CURRENT TRENDS IN PEDIATRIC ENDOCRINOLOGY AND DIABETES

Editors:

Iulian P. VELEA
Corina PAUL
Stuart J. BRINK

EDITURA MIRTON

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Preface

The mission of the Romanian Society of Diabetes, Nutrition and Pediatric Endocrinology (ENDOPED) is to promote cutting-edge research, quality education and superior medical care in the fields of diabetes, nutrition and pediatric endocrinology.

We strive to improve the quality of life for children by providing accurate information, effective treatments and support for professionals in the field.

As every year and this year, on the occasion of the 11th ENDOPED Congress, we offer the participants a large collection of materials provided by some of the lecturers, whom we thank in this way for their availability and effort.

If the intentions of the authors involved in the publication of this book live up to the expectations of the readers, we can say that the idea was good and proved its usefulness.
With friendship,

Professor Iulian P.Velea MD, PhD
President of Romanian Society of
Diabetes, Nutrition and Pediatric Endocrinology.

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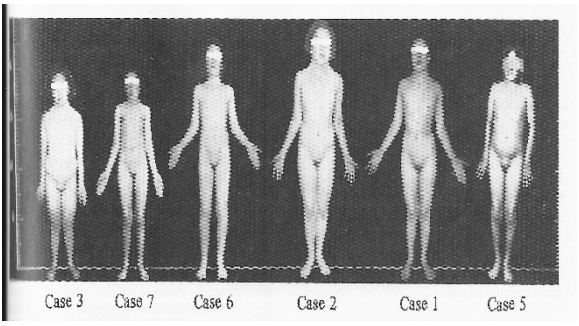
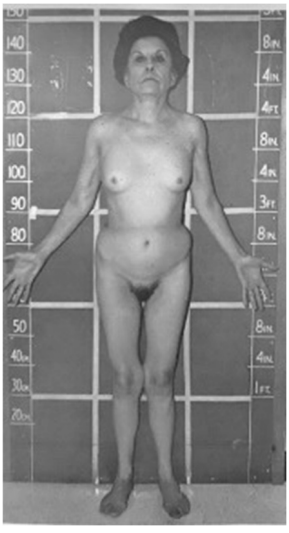
TURNER SYNDROME AND ENDOCRINOPATHIES

Stuart J. BRINK

Otto Ulrich in Germany described the syndrome in a case report in 1930 in the German medical literature[1] although other reports exist such as NA Shereshevki in 1925 or Giovanni Battista Morgagni[2] in 1768.

Henry Turner in Oklahoma then described seven patients (*Figure* 1: *six shown*) with short stature, congenital webbed neck, sexual infantilism and cubitus valgus in 1938[3] and actually treated them with a variety of pituitary and estrogen extracts available at that time. Some prefer Ullrich-Turner Syndrome (somehow ignoring Morgagni and Shereshevki) but more recently Turner Syndrome has predominated in the literature. The original index case of Turner (*figure 2*) was recently described, then 60 years old, in a follow-up article.[4] Subsequently, hypergonadotropic hypogonadism has been documented with increased gonadotropins plus low estrogen levels associated with rudimentary ovaries ("streak gonads") described by Wilkins and Fleishman in 1944[5] and absence of Barr bodies was then added to the description.[6] Documentation of the XO karyotype was first described by Ford and colleagues in 1959[7] with subsequent studies





describing a family of X chromosome abnormalities in females with Turner Syndrome (TS).
Complete or partial absence of the second X chromosome (45XO), with or without a mosaic or aberrant genetic pattern (ie. ring chromosome) is detected in approximately 1:2000-2500 live born females.[8]

Turner Syndrome

Karyotype	Approx frequency
45,X	60%
Chromosomal mosaicism • 45,X / 46,XX • 45,X / 46, XY • 45,X / 47, XXX • 45,X / 46,XX / 47, XXX	15%
Structural abnormality • 46,X,I (Xq) (ISOCHROMOSOME Xq) • 46,X,Xp (partial deletion Xp) • 46, x,r(X) (ring X)	10%
Mosaicism with structurally abnormal X • 45,X / 46,X,I (Xq) • 45,X / 46, X,Xp- • 45,X / 46,X,r(X)	10%
Other unusual patterns	5%

It has been estimated that about 1% of 45XO fetusus actually survive to term delivery and perhaps as many as 10-15% of all spontaneous miscarriages have the 45XO karyotype.[9] Such spontaneous abortions usually occur during the first trimester of pregnancy but some are recognized with fetal ultrasonography associated with cardiac anomalies, nuchal cysts, severe lymphedema or hydrops fetalis and a second trimester miscarriage in addition to elective termination in those fetuses most heavily affected.[10] In most case series, *figure 3*, about 50-60% of TS patients have sex chromosome aneuploidy, 15% have chromosomal mosaicisms (ie. 45X/46XX, 45XO/46XY, 45XO/47XXX), 10-20% have structural abnormalities of various types such as ring chromosomes, partial deletions or isochromosomes (ie. 46X,Xq, 46X,Xp) 46X,rX and mosaic variations in addition to structural abnormalities) and another 5% have other patterns.[11,12] Usually the loss of the second X chromosome occurs as a result of the nondisjunction error during paternal meiosis

since the single X chromosome is most often thought to be maternal in origin[13] while mosaicism likely comes from post-fertilization mitotic errors.[10]

More recently attempts to correlate TS phenotype with specific chromosomal patterns or with subpatterns has increased especially vis-à-vis studies of SHOX, the "short stature homeobox-containing gene."[14,15] Several studies show SHOX haplotype insufficiency associated not only with the short stature seen in virtually 100% of TS patients but also those with unrelated Leri-Weill dyschondrosteosis. In addition, SHOX abnormalities may occur in up to 15% of children with ISS (idiopathic short stature) who respond to growth hormone treatment even though these ISS patients, while significantly short compared to peers, do not have classical growth hormone deficiency as defined by stimulation testing. This may help explain the nice growth hormone treatment response (see below) seen in so many TS patients.

Most prenatally detected cases of TS are discovered incidentally during amniocentesis or chorionic villous sampling performed most commonly in association with advanced maternal age risk assessment.[16] Maternal and paternal age are not associated with increased TS risks. Prenatal ultrasonography also may detect nuchal edema, cystic hygroma as well as cardiac, aortic and renal anomalies, fetal growth retardation and other abnormalities as can maternal serum screening with alpha-fetoprotein, estriol etc. With increasing severity of some of these prenatal malformation, fetal demise is more likely. Karyotype confirmation is needed before establishing a diagnosis of TS under such circumstances.[15] The presence of Y chromosome material or probing for the SRY (sex determining region of the Y chromosome) gene or with fluorescent in situ hybridization (FISH) technology with Y-specific centromeric probes[11] is important if there are mosaicisms because of associations with gonadoblastoma in such TS mosaic patients (see below). Studies of HLA[17] looking for explanation of increases in autoimmune disorders like thyroiditis and celiac disease in TS[18] have not been positive. With all these complexities, the importance of appropriate genetic counseling cannot be overemphasized as well as involvement of a many different health professionals and especially a pediatric endocrinologist.

Overall, TS remains underdiagnosed or diagnosed much later than should be reasonable with many affected individuals having rather subtle phenotypes not easily recognized by parents or health

care professionals despite quite obvious early and persistent growth abnormalities, a myriad of nonspecific problems (feeding problems, eye, ear, dental, cardiac, gastrointestinal, renal, thyroid, reproductive, skin and skeletal) as well as a host of learning and psychosocial adaptive difficulties.[10,11] Many authorities[15] recommend a multidisciplinary team approach to maximize consistency and support.[19] In this author's personal experience, in fact, by the time TS patients are diagnosed, they invariably have so many of these problems that have **not** been tied together into a uniform diagnosis, that it becomes impossible to understand why this diagnosis was not at least considered months if not years earlier rather than the all too commonplace delay until short stature and pubertal delay/failure spur consultation. Savendahl and Davenport have proposed an excellent pediatric care change paradigm to attempt to correct this problem.[20]

Lymphedema

The lymphatic system is presumed to play a major role in the pathology of TS with severe abnormalities reflected in hydrops fetalis and increased fetal demise spontaneously. Not only does the TS infant present with swelling of the hands and/or feet and neck but web neck (pterygium coli), thickening and tightening of the skin, decreased flexibility of the limbs, ingrown toenails and soft tissue infections as well as nail dysplasia are all thought to be related to the aberrant lymphatic system itself.[21] Occasionally a TS infant presents with significant labial edema which is also thought to be related to lymphatic obstruction syndrome. Upward sweeping hair and low posterior hairline also may be a consequence of previous cystic hygroma or neck edema even if only recognized in childhood or adolescence. The bushier eyebrows of some with TS may also be explained by lymphatic changes prenatally. Widely spaced or hypoplastic inward turned nipples/shield chest could reflect similar undiagnosed prior lymphatic problems. While most neonatal lymphedema seems to dissipate spontaneously, occasionally with estrogen treatment initiation, there is also some peripheral edema.[11]

Unusual physical characteristics[10,11]

Several physical characteristics are present in TS infants, children, adolescents including micrognathia in up to 60% of those with TS and more frequent dental occlusion problems. Low set ears or posteriorly rotated ears also can be seen. Multiple pigmented nevi

may be twice as common in TS as in the general population and may also grow more in TS treated with growth hormone but without any apparent increase in malignancy.[10] More keloid formation is also present in TS individuals and this may be most important with ear and other body piercing decisions as well as when decisions about plastic surgery repair of micrognathia or web neck are considered. In many studies, hemangiomas as well as atopic dermatitis, seborrheic dermatitis, vitiligo, alopecia, psoriasis and keratosis pilaris are more common in TS as well but for most of these conditions, exact genetic or pathophysiologic explanations remain obscure.

Cardiac abnormalities and hypertension

Cardiovascular malformations are common anomalies in TS occurring in about 75% of fetuses and 30-40% of patients with TS.[22] Congenital heart disease in conjunction with aortic dissection and rupture, hypertension and ischemic heart disease makes cardiovascular disease the leading cause of premature morbidity and mortality in TS.[10] Cardiac problems are most often left-sided with coarctation of the aorta with or without bicuspid aortic valves present in up to 15-30%[10,11] with a variety of other cardiac anatomic abnormalities (ASD, VSD, aortic stenosis, aortic regurgitation and more rarely, hypoplastic left heart syndromes, mitral valve prolapse) as well as nonspecific electrocardiographic changes (ie. conduction and repolarization abnormalities as well as prolonged QT intervals) also more common than in the general population and also more common in those with TS who also have significant neck webbing. Embryologic and pathophysiologic speculation suggests that aberrant lymphatic systems may also be involved with such cardiac and aorta anomalies.[23] Hypertension from cardiac and/or renal origin as well as idiopathic hypertension may occur in up to 30-50% of TS patients.[11,24] Right and left arm blood pressure discordance may point to aortic arch constriction or other aortic arch abnormalities. Aortic arch dilatation, dissection or rupture are associated with one another in up to 1% of TS patients often with diagnosis in late childhood or adolescence so that the potential for direct counseling and ongoing cardiac surveillance exists to avoid morbidity and unexpected death from aortic rupture later. Some studies report aortic dissection occurring even without known cardiac anomalies, however.[25] Echocardiography as well as MRI helps in imaging of the entire aorta, in defining such anomalies and in appropriate counseling for surgical repair in other than an emergency situation.

Pregnancy adds further frisk of aortic dissection so that appropriate cardiac monitoring is imperative in those with TS planning pregnancy.[11] More cardiac mortality at an earlier age has also been reported involving heart attacks and strokes in young adult TS patients especially among females with XO or isochrome Xq.[26] Routine blood pressure monitoring, checking both the right and the left blood pressures and at least palpation of the femoral pulses should be standard care for TS patients from infancy and at least yearly. Baseline electrocardiograms and baseline echocardiograms is also generally considered prudent [15, 18] with periodic follow-up assessments and/or cardiology consultation. Cardiology clearance for competitive sports in TS patients should also be considered unless significant aortic enlargement exists.[11] Several studies of TS patients treated with growth hormone have provided reassuring data concerning no deleterious effects of GH treatment on aortic diameter or blood pressure.[11,27]

Renal anomalies

Congenital renal and urinary system anomalies are about 9x more common in TS than in the general population[28] with renal and renovascular abnormlatiies occurring in about 35-40% of the TS population.[29] There can be double collecting systems, absent kidneys, pelvic kidney, horseshoe kidney (up to 7%[11]) or other malrotations. Some of these anomalies are associated with increased risk of hypertension but some cases of hypertension occur without either cardiac or renovascular anomalies present. Hypertension should be treated according to standard hypertension treatment guidelines with an aim toward achieving normal blood pressure values for age appropriate standards. Previously intravenous pyelograms were used for earlier studies but more recently routine baseline renal ultrasonography is recommended in most TS guidelines with periodic follow-up as well as aggressive management of any urinary tract infections or hypertension.[15,18]

Orthopedic abnormalities

Short (as well as webbed) neck, scoliosis, Madelung (wrist) deformity, cubitus valgus (carrying angle), short 4th metacarpal, congenital hip dysplasia as well as short stature all may come to or require the attention of orthopedic consultants in those with TS. According to some authors,[11] the widely spaced nipples may be an illusion created by relative short and stocky appearance of the trunk

in TS patients. Relative hypoplasia of the cervical vertebrae may account for the shortened neck (40% of TS) whereas short stature (100%) is a generalized impairment of long bone growth over vertebral growth in TS patients (and usually without classical growth hormone deficiency [see below]). Individual bones may have varying degrees of differences from the general population including the increase in carrying angle (cubitus valgus) in about 50% of TS patients[10] as well as the abnormally small fourth (and sometimes also fifth) metacarpal heads (35-40% of TS) ("*knuckle dimple*" sign). Madelung deformity of the wrists (about 5-10% of TS) has genetic implications for SHOX gene abnormalities as one of its putative explanations. 20-40% of TS patients may have either kyphosis, scoliosis or combined kyphoscolisosis and these may increase with either growth hormone and/or estrogen treatment of short stature so that bracing is needed. Rarely is it necessary to require surgery for TS scoliosis.[9] Most cases of scoliosis are considered idiopathic and routine screening for scoliosis should occur even more often in those with TS treated with either growth hormone, estrogen or combination therapies.[10,11,15,18] Congenital dislocation of the hip can occur in 5-10% of TS patients, considerably more than the 1:1000 in the general population.[15] Unusual fishnet (coarse trabecular) appearance of radiographs of the carpal bones[30] is sometimes present as is an unusual ballooning of the tips of the terminal phalanges of the hand[31] so that radiologists and astute clinicians looking at hand and wrist bone ages may surmise the diagnosis of TS in a short girl at the very initial stages of her evaluation even when there are not many other stigmata apparent.

Ophthalmology:[10,11]

Ptosis (15-30%), epicanthal folds (10-45%), hypertelorism, antimongoloid slant and strabismus (10-35%) all occur more commonly in TS patients and most are easily diagnosed but without need for major interventions compared to non-TS patients either in the newborn or infancy time periods. Strabismus usually becomes apparent in toddlers as an accommodation for farsightedness and with early enough correction can help minimize or prevent amblyopia (loss of vision in the deviated eye). TS also is associated with increased red-green color blindness (about 10%). In some studies, iridocyclitis is also more common. All children with TS should have vision carefully checked and baseline ophthalmologic evaluation in

all TS patients should be started at about age 1-2 years and carried out periodically according to most guidelines.[15,18]

Ear and Hearing problems[10, 11]

Conductive, progressive hearing losses as well as sensorineural hearing losses are both more common in TS patients with the majority of children (60-80%) having such problems related to chronic otitis media and its consequences. Eustachian tube abnormalities (shorter and more horizontally oriented) results in poor drainage and/or ventilation of the middle ear is a likely explanatory factor. Up to 90% of TS women having a significant hearing loss and about 25% require hearing aids especially as they move into adulthood; such hearing problems seem to be progressive in adults with TS. Attempts to prevent and allow early diagnosis and treatment should include periodic consultation with audiologists and otolaryngologists for consideration of tympanostomy tubes and/or tonsillectomy or adenoidectomy[15,18] as well as continued auditory deficit screening for children, adolescents and adults.

Non-verbal learning problems, attention deficit, intelligence and psychosocial issues

Most girls with TS have normal or low-normal IQ but have specific neurocognitive and neurobehavioral problems including deficits in visual-spatial and perceptual abilities, nonverbal memory, motor functions, executive function, attention and social skills.[32] TS infants, toddlers and children are often described as less persistent, more avoidant socially and slower to adapt so that they may be at higher risk for educational problems when they enter formal schooling.[33] Teasing and bullying may also be more common with peers. In the middle and adolescent school years, special problems with mathematics surfaces and some degree of emotional immaturity has also been linked to delayed puberty, androgens and estrogens.[34] With some improvement in those TS patients provided low dose androgen therapy as an assist toward improved height and/or pubertal progression suggesting that androgens may have some specific functional interaction with brain and learning functions.[35] Estrogen replacement closer to the times of normal estrogen presence in peers also seems to help with some of these learning and brain function issues as well as maturity and self-image.[36] Most guidelines suggest a baseline developmental and/or academic evaluation to assess learning process difficulties, behavioral and neurologic issues

and to allow appropriate academic tutoring, occupational therapy and problem solving learning to help girls and adolescents with TS optimize their learning capabilities.[10,11,15,18] There is interesting research that suggests that earlier estrogen and/or androgen therapy in TS may help minimize such problems as well. There is also some other research in TS patients that has associated positive thyroid autoimmunity either in TS patients[37] or their mothers with lower IQ scores and more learning disability. Speculation suggests that perhaps either the TS patient or their mothers had some intermittent periods of hypothyroidism that were unrecognized and/or untreated and that this may explain this association. Alternatively, there may be some genetic linkage of autoimmunity and learning problems.[11] More recent attempts to correlate specific neurologic issues with EEG, MRI and PET scanning have shown some promise but also some conflicting results.[11] In some studies, anxiety and depression coupled with social isolation have been associated with TS with some improvement with growth hormone, androgen and/or estrogen replacement therapies. As endocrine interventions occur earlier and height and pubertal issues become less divergent from the general population, research may help to answer such questions as to whether these are inherent TS problems or specific to the height and pubertal status themselves. Some studies have associated different issues of these kinds with different genetic patterns so that TS mosaic patients had fewer identified neuropsychological issues than did those with 45XO karyotypes and those with isochromes or ring chromosomes had different characteristic as well but these studies also show some conflicting results not yet sorted out. How important is prenatal estrogen and androgen, how important is the timing of these hormones prior to puberty and how important is their replacement timing are all key research studies needing more work.[11] Impairments in self-esteem, emotional competence, higher degree of dependence all are described in TS studies. TS patients generally have a typical female pattern of psychosocial development with unambiguous female gender identifications even when puberty is severely delayed and estrogen treatment comparably delayed. While they appear to be more emotionally immature, leave home somewhat later than peers, date and initiate heterosexual activity somewhat later or less frequently than peers, they almost uniformly function in a traditional female and heterosexual sphere.[11]

Special training and attention to recognizing facial and social clues may be helpful as TS patients move into adolescence and

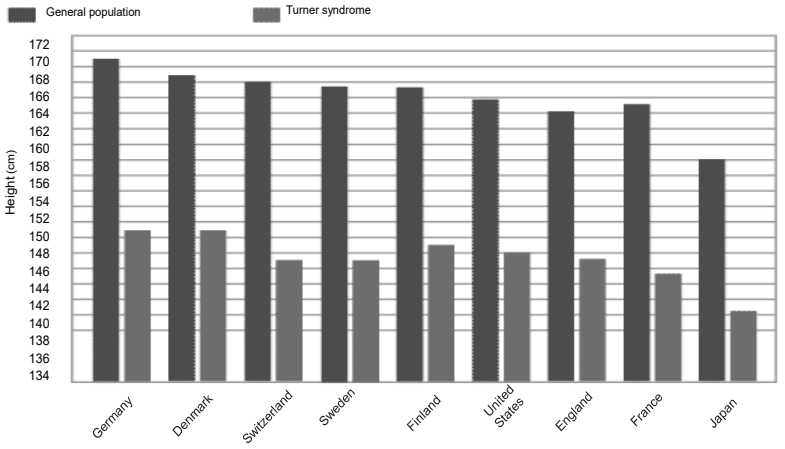
adulthood.[38] In the teenage years and into adulthood, transition may also require some attention for age-appropriate and not size-appropriate expectations especially if hormone treatments have not been available or helpful. When driving begins, many with TS will require attention to impaired navigational planning, visual-motor integration and spatial and directional abilities that are often less than optimal in many TS patients. As Saenger states rather eloquently[11] …"Whether this complex psychosocial behavior is endogenous or adaptive, as more aggressive and innovative endocrine therapies begin to alter the outlook for stature, timing of feminization and fertility, attention should still be paid to assessment of psychological adaptation. Because the factors that affect the quality of life are the same as those that affect the rest of society, psychological care should be provided within the context of helping to prevent difficulties and normalizing the developmental process rather than operating from an illness model. Plans for both medical and psychological intervention should be developed so as to reinforce and support the individual's self-esteem and to ensure that individual with Turner syndrome remains in the mainstream of social, educational and employment activities." Guidelines include psychosocial and learning assessments early and periodically with appropriate assistance provided according to outcomes of such assessments.[15,18] Age apropriate pubertal induction is recommended as much as is possible not only for the endocrine and statural benefits that may follow but also for the psychological benefits of remaining similar to peers. It is equally important to address issues surrounding diagnosis, height and secondary sexual characteristics, infertility and reproductive options in an honest and open manner to empower appropriate participation and optimize understanding. These include in-vitro fertilization, oocyte donation and adoption Such issues are readily discussed in a supportive fashion not only by the health care teams (primary care physicians and pediatric as well as adult endocrinologists, among others) but also through support systems more recently available through the internet such as the Turner Syndrome Societies that exist in many countries around the world. Transition from pediatric to adult care is a particularly difficult time when many of these issues of independence and reproductive health are still unsettled. Sexual function is normal in TS women who are receiving hormone treatment and counseling should introduce them to the concept that they should expect to have a healthy and satisfying sexual life. Teens and women with TS have

the same risks for sexually transmitted diseases as non TS peers and should be educated how to protect themselves from such risks when they become sexually active. Long term chronic health issues involving thyroid follow-up, bone mineralization, BP and cardiac disease, deafness and diabetes all require ongoing follow-up in a coordinated fashion.[10,11,15,18,39] Much of the research was done before current hormone optimization has taken place and so the newer generations of TS children may grow up with fewer or different concerns than those previously reported in the literature.

Growth

TS patients often grow poorly in utero.[10] They often have mild decrease in natal weight and length but usually they are without "dramatic" enough stigmata to be recognized in the nursery or for many years afterwards. Too often throughout childhood they have been significantly shorter than peers for many years yet no diagnostic

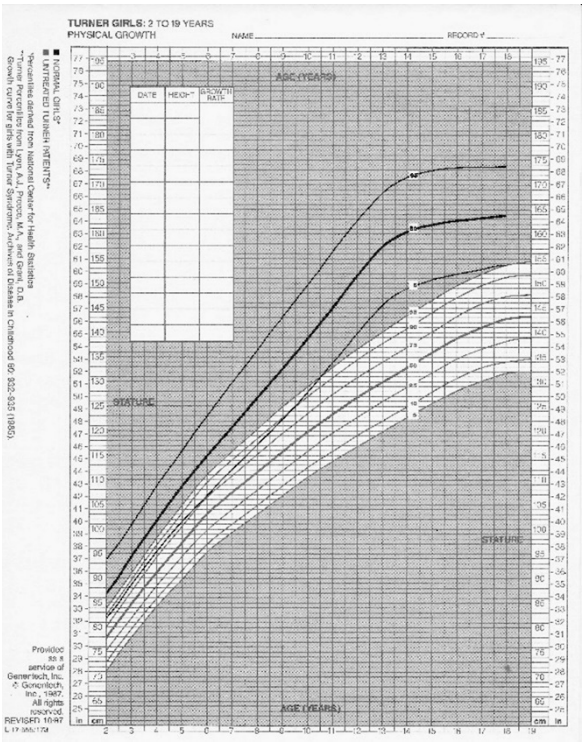
Adult height in Turner syndrome in various countries



evaluations have taken place except to send them for care of their recurrent otitis, engage them in their educational and psychological needs as they enter the school system but not notice that they exhibit decreased height velocities until delayed puberty or amenorrhea arises as an issue finally demanding medical attention. A variety of

gastrointestinal problems (see below) that are more commonly seen in TS patients also may interfere with a correct diagnosis. As a result of the obvious delay in puberty from estrogen deficiency and the height loss that eventually accompanies such lack of estrogen, undiagnosed and untreated adult TS patients are approximately 20 cm shorter than their country-comparison peers (*figure 4*) whether they come from tall countries like Scandinavia, Holland or Germany or from other countries around the world.[40]

Specific TS growth charts[41] (free computer download available from www.magicfoundation.org; *figure 5*) make such comparisons with the rest of the population rather obvious with significant short stature already present (but unrecognized) in the preschool years and actual height deceleration worsening compared to the general population from age 10 years onward - unless the diagnosis of TS is established and growth hormone with ultimate androgen and estrogen therapy instituted. As with most other growth patients, the earlier the diagnosis, the better and earlier the catch-up growth that occurs and the better the final height outcome. Average TS growth velocity in childhood untreated is 4.4 cm. TS girls who fall away from the TS charts should be investigated specifically for co-morbidities that results in such height deceleration including classical growth hormone deficiency, inflammatory bowel disease and particularly celiac disease, anemia, renal disorders and hypothyroidism, among others.[10] Girls with TS generally are not classical growth hormone deficient as defined by growth hormone stimulation tests.[10] The data are overwhelming that the administration of pharmacologic growth hormone doses

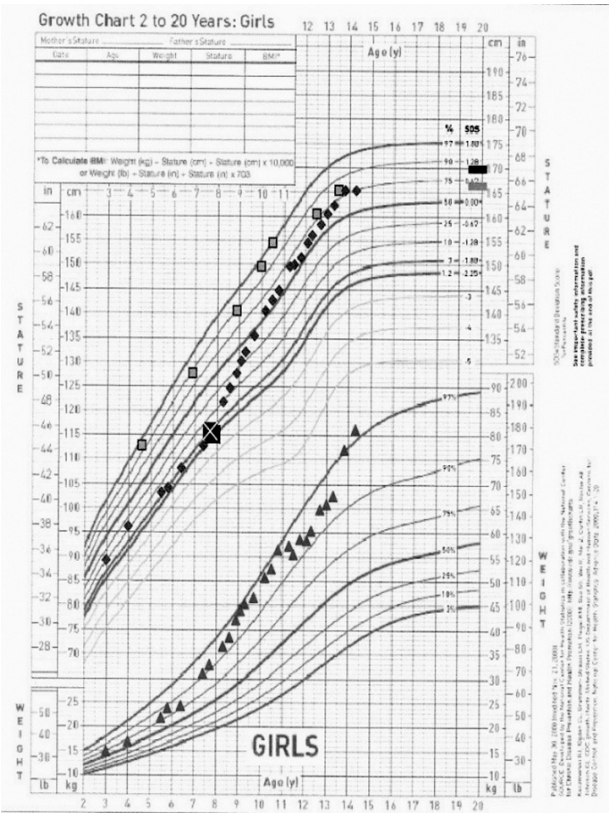


increases the growth velocity of TS patients and final adult stature increases as well in a classical dose-response fashion.[7,9,10,11,42] Higher GH doses work better than lower doses.[43,44,45] Early treatment with growth hormone also improves height velocity and final adult height achievement.[46,47]

Oxandrolone, a non-aromatizable anabolic steroid, can be used in combination with growth hormone to augment growth[48,49,50,51] especially in the first few years of treatment but, in contrast, early or late estrogen provision, despite some positive psychosocial benefits when attempting to mimic non-TS pubertal patterns (see below), does not seem to have an effect on final adult height.[52,53] In other studies, however, slightly delaying the initiation of estrogen therapy[54] has shown some benefit in final height achieved. Typical oxandrolone doses are 0.03-0.05 mg/kg/day with an expected final height increase compared to GH therapy alone of about 2.7 cm.

IGF-1 levels are somewhat lower than population means for TS patients but not usually diagnostic.[10] Growth hormone stimulation tests do not predict efficacy of GH therapy and are not different from the non-TS population as well although some overnight neurosecretory studies suggested that TS patients may have lower overall GH secretion when looking at hourly secretory studies.[55] Formal growth hormone stimulation tests are not necessary as a prerequisite for starting treatment with growth hormone in TS patients. GH should be started[10,11,15,18] as soon as there is growth failure, decreased growth velocity and/or there is a prediction of a subnormal final adult stature compared to non-TS peers or compared to mid-parental height percentiles. If a Y chromosome is present, GH treatment should generally be delayed until a gonadectomy has been performed so even a remote possibility of growth hormone potentiating a gonadoblastoma is eliminated (see below). The earlier initiation of growth hormone itself allows earlier and more age-appropriate use of estrogen in TS patients. Several studies have shown improved behavior and social interactions, increased independence and happiness as well as perceptions of being more intelligent, attractive and popular under such circumstances.[10,56]

Figure 6 presents an excellent example of (later-than-ideal) initial growth hormone treatment, then oxandrolone soon afterwards and finally estrogen therapy as the third step in the combination treatment paradigm presented earlier and with final height very near mid-parental height expectations. GH[10,11] is generally started at 0.05 mg/kg/day when growth drops below the 5th percentile on the normal growth charts (seen here and "overlooked" at age 5 years); this can occur as early as 2 years of age. GH is administered every night of the week subcutaneously by syringe or pen (or recently with weekly GH injectable formulations) with consideration for increasing the dose at or around puberty and with an aim of maintaining IGF-1 levels in the mid to slightly higher than mid-normal ranges for girls. Some authorities recommend starting at higher dosage (up to 68 ugm/kg/day) if a TS girl is significantly short and catch-up growth is desired while titrating the dose according to IGF-1 levels.[10,11] GH usually is continued until expected correction of height and/or documented epiphyseal closure occurs.



Oxandrolone at a dose of 0.0625 mg/kg/day orally is generally added at about age 8-10 years to provide a small dose of androgens that would usually also be produced by the ovary in non-TS girls and to improve growth velocity while growth hormone continues.[10,11] Rarely oxandrolone produces clitoromegaly as well as glucose intolerance. The longer the duration of estrogen-free GH treatment, the later epiphyseal closure and the taller the final height likely to be reached.[44] Most authorities believe that trying to match the timing of

puberty with non-TS peers is also a reasonable goal for psychosocial reasons and improvement in self-esteem but also for improvement of bone density as well as cardiac function[57] so some discussion between patient, parents and pediatric endocrinologists should attempt to balance the pros and cons of timing of estrogen initiation. Empowered open discussions with the TS patient as well as family can help with mutually agreeable decisions about such timing issues. Continued growth usually occurs for only about 18-24 months after initiation of estrogen treatment as the epiphyseal plates close.[11] Side effects from growth hormone treatment with or without oxandrolone and with or without estrogen therapy seem to be not much different than the general population and also not much different than other youngsters treated with growth hormone for classical growth hormone deficiency, idiopathic short stature or following small for gestational age births.[10,11,58] These include larger-than-expected hands and feet, growth of skin nevi, cerebral edema, slipped capital femoral epiphyses, possible potentiation of malignancies, scoliosis and some increase in hypertension, glucose intolerance/diabetes or hyperlipidemia in those otherwise predisposed. Not only must all these potential side effects be reviewed in advance of starting GH treatment but surveillance and monitoring of all these areas must be part of the specific treatment plan as well. Multicenter long duration national as well as international collaborative studies around the world such as Genentech's NCGS, Pfizer's KIGS, Lilly's Genesis, Serono's Saizen Growth Study and NovoNordisk's Answer Study have all been consistent in <u>not</u> demonstrating any other risks while documenting growth benefit in TS patient receiving growth hormone according to standard treatment protocols as listed above. Side effects generally occur in about 1-2 per 1000 TS patients treated with the above protocols.[54] A prospective Multicenter GH treatment trial[46] documented about 7 cm height gain in the combined oxandrolone and growth treatment cohort. Earlier diagnosis and therapeutic regimens aimed at early GH treatment and optimizing the dose of GH may allow more normalization of height during the school-age years and thus earlier introduction of estrogens with the expectation that this would lead to better fitting in with peers from a psychosocial perspective as well as improved final heights.[9,48,53,54]

Thyroid

The most prevalent autoimmune disorder in TS is chronic Hashimoto's lymphocytic thyroiditis[10,11] with different studies suggesting widely divergent 25-85% positive antibodies.[59] Presence of positive antibodies is thought to predict future clinical thyroid dysfunction especially after age 10 years but clinical thyroid disease may occur even in the absence of positive thyroid antibodies.[60] Many TS patients have rather subtle hypothyroidism with few overt symptoms or signs. Hypothyroidism occurs in TS in about 10-30% with prevalence increasing with age, peaking around 15 years of age and then plateauing.[57] Thyroid functions tests including total T4 and free T4, total T3 and sensitive TSH assays are extremely useful for diagnosing hypothyroidism and for follow-up of treatment as replacement levothyroxine is titrated to normalize thyroid functions (especially TSH) and eliminate any goiter or hypothyroid symptoms and signs.[61] Actual diagnosis and treatment of hypothyroidism, with or without documented Hashimoto's thyroiditis, is the same in TS patients as in non TS patients with follow-up thyroid blood work every 2-4 months after treatment is begun and until stabilization of medication is documented. During childhood and adolescence, more frequent follow-up is generally suggested because of changes in dose required. After cessation of growth and pubertal hormone replacement, more stability exists and dose changes are mostly dependent upon degree of obesity and compliance so that thyroid follow-up laboratory testing can be done at 6 months intervals, perhaps even at annual visits if there is documented normality by lab work and good compliance persists.

Hyperthyroidism as well can occur although exact prevalence remains elusive in TS, perhaps in the range of 2-8%.[10,11,57,58] Thyroid function testing to confirm diagnosis of hyperthyroidism in TS is no different than any other patient[59] with elevated total and free T4, elevated total T3 (and sometimes free T3) as well as suppressed TSH. Thyroglobulin and thyroid peroxidase (microsomal) antibodies are usually, but not invariably, positive and thyroid stimulating immunoglobulins (TSI) as well as thyroid binding immunglobulins (TBII) also are often positive. Some endocrinologists suggest following such TSI and TBII levels and when their levels drop, to consider that remission has occurred and adjust treatment accordingly. Usually, medical treatment of hyperthyroidism includes anti-thyroid hormone blockade using methimazole or carbamizole to normalize hyperthyroid symptoms and signs as thyroid functions are blocked

and then to attempt to induce a remission. Earlier, propylthiouracil was also a possible treatment medication but more recent concerns about liver function abnormalities has removed propylthiouracil from the preferred therapeutic armamentarium.[62] Radioiodine can also be used as first line treatment of hyperthyroidism or be reserved for when blockade failure does not occur, medication noncompliance is suspected, side effects from medical blockade occur (ie. allergy, liver enzyme abnormalities) or recurrence of hyperthyroidism occurs.[63] Ultrasonography and radioiodine as well as technetium thyroid scanning is usually not needed for diagnosis of hyperthyroidism unless there is a question of diagnosis with discordant symptoms, signs or lab results or if there is some suspicion of a hyperfunctioning nodule. Nuclear scanning may be helpful if radioiodine therapeutic treatment is considered to help determine appropriate dosage. Surgery[59] with removal of a hyperthyroid goiter is rarely needed for hyperthyroidism except when medical treatment is unavailable and similarly radioiodine treatment is also unavailable or not acceptable – or if there is a defined, hyperfunctioning thyroid nodule.

All TS guidelines suggest that annual thyroid screening occur usually with total T4, free T4 and TSH but sometimes also with periodic thyroid antibodies as well[10,11,15,18] with the hopes of detection before significant symptoms and signs develop or there is any further compromise in growth in youngsters or lipid and cardiac abnormalities in adults.[64]

Metabolic syndrome/glucose intolerance, diabetes mellitus, hyperlipidemia

Type 2 diabetes mellitus is 2-4x more common in women with TS[27] and glucose intolerance or the metabolic syndrome may be even more common, perhaps as much as 10-34% of TS patients.[10,11,65] Insulin resistance and hyperinsulinemia are defects in carbohydrate metabolism that may already be present during childhood in TS and with increasing age and/or excess weight, prevalence of glucose intolerance increases.[66]

Treatment with growth hormone and/or oxandrolone is thought to aggravate the glucose intolerance but not generally require any specific diabetes treatment clinically.[67]

Avoiding obesity and maintaining normal body weight throughout life with increased daily exercise and fewer daily calories in TS as well as more recent studies showing benefits of low carbohydrate/ketogenic dietary recommendations, is as important as

in non-TS patients with the hopes of preventing deterioration of glucose tolerance and progression to frank clinical diabetes that may occur in TS adults.[68]

Most TS studies do not suggest an increase in Type 1 diabetes mellitus in TS patients despite the increase in other endocrine autoimmunopathies such as Hashimoto's thyroiditis and celiac disease.[10,11] Lipid abnormalities in some studies are abnormal while in others there is not much difference from the general population except for what would be expected with comparable weight excess.[10,11,27,37,38]

Celiac

Celiac disease is another of the autoimmunopathies that is more common in TS than in the non-TS population without about a 10x increased prevalence and a diagnosis in about 5-6% of the TS patients.[69] Of those girls who had celiac disease, 40% also had concomitant Hashimoto's thyroiditis. Some of the TS patients had symptoms whereas many did not.

Diagnosis of celiac disease was based upon positive transglutaminase antibodies, endomysial antibodies and usually also with positive antigliadin antibodies as well with confirmation by small bowel biopsy. Treatment response to a strict gluten-free diet was as expected with celiac disease in the non TS population. Concomitant iron deficiencies as well as vitamin D deficiencies/insufficiencies (see below) can occur with untreated celiac disease and there can also be further height compromise. Quality of life also seems to increase when improvement and gluten free compliance takes place, as expected.[68-70]

Inflammatory bowel disorders and other gastrointestinal problems

Infantile feeding problems are more common in TS babies but usually without obvious explanation. There can be concomitant gastroesophageal reflux as well as outright dysmotility. Later, intestinal telangiectasia, hemangiomatosis and other gastrointestinal vascular malformations seem to be more common in TS patients[10,11] with an increase in gastrointestinal bleeding, sometimes massive. Inflammatory bowel disease (IBD), not just celiac disease, also seems to be more common in TS including about 3% of TS with Crohn's disease and ulcerative colitis.[70] Often such IBD[71] present in the late teenage years but there does not seem to be any correlation with

oxandrolone, growth hormone, estrogen or progesterone treatment, per se. Growth retardation is often seen and classical IBD symptoms such as severe bloody diarrhea, abdominal pain and weight loss are not always so obvious. There may be concomitant osteoporosis, depression and unexplained fatigue. (see below) TS patients who are undiagnosed and present with growth deceleration or short stature as well as delayed puberty must have an evaluation for inflammatory bowel disorders as well as evaluation of other endocrine disorders such as hypothyroidism and primary hypogonadism; all can co-exist, of course.[37,38,72]

Hypovitaminosis D, osteopenia, and osteoporosis

TS patients often will have an osteoporotic appearance of their bones even in childhood and some believe that this is primary related to SHOX deficiency while others suggest subtle estrogen deficiency is causal.[11] Both may occur. Comparison bone density studies show abnormalities in TS patients but when corrected for height of the TS patients there are fewer such abnormalities than when comparison studies are performed by age matching of patients.[73] Adult TS patients appear to have an increased risk of abnormal bone density by DXA scans, more osteoporosis and more fractures as well. Both growth hormone therapy as well as estrogen therapy may help these problems[74] particularly if they are started earlier than in past years with earlier diagnosis of TS in the first place.[10,11,37,38] Low calcium intake and low vitamin D levels are common in many parts of the world and perhaps likely to have more relevance in TS patients since short stature, thyroid and estrogen problems and also more celiac disease as well as IBD all may contribute to problems of hypovitaminosis D. Specific questions about dietary intake of calcium and vitamin D should be routine and measurements of blood vitamin D levels should be considered to document the need for appropriate supplementation. If not already done, then at the end of the adolescent years, a baseline bone density DXA of the hip and the lumbar spine as well as total vitamin D levels every 4-12 months should be helpful with 3-5 yearly follow-up DXA scans recommended by TS guidelines.[15,18,58,70]

Primary ovarian failure, delayed or absent pubarche and reproductive system

Lack of sexual development[7,9,10,11,75] (breast development, feminine body contours and menstruation) during adolescence is

another hallmark of TS with primary gonadal failure (low estrogen and high gonadotropins; hypergonadotropic hypogonadism). Most girls will need induction of puberty and treatment with sex hormones for the remainder of the normal reproductive period. The major function of the ovaries, production of female sex hormones, is abnormal in TS patients as are the number of eggs in the ovaries. The other female reproductive organs (fallopian tubes, uterus and vagina are present and function normally although are obviously under-stimulated without estrogen being available or provided. Pubic and axillary hair usually occurs since the adrenal glands produce normal amounts of androgens responsible for such effects. When there is a chromosomal defect in the germ cells, the process of oocyte loss is accelerated with more stromal fibrosis. The triggering mechanisms involve oocyte-specific apoptosis defects but the process of oocyte loss and fibrosis is neither absolute nor inevitable. Differences occur in relation to exact genetic deficits (45XO vs ring vs isochrome vs mosaicisms) and even from patient-to-patient. Older TS literature suggest that this is a universal phenomenon but some TS patients will have spontaneous breast development while others will have spontaneous menarche only to have menses stop in the later teenage years. Still other TS patients will have normal puberty, normal menses and have premature menopause or secondary amenorrhea as the main reason for gynecologic or endocrine evaluation. Many of these TS patients, of course, have many other physical stigmata of TS but usually these have not been recognized as such except retrospectively. The uterus and fallopian tubes as well as vagina are present and function normally although not having had the benefit of normal estrogen exposure so that size may be somewhat smaller until estrogen is provided. Rarely pregnancy may occur in TS including those with classical 45XO karyotypes. Counseling about the expectations and future management of TS needs to include the likelihood of gonadal failure and infertility but not its inevitability while realizing that reproductive failure is high.[11] The hypothalamic-pituitary-gonadal axis[76] seem to be very normal in TS patients, functionally consistent with primary agonadism or hypogonadism so that newborns often – but not inevitably – have elevated gonadotropins, FSH more than LH (sometimes both) suggesting intrauterine and neonatal hypo-estrogen status. Normal neonatal gonadotropin levels, however, also occur not infrequently so that measurement of gonadotropins and especially FSH in a neonatal screening sense would not always be helpful. This rise in pituitary

gonadotropins lasts for several weeks to months after birth and then the hypothalamic and pituitary regions "become quiet" during the school-age years until the normal pituitary "wake-up" in the pre-teenage years. Prior to about age 4 years, including in the newborn and infancy period, gonadotropin evaluation would be helpful but then not until the peripubertal second rise would there be "sufficient feedback" to expect low estradiol and elevated gonadotropins on random sampling.[77] Thereafter, in TS patients, rising gonadotropins may also be useful in establishing to help elucidate the diagnosis.[10,11,23] Usefulness of pelvic ultrasonography in the neonate or school-age child have been inconsistent with some studies suggesting that detectable ovaries may be associated with future preservation of some ovarian function at puberty.[78]

Ideally, if growth hormone is available and affordable, estrogens should not be given alone to TS patients since estrogens by themselves will not increase final height. Estrogens will produce a temporary increase in height velocity, of course, but ultimately by themselves will advance the bone age more quickly than desired producing lower final stature. The timing and dosing of estrogen replacement after growth hormone (and usually also after oxandrolone) should attempt to mimic normal pubertal development.[7,9,10,11,15,18,79] This allows growth of the breast tissue, changes in body habitus as well as growth and development of the uterus, fallopian tubes and vagina. Sexual interest is directly or indirectly related to estrogen availability and improvement in lipid levels as well as bone mineralization also are directly related to estrogen levels in the growing child, adolescent and adult woman. Improvement in IGF-1 levels also occur concomitantly with sex steroid rises in peripubertal/pubertal youngsters and body composition effects are notable.[10,11] Estrogen replacement treatment protocols as reported in pediatric endocrinology textbooks for TS are enormously variable[80] without many directly compared one-to-the-other in any prospective, randomized study. Conjugated estrogens such as Premarin® or synthetic estrogens such as ethinyl estradiol or 17-β-estradiol are available and used with some available in tablet as well as transdermal forms. Transdermal formulations[81] may be more physiologic since they do not pass through the liver for their metabolism and also can be more directly measured in the blood with modern hormonal assays. In recent years transdermal estrogen seems to be favored by patients, family and health care providers.[82] There is no data establishing the optimal initiation dose or regimen

nor the optimal adult dose and regimen to induce and maintain menses, breast and vaginal health, libido and at the same time minimize breast cancer and other estrogen-related cancer and liver risks. Most females with TS will need appropriate hormonal replacement therapy that should continue until a time of usual menopause, approximately 50-57 years with a goal of mimicking the normal menstrual pattern but this must be individualized for each patient. Similarly, when and how to add progesterone to estrogen therapy to help mimic more normal menstrual function, is not optimally known.[10,11] Typical starting estrogen doses (2-4 mg/day) have been approximately 25% of the usual adult dose with decisions about how quickly to advance estrogen levels based upon the timing of puberty desired, degree of breast enlargement wanted, actual height and bone age at the time of initiation of estrogen treatment and the potential for further height acceleration as well as the maturation of female identity. After about two years of unopposed estrogen treatment, progestin is usually added for about 2 weeks of each monthly cycle to help induce menses and diminish the risk of endometrial hyperplasia and carcinoma. 5 mg of medroxyprogesterone (Provera®) or 200 mg of micronized progesterone are generally used with some improvement in sleep and reduction in vaginal bleeding amounts with the micronized progesterone according to some authorities. Low dose combination estrogen/progesterone birth control pills have also been prescribed to help lower costs, improve compliance and allow menstruation. Transdermal estradiol[83,84,85] can also be used at initiation or after some time with oral treatment if this is desired and as the dose (50-200 ugm/day) is slowly increased levels of blood estradiol can be used to help with titration decisions. Some add a small dose of testosterone if there is persistent decreased energy or libido but evidence based dosing decisions for all of these hormone replacement options remains lacking.[9,10,11,15,18,49,50,51,54,82]

Spontaneous fertility is rare among patients with TS and more likely in women with mosaicism rather than classical 45XO karyotypes. But there may be more risk of spontaneous miscarriage, twins and other chromosomal problems (ie. more Down Syndrome, spina bifida and congenital heart disease) than in the general population.[37,8] 35% of such offspring may have TS.[86] Appropriate sexuality counseling[87]preconception counseling is extremely important and discussions about how and when and with whom to include re: infertility, fertility options are also important, frequently

overwhelming and almost always emotionally taxing. Individualizing such concepts appropriate for age and development, maturity, family desires need to be addressed by the health care team members so that a consistent, uniform approach for each patient can be advanced.[88] Discussions[89] about adoption as well as new methods of in-vitro fertilization, preservation of oocytes and donor eggs show promise but are complex and expensive. Particular attention to monitoring and treating blood pressure and subtle cardiac disease particular aortic dissection potential is very important during TS pregnancies because of such high risk consequences and this too must be addressed by the health care team. Experienced genetic counseling as well as cardiology and obstetrical staff must also be involved as appropriate for the circumstances. Discussions should include information about the higher risks of preterm birth, small for gestational age babies and fetal malformation and chromosomal abnormalities as well as transition from adolescent care to adult care.[90,91,92]

Girls or women with TS karyotypes that contain Y material such as mosaic 45XO/46XY appear to be at increased risk for development of gonadoblastomas and more associated malignant gonadal tumors.[10,11,15,18,37,38]

Some but not all develop virilizing syndromes with clitoromegaly or partial labial fusion. If about 5% of TS patients have a Y chromosome as part of their karyotype, estimates suggest that about 15-20% of this group will develop a gonadoblastoma.[93,94] Gonadoblastomas may be microscopic or may present as a mass, sometimes with calcifications. They may present in childhood or later. Most but not all authorities recommend prophylactic gonadectomy, usually laparoscopically if there is Y chromosomal material or markers and postponement of any growth hormone treatment until this is done to minimize any malignant potential.[95]

Perinatal steroid insufficiency in TS may also imprint differentiated functions in later life for TS women.[78] Research into the effect of estrogen deficiency in the fetus, in infancy and childhood and the possible long term consequences of such deficiencies may help answer some of these questions about brain function, psychosocial function and bone mineralization in the coming years.

Summary

Turner Syndrome resulting from a complete or partial absence of one X chromosome is the most common occurring chromosomal

abnormality in females. Up to 99% of fetuses with 45XO karyotype are believed to end in spontaneous abortions, primarily in the first trimester.[96] Numerous problems exists in the newborn period and continue into childhood and adolescence as well as adulthood and include short stature, multiple birth anomalies, cardiac and gastrointestinal problems (celiac disease and inflammatory bowel disorders) as well as renal anomalies and hypertension. Primary gonadal failure is a hallmark of Turner Syndrome and combination treatments with growth hormone, oxandrolone and eventually with estrogen and progesterone have produced great success in increasing height and improving quality of life.[97] Psychosocial issues add to the complexities as do skin, eye and ear difficulties, neuropsychosocial and orthopedic problems. It is important to keep in mind that non-diagnosis/misdiagnosis with median age of diagnosis estimated to be 15 years of age and "never diagnosed" occurs in approximately 20% according to the latest literature reviews.[98,99] Being aware of the potential diagnosis and making the diagnosis earlier than ever before remains a major challenge for health care professionals around the world but is do-able.

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MANAGEMENT OF CHILDREN WITH SILVER RUSSELL SYNDROME

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Introduction. Definition

Silver-Russell syndrome (SRS) is a rare congenital clinically and genetically heterogeneous entity, caused by (epi)genetic alternations. The disorder is characterized by prenatal and postnatal growth retardation, and some particular features.[1]

SRS was first mentioned by Silver et al [2] and Russell [3] who, independently described similar features in children born small for gestational age (SGA), with body asymmetry, particular facial features and postnatal growth retardation.

It is very important ot distiguish children with SRS from those with idiopathic intrauterine growth retardation (IUGR) or SGA and postnatal growth failure, aiming for the proper management of each patient.

IUGR diagnosis is based on at least two ultrasonography measurements, at least 2 weeks apart, with fetal weight below the 10th percentile for gestational age. IUGR doesn't mean that the newborn will be SGA, for sure.[4]

SGA means that, at birth, the weight and/or length of the newborsn are less than −2 SDS for gestational age, if accurate anthropometry (including weight, length and head circumference) and reference data from a relevant population are used at birth.[5]

Unlike the two mentioned categories, SRS is a syndromic growth disorder presenting, besides prenatal and postnatal growth failure, many particular features, which are more evident at young age and diminish during growth. The most frequently encountered characteristic features of children with SRS include relative macrocephaly at birth, proeminent forehead, with frontal bossing, triangular face, body asymmetry and feeding difficulties.[6-9]

SRS is a clinical diagnosis, but molecular testing could identify an underlying defect in around 60 % of the patients that clinically meet the criteria for this diagnosis.[6]

These findings might be of help, in order to define the subtype and choose the correct management for each patient.

The management of children with SRS requires a multidisciplinary approach with a multidisciplinary team, including the pediatric endocrinologist, genetics specialist, gastroenterologist and other specialists. The issues that should be solved include severe feeding difficulties, hypoglycemia, growth failure, motor and speech delay, body asymmetry, scoliosis etc. [7-9]

Because data from literature presenting results from controlled trials are limited, in 2016 a Consensus Meeting was organized to develop the guidelines for the diagnosis and management of the patients with SRS.[10]

Epidemiology

The exact incidence of SRS is still unknown, but it may be more common than it was previously estimated. The figures reported so far, are ranging between 1:30.000 to 1:100.000.[11]

Genetic testing

The molecular etiology remains unknown in a substantial proportion of SRS patients with characteristic clinical features. A negative result at the molecular test does not mean that the patient is not a SRS.

As already mentioned, an underlying molecular cause can currently be identified in around 60% of patients clinically diagnosed with SRS.[7] Chromosomes 7 and 11 are consistently involved in all individuals who meet the strict diagnostic criteria of SRS.

In 30-60% of the patients, loss of methylation on chromosome 11p15 (11p15 LOM) is the underlying mechanism, while maternal uniparental disomy for chromosome 7 (upd (7) mat) is seen in ~ 5–10% of patients. [7,12,13]

The recommendations are that, if testing of both 11p15 and chromosome 7 is negative, additional molecular testing can be considered (10).

Clinical diagnosis

The RSS diagnosis is, mainly clinical, according to the Netchine-Harbison Clinical Scoring System (NH-CSS). This scoring

system was proposed by Azzi and colleagues in 2015, [14] and adopted by the Consensus Guideline to be used for the clinical diagnosis of SRS. The following six features are included in this score:

- SGA at birth;
- Postnatal growth retardation;
- Relative macrocephaly at birth;
- Protruding forehead;
- Body asymmetry;
- Feeding difficulties / low BMI.

The NH–CSS includes four objective criteria, while the other two-protruding forehead and feeding difficulties- remain subjective.

It is recommended that only patients scoring at least four of six criteria, including both protruding forehead and relative macrocephaly, should be diagnosed as 'clinical SRS' (previously known as 'idiopathic SRS). [10]

Relative macrocephaly at birth is defined as a head circumference at birth ≥1.5 SDS above birth weight and/or length SDS. This feature and a protruding forehead are the two features in the NH-CSS that best distinguish SRS from non-SRS SGA. [7,10,14,15]

The NH-CSS has proved to be more sensitive than other previous scoring systems[7,16] and, also, had the highest negative predictive value (89%), which gives a high degree of confidence that patients who have less than four of the six clinical criteria for diagnosis, are truly unaffected by SRS.[10]

The system is easy to use in clinical settings (Tabel 1).[10]

Table 1. Netchine-Harbison clinical scoring system. [10]

Clinical criteria	Definition
SGA	≤-2 SD for gestational age
Postnatal growth failure	Height at 24 +/- 1 month ≤-2 SDS Height ≤-2 SDS below mid-parental target height
Relative macrocephaly at birth	Head circumference at birth ≥1.5 SDS above birth weight and/or length SDS
Protruding forehead	Forehead projecting beyond the facial plane on a side view as a toddler (1-3 years)
Body asymmetry	LLD of ≥0.5 cm or arm asymmetry or LLD<0.5 cm with at least two other asymmetrical body parts (one non-facial)
Feeding difficulties and/or a low BMI	BMI≤-2SDS at 24 months or current use of feeding tube or cryproheptadine for appetite stimulation

LLD - leg length discrepancy; SDS - standard deviation score;
SGA - small for gestational age.

Besides the main diagnosis criteria, included in the NH – CSS, many others have been described in SRS patients, even if some of them are present also in non- SRS SGA born children. Some of these are, still, much frequently encountered in SRS patients: triangular face, fifth - finger clinodactyly, micrognathia, low muscle mass, excessive sweating, low set/ rotated ears and down-turned mouth, high pitched voice, prominent heels and delayed closure of fontanelle, male genital anomalies, speech and motor delay, crowded or irregular teeth, hypoglycaemia, scoliosis and kyphosis.[10]

The diagnosis will start from the clinical score, but a positive molecular result will be of great help in choosing the correct management.

And that's because, different molecular findings are associated with various clinical expressions and the response to treatment will, also, be variable. This means, that molecular testing will enable the inclusion of SRS patients into subgroups, which can lead to more individualized management.

According to the guideline, molecular testing should be done if ≥3 of six clinical criteria are present in a patient.[10]

On the other hand, a performant clinical scoring system allows the establishment of a correct diagnosis, and, consequently the access to appropriate treatment, also in patients who do not have access to molecular testing or whose tests are negative.

Management of SRS patient

Because of the numerous specific issues in this syndrome, a multidisciplinary approach will be the most appropriate in order to manage the disease. These issues include growth failure and early, severe feeding difficulties, hypoglycemia and gastrointestinal problems, body asymmetry, scoliosis, motor and speech delay.[10]

The multidisciplinary team should include pediatric subspecialists like: endocrinologist (coordinator of the medical team), gastroenterologist, dietician, clinical geneticist, craniofacial team, orthopedic surgeon, neurologist, speech and language therapist and psychologist.

Early feeding problems

The SRS newborn baby has length deficit, but, soon after birth, weight SDS drops below length SDS, due to feeding difficulties. The SRS neonate presents early failure to thrive because of a poor appetite associated with gastrointestinal functional or structural

problems, including gastro-esophageal reflux, delayed gastric emptying, constipation, malrotation. [7,14,17]

Data from literature show that the gastrointestinal problems are present in more than 70% of the SRS patients, including severe gastroesophageal reflux (55%) which often results in persistent vomiting after the age of 1 year. Constipation is also common, particularly after the age of 2 years.[17]

The first two important issues during infancy and second year of life are: to avoid hypoglycemia and ensure a proper nutritional status and, consequently, an appropriate weight and height gain.

Meanwhile, health care professionals should be aware of the risk of metabolic and cardiovascular disorders later in life, if postnatal weight gain is too accelerated. That means rapid postnatal catch-up by overfeeding, should be avoided.[10,18]

These goals should be solved before the age of 2 years, when growth hormone (GH) therapy should be initiated.

Before starting GH therapy into these children, another issue should be taken into consideration: their abnormal body composition. SRS children have low muscle mass, being light for length.[6,14,19] That is why, normal BMI targets are excessive for this group of children, and, some authors[20] are suggesting to use other targets in the management of SRS children.

The following targets are currently used in some centers: Waterloo score 75-80 %; weight-for-length SDS (−2 to −1) *in the first year of life*; BMI target SDS between (−2 to −1) after the first year of life.

For children aged 2–4 years preparing for GH therapy, targets are: weight 75–85% of the 50th centile weight for length or height and/or BMI 12–14kg/m^2, (using height measurements on the longer side if significat leg length discrepancy is found).

If weight is below 70% of the ideal weight for length or height, growth velocity will be compromised, despite GH treatment.

For children older than 4 years, the optimal target BMI will depend on their muscle mass.[10]

Prevention of hypoglycemia

Hypoglycemia is frequently encountered in SRS children, especially during night, with asymptomatic episodes.[21] These episodes are explained by the low muscle and liver mass, and disproportionately large-for-size brain, associated with feeding problems, especially in children younger than 5 years.

It is required to evaluate hypoglycemia related to fasting physical activity or illness, by measuring ketonuria (the ketones in the urine). This will allow to evaluate the safe fasting time for the child.

Nocturnal hypoglycemic episodes can be avoided by adding high molecular weight glucose polymer (for infants under 10 months) or uncooked corn starch (for older infants and children) at the latest evening meal.[22]

During acute, febrile, illness or in preoperative states, intravenous glucose (10%) might be required. That is why, SRS children should be admitted in the hospital during acute illness, if hypoglycemia with ketonuria occurs.[10]

Glucagon is not useful to correct acute hypoglycemia in SRS children, firstly, because of the poor glycogen stores in the low muscle and liver mass, and, secondly, because of reduced gluconeogenesis capacity.[10]

In cases where these interventions are not effective, and other causes of hypoglycemia have been excluded, enteral feeding might be considered (gastrostomy or jejunostomy tube). When hypoglycemia remains a problem, early GH therapy should be considered.[23,24]

GH Therapy

SRS is an indication for growth-promoting GH treatment under the SGA registered license. SRS was the only syndrome to be included in the clinical trials of GH in short children born SGA that, later, led to the indication of GH therapy in patients with SRS.[25-30]

In 2007, according to the SGA consensus statement, early treatment with GH was recommended (from age 2yrs) in SGA born children, including those with RSS, with severe growth retardation (length SDS ≤-2.5).[5] Some cases make exception to this current SGA licensed indication (in some centers) with the indication of starting GH therapy below the age of 2 yrs: in case of severe hypoglycemia, severe malnutrition despite nutritional support, severe muscular hypotonia.[10]

Besides promoting growth, the expected benefits of GH therapy in SRS children include increased appetite, thus reducing the risk of hypoglycemia, increased body composition with increased lean body mass, and, consequently an improved motor function.[10,31,32]

The recommendations are to start GH treatment with the lowest dose of 35 μg/kg per day, as soon as possible, after correcting

caloric deficits. The lowest dose that results in catch-up growth should be maintained (the maximum dose accepted 70 μg/kg per day). Cessation of therapy is recommended if growth velocity decreases below 2 cm/ year (over a 6-months period) and, re-evaluation of the underlying diagnosis, adherence to treatment, associated pathologies, IGF1 and IGFBP3 responses. GH therapy should be stopped, also, if bone age is >14 years (girls) or >17 years (boys).[10]

Puberty and bone age advancement

A particularity of the child with SRS is the bone age advancement. Bone age is delayed in early childhood, but rapidly advancement in bone age occurs around the age of 8-9 years, or even earlier in children who were overfed at younger ages, aiming to recover weight and catch-up growth. [6,33,34]

The onset of puberty is, usually, within the normal range age, but, mostly, in the first half of the range and with a faster progression.[6,19,35-37] An early, and, sometimes, aggressive adrenarche can be seen, especially in some subgroups of patients (11p15LOM).[37-39]

These characteristics of the pubertal development will shorten the period for effective GH treatment in SRS patients compared to non SRS SGA children, meaning that the height gain will be lower that expected. Some studies focused on the use of GnRH analogues treatment along with GH treatment, at the beginning of puberty, in order to improve adult height in both SRS- SGA and non SRS SGA born children, but further studies focused on SRS patients are needed.[40,41]

The general recommendations are to monitor for bone age advancement, early pubarche, rapid progressive central puberty and insulin resistance in all SRS children, and, also to consider individualized GnRH analogues treatment, for minimum. 2 years, if precocious puberty occurs, in order to preserve adult height potential.[10]

Long-term metabolic complications

It is well known that SGA born children are at risk to develop metabolic and cardiovascular diseases in adulthood, including coronary heart disease, hypertension, and metabolic syndrome (obesity, insulin resistance, dyslipidemia). Moreover, those with rapid weight catch-up have a higher risk.[42-44]

Insulin resistance is evident in pubertal or post-pubertal SRS patients, with increased fasting levels of blood glucose and insulin,

which may progress even towards the development of type 2 diabetes mellitus. In pre-pubertal SRS children, insulin resistance can be confirmed on oral glucose tolerance test (OGTT) which may reveal impaired glucose tolerance.[45,46]

GH therapy has positive metabolic effects in SGA born children (reduces fat mass, improves lipid profile, increased lean body mass and decreases blood pressure) but data concerning these effects in SRS patients are lacking, so further studies are required on this topic.

The recommendations are[10]:

- to avoid overfeeding and rapid weight gain in SRS infants, in order to prevent insulin resistance (IR) and all its consequences later in life (early and rapid progressive adrenarche and central precocious puberty, and, even PCOS in girls).
- to screen for insulin resistance indicators during GH treatment (especially in children with low muscle mass and high baseline IGF1 levels); if clinical signs of IR are present, OGTT, serum insulin and C-peptide levels are recommended)
- to promote a healthy lifestyle and diet in older children to avoid unhealthy weight gain, particularly after discontinuation of GH treatment.

Neurocognitive problems

Motor delay in SRS patients is due mostly to reduced muscle mass, but also, to relative macrocephaly reported to body size. Speech delay, verbal dyspraxia, learning difficulties and even autistic spectrum disorder are sometimes encountered in SRS patients.[7,14,15,47,48] That is why, it is recommended[10] to refer SRS children for developmental assessment in order to ensure early intervention as early as possible. Patients should be monitored closely for speech and learning difficulties, cognitive delay and psychosocial challenges, and, appropriate interventions should be ensured.

Orthopedic problems

SRS patients present frequently with limb or body asymmetry as an early characteristic feature, Other orthopedic problems may be associated in these patients: scoliosis, hip dysplasia and hand and/or foot anomalies.

Scoliosis may be aggravated with GH therapy[49] and back pain might be associated.[8,50] Limb length asymmetry is not significantly affected by GH treatment, as some studies have shown.[51]

Surgery performed to equalize limb length has proved to be effective in SRS patients.

It is recommended that SRS patients should be examined for scoliosis and length asymmetry and referred to the orthopedic team while treated with GH. [10]

Maxillofacial abnormalities

The specific triangular- shaped face might be associated with delayed dental eruption, microdontia and absence of secondary teeth.[52,53]

Micrognathia associated with facial asymmetry may impair normal chewing, while otitis media which are frequent in young SRS children seems to be improved by orthodontic treatment.[10,48] Orthodontic intervention can also help to normalize oropharyngeal function and facial appearance. An ideal team will include orthodontists, plastic surgeons and ENT specialists.

Some of the SRS patients may complain of daytime fatigue due to sleep problems, including snoring and obstructive sleep apneas, that is why, patients should be periodically referred to ENT and sleep specialists, for evaluation.[10]

Other congenital anomalies

In a reduced number of patients, renal anomalies, congenital heart defects or genital abnormalities (cryptorchidism, hypospadias in boys, Mayer–Rokitansky–Kuster–Hauser syndrome in girls) have been reported. [7,47,48,54]

Adulthood with SRS and Genetic Counselling

As mentioned before, long-term complications in patients born SGA, including SRS-SGA born children, the major risk complications are cardiovascular and metabolic, including: hypertension, dilated cardiomyopathy, type 2 diabetes mellitus, hypercholesterolemia, fatty liver infiltration, elevated glucose levels and raised HbA_{1c} levels, however, these reports might not be representative of the population as a whole.[45,46,55]

Medical care should focus on screening for all these possible disorders in adult SRS patients.

Genetic Counselling should be provided by Specialists in Molecular and Clinical Genetics, with experience in imprinting disorders.

Data are limited regarding the risk of parents of children with clinically diagnosed SRS having another child with SRS; however, the overall risk is probably low. Similarly, the offspring risk for individuals with clinically diagnosed SRS is likely to be low.[10]

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CONGENITAL HYPOPITUITARISM CAUSED BY MUTATION IN PROP-1 GENE: THE STORY OF FOUR FAMILY MEMBERS

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Abstract

Introduction:

Congenital hypopituitarism refers to one or more pituitary hormone deficiency, resulting from abnormalities during foetal pituitary development. These problems may be due to genetic mutation or structure alteration during foetal period of life. PROP1 mutations are the most frequent genetic cause of multiple pituitary hormone deficiency. These mutations are characterized by clinical variability, regarding time of onset of hormonal deficiencies and hypophyseal morphology.

Case reports:

We describe 4 patients (1 male and 3 females) aged 51, 59, 66 and 69 years, members of the same family, diagnosed with combined pituitary hormone deficiency due to PROP1 mutation. No previous family history of pituitary deficiency was reported, and their parents were not consanguineous.

All the cases presented dwarfism due to GH deficiency, as initial sign. Due to lack of GH substitution therapy, their final adult height varies between 130 and 146 cm.

The diagnosis of pituitary insufficiency was established between 10 and 20 years-old, mainly due to growth retardation and absence of pubertal onset. At first admittance into our clinic, all the patients presented combined pituitary insufficiency, for all the anterior pituitary hormones, including corticotrope secretion. They were under substitution therapy for thyrotrope and corticotrope insufficiency, the male patient being substitute with testosterone, too. GH-deficiency was never treated in all these cases.

Imaging studies revealed anterior pituitary atrophy in two cases and pituitary enlargement due to a microcysts in the youngest sister.

The genetic study was performed in the youngest sibling, detecting a homozygous mutation in the PROP1 gene (c.301–302del).

Associated comorbidities, nowadays, are represented by many other diseases, including coronary artery disease, hypertension, osteoporosis, and obesity. Some of these comorbidities are, probably, in relation with missing substitution therapy for GH deficiency.

Conclusion:

These cases reflect the clinical expression of the pituitary functional impairment, including ACTH-secretion, due to PROP1 gene mutations. Some of the associated comorbidities are in relation with life-long absence of GH-substitution therapy.

Key words: pituitary insufficiency, familial, PROP1 gene, mutation

Introduction

Pituitary gland is one of the most important endocrine structures due to its role as a control centre for many others endocrine glands. Pituitary hormone deficiency may be isolated or multiple, acquired, or congenital.[1]

Congenital hypopituitarism refers to one or more pituitary hormone deficiency, resulting from abnormalities during foetal pituitary development.[2] These problems may be due to genetic mutation or structure alteration during foetal period of life.[3]

Congenital pituitary insufficiency has a broad clinical spectrum ranging from multiple severe hormone deficiency with life-threatening effects on neonates to a mild late-onset gradual development during childhood or adolescent period.[1,4,5]

In this chapter we discuss a familial form of PROP1 mutation resulting in the development of multiple pituitary insufficiency in four members of the same family.

Case-Reports

Case 1. Patient IS, male, aged 66-years-old, had unremarkable neonatal period and psychomotor development. Growth failure was noticed when he was 12 years. The diagnosis of combined pituitary hormone deficiency was established at the age of 14 years, when substitution therapy with glucocorticoid and thyroid hormones was recommended. During that period, GH substitution therapy was not possible, in Romania, so his final stature is 146 cm. He has now a body mass index of 33.1 kg/m^2. He is, currently, being supplemented with levothyroxine tablets, 50 mcg daily, prednisone tablets, 5 mg daily and testosterone undecanoate 1000 mg by intramuscular injections, every 3 months. His actual hormonal determinations (table 1) confirmed total pituitary insufficiency and revealed low values for FT4, the daily levothyroxine dose being

increased, accordingly. Total testosterone value is increased, being influenced by the testosterone injection. Biochemical lab results detected polyglobulia and hepatic cytolysis, alterations that could be in relation with testosterone therapy. Pituitary IRM indicated severe pituitary atrophy, with normal neurohypophysis and pituitary stalk. Associated comorbidities are represented by hypertension, type 2 diabetes mellitus, hypercholesterolemia, steato-hepatitis, obesity, osteoporosis with multiple vertebral fractures (T10, L1 and L4), bilateral coxarthrosis, bilateral gonarthrosis and hip dysplasia. Psychological evaluation revealed an IQ of 104, indicating an average level of intelligence.

Case 2. AI, female, aged 69, was also born by normal delivery, at term, and presented a normal neonatal and psychomotor development. Growth retardation and lack of pubertal development were noticed when she was 16 years-old and the diagnosis of combined pituitary hormones deficiency was established at the age of 20. She was treated with hormone replacement therapy for all pituitary insufficiency, except GH, currently being under treatment with levothyroxine 25 mcg daily and Prednisone 5 mg daily. Now her adult height is 130 cm and weight – 45 Kg. Her hormonal determinations (tab. 1) confirmed total pituitary insufficiency and revealed low values for FT4, the daily levothyroxine dose being increased, accordingly. Associated comorbidities are osteoporosis, coronary artery disease, steato-hepatitis, cervical spondylosis. Psychological evaluation indicated IQ above average: 113.

Case 3. VH, a 59-year-old woman, displayed typical neonatal and psychomotor development. At the age of 10, the patient was diagnosed with combined pituitary hormones deficiency after presenting with growth failure and, later, with the absence of pubertal development. Except for GH, all pituitary insufficiencies were treated with hormone replacement therapy. She is currently receiving treatment with levothyroxine tablet, 75 mcg daily and Prednisone tablet, 5 mg daily. Patient has a body mass index of 31 kg/m^2 with a final height of 130 cm. Her hormonal results (tab. 1) indicated total pituitary insufficiency with inadequate FT4 levels, so her daily levothyroxine dose was increased accordingly. A CT scan of the brain showed a suggestive aspect of empty sella. Associated comorbidities are represented by osteoporosis, hypertension, coronary artery disease, steato-hepatitis, mixed dyslipidaemia, bilateral gonarthrosis and psoriasis. Psychological evaluation revealed an IQ of 94, indicating an average/low level of intelligence.

Case 4. Patient BE, female, 51 years old, also did not have any complications throughout the neonatal period or regarding psychomotor development. The diagnosis of combined pituitary hormone insufficiency was established at the age of 5 by documenting deficiencies in GH, TSH, FSH/LH, prolactin and cortisol levels. The patient received hormone replacement therapy for all pituitary insufficiencies except GH. She is currently being treated with levothyroxine tablets (100 mcg daily) and prednisone tablets (5 mg daily). Her current height and weight are 138 cm and 51 kg, respectively. Her hormonal determinations (tab. 1) indicated complete pituitary insufficiency and revealed low values for FT4, thus the daily levothyroxine dose was increased accordingly. Pituitary MRI imaging detected features of pituitary nodular enlargement, with the presence of a microcyst. Associated comorbidities are represented by osteoporosis, asthma, hypertension, coronary artery disease, mixed dyslipidaemia and left gonarthrosis. Psychological evaluation revealed an IQ of 100, indicating average intelligence.

Tabel 1. Hormonal determinations on the first admittance in to our clinic

	Normal range	**Case 1 (M, 66y)**	**Case 2 (F, 69y)**	**Case 3 (F, 59y)**	**Case 4 (F, 51y)**
GH ng/mL	0.05-8	<0.05	<0.05	<0.05	<0.05
IGF-1 ng/mL	40-225	34.90	17.6	22.10	<15.00
PRL ng/mL	M:2.1-17.7 F: 2.8 - 29	1.05	0.64	0.38	0.63
TSH mUI/L	0.55-4.78	0.023	0.154	0.006	0.02
FT4 pmol/L	11.5-22.7	7.98	6.42	8.47	5.91
Cortisol mcg/dl	4.3-22	1.54	0.92	7.62	1.75
FSH mUI/mL	M:1.4-18.1	0	0.26	0.17	0.50
LH mUI/mL	M:1.5-9.3	0	0.09	0.08	4.94
Estradiol pg/mL	Menop: <32.2	-	35.99	18.07	21.82
Testosterone mcg/dl	187.72-684.19	1414.94 (under Testost. therapy)	-	-	-

Pituitary morphology

Pituitary morphology by imaging study was checked in cases 1, 3 and 4. Patients 1 had performed an MRI and patient 3, a CT scan, both revealing pituitary atrophy. On patient 4, MRI showed a pituitary nodular enlargement with the presence of a cystic lesion.

Molecular genetic analysis

The genetic study was performed in the youngest sister of affected siblings, BE, 51y. Sequencing of 4813 genes was performed, using the Illumina TruSight One Sequencing Panel. Genetic analysis identified a pathogenic homozygous frameshift variant c.301_302del in the PROP1 gene, located on chromosome 5q35.3. We assume that the same mutation is present in the other 3 siblings, all patients being part of the same family (*Fig.1*).

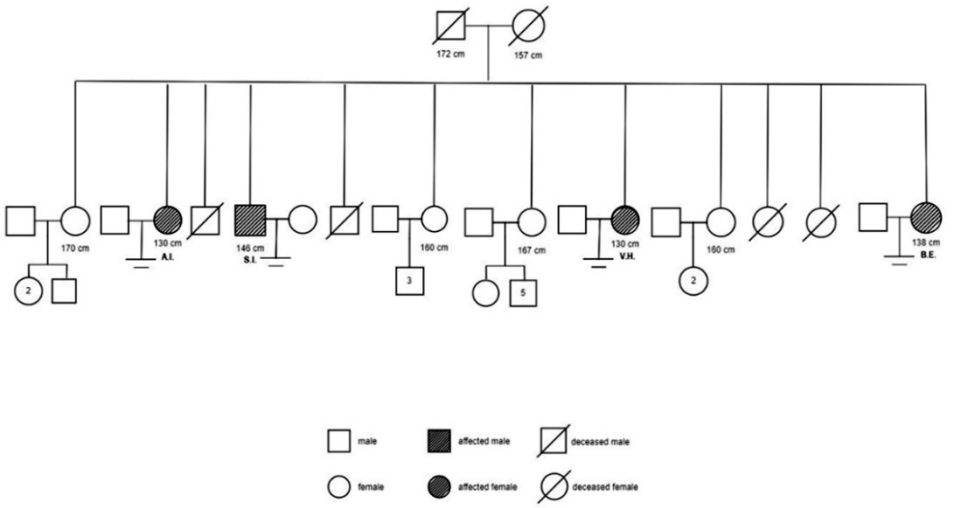

Fig.1. Genealogic tree of the family revealed affected siblings of 12 children born from the same non-consanguine parents

Discussions

The pituitary gland arises from the hypophyseal placode of the neural crest. Pituitary development is a very complex process, involving a sequential expression of many transcription factors, responsible for an adequate differentiation of the five types of cell populations – somatotrophs, lactotrophs, thyrotrophs, gonadotrophs, and corticotrophs. Embryology studies demonstrate that the anterior hypophysis is fully differentiated by 16 weeks of gestation.

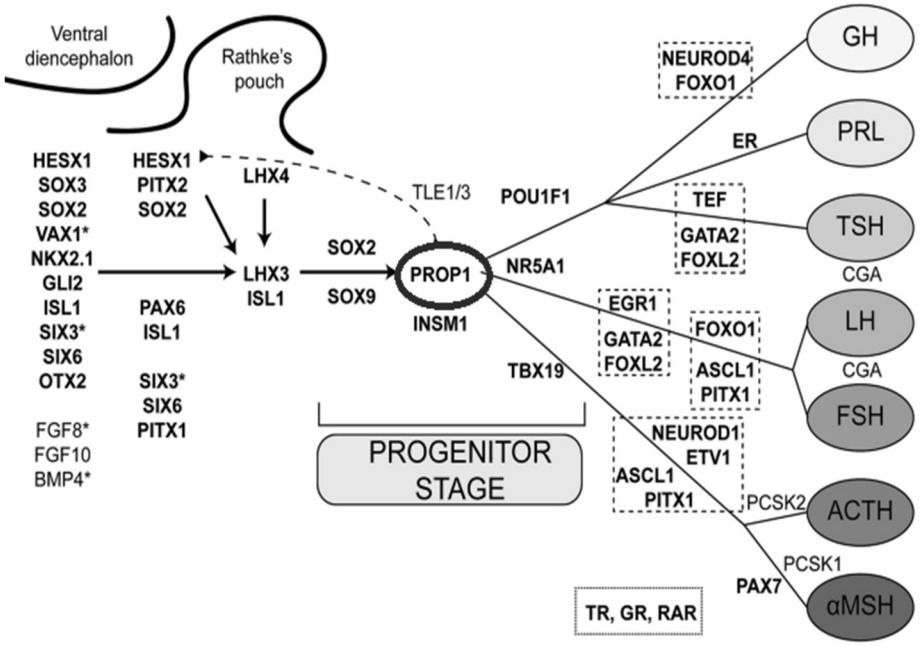

Fig. 2. Transcription factors involved in pituitary morphological and functional development (adapted after Gergics, P. Pituitary Transcription Factor Mutations Leading to Hypopituitarism, 2019.[6]

Various transcription factors are involved in coordinating these processes (*Figure 2*) and gene mutation can result in underdevelopment or arrest of pituitary development.

Any disturbances of these transcriptional factors and gene mutation will result in underdevelopment or arrest of pituitary development and reduce hormonal production.

Genetically determined forms of congenital pituitary deficiency may be caused by an abnormal gene encoding one of the transcription factors such as PROP1 (Prophet of Pit1), POU1F1 (Pit 1), HESX1, LHX3, and LHX4 that influence the formation of the pituitary gland, acting according to a specific temporal and spatial pattern (*Fig.2*).[3] Mutations of PROP1 are thought to be the most common cause of genetically determined combined pituitary insufficiency, being involved in up to 50% of cases.[7]

The PROP1 gene is located on the long arm of the fifth chromosome pair (5q35).[3,8] The most common mutation is a deletion of two base pairs described as c.301_302del, which was detected in

our case, too. The deletion results in a frame-shift mutation and premature termination of transcription. The resulting protein is deprived of the ability to bind to DNA, and, consequently, the activation of transcription is stopped.[8,9] Mutations inactivating PROP1 are manifested by both abnormal pituitary morphology and loss of its function. PROP1 expression is essential to allow Pit1-specific cells differentiation. PROP1 mutations are characterized by a variable phenotype presentation, depending on the age of the patient when the pituitary hormones become insufficient. The symptoms of combined pituitary hormones deficiency may have different clinical presentations, GH and TSH deficiency inducing frequently the first clinical manifestations.

Perinatal signs of hypopituitarism are rare in PROP1 mutation, comparing to other genetic forms of combined hormonal pituitary deficiency. The first sign is, usually, the growth failure that occur during childhood, but not before 6 years of age. In our cases, due to the lack of GH-substitution therapy their final height is between 130 and 146 cm, being obvious that they have a childhood-onset GH deficiency (CO-GHD).

According to published data in case-series, puberty onset in PROP1 mutation may vary from case to case: some of the patients have insufficient LH and FSH to initiate puberty, whereas others develop initial signs of puberty, follow by a progressive gonadotropin deficiency, so they do not complete puberty. In our patients, hormonal determinations reveal low LH and FSH. Some of secondary sexual characteristics were developed under substitution therapy with oestroprogestative or testosterone, but this therapy was not followed regularly, according to patients' reports.

Some reports have demonstrated that TSH deficiency in PROP1 mutations is variable, being reported as the first presenting insufficiency in some cases or delayed. All our patients presented TSH deficiency at diagnosis and substitution therapy was started since then. The adjustment of levothyroxine dose was done not very frequently, FT4 being low in all the cases at the first admission into our clinic.

Lactotroph function may be different among patients with PROP1 mutation. In our patients, PRL level is low, indicating lactotroph deficiency.

Regarding ACTH secretion, PROP1 seems not being directly involved in the transcription of genes necessary for the formation of corticotrophs. Nevertheless, some reports revealed a late onset of

ACTH deficiency in patients with PROP1 mutations.[8] This phenomenon could be explained by the dysfunction of PROP1 in initiating pituitary stem cell migration and differentiation. All our patients present total secondary adrenal insufficiency, being under substitution therapy with Prednisone. This aspect could be explained by the very-late evaluation after initial diagnosis.

Pituitary size or morphologic alterations in cases with PROP 1 mutation could be variable. While some patients present normal hypophysis on imaging studies, others have enlarged or hypoplasic anterior pituitary gland. One explanation for this is that anterior pituitary enlargement, presented in early childhood, will be followed by regression and involution later, resulting in an empty sella. Pituitary enlargement in patients with PROP1 gene mutations may represent a cystic hyperplasia of the intermediate pituitary lobe, as it was described by other authors.[10,11] One of our cases, the youngest sibling, had such a small pituitary cystic lesion detected on MRI. Two other patients from this family had pituitary atrophy.

About substitution therapy, we could underline that all these patients were replaced with glucocorticoids, thyroxine, and some gonadal steroids, but GH deficiency remained untreated lifelong. Nowadays, they developed a lot of comorbidities: coronary artery disease, hypertension, osteoporosis, obesity, hypercholesterolemia. Some of their comorbidities could be in relation with lifelong GH-deficiency, because today clinical manifestations of adult GH-deficiency are well-known and include cardiovascular disease, abdominal obesity, decreased bone mineral density, reduced psychological wellbeing, and quality of life.[12,13] However, this interpretation presumed that replacement of adrenal, thyroid, and gonadal deficiency was optimal. In fact, replacement, particularly with thyroxine was more likely underdosed, as it was revealed by hormonal determination from our clinic and oestrogen-deficiency was reported not systematically replaced in female-patients.

Conclusion:

These cases reflect the clinical expression and evolution of the anterior pituitary functional impairment, including ACTH-secretion, due to PROP1 gene mutations. All the patients, from the same family, developed in time many comorbidities, some of them being in relation with life-long absence of GH-substitution therapy.

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CONGENITAL ISOLATED ALDOSTERONE DEFICIENCY- A RARE CAUSE OF NEONATAL SALT-WASTING SYNDROME AND CHRONIC HYPONATREMIA

Rodica Elena CORNEAN, Mihaela VINTAN, Bianca SIMIONESCU

Abstract

Congenital isolated aldosterone deficiency (CIAD) is a very rare case of hyperreninemic hypoaldosteronism. Inherited as an autosomal recessive disorder, it is caused by aldosterone synthase deficiency (ASD).

The two different forms of aldosterone synthase deficiency (ASD) are also termed corticosterone methyl oxidase (CMO) deficiency type I and type II.

Both disorders are characterized clinically by salt-wasting, polyuria, vomiting, severe dehydration, failure to thrive and growth retardation.

In both CMO deficiency types, aldosterone biosynthesis is impaired, while corticosterone of zona glomerulosa origin is excessively produced. The two defects differ biochemically in that 18-OH-corticosterone is deficient in ASD/CMO deficiency type I, but overproduced in ASD/CMO deficiency type II.

Although hypoaldosteronism and chronic hyponatremia are reported in many other disorders, the isolated aldosterone deficiency should be suspected in all infants presenting with salt-wasting syndrome, hyponatremia, hyperkalemia but no signs of hyperandrogenism or low cortisol (hyperpigmentation, hypoglycemia, etc.).

Furthermore, as aldosterone synthase deficiency (ASD) is reported with very low incidence in the pediatric medical care (less than 70 cases worldwide) and sometimes we have to deal with limited ability to measure diagnostic steroids, genetic confirmation of the CYP11B2 mutations by using next generation sequencing (NGS) might be the shortcut to the accurate diagnosis which is mandatory for the successful treatment.

Keywords: hyperreninemic hypoaldosteronism, aldosterone synthase deficiency (ASD), CYP11B2 gene mutations, hyponatremia, salt-loss syndrome.

Introduction

Neonatal salt-wasting syndrome is a life-threatening medical condition due to the complex electrolyte and acid base imbalance (hyponatremia, hyperkalemia, metabolic acidosis, etc.)

The clinical hallmark of the salt-wasting syndromes regardless of their etiology, is the preservation of the diuresis even in the case of severe dehydration

Isolated aldosterone deficiency (IAD) as a cause of salt-wasting syndrome is a rather uncommon disorder in children with less than 70 cases reported worldwide.

Genetics

Congenital isolated aldosterone deficiency (IAD) is an extremely rare autosomal recessive inherited mendelian trait.

The main causes are the pathogenetic variants of the *CYP11B2* gene leading to aldosterone synthase deficiency.

Aldosterone synthase is coded by *CYP11B2* gene located on chromosome 8q24.3.

Two types of aldosterone synthase (AS I and II) initially known as corticosterone methyl oxidase deficiency type I and II (CMO I and II) are recognized (OMIM#203400 and 124080, respectively). They are responsible for the last 3 terminal steps of the aldosterone synthesis. Figure 1.

The common denominator is represented by the hyperreninemic hypoaldosteronism while the distinction between these two entities, resides in the level of 18-OH-corticosterone: it is deficient in ASD/CMO type I, but overproduced in ASD/CMO type II. (*Figure 1*).

Both type AS I and II deficiencies result from loss-of- function mutations of *CYP11B2* gene, but the two are not simply allelic variants.

To date, approximately 40 loss-of-function mutations (missense, nonsense, frameshift, gross deletions, etc.) in the *CYP11B2* gene have been identified.

The most common ones were the missense and nonsense mutations.

According to the autosomal pattern of transmission, both the genetic status of classic homozygous and that of the compound heterozygous have been displayed by the molecular assessment. This aspect is consonant with the reported heterogenous ASD phenotypic spectrum.

Figure 1. Pathways of steroid hormone synthesis.

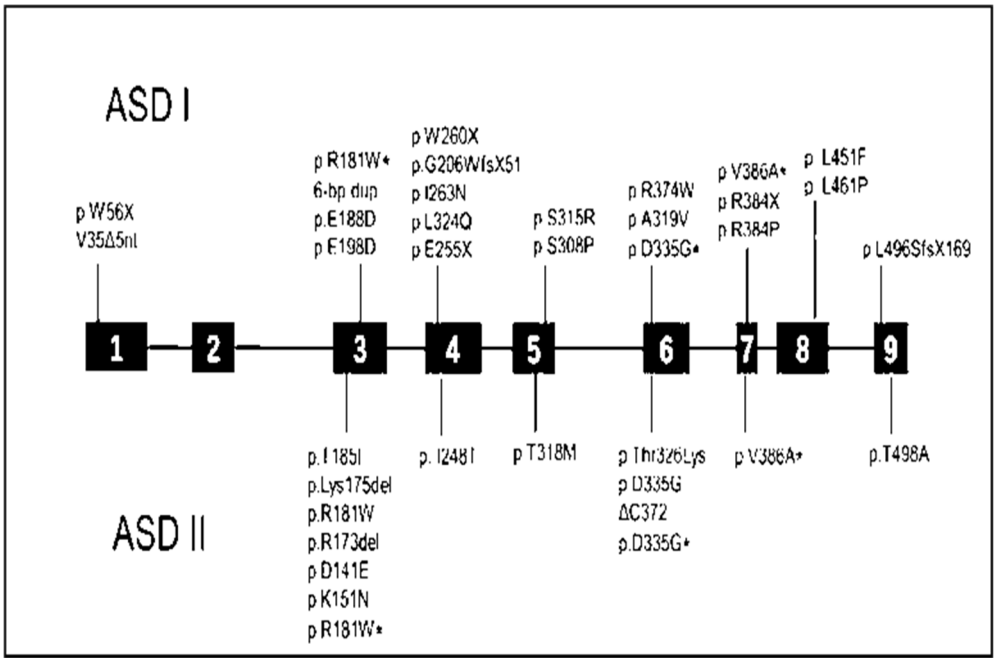

Figure 2. Analysis of novel heterozygous mutations in the CYP11B2 gene causing congenital aldosterone synthase deficiency and literature review. Hui Miao, Zhongxun Yu, Lin Lu, Huijuan Zhu, Richard J Auchus, Jiayan Liu, Jun Jiang, Hui Pan, Fengying Gong, Shi Chen, Zhaolin Lu. Steroids, Volume 150, October 2019, 108448.

Furthermore, wide variations in the severity of the clinical and biochemical evaluation seem to reside not only in the severity of the mutations or/and of their genetic combinations (e.g. compound heterozygous) but also due to the sum effects of other genetic loci or epigenetic factors (as suggested by the clinical heterogeneity reported even among patients with identical mutations in the *CYP11B2* gene).

Diagnostic criteria

Clinical and biochemical presentation

The severity of the clinical presentation of aldosterone synthase deficiency varies with the chronological age of the patient.

Young infants (within the first weeks up to the first 4 months after birth) are usually hospitalized for vomiting, dehydration and failure to gain weight (symptoms initially considered to be related to

the gastro-intestinal disorders) or for life-threatening severe hyponatremic dehydration, hypotension/decreased arterial pressure and hypovolemic shock.

However, biochemical assessment confirms the severe salt-wasting syndrome with the subsequent characteristic laboratory findings: hyponatremia, hyperkalemia, metabolic acidosis, inappropriate low or normal aldosterone concentration versus high renin activity.

NOTA BENE: as cortisol and sex steroid levels (androgens) are always normal, no hypoglycemia, hyperpigmentation or signs of virilization of the external genitalia are present!

During childhood, patients are presenting attenuated symptoms: anorexia, mild dehydration, delayed growth. Electrolyte abnormalities are reported only in early childhood while children more than 5 years old may have normal electrolytes values.

Theoretically, adults are asymptomatic but they may need salt-supplementation during intercurrent illness (e.g. gastroenteritis).

The cornerstone of the clinical diagnosis of isolated aldosterone deficiency is represented by the concomitant presence of dehydration with preserved diuresis, lack of hyperpigmentation and virilized or under virilized external genitalia, aspects which allow the differential diagnosis at a glance with any other cause of aldosterone deficiency combined with cortisol deficiency and hyper or hypoandrogenism (congenital adrenal hypoplasia, congenital adrenal hyperplasia). Table1.

Laboratory parameters

Due to aldosterone synthase (AS) deficient activity or secretion within the zona glomerulosa, conversion of corticosterone to aldosterone is impaired. Subsequently, decreased aldosterone is followed by salt-loss syndrome (due to impaired renal tubular reabsorption of sodium and its increased urinary excretion) and markedly elevated levels of plasma renin activity (PRA). Usually, aldosterone values are inappropriately low compared to these of PRA. Table II.

As a result, the common denominator is represented by the hyperreninemic hypoaldosteronism while the distinction between these two entities, resides in the level of 18-OH-corticosterone: it is deficient in ASD/CMO type I, but overproduced in ASD/CMO type II.

Similarly, the markedly elevated ratio of 18-hydroxycorticosterone to aldosterone distinguishes the patient with ASD II (higher than 40) from the patient with ASD I (lower than 10). This ratio is also reported to be the only ~~one~~ abnormal parameter in untreated adults. Table II.

Table 1. Causes of decreased mineralocorticoid synthesis or function

Causes	**OMIM**
Congenital adrenal hypoplasia	
DAX 1	300200
SF1	184757
Congenital adrenal hyperplasia	
StAR	600617
CYP11A	118485
HSD3B2	201810
CYP21	201910
CYP11B2	
Aldosterone Synthase Deficiency (ASD I/CMO I)	203400
Aldosterone Synthase Deficiency (ASD II/CMO II)	124080

OMIM: Online Mendelian Inheritance in Man, DAX1: Dosage-sensitive sex reversal-Adrenal hypoplasia congenita critical region on the X chromosome 1, SF1: Steroidogenic Factor 1, StAR: Steroidogenic Acute Regulatory protein, CYP11A: Cytochrome P450, family 11/Cholesterol side-chain cleavage enzyme, HSD3B2: 3-beta-hydroxy-steroid dehydrogenase (3β-HSD) enzyme, CYP21: CYP21A2 cytochrome P450 family 21 subfamily A member 2/21-hydroxylase, CYP11B2: aldosterone synthase, CMO type I and II: corticosterone methyl oxidase type I and II.

When salt-loss from aldosterone deficiency exceeds intake, as expected, high urine sodium (urine osmolarity >300mOsm/kg) is present. On the other hand, blood sodium deficit results in hypotonic hyponatremia confirmed by plasma osmolarity less than 275 mOsm/L.

Patients with impaired renal reabsorption of natrium due to aldosterone deficiency develop the special pattern of hypotonic hyponatremia with hyperkalemia.

This is why, evaluation of serum and urine osmolality are vital components of the evaluation of hyponatremia.

The steps of the diagnostic approach of hypotonic hyponatremia are described in *Figure 3*.

Table II. Biochemical assessment in ASD type I versus ASD type II. (Adapted from Allen W. Root, Dorothy I. Shulman. Clinical adrenal disorders, Ora H. Pescovitz and Erica A. Eugster. Pediatric Endocrinology, Mechanisms, Manifestations and Management, 2004; 593.)

Laboratory parameters	**ASD I**	**ASD II**
Cortisol	N	N
17 hydroxyprogesterone	N	N
Sex steroids (androgens)	N	N
Desoxycorticosterone	↑↑	↑↑
Corticosterone	N, ↑	N, ↑
18-Hydroxycorticosterone	↓	↑↑
Aldosterone	↓↓	↓
Corticosterone/18-hydroxy-corticosterone ratio	↑	↓
18-Hydroxycorticosterone/ aldosterone ratio	<10	<40
Plasma renin activity	↑↑	

N, normal; ↑, increased; ↑↑, markedly increased;
↓, decreased; ↓↓, markedly decreased.

Treatment

The therapeutic approach is distinctive during the acute phase of the severe salt-loss syndrome, hypovolemic shock, hyponatremia, hyperkalemia and metabolic acidosis which may require the intensive care standard protocol compared to the chronic phase in which the main goal is the mineralocorticoid substitution.

Until the hormonal results are obtained, the initial therapeutic protocol is similar to that of the most common forms of adrenal insufficiency which includes the starting of the glucocorticoid therapy immediately in order to avoid life-threatening complications.

Fludrocortisone therapy is considered the cornerstone of the long-term therapy strategy in these patients.

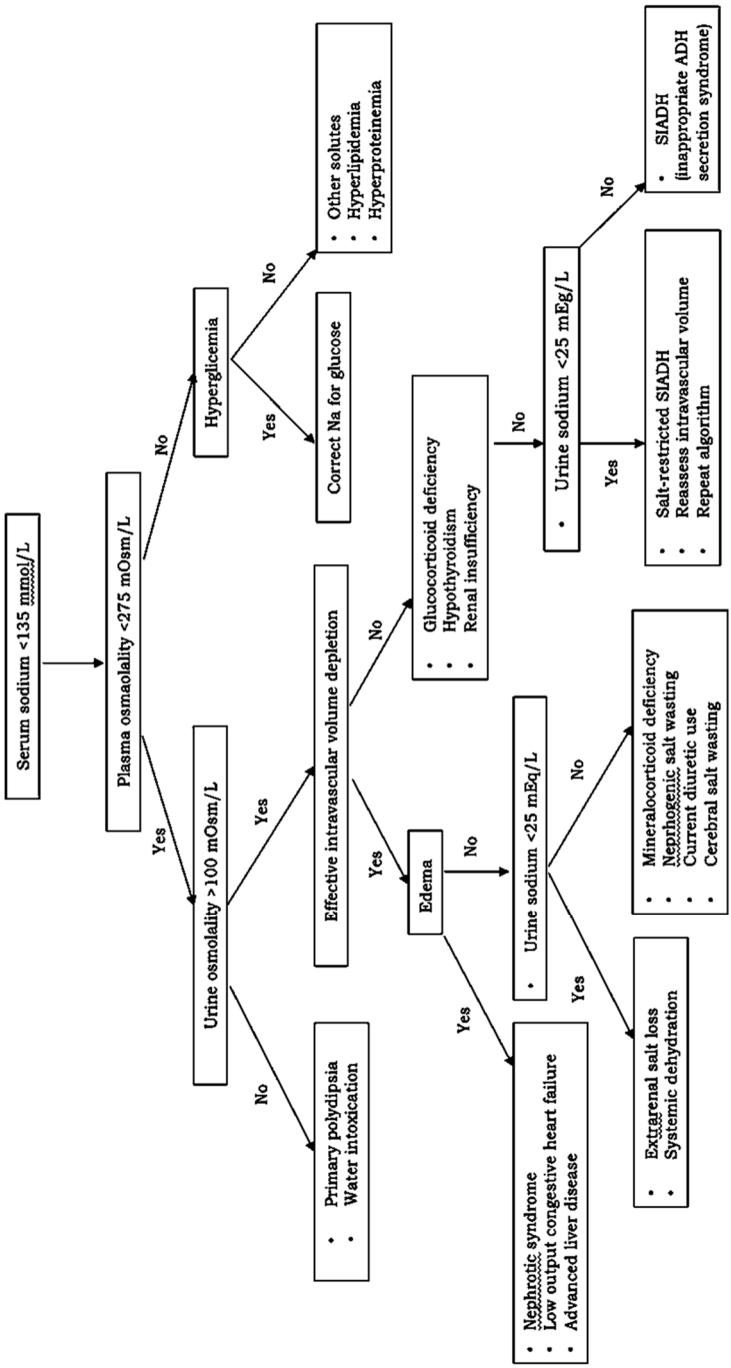

Figure 3. Diagnostic algorithm of hypotonic hyponatremia
Adapted from David R. Repaske. Disorders of water balance. Brook's Clinical Pediatric Endocrinology, 6th ed., Edited by C.G.D. Brook, Peter E. Clayton, Rosalind S. Brown. Wiley- Blackwell, 2009: 362.

Adequate fludrocortisone replacement should be adjusted based on the clinical condition, plasma sodium, potassium and renin value and not on body weight. The recommended initial dose of fludrocortisone (FC) is 0,1-0,2 mg/m^2/d. In addition, salt-supplementation is mandatory during infancy (2-2,2mmol/kg/day). Sodium chloride supplementation alone is able to maintain a serum sodium above 130mmol/l.

Even if the fludrocortisone therapy is vital during infancy for ASD patients, the need for mineralocorticoid replacement wanes with age. The potential reasons why the mineralocorticoid requirements of aldosterone synthase deficiency (ASD) children decline with age are:

- mineralocorticoid receptors are less expressed in newborns but they are better expressed with age with increased sodium reabsorption due to mature renal tubules
- mother's milk has low content of sodium but the dietary sodium is increasing with age and transition to "table foods"

Conclusion

Aldosterone deficiency due to ASD I or II is a very rare cause of hyperreninemic hypoaldosteronism in infants.

Severe salt-loss syndrome and hyponatremia in the neonatal period is responsible for the dramatic clinical presentation with hypovolemic shock and life-threatening episodes of seizure and coma.

It should always be suspected in infants with severe salt-wasting syndrome and failure to thrive but no signs of cortisol deficit or virilization.

The severity of the disease declines with the chronological age and subsequently, no fludrocortisone therapy is required. Some adult patients may be completely asymptomatic despite no mineralocorticoid substitution being given anymore while others may develop orthostatic hypotension and stress-induced hyperkalemia (e.g. dehydration, heat stroke or reduced salt intake).

Furthermore, the molecular genetic background of this disorder is far more complex and heterogenous than previously described. Despite significant progress in the understanding of this complex disorder and the fact that this medical condition has gained greater clinical recognition over time, diagnosis and treatment management remain an issue of concern in young infants.

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SHOULDN'T WE PAY MORE ATTENTION: VITAMIN D (HYPOVITAMINOSIS D), OSTEOPENIA AND OSTEOPOROSIS AND ASSOCIATED FUTURE CO-MORBIDITY RISKS IN CHILDREN AND ADOLESCENTS AND YOUNG ADULTS

Stuart J. Brink

Introduction

Problems with vitamin D[1] in healthy children, adolescents[2] and young adults as well as associated problems with calcium are much more common than appreciated by most health care professionals around the world but many times are overlooked or completely forgotten when it comes to quality of life and prevention of several important and preventable future medical complications. One out of every four men over 50 and one out of every two women over 59 will be diagnosed with osteoporosis in the USA. The importance of vitamin D[3] - in reality a hormone and not a vitamin - for prevention of osteopenia and osteoporosis has been known for many decades and, in its classical presentation as childhood rickets[4], hypovitaminosis D or vitamin D deficiency, can cause severe problems.[5] Vitamin D as well as calcium and other minerals, growth hormone, parathyroid and thyroid hormones and ultimately sex steroids all are needed for optimal growth and development, structure and functioning of bones. Yet the frequently assumption that only the elderly have osteoporosis difficulties and that's when fractures, vertebral collapse, "elder hunchback "and thromboembolic phenomenon show up.[6,7] There are numerous studies reflecting such problems and strongly hinting at the possibiities of decreasing and preventing osteopenia and/or osteoporosis[8] accordingly if identifying causative factors can be ameliorated and/or treated on a population basis earlier than is currently the case.[9,10]

The "*miracle of vitamin D*" is its presence via receptors on numerous cells throughout the body so that when vitamin D levels increase, improvement in stamina and energy as well as improvement in physical strength occur suggesting possible associations with muscle function.[11] Improved sleep, improved brain function, lessened anxiety, less depression and improvement in other mood disorders also have been documented in some studies as has association with attention deficit hyperactivity disorder (ADHD) and its variants without hyperactivity.[12] Metabolic syndrome itself has been linked with less than ideal vitamin D levels[13] with some studies suggesting demonstrable associations with improvement in vitamin D levels associated with less obesity[14], decreased (early) heart disease and also less hypertension.[15] Because of the associated functions of various forms of vitamin D and immune activity, not only is it suspected that increases in tuberculosis and viral respiratory infections are linked with lowered vitamin D levels[16] but also increases in autoimmune disorders such as type 1 diabetes[17,18,19], lupus[20,21], rheumatoid arthritis[22] and Hashimoto's thyroiditis[23] have been reported. Increased breast, colon, lung and other cancers also have been tied to insufficient vitamin D levels[24] as have all-cause mortality.[25] (*Figure 1: Mortality rates for women and men in the Dubbo Osteoporosis Epidemiology Study*)

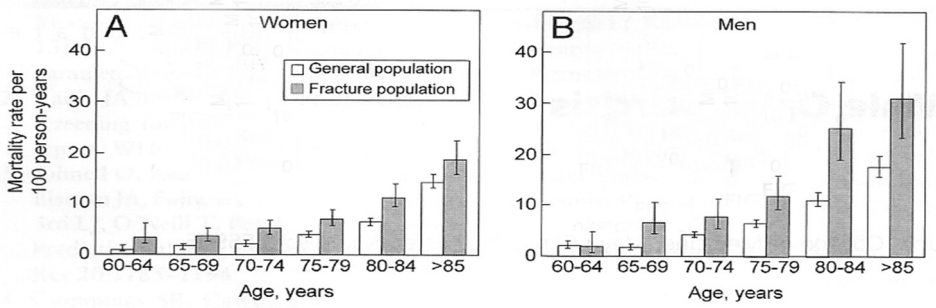


FIG. 1. Mortality rates for the general participants and fracture patients according to age from the Dubbo Osteoporosis Epidemiology Study in women (A) and men (B). In this population, there were 2245 women and 1760 men aged 60 yr and older. Of the fracture patients, 952 were women and 343 were men. *Error bars* indicate 95% CI. [Reproduced with permission from D. Bliuc *et al.*: *JAMA* 301:513, 2009 (10). ©American Medical Association.]

The hormone function of vitamin D is felt to be a higher evolutionary endocrine function whereas the more "primitive" function of vitamin D as that of a cytokine generated to protect the host from microbial invaders (ie. mycobacterium) is also thought responsible for some

vitamin D associated immune functions.[26] Intracrine modulation with the vitamin D receptor (VDR) in monocytes and macrophages of T and B lymphocytes is thought to act in the local inflammatory microenvironment. Vitamin D is also needed to help generate antimicrobial peptides in both monocytes and also macrophages.[17] Activation of the Toll-like (TLR) pathway in monocytes and macrophages initiates expression of the CYP27B1 (1-hydroxylase) as well as VDR genes, vitamin D expressors, in those cells. Therefore, this sets up activation of antimicrobial genes such as catheliciden and associated killing of an ingested microbe. This is especially obvious when studies are done in vitamin D deficient tuberculosis patients and the same seems to happen in the lung, gut, skin and placenta ibid as a possible explanation for hypovitaminosis and increased respiratory (viral) infections as well as the known specific increased tuberculosis risks.[27]

Osteoporosis changes the internal structure of bones especially the spongy matrix inside the ends of long bones in the hips and spine. Unchecked, large pores form in that matrix and so the disease's name came to be. Bones become fragile and can shatter even with minimal impact. Complications of hip fractures are associated with up to 25% of older aged patients with osteoporosis who may die within 6 months due to complications of hip fractures in some studies.[28] Calcium is a key participant in this process and needed for proper muscle contraction as well as neuronal signaling. Most of a person's calcium is locked up in hydoxyapatite, the crystals of calcium and phosphate that make bone hard. Remodeling cells on the surface of bones respond when the concentration of calcium dissolved in the blood dips too low, cells called osteoclasts are triggered to break down bone matrix and release minerals into the blood. Once calcium gets high enough in the circulation, osteoblasts are triggered and they grab calcium and phosphate from the bloodstream to rebuild bone. These negative feedback loops keep bone density and blood calcium and phosphate levels steady. In osteoporosis, the bones break down too quickly or rebuild too slowly. According to Professor Holick, in a brilliant editorial[29] some of these ongoing controversies and the rationale for further changes require larger and better designed scientific study but the importance of these minerals with vitamin D as potential factors which can help decrease many of these medical issues.

Differential diagnosis

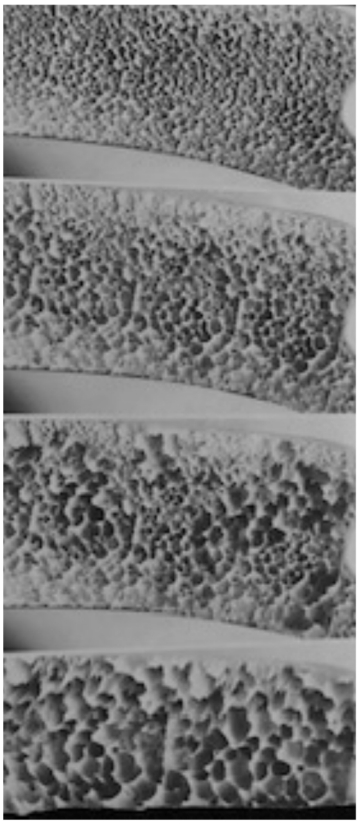


Figure 2. Four Stages of Osteoporosis [26]

Classical and severe pediatric and adolescent osteoporosis is quite rare if one only "waits" for symptomatic patients to present. Table 1 presents a differential diagnosis list for newborns and infants which is strikingly different from the list affecting older children and adolescents where not only are lack of sunlight and nutritional aspects common but the secondary association of vitamin D deficient problems either with the listed disorders or their treatment consequences - or both - have had little attention paid to until recently. A key change has been the wider availability of bone density dual energy x-radiation absorptive (DXA or DEXA) studies[30] with comparisons available utilizing age-matched control populations as well as the ability to actually measure circulating blood vitamin D levels and place these into perspective for this younger population. An example of sequential bone mass changes and varying stages of osteoporosis[31] consistent with worsening risks of fracture as severity increases is shown in *Figure 2*.

TABLE 1: CLASSICAL PEDIATRIC AND ADOLESCENT OSTEOPENIA/OSTEOPOROSIS DIFFERENTIAL DIAGNOSIS

- NEWBORN & INFANTS
  - Maternal vitamin D deficiency
  - Familial hypercalcemic hypercalciuria
  - Primary hyperparathyroidism
  - Renal disease
  - Vitamin D deficiency
  - Vitamin D resistant syndromes
  - Hypophosphatemic rickets
  - Osteogenesis imperfecta

- CHILDREN & ADOLESCENTS
    - Hyperparathyroidism
    - Hyperthyroidism
    - Hypogonadism
    - Cushing's and chronic glucocorticoid Rx
    - Rickets
    - Malabsorption/GI inflammatory syndromes
        - Celiac
        - Crohn's
        - Ulcerative colitis
    - AIDS
    - Chronic renal disease
    - Cancer, chemotherapy, radiation treatment
    - Malnutrition
    - Anorexia nervosa, diabulimia, bulimia
    - Osteogenesis imperfecta
    - Juvenile idiopathic osteoporosis
    - Polyostotic fibrous dysplasia
    - Immobilization
    - Chronic medications
        - Anticonvulsants
        - immunosuppresants

Vitamin D

Vitamin D is predominantly produced in the skin as its endogenous source and produced by an ultraviolet-B mediated photolytic nonenzymatic reaction that converts precursor 7-dehydro-cholesterol to pre-vitamin D3.[32] In North America, Europe and Asia highest 25 OH vitamin D levels are maximum about 30-60 days after peak summer sunlight exposure (ie. Fall) and measurable with current assays in ng/ml concentrations; some laboratories still report in nmol, however. (50 nmol/L vitamin D = 20 ng.ml). Pre-vitamin D3 then is non-enzymatically converted to vitamin D3 also in the skin. Thereafter, vitamin D3 enters the circulation and is measurable in pg/ml concentrations. From the general circulation, vitamin D3 is converted by liver cytochrome P450s to 25-hydroxy vitamin D3 and it is this form of the hormone/vitamin that acts as a pro-hormone – or immediate precursor metabolite – to the "active" form of vitamin D: 1,25 di-hydroxyvitamin D3 (1,25 di-OH-D_3 = calcitriol). Calcitriol is the product of a single enzyme, 1-hydroxy-mitochondrial CYP27B1-hydroxylase in the kidney proximal tubular epithelia. Calcitriol circulates in the serum at concentrations of approximately 0.1% of the prohormone 25-hydroxy-D and current

assays can measure calcitriol in pg/ml concentrations with reasonable accuracy and reliability. Calcitriol synthesis in the kidney by such 1-hydroxylation is primarily stimulated by parathyroid hormone and is inhibited by osteocyte fibroblast growth factor 23 (FGF23). Calcitriol serves as a ligand for the vitamin D receptor (VDR) in numerous target tissues throughout the body. Approximately 80% of vitamin D is thought to come from sun exposure whereas only 20% generally comes from dietary sources of vitamin D for most populations so lack of sun exposure because of sunscreen use, more indoor rather than outdoor activities, less sunny days, darker skin color as well as nutritional deficiencies (see below) can all be important explanation for increasing vitamin D deficiency situations in modern life.

Nutritional vitamin D

US pediatric calcium dietary trends Greer Pediatrics 2004

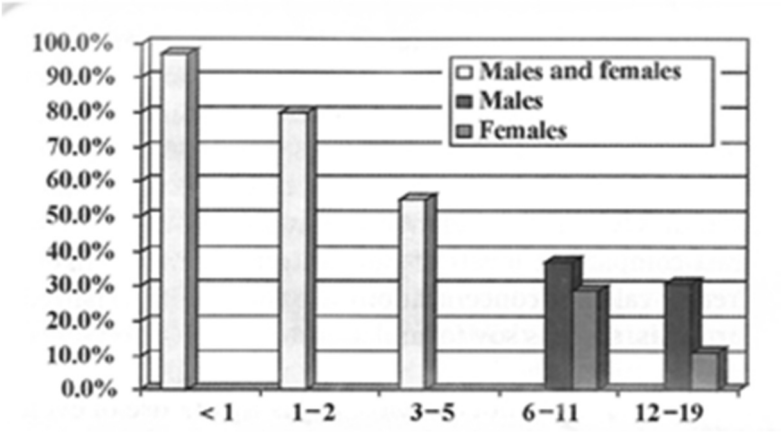


Figure 3 – Percentaje of children achieving the recommended daily adequate intake for calcium

Worldwide there appears to be a trend producing vitamin D insufficiency if not outright deficiency that couples less exposure to natural sunlight either because of less time spent outdoors and/or more time in school (for youngsters) or working (for adults) indoors coupled with lower access to foods naturally richer in calcium and vitamin D.[33] More free time spent on mobile phones or computer screens also are contributing factors in high resource as well as middle resource and low resource countries of the world. More use of sunscreens as a way to decrease skin cancer and also minimize

sunburn also is contributory. United States pediatric calcium dietary trends move downard quite dramatically when compared to recommended dietary allowances (RDA) starting in infancy, through the preschool years and into the school age and adolescent age brackets.[34] (*Figure 3: US pediatric calcium dietary trends*).

Advertising of sugary drinks contributes to the decrease in milk and milk-based products as well around the world. In the United States and Canada, vitamin D3 (cholecalciferol) is added as a nutrition supplement to milk and other dairy products so that milk, yogurt, cheeses, ice cream and pudding/custard foods as well as some orange juice products now serve as the main dietary source for vitamin D for most North Americans. In the UK, vitamin D2 (ergocalciferol) is used as a nutrition supplement but it is felt that both vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) can serve as precursors to bolster human vitamin D levels in equal manner.[35] Some vitamin D also is available in certain nuts such as almonds, in some wild fish such as salmon and sardines, some mushrooms as well as beans and green, leafy vegetables like broccoli. However it should be noted that these are often food choices that many children and adolescents - let alone many adults - may not prefer. Dietary vitamin D either naturally occurring in such foods, or added as a supplement through milk and its derivatives, is absorbed as a fat-soluble vitamin in the proximal intestine.[36] Vitamin D fortified orange juice as well as other vitamin D fortified drinks are also available and especially helpful in those who are not drinking milk or otherwise having sufficient dairy intake. This process is interfered with when various gut inflammatory disease processes – especially of the upper intestine - occur as in gluten-sensitive celiac disease and Crohn's disease.

Changes in hormonal vitamin D metabolism and action

Liver diseases decrease 25-hydroxylation. Kidney diseases decrease 1- hydroxylation. Both chronic liver disease and chronic kidney disease often are associated with lowered vitamin D levels and, if this persists, with associated bone mineralization abnomalities (osteopenia and osteoporosis) as well as other hypovitaminosis D-related conditions already mentioned previously. These are almost always asymptomatic. Gut diseases such as celiac disease and its variants as well as inflammatory bowel disorders such as Crohn's disease are associated with decreased absorption of dietary micronutrients including calcium and vitamin D as well as

other minerals and vitamins needed for optimal bone health. Coupled with less sunlight exposure, more suncreen use and naturally darkly pigmented skin in some populations around the world (ie. Africans, African-Americans, some Asian populations and many indigenous native populations compared to lighter-skin-colored Caucasians mainly from European countries originally), less ultraviolet exposure of vitamin D precursors and also decreased dietary intake of vitamin D all set up the "perfect storm" for vitamin D insufficiency and vitamin D deficiency syndromes and their sequelae to be increasing in prevalence in recent years.

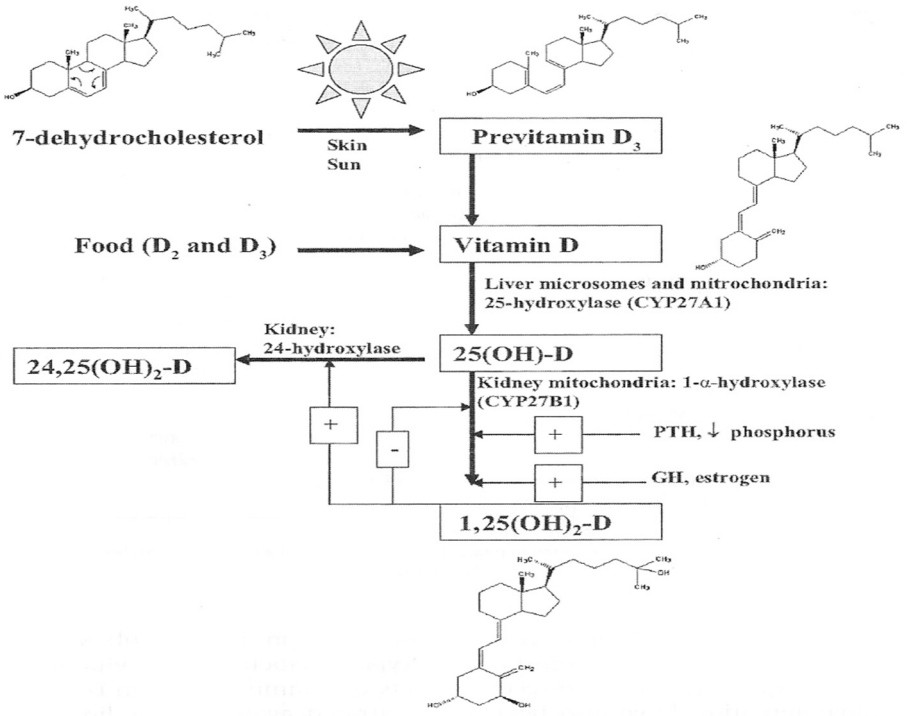


Figure 4 - Vitamin D, calcium, PTH, VDR and feedback loops interacting [37]

Whenever there are changes in dietary calcium, directly because of lowered intake or indirectly because of lowered synthesis of vitamin D levels, changes occur correspondingly with further decreases in serum calcium and this is detected by calcium sensing receptors of the parathyroid gland. Thereafter, parathyroid hormone (PTH) increases as the 25-hydroxy-D levels move downwards (<30 ng/ml) and before most experts would "call" full vitamin D deficiency.

PTH then travels to receptors in the renal proximal tubular epithelium to increase CYP27B1 and then 1-hydroxylation. This converts more 25-hydroxy-vitamin D to 1,25 di-hydroxy-vitamin D. The di-hydroxy-vitamin D then interacts with the VDR (vitamin D receptor) genes of the gut and the bones to attempt to increase intestinal calcium absorption further and free up stored calcium in the bones ("good for the calcium levels circulating but not so good for the bone matrix"). This allows bones to "respond" to such calcium level changes and release FGF23 to "feed back" and try to stop the process and attempt rebalancing.

In essence, and somewhat arbitrarily, vitamin D ***insufficiency*** may then be defined biochemically according to this cascade approach as ocurring when 25-OH-vitamin D total blood levels are approximately 30-50 ng/ml (~<125 nmol/L) and vitamin D ***deficiency*** may then be defined as occurring when the 25-OH-vitamin D total blood levels are < 30 ng/ml (~<75 nmol/L). As these levels lower, PTH levels secondarily rise in response. Vitamin D epidemiologic studies suggest the following:

- NHANES documented doubling of adult American population with 25OHD levels <30 ng/ml from 1994 to 2004[38]
- Frank vitamin D deficiency, previously (and also arbitrarily) defined statistically as < 20 g/ml currently occurs in 25-35% of the US population[32,39]
- In Black American and Latin-American populations (both dark skinned) using these same statistical cut-off points, 90% are deficient
- In US pediatric populations studied, the exact same trend occurs as in the adult populations [25, 28]
- Studies have also documented increased hazard risk for diabetes associated microvascular complications in general as well as diabetic nephropathy, diabetic retinopathy and diabetic neuropathy so that a potential beneficial role of maintaining adequate vitamin D status should be considered.[40]
- As the childhood, adolescent and adult population becomes more overweight and more obese and as body mass indices worsen, blood vitamin D levels also decrease presumably with the fat-soluble vitamin D no longer available for the rest of the body but "taken up" by the body fat mass instead. [i] Populations with more poverty and/or minority ethnic status may also be at increased risk.[42]

- All elements of the metabolic syndrome are correlated with vitamin D intake, sun exposure/latitude, and actual measured vitamin D levels:[13]
    - Insulin resistance
    - Glucose intolerance
    - Glucose levels/diabetes
    - Lipid levels
    - Hypertension
    - Weight and BMI in adults and teenagers

Coronary artery disease, overt heart failure, peripheral artery disease all show stepwise increases as serum 25OHD levels drop to less than 30 ngml and then to below 20 ng/ml

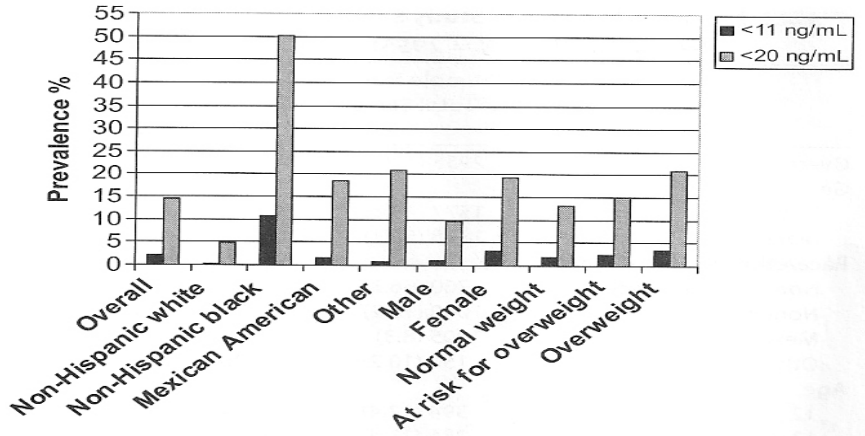
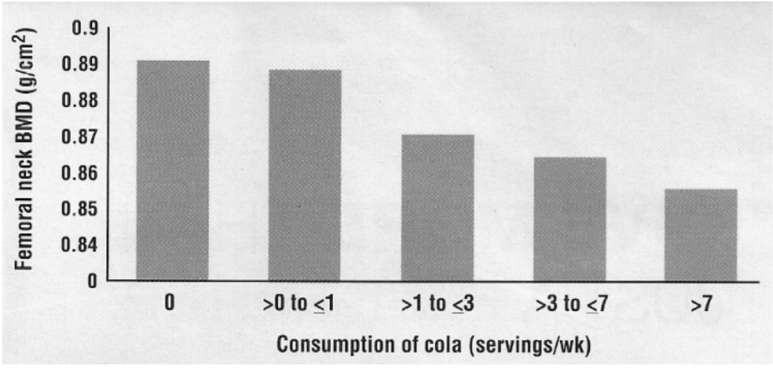


Figure 5, NHANES J Pediatr 2004 abnormal vitamin D prevalence data (Weight prevalenece of vitamin D deficiency (< 11ng/ml) versus recomanded (<20 ng/ml) levels according to race/ethnicity, gender and weight)



Cola Consumption and Bone Mineral Density in Women

- Phosphate intake, mostly in sugary carbonated beverages like colas and root beer, potentially "rob" the bones of calcium and in so doing, worsen the mineralization situation of the bones (in essense helping to produce bony "calcium deficiency" compounding the insufficiency of dietary calcium and the absolute deficiency of vitamin D production as well as nutritional intake. Studies show direct correlation of consumption of such beverages and DXA studies[43] (*figure 6*)
- Caffeine intake in some studies also acts on bone calcium in somewhat similar fashion perhaps also reflecting more renal calcium loss as well as producing negative bone calcium flux.

Fractures

Fracture risks are presumed to be increased in association with lowed calcium and other mineral intake as well as with lower vitamin D levels (production and/or intake), increasing obesity and subtle secondary hyperparathyroidism so that enhanced osteoclastogenesis helps to increase bone resorption outstripping osteo-blast directed bone formation that might otherwise occur.[44] Most studies of skeletal disease/fracture risks, however, are done in adults and especially in women because of more numbers of fracture occurrence in women as they age and lose estrogen bone protection. A study of bone mass and bone strength in young women involved young women over a a 10 year period of time and suggested that exercise was the predominant lifestyle determinant of bone strength in their cohort.[45] As men age, the same phenomen occurs as testosteone levels drop but this occurs relatively later in the life cycle compared to women, thus fewer fractures in men than in women. In populations with darker skin pigmentation and less daily activity, fracture rates may increase further. The same pattern occurs in children, teenagers and young adults although with lesser frequency than in the elderly.

DXA definitions and caveats

DXA [46] has become more available in recent years and with better age and race-based control standards available via computerized statistical analysis programs.[47,48] Originally, hip radiographs were analyzed for bone density assessment. These were then supplemented with bone density analysis of the first four lumbar vertebrae; L1-L4 summation values seemed to provide improved statistical discernment of normal vs osteopenia vs

osteoporosis definitions especially with the idea that spinal load bearing may be of more clinical relevance than the hips. More recently, total body skeleton bone density assessments have also become available for evaluation of child and adolescent bone densities. Most radiographers strongly urge interpretation based upon such age, sex and race-based standards and some newer studies suggest that pubertal Tanner staging may also be important as well as height or height standard deviation z-scores. (*Table 2: WHO Diagnostic Categories*[49]) DXA abnormalities clearly correlate with adult fracture risks when utilzing hip and lumbar spine standards.

Table 2. WHO Diagnostic Categories of Bone Mineral Density

Diagnostic Category	Criterion
Normal	A value for BMD o BMC that is within 1.0 SD of the reference mean for young adults
Low bone mass (osteopenia)	A value for BMD or BMC that is more than 1.0 but less than 2.5 SD below the mean for young adults
Osteoporosis	A value for BMD or BMC that is 2.5 SD or more below the mean for young adults
Severe osteoporosis (established osteoporosis)	A value for BMD or BMC that is 2.5 SD or more below the mean for young adults in combination with one or more fragility (low-trauma) fractures
BMD denotes bone mineral density and BMC bone mineral content.	

Standards must be utilized specific to the DXA machine used for measurements with results reported absolutely as well as with standardized z-scores for children and teenagers[50,51] and standardized T-score for adults. An important issue is the subtle differences in childhood and teenage DXA interpretations with frequent “errors” when adult radiologists aren’t aware of the specific differences in interpretation in these age groups - so ideally, having pediatric and adolescent radiology teams help with interpretation should be considered.[52] Comparison should also optimally be done on the same machine by the same operator. Such DXA reports for adults compare results to an arbitary optimally “healthy” 25-35 year old man or woman by consensus. *Figure 6-9* shows some typical DXA scans obtained on Hologic® scanners demonstrating normal bone density results for evaluation of dietary calcium insufficiency and/or hypovitaminosis D, osteopenia of hip and lumbar spine, osteopenia of the hip but osteoporosis of the lumbar spine and osteoporosis of

both hip and lumbar spine sites and also demonstrating sequential assessments of such DXA results over time. Assessing actual bone health with DXA scans[53] through detailed discussions with family members and, if developmentally appropriate with the child or adolescent and young adult with such issues is important and may assist with compliance options too. Follow up DXA decisions especially in those where initial DXA results are normal also need further studies and consideration to optimize recommendations.[54]

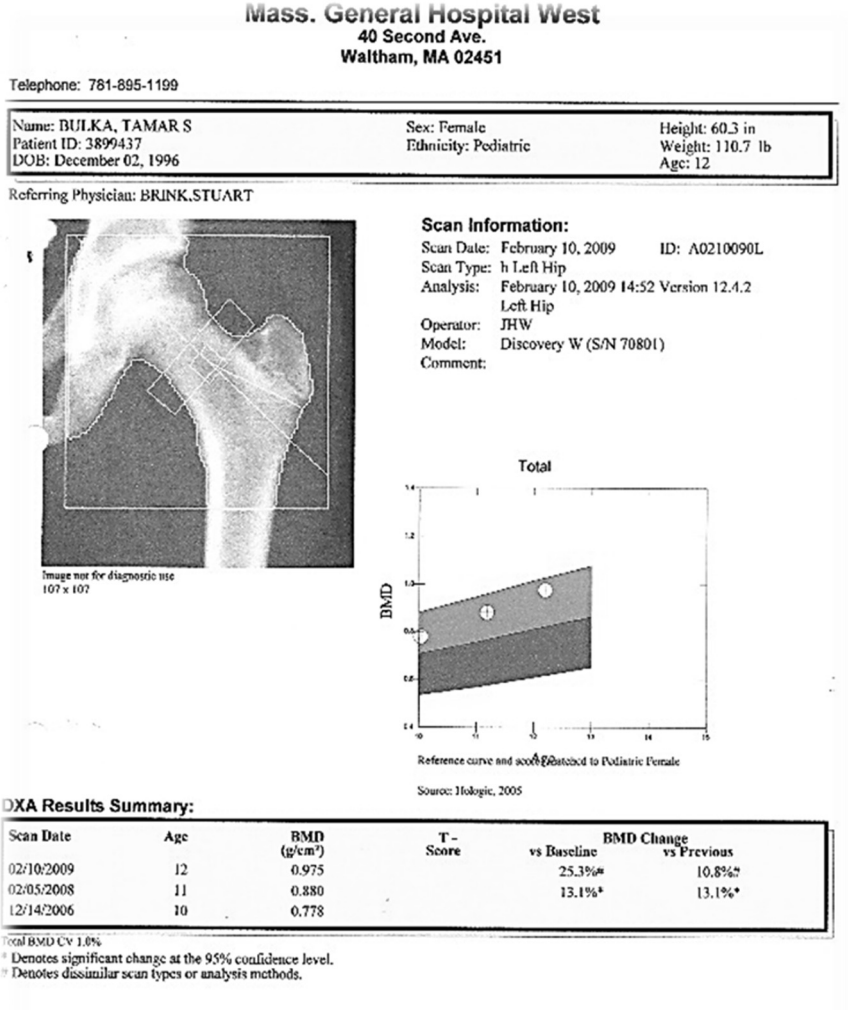


Figure 7: Normal hip DXA scan results despite hypovitaminosis D

Mass. General Hospital West
40 Second Ave.
Waltham, MA 02451

Telephone: 781-895-1199

Name: BULKA, TAMAR S	Sex: Female	Height: 60.3 in
Patient ID: 3899437	Ethnicity: Pediatric	Weight: 110.7 lb
DOB: December 02, 1996		Age: 12

Referring Physician: BRINK,STUART

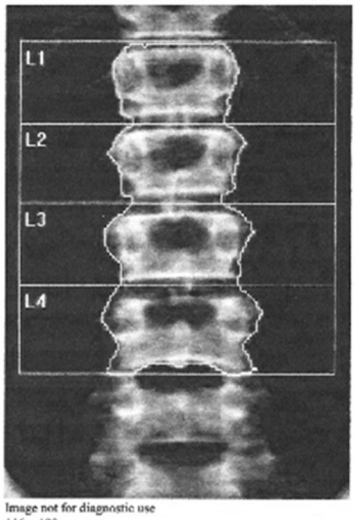
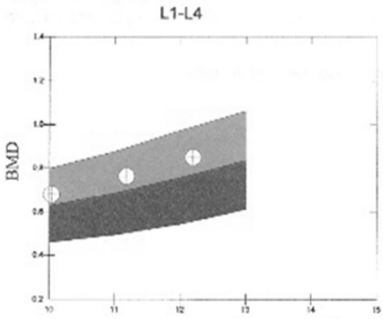


Image not for diagnostic use
116 x 122

Scan Information:

Scan Date: February 10, 2009 ID: A0210090K
Scan Type: h Lumbar Spine
Analysis: February 10, 2009 14:52 Version 12.4.2
Lumbar Spine
Operator: JHW
Model: Discovery W (S/N 70801)
Comment: [EXAM:NOLAT]



Reference curve and scores matched to Pediatric Female

Source: Hologic, 2005

DXA Results Summary: L1-L4

Scan Date	Age	BMD (g/cm²)	T-Score	BMD Change vs Baseline	BMD Change vs Previous
02/10/2009	12	0.851		25.3%#	11.5%#
02/05/2008	11	0.763		12.3%*	12.3%*
12/14/2006	10	0.679			

Total BMD CV 1.0%
* Denotes significant change at the 95% confidence level.
# Denotes dissimilar scan types or analysis methods.

Figure 8: Osteopenia of the hip and also the lumbar spine

Mass. General Hospital West
40 Second Ave.
Waltham, MA 02451

Telephone: 781-895-1199

Name:	MAXIM B	Sex: Male	Height: 67.0 in
Patient ID: 4479281		Ethnicity: Pediatric	Weight: 110.7 lb
DOB: June 28, 1996			Age: 13

Referring Physician: BRINK,STUART

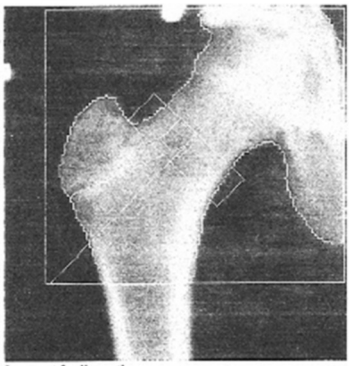
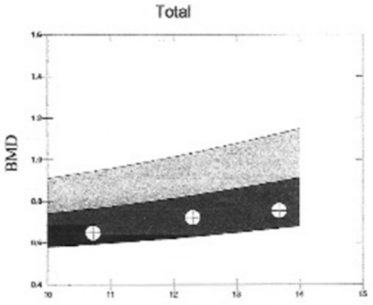


Image not for diagnostic use
111 x 103

Scan Information:

Scan Date: March 01, 2010 ID: A03011001
Scan Type: a Right Hip
Analysis: March 01, 2010 16:10 Version 12.4.2
Right Hip
Operator: JM
Model: Discovery W (S/N 70801)
Comment:



Reference curve and scores matched to Pediatric Male

Source: Hologic, 2005

DXA Results Summary:

Scan Date	Age	BMD (g/cm²)	T-Score	BMD Change vs Baseline	BMD Change vs Previous
03/01/2010	13	0.755		16.3%#	4.4%#
10/17/2008	12	0.723		11.4%#	11.4%#
03/19/2007	10	0.649			

Total BMD CV 1.0%
# Denotes significant change at the 95% confidence level.
† Denotes dissimilar scan types or analysis methods.

HOLOGIC

Figure 9a:

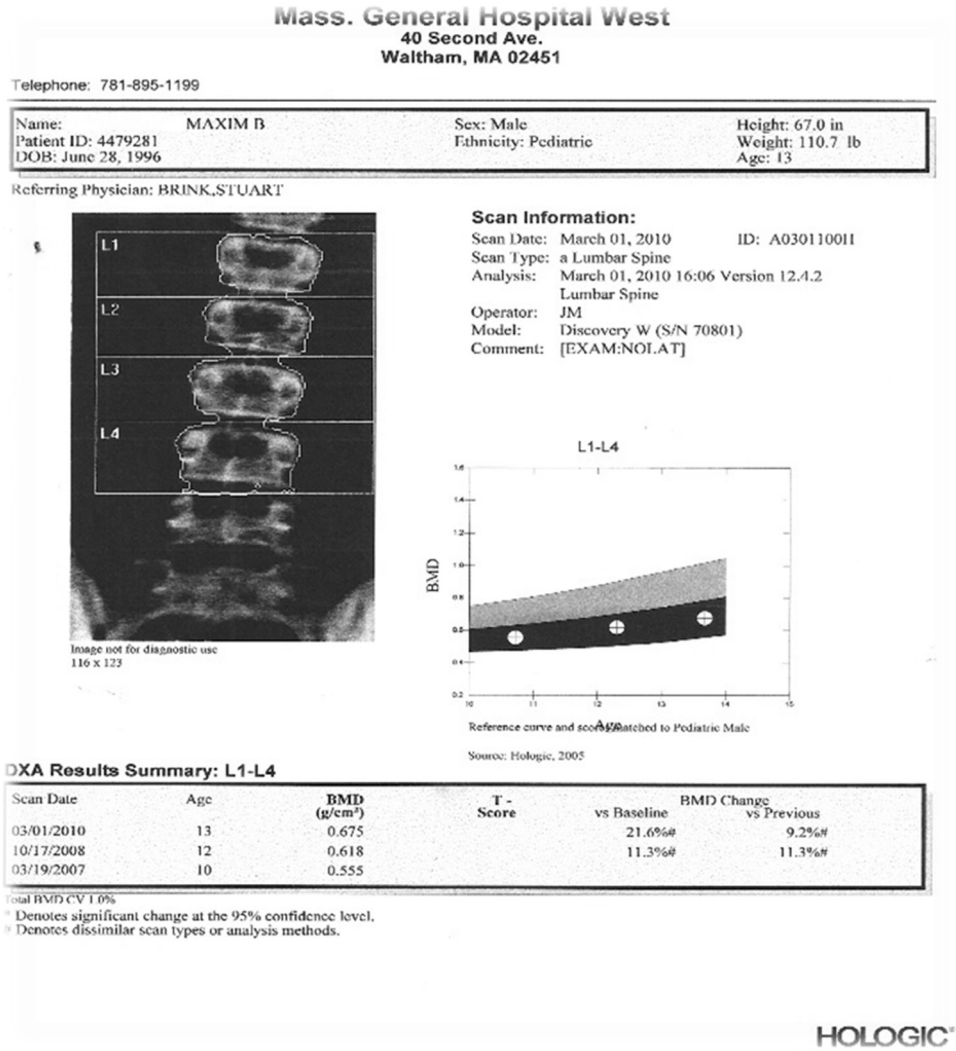


Fig 9 b.

Fig - Osteopenia of the hip and osteoporosis of the lumbar spine

Mass. General Hospital West
40 Second Ave.
Waltham, MA 02451

Telephone: 781-895-1199

Name: NASCIMENTO, KRISTIN	Sex: Female	Height: 62.3 in
Patient ID: 4133172	Ethnicity: Pediatric	Weight: 90.0 lb
DOB: July 27, 1995		Age: 13

Referring Physician: BRINK,STUART

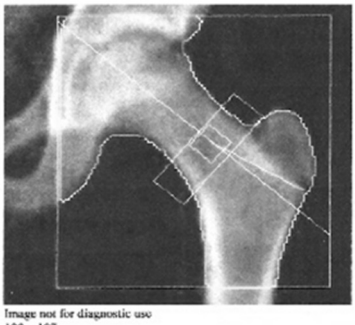
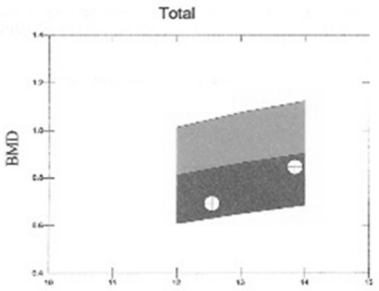


Image not for diagnostic use
100 x 107

Scan Information:

Scan Date: May 29, 2009 ID: A0529090A
Scan Type: x Left Hip
Analysis: May 29, 2009 13:46 Version 12.4.2
Left Hip
Operator: JHW
Model: Discovery W (S/N 70801)
Comment:



Reference curve and scores matched to Pediatric Female

Source: Hologic, 2005

DXA Results Summary:

Scan Date	Age	BMD (g/cm²)	T - Score	BMD Change vs Baseline	BMD Change vs Previous
05/29/2009	13	0.846		22.1%*	22.1%*
02/11/2008	12	0.692			

Total BMD CV 1.0%
* Denotes significant change at the 95% confidence level.

HOLOGIC

Fig. 10.a.

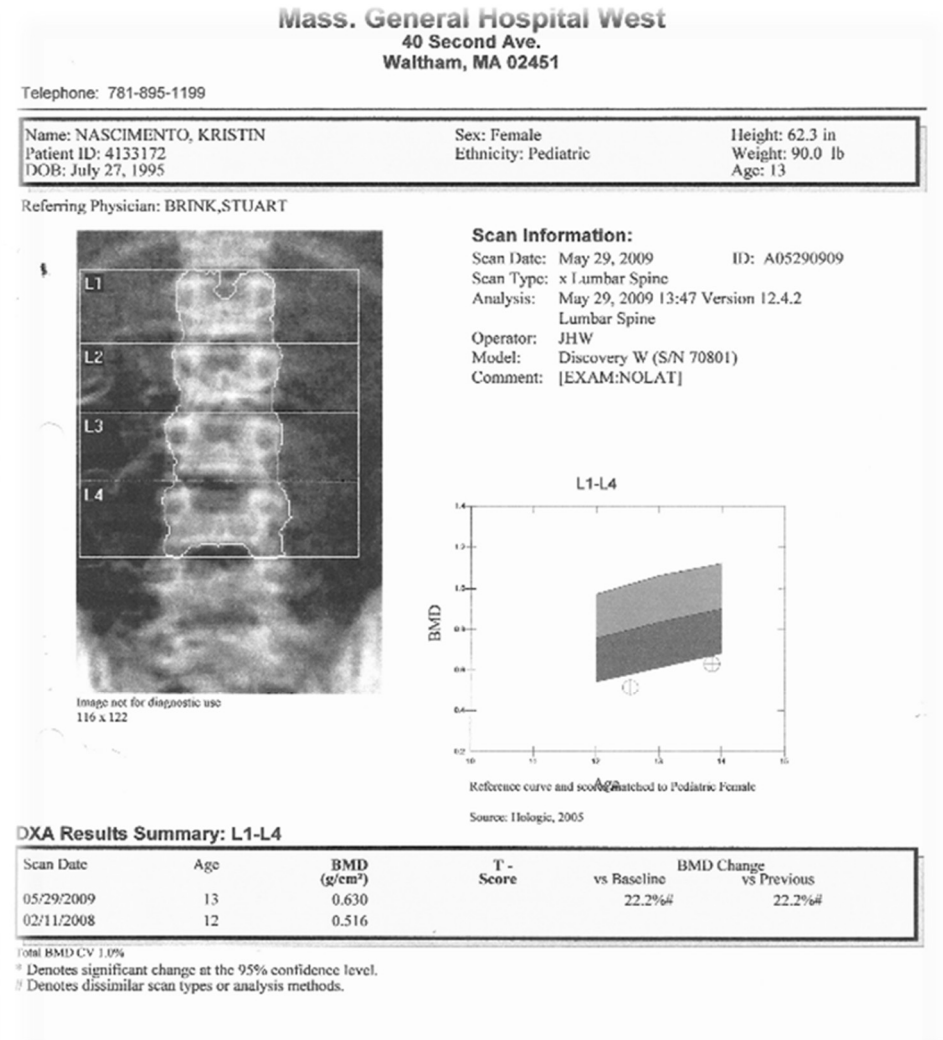


Fig. 10.b.

Figure 10: Osteoporosis of the hip and also the lumbar spine

Worsening bone mineral density (BMD) occurs in association with increasing (women's) fracture rate[55] (*figure 11 below*). BMD deteriorates with age (*figure 12 below*) and in comparative terms, moving from child, to adolescent, to young adult and then to menopausal adult women/elderly, peak bone mass (DXA, BMD) shows rather consistent general patterns of change[34] (*figure 13* below)

reflecting all the myriad nutritional, activity, hormonal and associated medical-condition correlates previously listed.

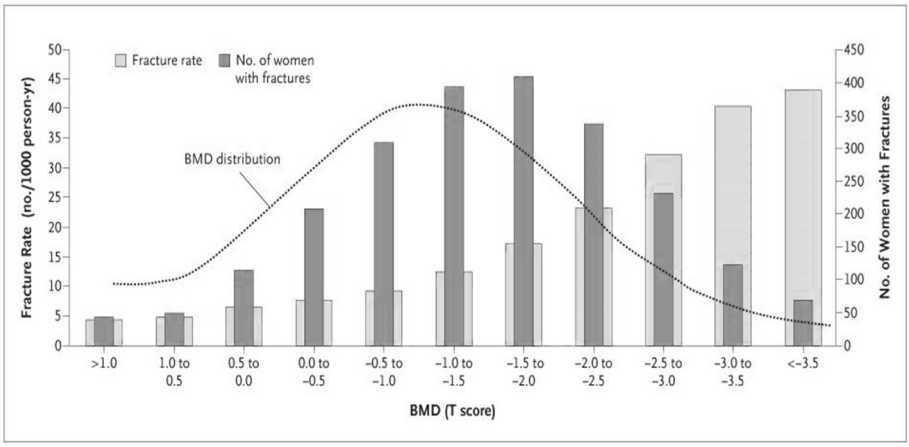


Figure 11.- Worsening BMD associated with increasing women's fracture

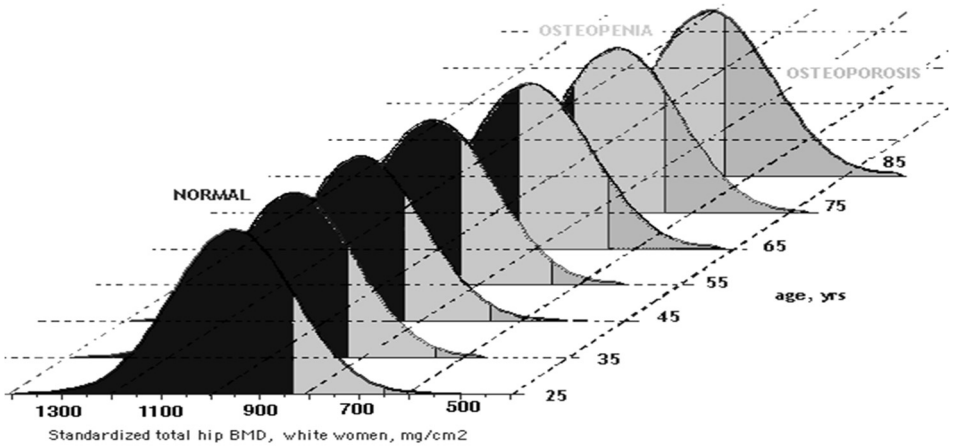


Figure 12.- Bone mineral density deterioration with age in women.

Who to question, what labs to consider: virtually all NEDEC endocrine patients and perhaps all patient

Because of how common low vitamin D levels seems to be around the world and because of the increasing prevalence of fractures after age 50 years - especially in the richer countries of the world and in those countries in more northern latitudes with less sunlight exposure –

medical history of calcium and vitamin D nutritional intake should become much more common as should sun exposure and sunscreen use history.

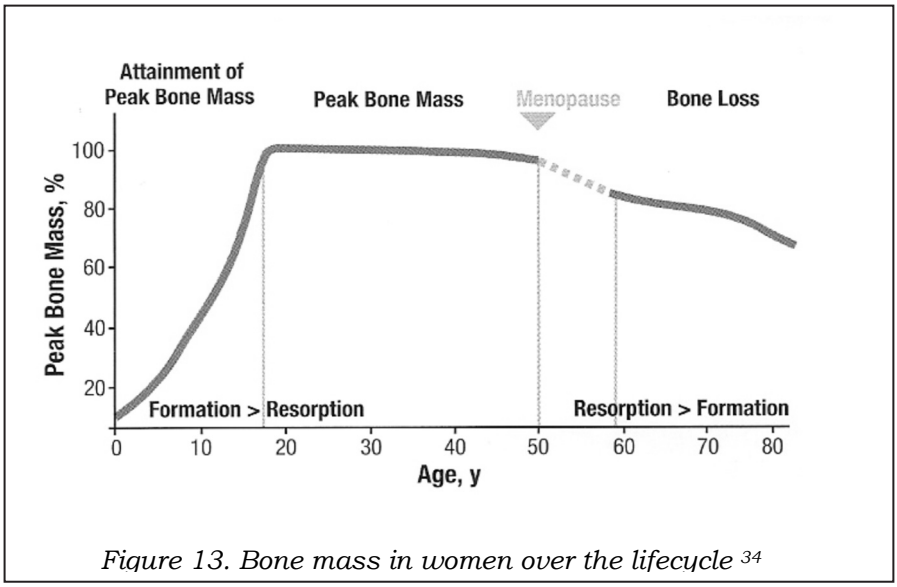


Figure 13. Bone mass in women over the lifecycle [34]

This would apply to healthy children and adolescents as well as adults. Certain associated medical conditions seem to have higher-than-average rates of dietary insufficiency or deficiency as well as actual hypovitaminosis D and osteopenia in the few studies currently available for evidence-based review. Encouraging radiologists to actually make a diagnosis of osteopenia (and not just report Z scores) should also be helpful since diagnostic insurance coding for osteopenia could then be legitimized and helpful in raising child, adolescent, parental as well as health care provider awareness and need for improved calcium, mineral and vitamin D compliance as well as follow up discussions, lab testing and repeat DXA scanning.

Listed are some of these conditions[56,57,58,59,60,61,62] that are especially pertinent to the pediatric/adolescent/young adult endocrinologist besides classical nutritional rickets and the more rare but more specific parathyroid and calcitonin abnormalities:

- Type 1 diabetes
- Type 2 diabetes
- Hyperlipidemia and cardiovascular diseases

- Hypertension
- Metabolic syndrome/prediabetes
- Obesity and its associated conditions especially acanthosis nigricans and premature adrenarche/androgen excess syndromes (most likely reflecting the metabolic syndrome)
- Pregnancy
- Celiac disease
- Other gastrointestinal inflammatory bowel conditions (ie. Crohn's, ulcerative colitis, eosinophilic or allergic bowel disorders
- Cystic fibrosis
- Lactose intolerance
- Thyroid disorders including euthyroid Hashimoto's thyroiditis, congenital and acquired hypothyroidism, hyperthyroidism
- Short stature including but not limited to actual growth hormone deficiency
- Cushing's Syndrome or Disease
- Those appropriately treated with chronic glucosteroid treatment including congenital adrenal hyperplasia, Addison's disease or for other conditions (cancer treatment protocols, rheumatologic or other systemic inflammatory illnesses such as juvenile idiopathic arthritis)
- Hypogonadal conditions including Klinefelter's Syndrome, Prader Willi Syndrome, Kallman's Syndrome and testicular as well as ovarian hypofunctioning conditions
- Turner's Syndrome
- Noonan's Syndrome
- Ehler-Danlos Syndrome
- Down Syndrome
- Galactosemia
- Marfan Syndrome
- Hematologic disorders: sickle cell disease, thalassemia
- Anyone with unexplained recurrent fractures
- Anorexia nervosa, diabulimia, bulimia
- Parathyroid and calcitonin abnormalities including congenital disorders of calcium, parathyroid and vitamin D synthesis, hypoparathyroidism, pseudohypoparathyroidism, pseudopseudohypoparathyroidism
- Post-cancer treatment survivors especially those who have had x-radiation treatment as part of their treatment protocols

including acute lymphoblastic leukemia, anyone receiving chemotherapy for childhood, adolescent or adult cancer, post-transplantation patients
- Chronic immobilization conditions such as cerebral palsy, myopathic disease, epidermolysis bullosa

Laboratory assessment

Once awareness of the possible diagnosis is heightened, then measurement of blood circulating vitamin D levels become the next step to consider. Actual measurement of serum calcium, phosphate, alkaline phosphatase and parathyroid hormone are generally not very helpful except in the rare circumstances where classical rickets or specific parathyroid hormone or vitamin D dysmetabolism is thought to be a high diagnostic possibility. Rather, the history can be utilized to move straight to total vitamin D levels as the best current diagnostic tool towards deciding about supplementation as well as baseline DXA. Of course, there will always be some circumstances where history and physical examination alone does not allow such decisions to be made without more basic laboratory investigation and thus the discerning clinician and diagnostician must always make such decisions for the individual patient as well as based upon availability and cost of such testing. Vitamin D assays remain expensive and somewhat difficult to standardize so knowledge of the laboratory availability and reliability are also important factors to consider especially if sequential testing will be done for comparison of treatment compliance and efficacy/dose adjustments. Fractionating vitamin D into 1,25 vitamin D3 as well as 25 vitamin D total and D3/D2 fractions is usually not particularly helpful and adds further expense so this author would suggest utilizing total vitamin D levels to be measured as a baseline value and then followed sequentially, perhaps every 3-4 months after supplementation is started and then sequentially twice-a-year thereafter to document reaching therapeutic goals and helping provide treatment feedback to the patient and family with sequential data. Similarly, PTH and calcitonin levels rarely are useful for most cases of sunlight and/or nutritional deficiency associated hypovitaminosis states with or without osteopenia/osteoporosis. After “screening” with detailed dietary history and sun exposure history, and perhaps after baseline total 25 hydroxy vitamin D levels are obtained, then the decision to order DXA testing also must be considered, modified by availability, reliability and expense of DXA

as well as sequential vitamin D results. It is possible that compliance for supplementation would increase if the child, teen, adult and/or family members get to see the actual DXA results for themselves and then also see the follow-up comparative DXA studies.

From a public health standpoint, considering how common will be abnormal findings, it may be more prudent - and certainly likely would be more cost effective - to not only obtain dietary and sun exposure history from all children and adolescents periodically (ie. every 5 years) using such a diagnostic paradigm. Increasing dietary sources of calcium and vitamin D for almost everyone (children, adolescents, adults and the elderly) would likely be reasonable for many parts of the world as may specific recommendations for increasing recommended dietary supplements above what are currently recommended by authorities.[63] Some such guidelines[64] from authorities have recently changed recommended dietary allowances (RDA) in the USA increasing from from 400 to 800 or even 1000 IU daily. This author suspects that these will remain insufficient, however, but controversies about agreed-upon definitions of insufficiency and deficiency, specifics of laboratory assays, skeletal and extra-skeletal effects of vitamin D as well as its therapeutics are still problematic[65] and it may be necessary to get some blood total vitamin D levels when clinical history so dictates.

Another reasonable approach suggests that a modified physical education approach may also be useful to increase bone mineral density coupled with increased awareness of students, families and school staff.[66]

Once low levels of vitamin D are documented and certainly when and if osteopenia or osteoporosis is diagnosed by DXA[67], then further supplementation is reasonable. Supplements of multiple vitamins containing multiple minerals may be used since as it is likely if dietary insufficiency of calcium and vitamin D exists, there is greater probability of other bone-health-promoting minerals also being insufficient (ie. potassium, chromium, selenium, magnesium etc).[68] Specific supplementation with calcium in forms that often contain extra vitamin D and/or potassium and/or magnesium also may be helpful to try to bring children and adolescents with osteopenia or osteoporosis closer to RDAs of 1500 mg for daily calcium. Most tablets and pills are available without prescription and alternatives such as sugary-“gummy” vitamins, “bears,” chocolates and “chews” also can be helpful to provide sufficient nutritional calcium intake adapted to individual likes and taste preferences.

Noncompliance with such calcium supplements is common and should be explored openly and honestly. Chronicity of the need for daily supplementation is also a consideration vis-à-vis compliance issues.

Specific vitamin D can be supplemented either as tablets, softgels or liquid preparations and these are available in high potency (50,000 IU) weekly or twice-a-week supplements as well as daily supplements (400, 800, 1000, 2000 and 5000 IU doses). Both vitamin D2 and vitamin D3 supplements seem to equally work well for boosting circulating levels of needed vitamin D hormones and act as appropriate substrates for the body to convert to active forms internally. As with calcium, however, compliance issues are important to discuss with an empowerment and motivational interviewing style designed to focus on awareness and improvement. If measured blood vitamin D levels remain suboptimal or if there is no improvement over time - or worsening over time - with follow-up DXA measurements or honest compliance discussions, then enlisting other family members should also be the considered. Exactly which blood levels to target for therapeutic/optimal efficacy is also not well agreed upon or known. Aiming for targeted blood total 25 hydroxyvitamin D levels of approximately 50 ng/ml is this author's recommended therapeutic level to use as a guide to increasing or decreasing such supplements and hopefully not only maintain these blood vitamin D levels but also allow bone density improvement to take place. Some patients need such supplements maintained for long periods of time while others need such supplements for a shorter duration of time and then doses adjusted downwards to maintain these levels and allow mineralization improvement based on actual follow-up DXA scans. Table 4 presents this author's NEDEC diagnostic and therapeutic protocol:

Table 4: Calcium and vitamin D diagnostic and therapeutic NEDEC protocol

1. Start with detailed dietary and sun exposure history focusing on calcium and vitamin D estimates
2. Take specific family history of fractures, osteopenia, osteoporosis
3. Measure baseline total 25 hydroxyvitamin D levels
4. Add calcium supplements once or twice-a-day to bring total dietary and supplement near to desired goal of approximately 1500 mg/day calcium intake. Some absorption studies suggest that calcium is better absorbed in two smaller doses rather than one larger dose but these studies are not conclusive

5. Add multiple vitamins with minerals in a form palatable and acceptable on the assumption that if there is calcium and/or vitamin D insufficiency then there may also be other minerals also insufficient or deficient that are difficult to measure
6. Add pure vitamin D3, cholecalciferol, either in liquid, softgel or tablet format on a daily basis stepwise increasing from 2000 – 5000 IU/day depending upon how low the initital blood vitamin D levels are. Most of NEDEC's child and adolescent-aged patients seem to require about 4000-6000 IU daily supplements to bring and sustain blood total 25 hydroxyvitmain D levels to about 50 ng/ml.
7. When such levels are not maintained and/or sustained for reasons of malabsorption and/or noncompliance, then a change to the more expensive higher potency 50,000 IU vitamin D preparation weekly can be considered. Rare patients require this twice-a-week and some patients find it easier to remember a once-a-week higher potency preparation rather than a daily supplementation.
8. Clinical experience at NEDEC suggests that abnormal DXA osteopenia and osteoporosis shows improvement in about 2-4 years in most such patients who comply with recommended suplementation and have documented improved vitamin D blood levels.

Conclusion

Long term prospective studies of children, adolescents and young adults will be required to document that such problems not only are being identified but also treated correctly and that such treatments are sustained to resolve the issues of abnormal bone mineralization and, ultimately, to prevent future fractures from occurring. Whether or not this will be sufficient to decrease the hypovitaminosis D associated with other conditions such as obesity, heart and blood pressure problems, lipid abnormalities, diabetes, respiratory infections, gastrointestinal disorders, autoimmunopathies, brain and mood disorders and cancer will also require long-term, prosepectvie studies. Until such studies are undertaken and completed, clinicians will be required to make prudent, cost-effective decisions about who to question, who to screen, which screening methods to utilize, when to order DXA scans and which types of treatments to recommend.

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HYPOGLYCEMIA FROM INBORN DISEASE OF METABOLISM

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INTRODUCTION

Hypoglycemia is frequently encountered in children and needs prompt treatment (paying attention to possible neurological sequelae)- the brain uses glucose as a source of energy, but also ketones or lactate – in certain situations. The rate of glucose utilization is higher in children compared to adults, because the children`s brain has a disproportionately larger volume compared to body mass.

DEFINITION

Hypoglycemia is defined:

- in newborns, when the plasma glucose concentration is < 40 mg/dL (2.2 mmol/L)
- in infants or children, when the plasma glucose concentration is < 60 mg/dL (3.3 mmol/L)[1]

Because glucose meters cannot determine low values of blood sugar, any determination < 54 mg/dL is considered hypoglycemia.[1] Hypoglycaemia is a medical emergency. Untreated, it can lead to:

- seizures,
- coma,
- irreversible brain damage,
- death.[1]

Blood glucose homeostasis is achieved through the balance between insulin and its counterregulatory hormones[2].

Fast-acting hormones are critical for countering the early phase of hypoglycaemia.

- Glucagon
- Adrenaline[3]

Slow-acting hormone - their release will begin 30 minutes after hypoglycemia and their counter-regulatory role cannot be appreciated until 3 hours after the onset of hypoglycemia.

- Growth hormone
- Cortisol[4]

ETIOLOGY OF HYPOGLYEMIA

Hypoglycemia can be an integral part of certain inborn metabolic diseases and endocrinological disorders. However, it can also occur in children who do not have these pathologies. The most common is ketotic hypoglycemia, which occurs in the age group of 18 months to 5 years (diagnosis of exclusion)[5].

Table 1: The etiology of hypoglycemia[6]

Endocrinological disease	Congenital hyperinsulinism Insulinoma Adrenal insufficiency Growth hormone deficiency Hypopituitarism Dumping syndrome hypothyroidism
Metabolic disease	Glyxcogen storage diseases Fatty acid oxidation defects Organic acidemia Disorders of gluconeogenesis Disorders in carbohydrate metabolism Disorders in ketone metabolism Mitochondrial diseases
Systemic Conditions	Idiopathic ketotic hypoglycemia Infections - sepsis, gastroenteritis Malnutrition Eating disorders Liver diseases
Toxicities	Medications (Insulin, beta-blockers, salicylates) Ethyl, methyl alcohol

Important data from medical history are:

- Age
- Birth weight and length
- Neonatal jaundice
- Neonatal history of hypoglycaemia
- Fasting tolerance / illness
- Family history: consanguinity, deaths of newborns without a known cause (inborn errors of metabolism), hGH deficiency, and hyperinsulinism.
- Episodes suggestive of hypoglycaemia, e.g., undiagnosed seizures.
- Dietary history, based on intake of:
  - Milk-based products ⟶ galactosemia
  - Fructose (juices) ⟶ hereditary fructose intolerance
  - Proteins ⟶ disorders in amino acid or organic acid metabolism
- Ingestion of medications/toxic substances: hypoglycaemic agents (Sulfonylureas), Aspirin, Beta blockers, accidental ingestion of ethyl and methyl alcohol.[7]

SIGNS/SYMPTOMS OF HYPOGLYCEMIA

Clinical examinations have to assess quickly signs/symptoms of hypoglycemia

Tabel 2: Signs/symptoms of hypoglycemia [8]

Autonome	**Neuroglycopenic**
- **Adrenergic response**	Lehargy
Palpitations	Iritability
Tremor	Hypotonia
Anxiety	Confused state
- **Colinergic response**	Headache
Swesting	Dizziness
Intense hunger sensation	Hypothermia
Nausea/vomiting	Seizures
Abdominal pains	Coma

There are some important clues that can guide to diagnose:

- Consciousness state

  - Vital signs* tachypnea ⟶ metabolic acidosis
  - High fever ⟶ sepsis
- Measuring stature, weight
  - Small stature ⟶ GH deficiency
  - Low weight ⟶ risk of ketotic hypoglycemia
- Hemihypertrophy, Macroglossia, Omphalocele ⟶Beckwith Wiedemann Syndrome
- Hepatomegaly ⟶Defect in gluconeogenesis, Galactosemia
- Defects of midline ⟶Hypopituitarism
- Hyperpigmentation of the skin and mucous membranes ⟶ Adrenal Insufficiency[9]

HOW TO EVALUATE A HYPOGLYCEMIC EPISODE

A hypoglycemic episode we will evaluate when is the first episode when blood glucose is ≤ 50 mg/dL, in case of hypoglycemic seizures or when the patient presents with unexplained hypoglycemia with neurological impairment[10].

In an emergency situation, we use the glucometer, but we do not wait for laboratory results to treat the hypoglycemic child. A value from the glucometer must be confirmed with the value from venous blood[10].

It is preferable to collect blood/urine samples before treatment. These initial analyses are complemented by others, which will be labelled 'post-treatment'.

According to priority, we have 2 types of investigations[11]:

1. First-line:

- Blood glucose level
- Insulinemia
- Peptide-C
- Ketones (acetoacetate, beta-hydroxybutyrate)
- Cortisol
- Acid-Base Status
- Human Growth Hormone (hGH)
- Acyl-carnitine profile
- Urine analysis (The first urine after the hypoglycaemic episode is the critical sample: urinary organic acids and ketones)

2. Second line:

- Electrolytes (Cl-, Na, K), Creatinine, Urea
- AST, ALT, GGT, Alkaline phosphatase

- Ionic Calcium, Magnesium, Phosphorus
- Uric Acid
- Creatine Kinase (CK)
- Plasma Amino Acids
- Ammonia
- Adrenocorticotropic Hormone (ACTH)

When we classify hypoglycemia etiologically, it is important to specify:

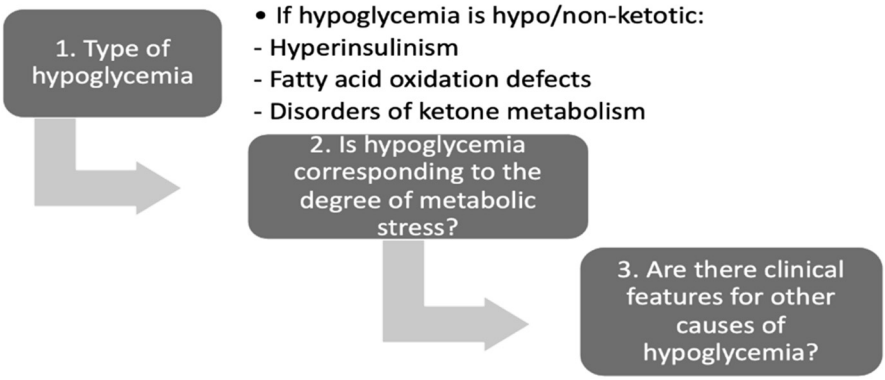

Figure 1: How to evaluate hypoglycemia[12]

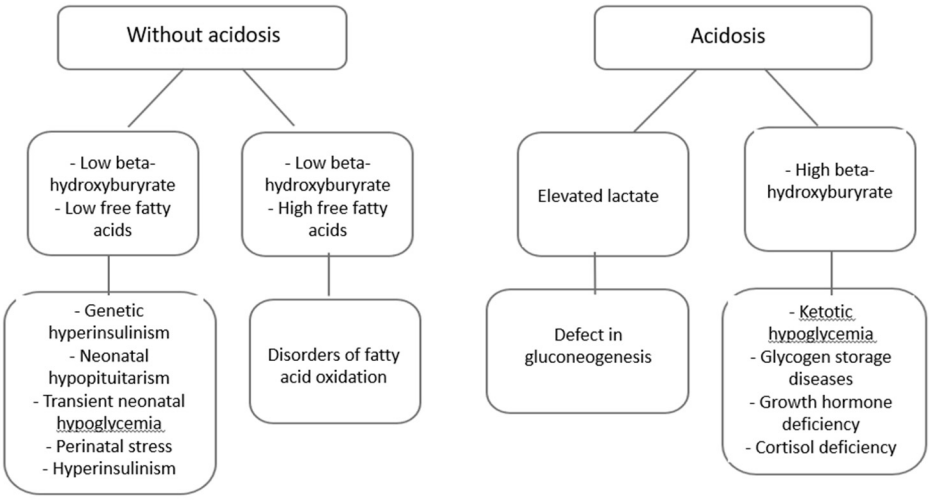

Figure 2: Investigation of causes of hypoglycemia
(Blood glucose <50 mg/dl) in relation to the biochemical profile[13]

CONGENITAL HYPERINSULINISM

Congenital hyperinsulinism (HI) is the most frequent cause of severe, persistent hypoglycaemia in newborn and children. When HI is not recognized and diagnosed or if treatment is ineffective in preventing hypoglycaemia, brain damage or death can occur[14].

The diagnosis of HI may be quite difficult if one relies on demonstrating an elevated blood insulin concentration at the time of hypoglycaemia because insulin levels fluctuate widely over time in patients with HI[15].

Congenital Hyperinsulinism International (CHI) created the HI Global Registry (HIGR) in October 2018 to collect patient-powered HI insights. HIGR provides the foundation for new research areas into the natural history and burden of the disease[16].

In most countries, HI occurs in approximately 1/25,000 to 1/50,000 births. About 60% of patients with HI develop hypoglycaemia during the first month of life. An additional 30% will be diagnosed later in the first year and the remainder after that[17].

Hyperinsulinism most commonly presents in the newborn period, but can also present during infancy and childhood. Hypoglycaemic symptoms vary from non-specific symptoms such as poor feeding, lethargy and irritability to apnoea, seizures or coma in the most severe cases[18].

Transient hyperinsulinism can occur in infants from diabetic mothers who have been exposed to maternal hyperglycaemia before birth. Infants who have sustained perinatal asphyxia and those with intrauterine growth restriction are also at increased risk of transient hyperinsulinism. In a few rare cases transient hyperinsulinism can result from a monogenic aetiology[19].

Permanent or persistent HI, also named “nesidioblastosis” is often associated with macrosomia, due to intrauterine exposure to high levels of insulin. The majority of cases with persistent hyperinsulinism are due to mutations of a single gene. HI may also occur as a feature of a genetic syndrome such as Beckwith–Wiedemann[20].

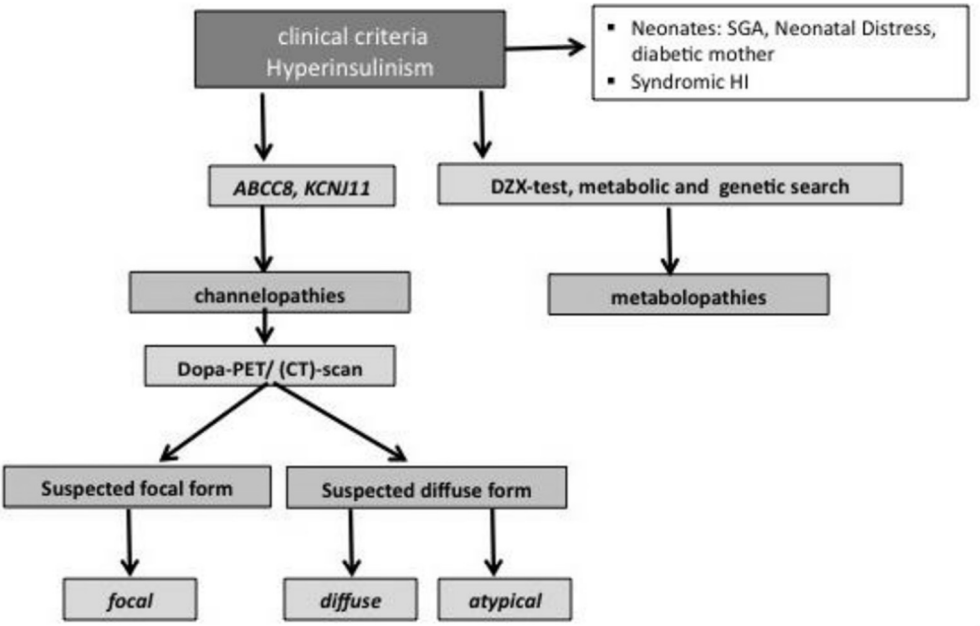

Figure 3: Hyperinsulinism algorithm[21]

Two main histological subtypes have been identified in individuals with hyperinsulinism:

- focal disease and
- diffuse disease[22]

A few patients have been reported to have 'atypical' histology. A number of different genetic mechanisms have been reported to cause atypical disease[23].

Focal hyperinsulinism arises when an infant inherits a pathogenic paternal *ABCC8* or *KCNJ11* variant and there is also loss of the maternal allele within the focal lesion[24].

Loss of heterozygosity and confirmation of focal disease can be confirmed in DNA extracted from the resected tissue using microsatellite markers within the chromosome 11p15 region[24].

Diffuse pancreatic disease affects all the beta cells within the islets of Langerhans. Diffuse hyperinsulinism is most often caused by autosomal recessive inheritance with disease-causing variants

being inherited from both unaffected parents although dominant inheritance has also been reported[25].

Diffuse hyperinsulinism is treated medically where possible with sub-total pancreatectomy only as a last resort since 75% of patients then develop iatrogenic diabetes[25]

It is important to differentiate between these two types as ^{18}F-DOPA PET-CT scanning is recommended for patients with a paternally inherited variant to locate a possible focal lesion within the pancreas as lesionectomy or partial pancreatectomy can cure focal hyperinsulinism[26].

The improvement in the understanding of the pathogenesis of congenital hyperinsulinism and the development of diagnostic modalities have helped in deciding the optimal management strategy for each patient

1) Molecular and pathological basis of congenital hyperinsulinism
2) 18F-DOPA PET/CT
3) Conservative treatment: diazoxide, octeotrid, lanreotide
4) Conservative surgery[27]

Type	Drug name	Mode of action
Diazoxide		Activates K_{ATP} channels of pancreatic β cells and maintains them in an open state, inhibiting insulin secretion
Somatostatin analogue	Octreotide LAR Lanreotide	Decreases secretion of insulin through hyperpolarization of β cells and inhibition of calcium channels
Sirolimus (formerly rapamycin)		Inhibits the mTOR signaling pathway, potentially limiting the production of insulin from β cells
Glucagon		Promotes hepatic glucose production and increases blood glucose levels

CHI, congenital hyperinsulinism; K_{ATP}, adenosine triphosphate-sensitive potassium; LAR, long-acting release; mTOR, mammalian target of rapamycin

Figure 4: Treatment options in congenital hyperinsulinism[17]

CASE REPORT

We report a case of a male infant who presented at the age of 2 months to the hospital for limb clones, later associated with drowsiness and hypotonia. In the emergency department we found a blood sugar level of 23 mg/dl. The value of insulinemia in the context

of hypoglycaemia was 25.7 IU/ml. The entire pregnancy was monitored at the gynecologist.

The morphology tests indicated ventriculomegaly and amniocentesis was recommended. The procedure could not be performed because of the position of the fetus and placenta. Screening test for metabolic disorders was not performed at birth.

At the clinical examination we notice macroglossia, hepatomegaly and earlobe creases.

The glucose requirement (GIR) administered by intravenous infusion was initially 15 mg/kg/min, with decrease in next days at 10 mg/kg/min, and persist > 8 mg / kg / min for next 3 weeks. Due to the constantly increased values of insulinemia (over 6 uU/ml) and the increased glucose requirement, the causes of hypoglycaemia with hyperinsulinism are discussed, but considering the association of hypoglycaemia with the absence of ketones and hyperinsulinism, the clinical-paraclinical and imaging investigations are directed in this sense.

As a result we contacted the Exeter center for genetic testing for congenital hyperinsulinism. Genetic testing has confirmed that the patient is heterozygous for a paternally inherited pathogenic ABCC8 missense variant.

Diazoxide treatment was tried but the response was not favorable, with persistent hypoglycaemia, which is why we considered this case as an unresponsive congenital hyperinsulinism to Diazoxide.

Glucagon test was not performed because in the meantime we received the genetic result of pathogenic ABCC8 missense variant. Considering the mutation found, the next step is to perform 18 FDOPA PET-CT.

During hospitalization, we applied a sensor for continuous monitoring of blood glucose – CGM. After 4 weeks, he was discharged in good condition, weight curve increasing, feeding with milk formula, to which Polycal should be added.

The mother was instructed on the administration of glucagon in case of severe hypoglycaemia (hypoglycaemic convulsions or unresponsive patient) Glucagon - Glucagen Hypokit - 0.5 ml subcutaneous or intramuscular will be administered.

CONCLUSIONS

Future studies are necessary to optimally define the normal blood glucose thresholds in newborns and children.

Regardless of the specific diagnosis, prompt recognition and treatment of acute hypoglycemic episodes are essential to prevent its complications.

Diagnosis should begin with the collection of critical samples at the time of hypoglycemia, providing specialists with clues to properly address clinical suspicion.

Based on recognized risks associated with some tests, the traditional diagnostic process, including fasting or dynamic tests, is currently controversial and likely to be replaced by modern molecular diagnostic techniques.

Next generation sequencing (NGS) approach also has the potential to diagnose disorders with mild biochemical abnormalities or atypical presentations or even to identify new diseases, altering the epidemiology of many conditions.

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THE BRAIN AND DIABETES IN CHILDREN

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Oana-Alexandra Velea-Barta

Introduction

Brain development affects the ability of children and young people to form healthy and satisfying relationships and succeed later in life both professionally and socially.

The first years of a child's life are very important for mental health and subsequent psychological development.

The development of the brain takes place in stages, with the lower and more primitive areas developing since the intrauterine period (the areas that regulate vital functions: breathing, heart rate, temperature).

At birth, the newborn already has billions of neurons, and during the first 18-24 months of life, there will be an extraordinary increase in the neural connections that form the communication paths between neurons that will direct all future functions and behaviors.

The brain continues to develop and transform even in adulthood, but the first 8 years of life represent the basis for good memory capacity and cognitive function in full accordance with genetic information.

The prefrontal cortex, which controls "executive" function, is the last area of the brain to mature in adulthood.

The degree of development of the child's brain depends, in addition to genetic factors, on a number of factors that can influence and even disrupt further development, such as: the mother's diet during pregnancy, exposure to a number of epigenetic factors (environmental, toxins, infections, etc.) but also the experiences acquired by the child through the interaction with the environment.

In this context, given the continuously increasing incidence of DM type 1 in children and especially the increasing number of cases with onset in the first years of life, there are more and more authors who pay attention to the impact of DM type 1 on brain development and of the cognitive function of these children. Although the results of the DCCT study showed conditions to reduce chronic microvascular complications, there is currently a growing interest in the effects of type 1 diabetes (T1D) on brain development, cognitive function and quality of life in patients with diabetes onset in childhood period.[1-3]

Stages of brain development

The basic structure of the brain is established during the intrauterine birth (at about 2 weeks of life), continues during early childhood, and the formation and refinement of neural networks continues until adulthood (around the age of 20 or even beyond). going through several stages.

- formation of the neural tube

During the embryonic period (approximately 2 weeks after conception) the primitive neural tube is formed. The cells inside the tube will later form the central nervous system (brain and spinal cord) and the cells outside the neural tube are the ones that will give rise to the autonomic nervous system.

- cell proliferation

The cells that line the innermost part of the tube, called the ventricular zone, proliferate and multiply, they form a second zone, the marginal zone, which will contain axons and dendrites. This marked proliferation explains why the newborn brain has more neurons at birth than the adult brain.

- cell migration

Cell proliferation is followed by the so-called cell migration through which the cells migrate to other areas that will become the final destinations. Following this migration around the age of 25 weeks of gestation, the fetal cortex has all 6 layers formed.

- differentiation

The neuron that has reached its target destination generally continues its evolution along one of two paths: it can either differentiate into a mature neuron, complete with axons and dendrites, or undergo apoptosis. Approximately 40 - 60% of neurons do not end up as mature neurons but undergo the phenomenon of apoptosis.[4]

Dendrite formation occurs by a slightly different process, driven by certain genes that control calcium-regulated transcription factors.[5]

- Synaptogenesis

The first synapses appear around the 23rd week of gestation[6], but maximum production occurs in the first year of life.

Different brain structures produce a maximum of synapses at different times. In the visual cortex, for example, peak production is postnatally between the 4th and 8th month, while in the prefrontal cortex the peak is reached at the 15th postnatal month.

- myelination

It represents the final process that facilitates neuronal activity and communication because this insulation allows myelinated axons to transmit electrical signals faster than unmyelinated axons.

In the prefrontal cortex (one of the regions involved in the acquisition of higher cognitive abilities), the process evolves in adolescence and continues until around young adulthood when it is finalized.[7,8]

The relationship between glucose and brain development

The newborn brain is about one-quarter to one-third the size of the adult brain, and it grows and specializes according to a precise genetic program.[9.10]

In the brain, glucose requires specific glucose transporters (GLUTs) contributing to the transport of at least 95% of glucose to nerve tissues[11.12] called GLUT 1 to GLUT 6 (more recently). Among them, GLUT 1 and GLUT 3 play the most important role in the brain.[13,14]

The expression of these receptors is regulated on the one hand by the demand for glucose in the brain (raised in children) [15,16] and on the other hand by a series of factors such as: hypoxia, insulinemia, hypo and respectively hyperglycemia. But, the absorption of glucose at the neuron level is carried out without the help of insulin, being influenced directly proportionally by the extracellular concentration of glucose, which exposes the brain to a much greater risk of deterioration compared to other cells in the human body.[17]

Glucose metabolism in the brain

Approximately 30% of circulating glucose is found in the interstitial fluid of the brain, requiring approximately 20–30 minutes

of concentration stabilization during periods of glycemic variability.[18,19]

Although there is such a large amount of glucose in the brain, not all glucose is metabolized immediately, much of it is stored as glycogen in astrocytes.[20] The energy provided by glucose metabolism in the brain is mostly used (about 70%) for signal transmission at the neuron level (action potentials, calcium activities, synaptic transmission and the glutamate cycle); the difference is used for axonal transport, resting potential and cytoskeletal remodeling.

On the other hand, glucose metabolism provides the carbon necessary for the synthesis of nucleic acids, fatty acids and amino acids and the production of metabolites involved in inflammatory reactions and redox phenomena.[20-21] There are few studies of glucose utilization in the human brain during development due to limitations imposed by the use of radioactive markers in healthy children.[22] In these conditions, the cerebral metabolic rate for glucose (CMRGIc) is accepted in children.[23,24]

Through this technique it was proven that in the first 2 years of life the CMRGIc is similar to that of the young adult (23-25 µmol/100g/min). [24,25] These values increase in the following years until doubling the value for adults around the age of 5, decreasing gradually in the preadolescence period to reach the adult values at the age of 15 (see *Table 1*)

TABLE 1 - Quantitative estimates of CMRGlu in children.[22]

Age	**n**	**CMRGIu (µmol/100g/min)**	**Reference**
Preterm	6	8,8	Powers et al
Preterm	8	5,5	Kinnala et al
Term – 2m	8	7,3	Kinnala et al
2m – 6 m	6	16,5	Kinnala et al
0 – 1 y	7	20,4	Chugani et al
1y – 2 y	4	27,4	Chugani et al
3 y – 8 y	12	48,1	Chugani et al
9 y – 15 y	6	39,2	Chugani et al
19y – 20 y	7	24,3	Chugani et al
21y – 28 y	8	23,3	Madsen et al

Studies have shown that not only does glucose utilization change with age, but glucose utilization is different from one area of the brain to another with age. In addition, it has been proven that

oxygen consumption also increases in children, but not as much as glucose consumption.

These data suggest that part of the increase in brain glucose consumption during prepuberty probably occurs through non-oxidative pathways, i.e., aerobic glycolysis.[26]

Aerobic glycolysis

It is a metabolic pathway characteristic of tissues in periods of proliferation/growth.

Oxidative phosphorylation is the main supplier of ATP (adenosine-tri-phosphate) producing up to 36 molecules of ATP / 1 molecule of glucose. This explains why aerobic metabolism is favored in the developing brain.[27]

Due to the various biosynthesis processes required for synaptic development and myelination, aerobic glycolysis is one of the main reasons for the increased glucose requirement in the developing child's brain.[28,29]

During different developmental stages, brain structures with high CMRGIc determine the predominant behavioral pattern at the particular developmental stage (*Table 2*) being closely correlated with postnatal age.

Table 2 – Age relationship – brain areas and neurological characteristics (after 27)

Age	FDG – PET pattern	Neurological features
Newborns	Subcortical brain structure	Intrinsec reflexes
3 months	Parietal cortex Primary visual cortex Cerebellar hemisphere	Visual – spatial and Visual – motor integration
8 months	Dorsolateral and frontal occipital cortex	Greater interaction with the environment
> 2 years	Global increased glucose consumption	
3 – 5 years	Twice the value compared to adults	

The impact of diabetes on the developing brain

Studies in adults with type 1 DM show regional reductions in brain volume. In those with childhood-onset DM, these volume reductions appear to reflect the sum of changes that occur during

brain development and changes that occur later in life that result from exposure to diabetes-related factors.[28]

Advanced imaging techniques detect in type 1 DM patients microstructural lesions both in the cerebral gray matter and in the white matter, lesions that affect structural and functional connectivity.

It is now clear that type 1 diabetes is associated with modest declines in cognitive functioning, which are most marked in patients with childhood-onset diabetes.[29] The exact localization of brain volume changes varies greatly between studies, highlighting the difficulty to detect small regional differences with current techniques and which should be sensitive to inter-individual differences in head size and shape.

Research has shown that there is a relationship between decreased brain volume and slower information processing speed, reduced attention, and even lower IQ test scores.[30,31]
Smaller brain volumes in patients have been linked to poor metabolic control secondary to chronic hyperglycemia[32] and hypoglycemic events.[33]

The reduction in brain volume in adults may thus reflect changes that have already occurred during brain development.
Recently, studies have shown that changes in brain volume can be detected in childhood[34], due to the interaction of DM type 1 with periods of neuronal growth.[34] The fact that smaller brain volumes are observed in adults with early-onset diabetes compared to those with later-onset diabetes supports the idea that brain changes in childhood persist into adulthood.[30,31].

Effects of hyperglycemia

In the pediatric population type 1 DM is the pathology with the most severe impact on glycemic homeostasis.[32] The negative impact of dysglycemia on brain structure and function has been confirmed by volumetric and structural changes in the brain.[33]

Hyperglycemia induces an increase in the permeability of the blood-brain barrier, leading to mitochondrial dysfunction and cellular damage.[17]

Indeed, chronic hyperglycemia can increase oxidative stress and even neurodegeneration,[34,35,36] induce changes in the composition of brain sphingolipids (ceramides and sphingomyelin) causing membrane rearrangements in some cell populations.[37]

Brain imaging in type 1 DM patients has shown total and regional reduction in gray matter and white matter volumes,[38,39] a reduction proportional to the time of onset of diabetes. With early onset at young ages, the brain parenchyma underwent a more drastic change compared to the later onset group.[40]

A decrease in gray matter was found in the precentral and temporal frontal regions, but also in the thalamus and insular cortex.[41] Regional differences can be explained by a greater need for glucose in the frontal and temporal lobes.[42]

Recently, a spectrometric imaging study in children with type 1 DM found decreased choline and N-acetylaspartate suggesting neuronal loss. or impaired function in that area, with changes in membrane lipids and/or a decrease in membrane turnover.[43] Reduction of N-acetylaspartate has been reported as a marker of neuronal density, thus linking chronic hyperglycemia, demyelination, and neuronal loss.[44] Increased myoinositol and choline have also been reported in T1D patients, demonstrating changes in osmolarity, demyelination, and hypoxic glial injury.[45]

Effects of hypoglycemia

Severe hypoglycemia is a real neuronal insult causing altered state of consciousness, with convulsions or coma. Progressive cognitive dysfunction appears to occur at a blood glucose level below 3.0 - 3.5 mmol/L.[46] Once established, hypoglycemia can trigger excessive synaptic release of glutamate, causing intracellular calcium toxicity and ultimately cell damage;[47] in severe hypoglycemia, an overstimulation of N-methyl-D-aspartate receptors can occur, initially resulting in excitotoxicity with later cell damage.[17] Under energy-restricted conditions (fasting conditions or after prolonged exercise), brain cells begin to use alternative resources in metabolism such as ketone bodies, which can provide up to 60% of metabolic requirements.[46]

Rapid fluctuations in blood glucose (glycemic variability) can affect cellular metabolism by decreasing mitochondrial activity and activating intrinsic apoptotic pathways.[46,48]

MRI examination after hypoglycemia has described manifestations of gliosis and reactive neurogenesis with compromise of normal hippocampal development.[49]

Another aspect worthy of consideration is the frequency of hypoglycemic episodes.

A meta-analysis studying the effect of recurrent severe hypoglycemia in children with T1D reported a slight but significant decline in cognitive performance in T1D children with episodes of severe hypoglycemia compared with those without such episodes.[50]

Significant differences were found in the four domains of cognitive function: memory, intelligence, learning and verbal fluency.[50] Neuroimaging studies support these findings, showing that severe hypoglycemia specifically targets neurons in the cerebral cortex, especially in the medial temporal region, including the hippocampus (involved in memory functions), basal ganglia, and brainstem.

At the same time, a reduction in the volume of gray matter at the left temporo-occipital junction in diabetic children with one or more severe episodes of hypoglycemia,[51] a relatively lower density of gray matter in children with a history of severe hypoglycemia,[52] was proven. with a predilection of the hippocampus.[50]

Effect of insulin deprivation on the brain

Recently, more and more voices are discussing the impact of diabetes and the degree of structural changes on the patient's brain.

A different approach from those presented is depriving the brain of insulin.

The lack of circulating insulin in a patient with DM type 1 (with an average duration of 5.4 hours) leads to an increase in blood glucose with an increase in the concentration of b-hydroxybutyrate and consequently to a decrease in the level of bicarbonate. In this sense, there are studies that sought to prove whether there are differences between type 1 DM patients who were induced with a transient insulin deprivation and non-diabetic patients.

The results showed that biochemical changes occurred in type 1 DM patients in whom insulin deprivation was induced. Thus, if at the beginning of the study there were no significant differences in the concentration of beta-hydroxybutyrate between the two groups (patients with DM type 1 and the control group of non-diabetics), after insulin deprivation the concentration of beta-hydroxybutyrate and osmolarity was much higher in patients with DZ type 1 compared to the control group.

Under these conditions, it was analyzed whether transient insulin deprivation would have an effect on cognitive function. Studies have shown poorer outcomes in insulin-deprived type 1 DM patients compared to controls in terms of attention and memory.

Interestingly, in the memory test, adolescents from both groups had poor results at the time of insulin deprivation, which draws attention to the possible negative effects of fasting in non-diabetic patients as well.

Vis a vis the metabolite changes, it was proven that the ratio N-acetyl acetate/creatinine (NAA/Cr) as well as myoinositol/Cr (mI/Cr) did not undergo significant changes after insulin deprivation compared to the initial moment. What was found was the decrease in the level of ATP after insulin deprivation in patients with type 1 DM compared to the control group.

Studies have shown that in patients with DM type 1, insulin deprivation is associated with a lower functional connectivity compared to the initial moment. Thus, although the type 1 DM group had baseline differences in brain functional connectivity (FC) compared to controls, more important changes occurred following insulin deprivation, particularly between hippocampal caudate-putamen regions and early visual and motor sensory areas.

In the study conducted by Ana L. Creo et al [53] between the different regions involved in memory FC was different in the group with DM type 1 compared to the control group, and the accumulation of neurometabolites (harmful), changed FC, which then led to cognitive changes.

The vigilance and attention required for learning success are mediated by the interaction between brainstem-diencephalic structures, with structures involved in memory and sensory-motor functions (30). All areas causing cognitive decline have been shown to be affected in type 1 DM patients during insulin deprivation of insulin receptor-rich brain regions.[54] It is known that there is a different distribution of receptors for insulin and IGF1 in the brain.[54]

In conclusion, it seems very likely that insulin deficiency produces, independently of hyperglycemia and/or glycemic variability, neurometabolic changes in the brain.

In an experimental study in diabetic mice, intranasal insulin administration resulted in increased insulin concentrations in the brain that activated downstream signaling with an effect on mitochondrial function in the hippocampus. These results support the idea that insulin acts directly on brain regions involved in memory, most likely by increasing local energy metabolism.

These results lead us to think about patients with DM type 1 who benefit from insulin substitution through an augmented insulin pump with a glucose sensor and who do not make a correct

adaptation of insulin doses, ending up relying on the pump algorithm that stops the discharge of the basal rate.

We consider a vigilant approach to this category of patients necessary through the intensive and repeated analysis of the reports and the identification of the causes that lead to the cessation of the discharge of the basal rate in order to reduce the time that the brain is deprived of insulin.

As an example, we present below a case, male, adolescent (17 years old) wearing an augmented insulin pump with a glucose sensor, and who has an unsatisfactory metabolic control (TIR with values between 64% - 66%, CV between 42, 5% - 44.7%, GMI = 66% - 6.8% (*Figure 1*). The analysis of the reports shows days with a very long duration of insulin deprivation throughout the analyzed period with a total of insulin deprivation in the 2 weeks shown in figure 2 of: 2 days, 6 hours and 8 minutes (*Figure 2*).

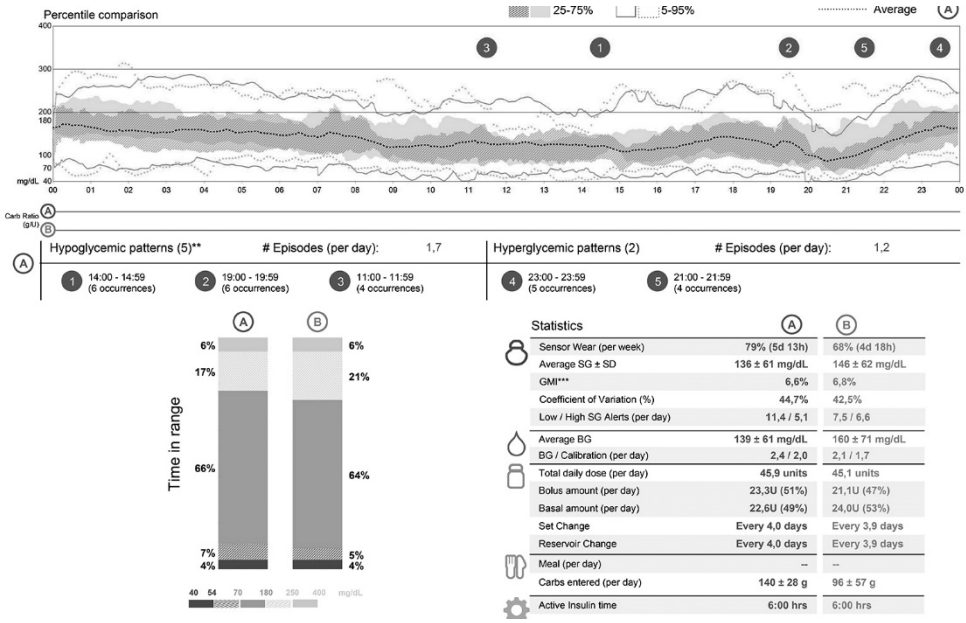

Figure 1 – Male, 17 years old with unsatisfactory metabolic control

DKA at diagnosis

The onset of DM with significant hyperglycemia triggers a series of structural and functional changes in the brain with increased permeability of the blood-brain barrier to larger molecules and potential neurotoxins.

If chronic hyperglycemia or CAD occurs during critical periods (especially in the first 4-5 years of life) "*alterations in brain organization may induce a diathesis of stress or predisposition that increases the individual's vulnerability to further further insults to the brain*".[55]

	Glucose Measurements		Bolus Events					Fill Events					
	BG Readings	Sensor Duration (h:mm)	Manual Boluses	Bolus Wizard Events	With Food	With Correction	Overridden	Rewind	Cannula Fills	Cannula Amount (U)	Tubing Fills	Tubing Amount (U)	Suspend Duration (h:mm)
Sunday 04.02.2024	2	23:50	3					1	1	0,7	1	8,9	3:48
Monday 05.02.2024	2	22:50	3										3:26
Tuesday 06.02.2024	2	23:45	3										5:55
Wednesday 07.02.2024	1	18:45	4					1	1	0,7	1	9,3	0:08
Thursday 08.02.2024	2	12:00	3										3:40
Friday 09.02.2024	2	23:35	3										6:45
Saturday 10.02.2024	3	23:50	4										5:32
Sunday 11.02.2024	3	23:05	3					1	1	0,7	1	5,9	3:56
Monday 12.02.2024	4	17:30	3										6:09
Tuesday 13.02.2024	3	13:10	5										
Wednesday 14.02.2024	2	23:50	3										2:01
Thursday 15.02.2024	2	23:05	5					1	1	0,7	1	4,1	2:31
Friday 16.02.2024	4	23:10	4										3:11
Saturday 17.02.2024	2	21:35	3										
Summary	2,4/day	12d 06h 00m	3,5/day	0,0/day	--	--	--	4	4	0,7U /fill	4	7,1U /fill	1d 23h 02m

Partial day Suspend Suspend On Low Suspend Before Low

Figure 2 – Male, 17 years old with days with a very long duration of insulin deprivation

In a larger prospective study that included 144 patients aged 4-10 years, followed for > 18 months, young children with Type 1 DM were followed and significant differences were reported: in brain development with reduced volume of regional white and gray matter but also in neurocognitive function.

Full-scale IQ (Cohen d = -0.47), memory (d = -0.41) and attention (d = -0.52) were significantly lower in children with moderate/severe CAD at baseline, compared to children with mild CAD or no CAD at the time of onset of DM type 1.[56]

The adolescent brain

During adolescence, hormonal and neurodevelopmental changes are mediated by the growth of neuronal processes,

remodeling of synaptic connections, increased myelination in prefrontal areas, and maturation of subcortical connecting regions.[57]

Myelin development during adolescence is an area of increasing interest given its potential relationship to cognition, behavior, and learning.

Recent investigations suggest that both white matter (WM) and gray matter (GM) undergo prolonged myelination during adolescence, Stage of puberty was significantly correlated with myelin density in several cortical areas and in the subcortical GM. These findings indicate significant differences in GM and WM myelination trajectories across brain regions and suggest that cortical GM myelination plays a dominant role during adolescent development.[58]

This information is to be kept in mind, especially since the adolescent period is characterized by an unsatisfactory metabolic control in a large percentage of patients (according to some studies, about 75% of adolescents have an average of HbA1c = 9%, which denotes a metabolic balance unsatisfactory.)

The best evidence for functional and neuroanatomical changes in the human adolescent brain that may underlie adolescent psychopathology has been obtained using various applications of structural and functional MRI.

Exploring the brain by MRI

The different studies carried out with the aim of exploring the imaging of the brain of patients with DM type 1 brought into discussion the relationship between hyperglycemia and respectively hypoglycemia and the reduction in brain volume.

Thus, it was proven that hyperglycemia accentuated the gray matter reductions of the brain, possibly through accelerated "frying" or direct cell damage.

Severe and especially repeated hypoglycemia slowed down the normal increase in white matter volume in the occipital and parietal regions, possibly by interfering with normal myelination or even by directly damaging the white matter.[59]

One of the potentially modifiable protective factors in children with DM type 1 and especially in adolescence is sleep.

Sleep disorders due to age and glycemic imbalance contribute to worsening glycemic control in type 1 DM [60,61] with indirect impacts on the management of DZ by changing children's behavior.[62] The increased incidence of DKA episodes leads to a decrease in the quality

of parental sleep and implicitly to a decrease in the parents' quality of life.[63]

More and more authors argue that executive function skills (cognitive flexibility and sustained attention) are particularly vulnerable in the case of patients with sleep disorders.[64]

In this sense, it was proven that extending sleep by 30 minutes led to the improvement of neuro-behavioral function in school-age children.[65]

Conclusions

In early childhood, increased vulnerability to insults is due to dynamic brain development, including maturation and pruning of synapses and increased myelination of neurons in the white matter.

Glycemic variability can affect developing neurons and myelin due on the one hand to the disorders produced at the level of the myelin sheaths, but also by reducing the myelin content secondary to hyperglycemia.[66,67]

Compared to control subjects, children with DM have poorer performances, if the onset of DM is earlier, performances that can worsen starting with puberty.[68]

A better glycemic control of blood sugar in these age groups, characterized by maximum vulnerability, is imperative.

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THE CHALLENGES OF ESTABLISHING THE DIAGNOSIS OF DIABETES

Andreea AVRAMOVICI, Iulian VELEA

Introduction

In the realm of chronic pathologies under long-standing scrutiny, there is a tendency to approach them without a sense of curiosity, often under the assumption of comprehensive mastery and exhaustive knowledge. However, like everything else, even well-established diseases such as diabetes are subject to ongoing evolution.

Returning to fundamentals, it's understood that diabetes stands as the most prevalent endocrine-metabolic disorder, characterized by heterogeneity from an etiopathogenic perspective. It arises due to deficiencies in insulin secretion, action, or both.[1]

The diminished response of the tissues to insulin and the inadequate secretion result in an abnormal response in the metabolism of carbohydrates, proteins and lipids.

It can happen that deficiency and inadequate insulin secretion coexist in the same individual.[2,3]

Given the multifactorial and heterogeneous nature of diabetes etiology, it is commonly classified into two broad etiopathogenic categories: type 1 diabetes and type 2 diabetes.

Type 1 diabetes (T1D) is distinguished by the near-complete or complete destruction of pancreatic β cells via an autoimmune mechanism, leading to the cessation of endogenous insulin synthesis. The onset of these autoimmune disorders is often precipitated by external factors acting upon a genetically susceptible background.[1,4]

It is one of the most common metabolic pathologies found in children/adolescents, with a maximum incidence around the puberty period, currently over 1 million children and adolescents

have T1D, the highest rates being in the Middle East and Northern Europe.[5]

The defining mark of type 1 diabetes is the presence of autoantibodies: anti-glutamic acid-decarboxylase (GAD), anti-islet cells (ICA), anti-insulin (IAA), anti-islet cell surface (ICSA), anti-carboxypeptidase H, anti-insulin receptor, anti-cationic transporter ZnT8. [4,5]

T1D can have several stages of development which can be seen in *figure 1.*

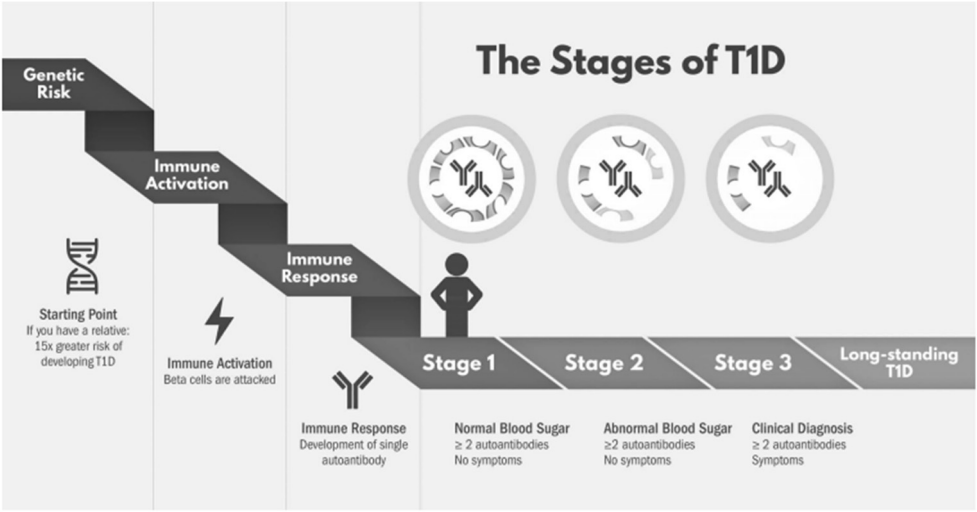

Figure 1. The stages of T1D (*DiabetesTrialNet.org*)

Individuals who have a first-degree relative with type 1 diabetes experience a relative risk of developing T1D that is 15 times higher compared to those without such familial history.

Those who possess two or more islet autoantibodies alongside normoglycemia are classified as having stage 1 T1D.

The majority (80% to 90%) of children who exhibit multiple islet autoantibodies progress to stage 3 T1D within 15 years, contrasting with approximately 15% of those who possess a single islet autoantibody. Ultimately, nearly all children with multiple autoantibodies will transition to stage 3 T1D.

In the context of multiple autoantibodies, the standard 2-hour oral glucose tolerance test (OGTT) remains the established diagnostic tool for staging the disease. This test involves administering 1.75 g/kg of oral glucose (up to a maximum of 75 g) and assessing glucose

levels at various time points. Glucose values equal to or exceeding 11.1 mmol/L (200 mg/dL) obtained at 30, 60, and 90 minutes post-glucose administration have been utilized in research settings to evaluate the risk of disease progression.

Moreover, mid-OGTT glucose values of 11.1 mmol/L (200 mg/dL) or higher can be employed to formally diagnose Stage 3 T1D, particularly when accompanied by elevated HbA1c levels or fasting glucose measurements. [1]

Type 2 diabetes (T2D) is defined by the lack of an adequate response to insulin in the presence of insulin resistance. What is worrying is the fact that its incidence is increasing globally, especially among young people.[1]

The diagnostic criteria for T2D are based on the determination of glycemic values, the presence of symptoms and the absence of autoantibodies. According to ISPAD, these criteria are:

- Glucose a jeune ≥ 7.0 mmol/l (126 mg/dl);
- Blood sugar at 2 hours during the glucose tolerance test ≥ 11.1 mmol/l (200 mg/dl);
- Symptoms suggestive of diabetes and occasionally determined blood glucose ≥ 11.1 mmol/l (200 mg/dl)
- HbA1c ≥ 6.5% (48 mmol/mol). [6]

In clinical practice, the differentiation of diabetes types may seem straightforward; however, the complexity of medicine becomes evident when encountering patients who defy conventional patterns and classifications. Such instances prompt healthcare professionals to delve deeper into individual patient characteristics, seeking the most appropriate diagnostic framework and treatment regime.

Material and method

As part of our study material, we will track the progression of two adolescents whose diagnosis and clinical course posed a significant challenge.

CASE 1

Patient aged 13 years and 10 months, female, with significant myopia and astigmatism, was diagnosed with fasting hyperglycemia in January 2022 (177 mg/dl) and an HbA1c level of 7,75%.

Apart from elevated fasting blood glucose and glucosuria, she does not present any clinical symptoms. In the familial medical history, the mother has a medical record of follicular thyroid cancer, while the father has a history of hepatitis B infection. There is no

known occurrence of diabetes among immediate or extended family members

The diagnosis of T2D is established and the treatment by changing the diet, with clinical and paraclinical follow-up is start. 7 months after the diagnosis, she presents herself to our clinic for more detailed investigations, having elevated glycemic values a jeun.

Clinical examination revealed: stature= 174 cm, weight= 55 kg, BMI= 18,17 kg/m2.

Glycemic values are increased postprandial (>200 mg/dl), but also during the night, and HbA1c = 7.54%.

The glucose tolerance test was performed in which the blood glucose value at 2 hours was 308 mg/dl. Laboratory investigations revealed the presence of islet autoantibody for Glutamic Acid Decarboxylase (GAD) (77,600UI/mL), and Islet Antigen Insulin (IAA) positive (1,06). C peptide value is normal.

The established diagnosis was type 2 diabetes with autoimmunity, and the established therapy was with metformin 1500 mg/day (morning 1000 mg + lunch 500 mg), to which the patient responds well, glycemic values normalizing. After three months, at the paraclinical re-evaluation, HbA 1c was 7,54% and blood glucose during the night is found to be elevated, which is why treatment with Metformin adjust to 2000 mg/day (morning 1000 mg + lunch 1000 mg) and insulin therapy with Detemir is initiated in the evening at 7 pm, with the dose adjusted according to the blood glucose values. After another six months, the patient returns to the control, HbA1c having the value of 6,9%,where the trend of hyperglycemia (>200 mg/dl) is observed, diminished postprandial, reason for which a new dose of Detemir is initiated in the morning. The total dose of insulin Detemir was 0.25 IU/kg/day.

Five months subsequent to the patient's previous clinic visit, there is a recurrence of postprandial hyperglycemia. Consequently, fast-acting insulin therapy with aspart 0.12 U/kg divided into two doses, administered in the morning and at lunch is initiated, resulting in the normalization of glycemic levels.

CASE 2

A 16-year-old male patient, diagnosed with Type 2 Diabetes and obesity in June 2020, commenced treatment involving insulin therapy and Metformin. He presented to our Clinic with complaints of polydipsia, polyuria, and a glycemic value of 500 mg/dl.

Subsequently, the patient entered a period of remission the following year, during which he continued treatment with Metformin and adhered to dietary measures, resulting in normalization of glycemic values. Notably, over the past three years, he has experienced a weight loss of 38 kilograms.

From the medical history, it's evident that tests for islet autoantibodies targeting GAD , IAA, and ZnT8 antibodies returned negative results. Additionally, the C-peptide level was within the normal range.

After 3 years of disease progression, the patient presents with diabetic ketoacidosis, exhibiting a glycemic value of 500 mg/dl, prominent glycosuria and ketonuria, and metabolic acidosis (venous pH = 7.30).

Clinical examination revealed the following measurements: stature= 183 cm, weight= 77 kg, resulting in a BMI= 22.99 kg/m^2. Laboratory investigations unveiled the presence of anti-glutamic acid decarboxylase antibodies (15.800 UI/mL) and a low C-peptide value (0,610 ng/mL), alongside an HbA1c level of 13.12%.

Elevated levels of triglycerides (205 mg/dl), increased abdominal circumference (105 cm), and low HDL cholesterol levels (26 mg/dl) were observed. Also presenting high glycemic values, the diagnosis of metabolic syndrome is made, the patient meeting 4 of the 5 criteria.

Following the initiation of insulin therapy, an elevated resistance to insulin was observed, with the HOMA index ranging between 5 and 8, surpassing the normal threshold of below 1.

The required insulin dosage post-diabetic ketoacidosis resolution ranged from 1.3 to 1.5 U/kg.

Can T2D in teenagers progress to T1D? This is the question that has always been following this cases. Research indicates a notable occurrence of auto-antibodies in patients initially diagnosed with type 2 diabetes. These unconventional cases, despite receiving appropriate treatment, exhibit an inadequate disease progression, warranting frequent monitoring.[6]

Patients transitioning from a T2D diagnosis to insulin-dependent T1D due to insulin resistance necessitate close surveillance. This subgroup faces heightened risks of micro and macrovascular complications.[7]

In such instances, the question arises as to whether individuals manifesting features consistent with both types of diabetes can be classified as having "double diabetes." This diagnosis requires a heightened level of attentiveness in disease management.[8]

Ketoacidosis at the onset of diabetes

Both cases exhibited a remarkably silent clinical onset of diabetes, a presentation more characteristic of the onset of T2D.

Children newly diagnosed with T1D commonly exhibit diabetic ketoacidosis (DKA) upon presentation. Frequencies of DKA at diagnosis vary widely, ranging from approximately 15% to 70% in Europe and North America. Recent reports from various countries indicate a rising trend in the frequency of DKA cases at T1D diagnosis. Particularly vulnerable populations include very young children and those from underserved ethnic groups, who are at heightened risk of presenting with DKA.[9]

A study from Finland demonstrated that the incidence of diabetic ketoacidosis is 19.4% of subjects who presented with diabetes at the onset, with a higher prevalence in adolescents and children <2 years old compared to other age categories.[10]

The prevalence of type 2 diabetes among children is on the rise globally. Approximately 5% to 25% of children diagnosed with type 2 diabetes present with diabetic ketoacidosis (DKA) at the time of diagnosis. According to findings from the SEARCH for Diabetes in Youth Study conducted in the USA, DKA was reported in nearly 6% of young individuals with T2D.[11]

Autoantibodies

What makes it difficult and "confounds" establishing a concrete diagnosis in this cases is the presence of autoantibodies.

Studies indicate that autoantibodies are detected in 10% to 20% of adolescents clinically diagnosed with T2D. The presence of these antibodies is associated with an accelerated onset of insulin dependency and an increased risk of developing other autoimmune conditions. It is recommended to conduct diabetes autoantibody testing in overweight or obese pubertal children displaying clinical symptoms of T1D, such as weight loss or ketosis/ketoacidosis. Some of these individuals initially diagnosed with T2D may exhibit a response to insulin therapy and achieve extended periods of glycemic control without insulin dependence.[6]

A study in Diabetes Care suggested that up to 30% of individuals diagnosed with T2D may have evidence of autoimmune beta-cell destruction, indicating potential overlap between T1D and T2D.[12]

The presence of antibodies in type 2 diabetes is an intriguing phenomenon that challenges the traditional classification of diabetes as solely either type 1 or type 2.

The majority of reports documenting GAD positivity in type 2 diabetes originate from Europe, with two notable studies examining large cohorts. In the UK Prospective Diabetes Study (UKPDS), the overall prevalence of GAD antibodies was 10%, while in the Botnia Study conducted in western Finland, the prevalence of GAD positivity was 9%.

Among the 4,134 participants enrolled in Diabetes Outcome Progression Trial who underwent GAD testing, 174 individuals (4.2%) were found to be GAD positive. The prevalence of GAD antibodies was comparable between North America (4.7%) and Europe (3.7%). [13] However, adolescents presenting with hyperglycemia and the presence of islet autoantibodies are most accurately categorized as having T1D.[6]

C-Peptide

Although T1D is commonly linked with low or undetectable C-peptide levels, research indicates that a portion of individuals with T1D may exhibit normal or elevated C-peptide levels. While the frequency of this occurrence varies among studies, it's estimated to be approximately 6-10%.[14]

It's widely acknowledged that individuals with T1D typically experience eventual complete destruction of beta cells, impacting treatment strategies and insurance coverage. However, the prevalence of residual insulin secretion in a diverse cohort of individuals across different ages of diagnosis and durations of T1D remains uncertain.

The American Diabetes Association characterizes T1D as typically resulting in absolute insulin deficiency, leading clinicians to view residual insulin secretion as uncommon in this group. However, our findings indicate that nearly one in three individuals diagnosed with T1D three or more years ago still exhibit residual secretion. Although the frequency of residual C-peptide declines over time from diagnosis regardless of age at diagnosis, individuals diagnosed during adulthood consistently demonstrate a higher frequency and greater levels of C-peptide secretion across all disease durations.[15] Urine C-peptide:creatinine ratio (UCPCR) and islet autoantibodies were assessed in samples obtained from 144 participants (median

age at diagnosis: 11.7 years; 47% male), approximately 23 years after diagnosis, with a range of 12 to 29 years.

Endogenous C-peptide secretion was observed in 51 participants (35.4%), with residual secretion noted in seven individuals (4.9%) and minimal secretion in 14 individuals (9.7%). Among the 132 samples collected more than 10 years after diagnosis, 86 participants (65.2%) tested positive for at least one islet autoantibody: 42 (31.8%) for GAD, 69 (52.3%) for IAA, and 14 out of 104 tested positive for ZnT8A (13.5%).[16]

Double diabetes

In recent years, the concept of "*double diabetes*" has garnered increasing attention within the medical community. This term denotes a condition characterized by a confluence of features from both type 1 and type 2 diabetes, manifesting as a co-occurrence of symptoms traditionally associated with each diabetes subtype within certain individuals.

The idea of double diabetes was first formulated in 1991 when it was observed that patients with T1D and who had a family history of T2D were most frequently overweight or obese and rarely had adequate glycemic results even with high doses of insulin therapy.[17]

***Characteristics of Double Diabetes**:*

- *Insulin Resistance*:

Patients with T1D generally have an autoimmune destruction of beta cells at the pancreatic level. In double diabetes, insulin resistance is associated with this destruction, which reduces the efficiency of both endogenous insulin (if there is still its own secretion) and exogenous insulin. The more the patient had a family history of diabetes, the higher doses of insulin he received.[18]

- *Autoimmune Destruction*:

This type of diabetes occurs in individuals who have a predisposition to both type 1 and type 2 diabetes. The autoimmune process that occurs in T1D can also contribute to insulin resistance.

- *Obesity*:

It is an important risk factor present in T2D, being overweight and obesity can amplify insulin resistance, which makes it difficult to obtain optimal glycemic control. It is worth noting that the incidence of obesity also increases in patients with T1D, which is also a side effect of intensive insulin therapy.[19]

- *Beta cell Dysfunction*:

In addition to the autoimmune-mediated destruction of pancreatic beta cells, their dysfunction emerges as a significant factor in the pathogenesis of double diabetes. Even when not entirely eradicated, beta cells exhibit impaired function, failing to operate at their typical capacity due to influences such as insulin resistance, genetic predispositions, or alternative mechanisms. (20)

The accelerator hypothesis

This hypothesis is a unifying, singular concept, which claims that type I and type II diabetes are the same disorder based on insulin resistance, stability on a different genetic ground. The role of autoimmunity is not denied, but only its primary appearance in the process. This theory distinguishes type 1 from type 2 diabetes only by the time period, so its appearance as early as possible reflects the genotype more susceptible to its appearance. Insulin-resistance is directly related to the presence of excess weight and obesity, being a trend that this hypothesis considers primary for the increase in the incidence of diabetes in developed countries. Type 1 and 2 diabetes is considered a continuum, in which the infinite interaction between genetic response and insulin resistance determines the age at which beta cell loss becomes severe. These are seen (most commonly) as two separate entities with a degree of overlap in those with type T2D who become insulin dependent over time.[21]

As outlined in Wilkin's accelerator hypothesis a few years ago, insulin resistance plays a significant role in the increasing incidence of both type 1 and type 2 diabetes. The key distinction between these diabetes types lies in the rate of progression to overt disease and the genetic predisposition to autoimmunity seen in individuals with T1D.[22]

Puberty is another critical period associated with hormonal changes and increased insulin resistance. The hormonal changes during puberty may exacerbate the autoimmune destruction of beta cells in genetically predisposed individuals, leading to an earlier onset of T1D or a more rapid decline in beta-cell function.[23]

Treatement

The management of adolescents with both autoimmunity and insulin resistance necessitates a exhaustive approach that addresses

each individual factor. Treatment strategies typically encompass lifestyle modifications, pharmacotherapy, and diligent monitoring aimed at preventing complications.

The most effective treatment strategy for double diabetes, entails a personalized and comprehensive management plan targeting. Here are some key components of treatment:

1. Insulin Therapy:

Due to the coexistence of insulin deficiency and resistance, insulin therapy frequently serves as a fundamental aspect of managing double diabetes. Basal-bolus insulin regimens or insulin pump therapy may be required to manage both fasting and postprandial hyperglycemia effectively.[24]

2. *Lifestyle Interventions*

Highlight the significance of consuming a well-rounded diet comprising whole foods, including fruits, vegetables, and lean proteins, while limiting intake of processed foods, sugary beverages, and high-fat items.

Promote consistent engagement in physical activity, targeting a minimum of 60 minutes of moderate to vigorous exercise on most days of the week.[25]

3. Medications Targeting Insulin Resistance:

Explore the possibility of additional medication options, like metformin or GLP-1 receptor agonists, to enhance insulin sensitivity and regulate glycemic levels.

Regularly assess for any potential adverse effects of medications and make appropriate adjustments to the treatment plan as required. [24]

4. Regular Monitoring and Follow-Up

Monitor blood glucose levels regularly, including fasting, pre-meal, postprandial, and bedtime readings. Perform periodic assessments of HbA1c, lipid profile, blood pressure, and renal function.[26]

5. Multidisciplinary Care

Engage in a collaborative approach by assembling a diverse team comprising endocrinologists, diabetes educators, dietitians, and mental health professionals to deliver holistic care and assistance.

Empower adolescents and their families by involving them in collaborative decision-making processes and setting shared goals to optimize treatment outcomes and enhance overall well-being.[27]

Behaviors that pose challenges in diabetes management encompass staying out late, irregular sleeping patterns, neglecting

insulin administration, and skipping meals. In certain cultural contexts, alcohol consumption may also be a factor. It is crucial to underscore the significance of maintaining regular and nutritious meals, especially during phases of rapid growth, to discourage excessive snacking later in the day. Adjustments in insulin dosages and meal timings may be necessary to accommodate variable schedules, considering commitments such as school, exercise, and work.

Regular weight monitoring is advised to promptly identify either weight loss or inappropriate weight gain. Excessive weight gain warrants thorough evaluation of insulin dosage, dietary habits, blood sugar control, and physical activity levels. Conversely, weight loss or failure to gain weight may signal insulin omission for weight management and could potentially indicate disordered eating behaviors.

Diabetes management encompasses a multifaceted interplay involving the family dynamics, broader social influences, peer dynamics, and the pursuit of maintaining a high quality of life. It necessitates a nuanced comprehension of how treatment protocols intersect with evolving physiological needs, such as growth spurts, fluctuations in appetite linked to changes in growth rate, varying nutritional demands, and levels of physical activity.

Evidence indicates the potential for enhancing diabetes outcomes by prioritizing nutritional management and adopting a personalized approach to education. This entails a deliberate focus on dietary objectives concerning glycemic control and the mitigation of cardiovascular disease risk.

Central to achieving successful dietary outcomes is the cultivation of a trusting rapport between the child or adolescent and healthcare providers. This bond facilitates the adoption of behavioral changes amid the unique challenges posed by childhood and adolescent development.[28]

Challenges in the management of adolescents

Adolescence marks the transitional period between childhood and emerging adulthood, characterized by distinct healthcare and emotional needs that differ from those of both younger children and mature adults.

Adolescence represents a pivotal stage characterized by peak physical development, alongside psychological and cognitive maturation, fostering autonomy and social independence. The rapid

physical and sexual maturation, coupled with subsequent neurodevelopment, renders this period one of physiological and behavioral vulnerability. This vulnerability is particularly pertinent in managing chronic conditions like diabetes, wherein the hormonal fluctuations of puberty directly influence glycemic control.[29,30]

Adolescents exhibit diverse adaptations and responses to change, with attitudes often marked by impulsivity, inquiry, and disruption, which may elicit negative reactions from adults. Similarly, in pediatric diabetes care, the acknowledgment of challenges in interacting with adolescents and navigating their behaviors is widespread.

During adolescence, glycemic control often experiences deterioration. This can be attributed to a blend of non-modifiable factors, including physiological insulin resistance (IR), the impact of gonadal steroids, an increase in lean body mass necessitating higher insulin doses, and modifiable factors such as psychosocial and behavioral changes leading to reduced adherence to treatment protocols, coupled with a decline in physical activity.

Furthermore, puberty introduces an additional risk for the development of diabetic complications, independent of glycemic control. Recent evidence indicates an elevated risk of vascular complications such as proliferative retinopathy and nephropathy in individuals with diabetes onset during puberty compared to those diagnosed after puberty. This increased risk is attributed to the effects of puberty-related insulin resistance (IR), alterations in growth hormone (GH) and insulin-like growth factor 1 (IGF1), androgens, and the heightened adiposity characteristic of adolescence.[29]

Periodic follow-up

Baseline and routine screenings for complications are imperative and should be meticulously documented in medical records annually, along with interpretations and reasons for any omissions. Collaboration with governmental bodies or non-governmental organizations can help alleviate financial barriers, facilitating screenings and ensuring early detection and prevention of micro- and macrovascular complications, which ultimately proves cost-effective in the long run.

In all healthcare settings, monitoring and charting of growth, physical development, and puberty are essential. Height and weight should be plotted on standardized growth charts during each visit.

During clinic appointments, individuals with diabetes should undergo a comprehensive physical examination, including blood pressure measurement using an appropriately sized cuff, inspection of injection sites, and examination of the feet for signs of cracks and calluses.

Thyroid function should be regularly monitored, with thyroid-stimulating hormone (TSH) levels assessed at diagnosis and subsequently every 1–2 years. In the absence of annual TSH screening, physical examination and specific evaluation of thyroid function become paramount, with TSH testing warranted for individuals exhibiting signs such as slow height velocity, delayed puberty, unexplained weight gain, constipation, or fatigue.

Screening for other comorbid conditions, such as celiac disease, should be conducted as needed, with documentation of any possible symptoms and utilization of laboratory investigations where available and feasible.[31]

Nephropathy, retinopathy, neuropathy, and dyslipidemia should be screened for regularly, particularly in cases of suboptimal glycemic control or in individuals with a medical or family history of diabetes or associated complications. The frequency and scope of these screenings will vary based on the resources and affordability available.[32]

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ADVANCES IN UNDERSTANDING THE PATHOGENESIS OF THE ENDOCRINE CONDITIONS ASSOCIATED WITH CELIAC DISEASE: RISK FACTORS, DECRYPTED AND POSTULATED MECHANISMS.

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Abstract:

Background:

Coeliac disease (CD) is a systemic immune-mediated disorder triggered by the dietary gluten in genetically susceptible individuals.

CD it's not only a malabsorption syndrome, but it's related with a multitude of extraintestinal conditions, especially endocrine and metabolic e.g. type 1 diabetes mellitus (DM), autoimmune thyroiditis, Addison disease, osteomalacia, secondary hyperparathyroidism, iron and vitamin D deficiencies, short stature, delayed puberty, fertility problems, and autoimmune hypophysitis.

Aims: A deep understanding of the CD pathogenesis may contribute to avoid definite complications.

Material and method: A systematic search was performed in the PubMed database utilising the keywords "celiac disease", "endocrine", "risk factors", "thyroiditis", "autoimmune" "diabetes", "Addison", "growth", "gluten", "hypophysitis".

Results: There is a shift in the clinical presentation of CD, the extraintestinal symptoms appearing more prevalent than the classical symptoms. The guidelines for CD recommend screening for type 1 diabetes and autoimmune thyroiditis. CD should be screened when type 1 DM is diagnosed.

The pathophysiology of CD has been broadly investigated in the past 20 years.

The pathogenesis of extraintestinal CD symptoms can be attributed to a larger adaptive immune response.

In some situations, the common denominator are the genetic factors.

A delay in the onset of puberty and fertility in CD involves not only the malabsorption, but also autoimmunity phenomena.
The autoimmune hypophysitis explains the CD-associated growth hormone deficiency.

Conclusion: It is necessary to further elucidate the processes involved in these CD related autoimmune diseases. The only existing available treatment for CD remains the complete gluten avoidance.
Keywords: Addison, celiac disease, diabetes, pathogenesis, thyroiditis

Background

Coeliac disease (CD) is a common autoimmune disease of the small intestine, affecting the small intestine in 1–2% of Caucasian individuals.[1] It' a unique autoimmune condition, because the dysregulation of the intestinal immune system is driven by T-cell reactivity towards a specific external factor, the dietary gluten, in genetically susceptible individuals. CD is a classic example of a multifactorial disease, involving both genetic and environmental factors.

CD typically manifests in the small intestine, but the paradigm shifted, CD to be considered nowadays as a systemic disease.

The recent celiac disease diagnostic guideline published by the European Society Paediatric Gastroenterology, Hepatology and Nutrition[2] recommends a simplified, but safe methodology, so called "the no biopsy approach". If CD is suspected in a paediatric patient, the measurement of total serum IgA (knowing the possible association between CD and the selective IgA immunodeficiency) and IgA-antibodies against transglutaminase 2 (TGA-IgA) is superior to other combination. High serum IgA class antibody concentration against transglutaminase 2 values (10 times the upper limit of normal) confirms the CD diagnosis. A subsequent verification key is the proof of positive endomysium antibodies (EMA-IgA) in an additional serum test.

Children with positive IgA class antibodies against transglutaminase 2, but lower titres (<10 times upper limit of normal) should undergo biopsies to decrease the risk of false positive diagnosis.

Human leukocyte antigen testing and presence of symptoms are not anymore mandatory criteria for the "no biopsies" procedure.

Various organ and systems are affected by either active coeliac disease or are prone to associated diseases due to the obvious genetic overlap between these disorders[1,2]. CD is still underdiagnosed

because of the heterogeneous presentation of clinical signs and symptoms, often extraintestinal.

The classical, typical symptoms suggesting CD are chronic or recurrent diarrhoea, but also chronic constipation not responsive to usual treatment, chronic abdominal pain, bloated abdomen, recurrent nausea, recurrent vomiting.[2] The consequences are the stigma of malabsorption – malnutrition syndrome: weight loss, failure to thrive, nutritional deficits, stunted growth.

A (perfectible) list of the extraintestinal or atypical symptoms and associated specific conditions encountered in CD or linked to it includes: nervous system disturbances (irritability, chronic fatigue, ataxia, depression, neuropathy), hypophysitis, recurrent aphthous stomatitis, dental enamel defects, dermatitis herpetiformis, psoriasis, alopecia, vitiligo, autoimmune thyroid diseases, arthritis/arthralgia, chronic iron-deficiency anaemia refractory to treatment, decreased bone mineralization, (rickets osteopenia/osteoporosis), repetitive fractures, delayed puberty, amenorrhea, infertility, abnormal liver biochemistry, type1 Diabetes Mellitus (T1DM), Addison disease, Down syndrome, Turner syndrome, Williams-Beuren syndrome, IgA deficiency.[1,2]

Because of the frequent association between CD and a variety of endocrine autoimmune conditions, the recent clinical guidelines should answer to questions like: “When and how often should monitor the paediatricians the possible coexistence of endocrine diseases related to celiac disease and why should adult endocrinologists recognize celiac disease?”

Aims:

The achievement of more understandings into the mechanisms that control these symptoms might be instrumental for the prevention of future complications.

Material and method:

A systematic search was performed in the PubMed database employing the keywords “celiac disease”, “endocrine”, “risk factors”, “thyroiditis”, “autoimmune” “diabetes”, “Addison”, “growth”, “gluten”, “hypophysitis.

Results:

Recent observations signalled a major change in the clinical presentation of coeliac disease. Nowadays the extraintestinal

symptoms appear more prevalent than the classical gastrointestinal symptoms.

The pathophysiology of celiac disease has been broadly investigated in the past 20 years. The pathogenesis of extraintestinal coeliac disease symptoms can, at least in part, be attributed to the spreading of the adaptive immune response to tissues other than the intestinal mucosa. In some situations, the common denominator are the genetic factors, e.g. T1DM, but the genetic risk alone cannot explain the increase in incidence rates by approximatively 3% per year in the last decades for type 1 diabetes and the increase in incidence of celiac disease. The increased reported incidence of CD could be linked to the raising awareness concerning the atypical or extraintestinal signs of CD.

CD and T1DM

T1DM is a glandular autoimmune disease that develops in genetically susceptible subjects and results in destruction of the beta islet cells. There occurs a combined action T cell mediated and an IgG-mediated pancreatic B- islet cell destruction by autoantibodies (glutamic acid decarboxylase antibodies - GAD) and a consequent insulin deficiency with rising of the glycemia.

Development of T1DM, like other autoimmune conditions, involves a series of unfortunate events involving association of genetic, environmental, and immunological factors.

15–30% of TDM1 patients have autoimmune thyroiditis and 3–12% associate CD.[3]

The close relationship between CD and endocrine autoimmunity, particularly T1DM, can be broadly explained by sharing of a common genetic background.

Identical twins had almost a 50% and 75% chance of developing T1DM and CD, respectively, compared to the general population.[4]

First-degree relatives had an increased risk of developing both the disorders compared to the general population. [4]

The genome-wide association studies (GWAS) identified 50 regions in the genome associated with susceptibility to T1DM. The human leukocyte antigen (HLA) remains one of the strongest genetic factors influencing the aparition of T1DM.[5]

The extent of T1DM risk is determined by specific combinations of DRB1, DQA1, and DQB1 alleles.

The DR-DQ haplotypes conferring the highest risk are DRB1*03:01-DQA1*05:01-DQB1*02:01(abbreviated "DR3") and DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/04 (abbreviated "DR4")[5]

In a similar manner, autoimmune disease occurs in 35% of patients with CD, including T1DM. The association between T1D and CD is one of the most intensely studied. The concept of linkage disequilibrium comes into play in this scenario where the sharing of HLA, specifically DR3 and DQ2, is evidently exhibited by both these conditions.[4] Major genetic risk factors in CD are DQ2 (DQA1*05:01-DQB1*02:01) and DQ8 (DQA1*03:01-DQB1*03:02), due to their properties to bind the gliadin derived peptides deamidated by tissue transglutaminase 2 (tTG2) [5]

Briefly, the presence of HLA-DQ2 and HLA-DQ8, confer high risk to develop CD and T1DMs, their presence was found in 98% of patients with CD and 95% of patients with T1DM.[6,7] However, there is rising evidence suggesting that there are some other genetic, immunological, environmental, viral infections[8] and nutritional factors that may contribute some to the pathogenesis and coexistence of these related diseases.

Enteroviruses and Rotavirus have been noted as culprits in the gut mucosa alteration, favourising an increased permeability.[9]

Troubles in the immune functioning of the gut have been postulated to alter finally the intestinal permeability. The intestinal microbiome may also play a critical environmental role in genetically-predisposed individuals. An altered composition of the gut microflora has been reported in CD, including a decreased ratio of Firmicutes and Bacteroidetes.[10]

The clinical picture, in case of association of CD with T1DM, is not typical and it is often confounded with the complications of diabetes. Diarrhoea and the villous atrophy can be a consequence of autonomic nervous system enteropathy or exocrine pancreatic insufficiency.

Patients with untreated or unknown CD associated with T1DM risk more episodes of symptomatic hypoglycaemia and a poor control of glycemia.[11]

An active screening for CD in patients with newly diagnosed T1DM is important at the time of diagnosis and during follow up: every 1-2 years because the succession of appearance of CD in T1DM patients cannot be predicted.[12]

In 3–4% of patients already at the onset of T1DM a temporary rise in antigliadin antibodies (AGAs) is described.[13] CD is usually diagnosed by screening after diabetes onset. Coeliac disease is often asymptomatic at diabetes diagnosis.[14]

In studies where patients with T1DM are followed yearly through a CD screening, CD is frequently diagnosed during the first year after T1DM onset.[13]

The precise mechanism for a possible pathogenic effect of gluten in type 1 diabetes is not known, but the effects of a gluten-free diet on control of T1DM have been studied. The gluten-free diet in CD associated with T1DM may contribute to better glycemic control and protect against further complications, including vascular complications.[15]

T1DM clinical studies have not shown an association between introduction of gluten in the diet and diabetes development.[16]

Coexistence of type 1 diabetes and coeliac disease raises important problems of diet compliance, especially in adolescence. The associations of these two related diseases increases the risk of further complications. A Swedish study found that in long-term follow-up (more than 15 years) CD is associated with a 2.8 times increased mortality risk in patients with T1DM.[17]

CD and thyroid disorders

The two main autoimmune thyroid disorders (AITD) are Hashimoto's thyroiditis and Graves' disease. In case of Hashimoto's thyroiditis, the anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin antibodies dare disturbing the functioning of the thyroid gland causing transient hyperthyroidism and finally, hypothyroidism. Graves' disease is a hypersensitivity reaction mediated by IgG thyroid-stimulating globulins, leading to a state of thyrotoxicosis.[4]

To explain the linkage between CD and autoimmune thyroiditis there are some postulates[4]:

1. Similarly to the case of T1DM, CD and autoimmune thyroiditis (AT) are linked by the HLA subtypes as: HLA-DQ2, HLA-DR3, and B8. This HLA varies largely between individuals and probably this disease association is more of a consequence of the multifactorial genetic inheritance. Although there is a weak association between HLA-DQ2, DQ8, and Hashimoto's thyroiditis, the relationship between HLA-DQ2 and Graves' disease is less evident.[18] CD and AITD have also been

connected outside the HLA region to the gene encoding cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), a potential gene for thyroid autoimmunity susceptibility.[19] There is a significant link between the CTLA4 haplotype and celiac disease. It is known that variations in the CTLA4 30 area impact autoimmune responses in Graves' disease and Hashimoto's thyroiditis.[20] In contrast to Grave's disease, CD exhibits distinct correlations at the CTLA4 exon 1 single nucleotide polymorphism (SNP) +49G>A. [21]

2. Thyroid gland and the gut are differentiated from a common origin: the pharyngeal gut.[12]
3. Increased permeability of the affected gut in the CD leads to penetration of great amounts of antigens in the bloodstream, which leads to a cross-reaction elsewhere in the body, thyroid included.
4. Tissue transglutaminase cross-reacts directly with the thyroid gland. [22]

During the diagnostic algorithm analysis, the clinicians, confront a lot of overlap symptoms for thyroiditis and celiac disease which can mimic each other: diarrhoea, weight loss, malabsorption, abnormality of menstrual cycle and osteoporosis.[4]

Approximately 5%-10% of patients with celiac disease have been found to suffer from hyper- or hypothyroidism, even though the percentages are highly variable. [23]

In studies, similarly, a prevalence of celiac disease is around 2-4% in patients with autoimmune thyroid diseases, higher than in controls. [23]

It seems that a gluten-free diet has some favourable effect on the thyroid functioning in patients with CD.

The clinical guidelines for celiac disease recommend screening for type 1 diabetes and autoimmune thyroiditis. CD should be screened soon after T1DM is diagnosed, and there is a recommended follow-up after two years. Data on long-term follow-up of patients with both CS and T1DM show that they have an increased risk of thyroid pathology compared with isolated T1DM [24] or to isolated CD. [25]

In the recent ESPGHAN Position Paper on Management and Follow-up of Children and Adolescents with Celiac Disease the Societal recommendations are as following:" the screening for thyroid disease with TSH and thyroxine (and autoantibodies if indicated) is

considered during follow-up after clinical evaluation at the discretion of the clinician"[26]

CD and Addison's disease

Adrenal insufficiency and CD may occur in some patients. In adults may be present an association of CD with autoimmune adreno cortical failure (autoimmune Addison's disease). Many appear in the scenery of polyendocrine failure that may include Addison's disease, thyroiditis, ovarian failure and CD.[9] CD is a strong predictor not only of glandular but also polyglandular autoimmunity. (PAS).[27]

It was recommended that patients with adrenal insufficiency should be screened for CD specially if there is failure to respond to hormonal substitution.

The CD patients should be investigated for adrenal insufficiency especially if associated with recurrent hypoglycaemia.[12]

CD and pituitary gland diseases

A growing attention is dedicated to the growth hormone (GH) deficiency in CD, knowing that the short stature can be the only presenting clinical feature of atypical CD in children, even when the gastrointestinal symptoms are absent.[13]

CD has a negative influence on growth, not only because of the malabsorption, but also because of an impaired pituitary function. Several theories have been advanced to explain this association (i.e., hypothalamic dysfunction and abnormal brain monoamines metabolism), but there is no convincing evidence.

In unselected cases admitted for short stature, the prevalence of CD varies from 2.9 to 8.3% and it is associated with delayed bone maturation.[28]

The catch-up growth with recovery of the height occurs in approximately 2 years after de initiation of the gluten free diet. The growth velocity after the gluten free diet (GFD) depends on the extent of the disease, the age at diagnosis and the degree of the height deficit compared with the target height.[29] More than 50 years ago, an insufficient GH response to hypoglycaemia was found in celiac children.

The GH secretion is restored after the initiation of a GFD. In untreated celiac disease children, not only a decreased peak of GH (less than -2 standard deviations) was observed in 69% of cases, but also a low IGF-1 level has been shown, unrelated to a GH deficiency and resistant to GH therapy, with normalization only under a GFD.[13]

Sometimes, a GFD is not enough, and GH deficiency persists. Growth hormone replacement may play role in children with CD and short stature, despite a gluten-free diet over a 1-year period.[30]

The autoimmune hypophysitis, detectable with the evaluation of antipituitary and antihypothalamus autoantibodies is one of the explanations of the CD- associated growth hormone deficiency, causing the absence of catch-up growth after the introduction of a strict gluten free diet. There are increasing prerogatives to search the antipituitary and antihypothalamus antibodies in all patients with GH deficiency associated to CD.

Hypoparathyroidism

Hypoparathyroidism has been rarely noted with CD, however in celiac patients with severe hypocalcemia or tetania this rare association should be considered. The gluten-free diet has a beneficial effect on blood calcium restoration in patients with concomitant CD and hypoparathyroidism. [31]

CD, puberty delay and fertility problems

Menarche appears later and menopause debuts earlier in celiac women, in consequence the fertility period is shortened.

The women with CD may present an ovarian failure causing infertility. Serologically based studies showed that over 4% of infertile females proved to have CD. There is a need to increase the awareness regarding the silent, undiagnosed, or atypical CD linked to infertility, because, after the introduction of the therapeutic GFD, a successful pregnancy becomes possible.[32]

The presence of CD could be also related to hypogonadism in males, leading to a delay in the onset of puberty. The boys are affected by a state of tissue resistance to androgens characterized by reduced serum level of dihydrotestosterone and by an increased serum level of luteinizing hormone.[13]

The pathogenesis of these problems is still unknown, but the implication of the selective malabsorption of essential micronutrients (e.g., zinc, iron, folic acid, and fat-soluble vitamins) is undeniable. Autoimmune mechanisms have been already hypothesized.

Despite the complex research, the only existing available treatment for CD remains the complete gluten avoidance.

In “celiac families”, with known genetic predisposition for CD there was the concern regarding the right moment of gluten introduction as complementary feeding, to avoid the disease clinical

manifestations at a young age. The studies showed that the introduction of gluten at 12 instead of 6 months of age only delayed the onset of CD, with similar prevalence at age 5 years.[33]

The present European guidelines recommend the introduction of small amounts of gluten gradually while the child is breast-fed and the avoidance of both the early (<4 months) or late (>7 months) introduction of gluten.[34]

The celiac disease is viewed nowadays like a multisystemic autoimmune disorder.

There is a new raising question:"*Can the gluten free diet for celiac disease prevent the development of autoimmune endocrinological disorders?*". T1DM appears to precede the development of CD, as determined by IgA-TG2 Antibodies positivity, which would allocate a less important role to the gluten free diet in the prevention of endocrine autoimmunity.[35]

Conclusion: It is necessary to further elucidate the processes involved in the CD and the related autoimmune diseases. The multidisciplinary approach and the teamwork are essential, to avoid a late diagnosis and an imprecise follow -up or treatment.

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THE ROLE OF GUT MICROBIOTA IN CHILDHOOD OBESITY

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Introduction

Over the past few decades, the global incidence of childhood obesity has surged to pandemic levels. [1] This condition brings about a plethora of immediate and long-lasting health implications, including heightened susceptibility to metabolic and cardiovascular ailments,[2-6] along with an increased likelihood of psychological comorbidities persisting into both childhood and adulthood.[7] While the correlation between dietary habits, lifestyle, and alterations in weight and body composition is well established, factors such as biology, behaviour, and environment, as well as their interplay, may also contribute to individual differences in susceptibility to obesity. Among these factors, the gut microbiota seems to be a significant influencer.[8]

The connections between gut microbiota and obesity have been extensively debated in recent years. However, substantial evidence supporting a causative relationship is still lacking for thorough investigation.[9] Pertinent research examining both adult and childhood obesity indicates that alterations in gut microbiota occur during the onset and management of obesity.[10-13] In adults, the composition of gut microbiota reflects various subgroups within the population, encompassing dietary preferences, lifestyle, and predisposing factors for metabolic disorders.[14] Consequently, the composition of gut microbiota has emerged as a promising predictor of metabolic disorders, including obesity. In contrast to adults, children's gut microbiota is undergoing maturation, making factors and conditions associated with childhood obesity crucial

determinants in shaping gut microbiota and potentially influencing long-term health outcomes.[15]

2. Gut microbiota development during childhood

The gut microbiota is a diverse community primarily composed of bacteria, but also including archaea, microeukaryotes, and viruses, which resides in the gastrointestinal tract and is thought to significantly impact various aspects of physiology such as energy regulation, circulation, and immunity.[8]

The gut microbiome undergoes constant changes from birth through adulthood, influenced by various factors. These features of the intestinal microbiota can impact the development of vital bodily systems such as the brain, immune system, and lungs, as well as overall growth. Environmental factors significantly affect the development of the microbiome.[16]

2.1. Perinatal colonization

The colonization of the gut might begin **in utero**, as reports show that bacteria could be found in the amniotic fluid, meconium, and placenta of healthy full-term infants,[17] but further research is needed to support this claim. Nevertheless, there is consensus on a substantial increase in **microbial colonization immediately after birth**, initially by facultative anaerobes, followed by obligate anaerobes. Exposure to bacteria and bacterial products via cord blood can affect the risk for childhood wheezing and prime certain immune responses.[16]

For full-term infants, **delivery mode** plays a crucial role in early intestinal colonization. Vaginally delivered infants acquire a microbiome resembling the mother's vaginal tract (*Lactobacillus, Prevotella, Sneathia spp*), while cesarean-born infants harbor microbes akin to maternal skin and oral flora (*Enterobacter hormaechei, E. cancerogenus, Haemophilus parainfluenzae, H. aegyptius, H. influenza, H. haemolyticus, Staphylococcus saprophyticus, S. lugdunensis, S. aureus, Streptococcus australis, Veillonella dispar, V. parvula*), missing out on crucial microbes from the vaginal canal and stool (*Escherichia/Shigella*, *Bifidobacterium longum*, *Enterococcus faecalis*, *Bacteroides fragilis*, *B. thetaiomaomicron*, *Bilophila wadsworthia*).[18,19] This disparity has implications, as C-section-delivered infants show lower abundance of beneficial bacteria like Bacteroides over time, regardless of

subsequent diet, and are associated with a higher need for antibiotics due to respiratory infections in the first year of life. [20]

Gestational age is a significant factor for microbiom development in preterm infants, the most specific types for this category being *Enterobacter*, *Staphyloccoccus*, and *Enterococcus*.[21] In infants born at full term, *Bacteroides*, *Bifidobacterium*, *Parabacteroides*, and *Escherichia* are more specific. [22]

2.2. Feeding practices

Infant diet also significantly impacts microbiome development, with **breast-fed infants** having a different microbiome composition (*Lactobacillus, Staphylococcus, Bifidobacterium*) compared to **formula-fed** ones (*Roseburia, Clostridium, Anaerostipes*), although this disparity tends to decrease with age.[21] The microbiome of a one-year-old infant transitioning from breastfeeding to other foods shifts towards a composition resembling that of adults, characterized by microbes capable of breaking down dietary fibers and generating short-chain fatty acids (SCFAs).[22] In contrast, formula-fed infants may experience a quicker development of their microbiome towards an adult-like profile, which has been linked to a higher presence of organisms associated with inflammation.[21]

2.3. Environmental factors

Additionally, factors like **antibiotic exposure, siblings, and household pets** can influence microbiome development during the first year of life. Antibiotics, in particular, have been associated with various health risks such as obesity, asthma, and allergies, and can also alter the gut virome.[16]

The infant microbiome typically matures into an **adult-like pattern by age three**,[23] but the development continues through childhood and adolescence being continuously affected by various factors.[21] In their case, *Bifidobacterium, Faecalibacterium* and *Lachnospiraceae* will be the more abundant phyla, while in adults, the gut microbiota will be predominantly consisting of *Firmicutes* and *Bacteroidetes*.[24]

Referring to a mature microbiome, a diverse bacterial population in the gut is linked to good health. In contrast, reduced diversity is observed in those who are diseased or experiencing abnormal physiological changes. When pathogenic strains dominate over commensals, it exacerbates these physiological alterations. The

trillions of microorganisms residing in the gut are not evenly distributed based on biochemical and physiological properties throughout the human body.[25]

Acidophilic genera like *Streptococcus, Lactobacillus, Helicobacter pylori, Candida*, and *Peptostreptococcus* are found in the **stomach**. Studies of the gut microbiome in health and disease typically focus on clusters of bacteria forming genera rather than specific strains. Almost every part of the human body, except sterile organs, hosts various bacterial populations. *Firmicutes* and *Bacteroidetes* are the most common genera in the human **gut**, accounting for 64% and 23% respectively, with *Actinobacteria* and *Proteobacteria* occurring in varying proportions.[25]

3. The main functions of gut microbiota

3.1. General notions

The gut microbiota performs vital functions across various aspects of human health, including immunological, metabolic, structural, and neurological domains, significantly impacting both physical and mental well-being.[26] It plays a crucial role in the normal development of the gastrointestinal tract, facilitating the maturation and differentiation of gut mucosa and its immune system. Additionally, it acts to curb the proliferation of pathogenic and potentially pathogenic microbes, engaging in competition and inhibiting their ability to colonize.

Certain strains of microbiota possess the capability to ferment nondigestible carbohydrates, fibers, and intestinal mucus, yielding gases and short-chain fatty acids like acetate, propionate, and butyrate. These SCFAs modulate diverse activities within the gastrointestinal tract, including cell proliferation, electrolyte absorption, hormonal secretion, and immune system activation.[27,28] Moreover, SCFAs can serve as substrates for colon cells, regulate leukocyte function, and induce a balance between pro-inflammatory and anti-inflammatory mechanisms. They also influence leukocyte chemotaxis, affecting their ability to target microbes in infection or inflammation sites.[29]

The deficiency of SCFAs can lead to conditions like leaky gut and local gut inflammation, facilitating microbial invasion. Butyrate, in particular, can trigger apoptosis in colon cancer cells and activate intestinal gluconeogenesis to enhance energy balance. It plays a crucial role in glucose homeostasis, regulating hepatic

gluconeogenesis and satiety signaling. The metabolic effects of SCFAs extend beyond the intestine, including the regulation of cholesterol metabolism and lipogenesis.[30]

Furthermore, the gut microbiota influences the host's energy balance through various mechanisms, including the extraction of energy from indigestible dietary components and the modification of bile acid pools, affecting their composition and abundance.[31,32] Gut microbiota-derived enzymes metabolize bile acids, essential for maintaining a healthy gut microbiota, enhancing lipid and carbohydrate metabolism, insulin sensitivity, and innate immunity.[33]

3.2. Involvement of specific organs and systems

To simplify the notions regarding the functions of the gut microbiota, one should think about them as several axes which include bidirectional actions.

3.2.1. The brain-gut-microbiota axis.

While the brain regulates the gut through serotonin, dopamine, and cortisol,[34] the gut influences the brain via the vagus nerve, neuropeptides, and neurotransmitters like leptin and serotonin, all which regulate fundamental physiological processes and mental functions such as learning, memory, and mood.[35,36] Immune signaling through secretory IgA and maintenance of mucosal integrity with Zonulin protein are additional factors involved, alongside the presence of SCFAs like butyrate.[37] Furthermore, the microbiota impacts the brain through various pathways. Certain bacteria, including *Lactobacillus* and *Bifidobacterium*, produce gamma-aminobutyric acid (GABA), while others like *Enterococcus, Escherichia*, and certain *candida* strains synthesize serotonin. Certain *bacillus* species are capable of dopamine production. Additionally, bacteria generate SCFAs like butyric acid, propionic acid, and acetic acid, stimulating the sympathetic nervous system and inducing mucosal serotonin release, thereby influencing brain functions such as memory and learning.[38]

3.2.2. The liver-gut-microbiota axis.

Gut-derived products are directly transported to the liver via the portal veins, while the liver synthesizes bile and antibodies, which are then sent back to the intestine. Preserving immune homeostasis within the liver-gut-microbiota axis relies heavily on the gut

microbiota. Metabolites produced by microbes, including SCFAs, trimethylamine, secondary bile acids, and ethanol, contribute to the pathogenesis of non-alcoholic fatty liver disease. Liver dysfunction such as cirrhosis induces significant alterations in gut microbiota and compromises the integrity of intestinal epithelial, vascular, and immune barriers.[39] Changes in the structure of gut microbiota can trigger mucosal immune responses, disrupting homeostasis and leading to bacterial translocation and migration of immune cells to the liver. This cascade of events ultimately results in inflammation-mediated liver injury and the progression of tumors.[40]

3.2.3. The cardiovascular-gut-microbiota axis.

Gut dysbiosis is associated with systemic inflammation, elevating the risk of obesity and type II diabetes mellitus, both significant cardiovascular risk factors, particularly for conditions like atherosclerosis and heart failure. Dietary habits that promote dysbiosis, such as high-fat diets, can contribute to the development of metabolic syndrome. Moreover, many risk factors for cardiovascular disease, including aging, dietary patterns, obesity, and sedentary lifestyles, can lead to gut dysbiosis. Dysbiosis, in turn, can increase gut permeability, leading to conditions like leaky gut syndrome and bacterial translocation, which are recognized risk factors for cardiovascular disease. Additionally, congestive heart failure can impair intestinal microcirculation, exacerbating leaky gut syndrome and promoting bacterial translocation, thus creating a vicious cycle that worsens heart failure.[41,42]

Trimethylamine N-oxide, a gut-derived metabolite is more elevated[43] and is linked to arterial dysfunction in obese children, [43,44] several studies even having acknowledged it as a marker of cardio-metabolic risk.[45,46]

3.2.4. The lung-gut-microbiota axis.

Recent findings indicate a reciprocal relationship between lung and gut microbiotas, with significant implications for respiratory health.[47] Compared to the gut microbiota, the lung microbiome is notably less abundant. Its composition is influenced by microbial colonization originating from the oropharynx and upper respiratory tract, as well as host mechanisms for microbial clearance, such as coughing and mucociliary clearance. Additionally, interactions with the host immune system and local environmental factors, including oxygen levels and pH, play crucial roles in shaping

the lung microbiota.[48] The gut microbiota can influence the enrichment of lung bacteria, thereby impacting the composition of the gut microbiota itself.

One potential mechanism for this inter-organ connection is the inhalation of gastroesophageal contents, such as through gastroesophageal reflux, and the subsequent swallowing of sputum. The lung-gut-microbiota axis may also involve indirect communication via modulation of host immunity, either locally within the gut or systemically, particularly affecting the pulmonary immune system.[49]

3.2.5. The renal-gut-microbiota axis.

The gut microbiota plays a pivotal role in hypertension and chronic kidney disease, by interfacing with the nervous, endocrine, and immune systems to regulate host homeostasis, including blood pressure and renal functions. This gut-kidney axis operates through metabolism-dependent mechanisms and immune pathways.[50]

SCFAs produced by commensal gut microbiota can influence the kidneys through immune system modulation and interactions with renal receptors and transporters.[51]

Kidney injury results in the accumulation of uremic toxins in the intestine, leading to increased intestinal permeability and a systemic inflammatory response.[52]

Uremia promotes bacterial translocation and compromises immunity by impairing T and B cell responses to vaccination and reducing the memory of these cells. Additionally, increased nitrogen waste products in uremia foster the proliferation of proteolytic bacteria.[53]

3.2.6. The skin-gut-microbiota axis.

The gut microbiota exerts influence on the skin microbiome through the SCFAs produced from fiber fermentation, which significantly contribute to the composition of the skin microbiota and immune defense mechanisms.[54] Propionic acid, a type of SCFA, exhibits potent antimicrobial properties, particularly against community-acquired methicillin-resistant *Staphylococcus aureus*.[55] Moreover, the gut microbiota aids in skin restoration and regeneration by modulating both innate and adaptive immune responses, thereby enhancing the skin barrier function through the regulation of T cell differentiation in response to various immune stimuli.[56]

4. Connections to obesity

Modified brain-gut-microbiota interactions establish a *persistent circular system*, contributing to *dysregulated eating behaviors* and obesity.[57] Early research comparing obese and lean individuals identified differences in gut microbiota composition, with obese subjects exhibiting a *higher abundance of Firmicutes and a lower abundance of Bacteroidetes.* This imbalance was found to reverse with weight loss induced by dietary interventions. The altered microbiota in obese individuals, characterized by an increased *Firmicutes* to *Bacteroidetes* ratio, may enhance the capacity to ferment dietary polysaccharides into SCFAs, including butyrate, propionate, and acetate. These SCFAs play vital roles in gut-barrier function and appetite regulation. Given that *Firmicutes* contains many SCFA-producing species and SCFAs contribute to a portion of dietary energy, it was hypothesized that obese individuals' gut microbiota may be enriched with species specialized in energy extraction.[58]

This cycle involves dietary factors, signals from the gut microbiota, satiety mechanisms, inflammation, and disturbances in brain equilibrium, leading to *increased reliance on pleasurable eating, diminished control, and a preference for high-calorie foods,* thereby exacerbating gut imbalances. Early-life gut dysbiosis, often induced by antibiotic use, results in heightened production of SCFAs, affecting liver lipid regulation and predisposing to obesity [58]. This dysbiosis also influences immunity and elevates the risk of cardio-metabolic diseases.[59-61] A study involving children with simple obesity and Prader-Willi syndrome-related obesity found comparable dysbiosis in both groups, which improved with a diet rich in non-digestible carbohydrates, leading to a shift toward a healthier microbiota state and reduced production of detrimental metabolites implicated in metabolic issues.[62]

As to the cardiovascular risk associated to weight excess, the aforementioned gut-derived metabolite, *trimethylamine N-oxide (TMAO), has emerged as a biomarker of both obesity and vascular alterations in children*[43] and adults.[63] Significant correlations have been observed between TMAO and atherosclerosis biomarkers markers such as the carotid intima-media thickness (CIMT), the pulse wave velocity (PWV), and peripheral blood pressure (BP) levels.[43] TMAO is derived from a variety of dietary sources including red meat, poultry, eggs, milk, and whole grains. These foods contain

notable quantities of choline, L-carnitine, γ-butyrobetaine, trimethyllysine, betaine, and δ-valerobetaine, all of which act as precursors for TMAO. Following ingestion, these substances undergo digestion and are primarily absorbed through the intestinal mucosa. In instances of excess precursor intake, the gut microbiota metabolizes them into trimethylamine. Trimethylamine is then absorbed from the intestinal lumen into the bloodstream and subsequently metabolized by hepatic flavin monooxygenases resulting in the production of TMAO.[43]

Regarding TMAO and atherosclerotic changes in obese children, Andraos et al. found higher TMAO levels in older adults, showing that TMAO precursors, not TMAO itself, were linked to metabolic syndrome, cardiovascular phenotypes, and inflammatory biomarkers in both children and adults.[64] Randrianarisoa et al. revealed an independent link between serum TMAO levels and increased CIMT, even after adjusting for cardiovascular risk factors, and noted that CIMT significantly decreased only in patients with the largest reduction in TMAO levels after a weight loss intervention [65]. However, most studies agree that CIMT does not decrease significantly following lifestyle or medical weight-loss interventions due to the low reversibility of arterial structure.[65,66] Regarding arterial stiffness, Brunt et al. found a correlation between circulating TMAO levels and age-related increases in aortic stiffness and blood pressure, suggesting potential preventive and therapeutic strategies, although further human studies are needed to confirm these findings.[67,68]. In obese children, TMAO correlates with PWV and peripheral BP levels and serves as an independent predictor for PWV, peripheral BP, and central systolic BP levels, even after adjusting for various variables.[43]

5. Solutions - Modulators of gut microbiota

Numerous investigations have highlighted the efficacy of probiotic-related products, such as prebiotics, dairy items containing probiotics, synbiotics, or direct intake of probiotic bacteria, in managing body weight among obese adults.

5.1. Diet

Clinical and preclinical research findings indicate that *maintaining an appropriate and regular intake of fruits and vegetables, abundant in essential vitamins, minerals, fibers, and*

bioactive compounds, contributes to overall physical well-being, immune function, and normal physiological processes.[69-72]
Rich in antioxidants, fruits and vegetables play a crucial role in neutralizing the harmful effects of free radicals, pervasive in various detrimental substances, thereby mitigating cellular damage and reducing the risk of numerous diseases. Nutrients found in fruits and vegetables, such as fiber and polyphenols, are recognized for their influence on gut microbial composition.[72,73]

Presently, a significant portion of the global population adheres to Western dietary patterns characterized by excessive intake of saturated and omega-6 fatty acids, coupled with insufficient consumption of omega-3 fatty acids, fruits, vegetables, and dietary fibers,[74] which contributes to alterations in microbiota composition, characterized by decreased bacterial richness and diversity.[75]

Against this backdrop, the **Mediterranean diet** emerges as a paradigm of healthy eating, renowned for its emphasis on fiber-rich foods, antioxidants, and polyphenols present in vegetables, fruits, legumes, and extra-virgin olive oil. These dietary components are strongly associated with a diminished risk of non-communicable diseases commonly associated with Western dietary and lifestyle patterns.[76]

Bacteroides and *Prevotella* can be modulated by a diet high in animal protein and saturated fat or a plant-based diet rich in fiber and simple carbohydrates.[77] Exercise is supposed to reduce inflammatory infiltration but increase microbial diversity in the gut.[78] A daily supplement of 7 g psyllium husk can increase *Veillonella* but decrease *Subdoligranulum* in healthy adults.[79]

5.2. Prebiotics

Numerous clinical trials have underscored the significant association between prebiotic-induced alterations in gut microbiota and obesity.

Prebiotics are non-digestible food ingredients that positively impact the host by selectively stimulating the growth and/or activity of specific bacterial species in the colon, thus enhancing host health.[80] Typically classified as nondigestible fibers, dietary prebiotics pass through the upper gastrointestinal tract intact, promoting the growth of beneficial microorganisms. Prebiotic ingredients include inulin, fructooligosaccharides, galactooligosaccharides, and human milk oligosaccharides.[81]

Prebiotics, found in fruits and vegetables, benefit health by improving nutrient absorption (calcium, iron, and magnesium), boosting immune function, and regulating gut microbiota. They stimulate the growth of beneficial bacteria, producing SCFAs that support intestinal health and influence metabolism. Prebiotics also help control appetite and promote weight management by affecting hormone levels and lipid metabolism.[82]

5.3. Probiotics

It is crucial to note that **the impact of probiotics on obese children might not mirror those observed in adults.** Research in this area remains limited. For instance, in 2013,

Recent studies indicate that certain strains of *Bifidobacterium* (*B. breve B3, B. infantis*, and *B. longum*) and *Lactobacillus* (*L. rhamnosus, L. casei strain Shirota* [LAB13], *L. gasseri,* and *L. plantarum*) have been used as probiotics in obese animal models. These strains have shown to suppress weight gain, fat depots, and white adipose tissue in animals compared to controls. However, the effects vary based on the duration and dosage of probiotic administration.[83]

In obese adults, *Lactobacillus* and *Bifidobacterium* strains, alone or in combination, have demonstrated reductions in body weight, waist circumference, and fat depots.[79,84-89] For instance, *L. gasseri* (SBT2055 and BNR17) treatment reduced visceral adipose tissue and waist circumference in individuals with obesity tendencies.[85,86]

Other studies have reported significant reductions in waist circumference, waist circumference/height ratio, and BMI with interventions such as *Bifidobacterium animalis* subspecies *Lactis CECT 8145* and *L. rhamnosus CGMCC1.3724.*[87,88]

High- and low-dose probiotic mixtures have shown beneficial effects on weight, BMI, and body fat mass in obese postmenopausal women, with the high-dose group also experiencing improvements in lipid profile.[89]

An interesting study on obese children has shown that the supplementation of multi-strain probiotics, *L. salivarius* AP-32, *L. rhamnosus* bv-77, and *B. animalis* CP-9, significantly decreased body fat, BMI, total cholesterol, LDL-cholesterol, leptin, and TNF-α, and increased the HDL-c fraction in overweight/obese children.[90]

5.4. Physical activity

The dynamic ecosystem of gut microbiota is also subject to various influences, including environmental factors like exercise, stress, and even altitude, temperature, pollutants, and noise.[91]

The impact of exercise is generally positive, including enhanced colon health, increased microbial diversity, and improved balance between beneficial and pathogenic bacteria.[92,93] *Firmicutes* and *Actinobacteria* are identified as the main phyla responsive to exercise.[94,95] Notably, exercise increases butyrate-producing bacteria such as *Roseburia hominis, Faecalibacterium pausnitzii*, and *Ruminococcaceae,* leading to elevated butyrate concentration in both rodents and humans.[96,97] Exercise also reduces transient stool time in the gastrointestinal tract, decreasing contact between pathogens and the circulatory system. Recent studies demonstrate a causal relationship between exercise and gut microbiome modulation, with improved gut morphology, inflammatory profile, and response to induced colitis in germ-free mice colonized with microbiota from exercised mice.[96] Furthermore, *Veillonella atypica* has been linked to exercise performance enhancement, suggesting its role as a performance-enhancer microbe.[98] While voluntary or moderate physical activity positively influences gastrointestinal motility and reduces inflammation, strenuous exercise triggers a stress response, leading to increased cortisol and epinephrine levels and reduced blood supply to the intestinal epithelium. This can damage the gut barrier, increase permeability, and promote inflammation and gastrointestinal distress.[99]

5.5. Stress management

Animal and clinical investigations have revealed that stressors exert a detrimental effect on the gut microbiota. Various models of stress in animals, including maternal separation, restraint conditions, heat stress, noise, and crowding, have been shown to alter the composition of the gut microbiota.[100] Notably, studies have consistently demonstrated lower levels of *Lactobacillus* following maternal separation and chronic restraint stress.[101] This reduction in abundance was associated with stress levels independent of cortisol levels, suggesting a distinct modulation pathway.[102] Administration of oral Lactobacillus in rodent models of stress improved behavior, cognition, and biochemical parameters while reducing corticosterone levels and preventing barrier leakiness.[103] Chronic stress paradigms have also been linked to an increase in the

Clostridiales family and a decrease in *Bacteriodes* abundance, both correlating with altered proinflammatory cytokine levels.[100] These findings collectively underscore the significance of the gut-brain axis in modulating the stress response.[104]

6. Conclusion

In conclusion, the role of gut microbiota in childhood obesity underscores its heightened susceptibility to obesity-related factors during this critical developmental stage compared to adulthood. Prioritizing early intervention and increased attention to childhood obesity are imperative for both immediate and long-term health outcomes, particularly in relation to gut microbiota-related health. The intricate interplay between the diversity, composition, and metabolic activity of the gut microbiota is intricately linked to nutrient intake and dietary patterns. Specific dietary factors and patterns have been shown to influence gut microbiota profiles, thereby potentially modulating the progression of obesity. However, further comprehensive studies and longitudinal trials are warranted to fully elucidate the effects of dietary patterns on gut microbiota alterations associated with obesity.

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