childhood. Universal screening is recommended for all children aged 9—11 years, regardless of general health or the presence/ absence of risk factors for cardiovascular disease. Screening should be repeated between 17 and 21 years of age.

Health care providers should realize the burden of the CVD and the benefits of prevention. The primary prevention will aim for extending the interval between the identification of the CVD risk factors and the expression of the CV disease.

Early intervention in childhood, through diet and lifestyle modifications, has proven to be effective in reducing the cardiovascular risk factors later in adulthood. Drug therapy is rarely needed to treat pediatric dyslipidemia.

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