PEDIATRIC ENDOCRINOLOGY AND DIABETES

2019 UPDATE

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EDITURA MIRTON

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Preface

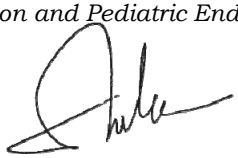
The increasing prevalence of endocrino-metabolic diseases, as well as their impact on the health of the population from the very early age, requires the training of pediatricians in endocrinology and pediatric diabetes.

Pediatric endocrinology is, unfortunately, not a recognized medical specialty in our country as it is in most of the developed countrys.

That is why, in our country, the diabetes and endocrinological diseases of children and adolescents are shared between adult endocrinologists, diabetologists, and some pediatricians with interest in this pathology.

Respecting the purpose of ”ENDOPED”, to the implicate in the professional training of all physicians who are in contact to the pediatric patient, we offer participants at the 6th National Congress of Diabetes Nutrition and Pediatric Endocrinology a new volume with “Pediatric Endocrinology and Diabetes – 2019 Update”.

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



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SYNDROMIC ENDOCRINE HYPOGONADISM: TURNER, KLINEFELTER, KALLMAN and PRADER WILLI SYNDROMES

Stuart J. Brink

Turner Syndrome

Otto Ulrich in Germany first described what is now commonly called Turner Syndrome with a case report in 1930.[1] Henry Turner in Oklahoma then described seven patients with short stature, congenital webbed neck, sexual infantilism and cubitus valgus in 1938 [2] and actually treated them with variety of pituitary and estrogen extracts. Some prefer the more historically correct name Ullrich-Turner Syndrome but more recently Turner Syndrome has predominated in the literature. The original index case of Turner was recently described in a follow-up article.[3] Association with increased gonadotropins plus low estrogen levels occurred with rudimentary ovaries ("streak gonads") described by Wilkins and Fleishman in 1944[4] and absence of Barr bodies was then also added to the description.[5] Documentation of the XO karyotype was first described by Ford and colleagues in 1959[6] with subsequent studies describing a variety 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.[7] It has been estimated that about 1% of 45XO fetuses actually survive to term delivery and perhaps as many as 10-15% of all spontaneous miscarriages have the 45XO karyotype.[8] Such spontaneous abortions usually occur during the first trimester of pregnancy but some now are being recognized with fetal ultrasonography associated with nuchal cysts, severe lymphedema or hydrops fetalis and a second trimester miscarriage. With such ultrasound availability, the family may choose elective termination in a fetus heavily affected.[9] In most case series, 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.[10,11] 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[12] while mosaicism likely comes from post-fertilization mitotic errors.[10]

While most prenatally detected cases of TS are discovered incidentally during amniocentesis or chorionic villous sampling performed because of advanced maternal age risk assessment [13], maternal and paternal age are not associated with increased TS risks. 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. Studies of HLA genes[14] looking for explanation of increases in autoimmune disorders like thyroiditis and celiac disease in TS[15] have not been positive. With all these complexities, the importance of appropriate genetic counseling cannot be overemphasized and involvement of a many different health

professionals and especially a pediatric endocrinologist can be very helpful.

Overall, TS remains underdiagnosed or diagnosed much later than should be reasonable. Many affected individuals have subtle phenotypes not easily recognized by parents or health care professionals unfamiliar with such groupings of co-morbidities: 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 an experienced multidisciplinary team approach so maximize consistency and support.[16] 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 is 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. [17]

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 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 demanding medical attention.

A variety of gastrointestinal problems 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 whether they come from tall countries like Scandinavia, Holland or Germany or from other countries around the world. [18]

Table 1.1: Clinical Characteristics of Turner Syndrome Patients [10,11,15,19,20,21,22,23,24]

- Lymphedema: web neck, cystic hygroma, edema of hands and feet, labial edema
- Ptosis, hypertelorism, strabismus, red-green color blindness, iridocyclitis
- Micrognathia, dental occlusion problems
- Conductive and sensorineural hearing loss
- Low set ears, posterior rotated ears
- Bushy eyebrows
- Widely spaced ("shield chest"), hypoplastic nipples
- Multiple pigmented nevi and keloid formation
- Hemangioma, atopic dermatitis, seborrheic dermatitis, vitiligo, alopecia, psoriasis and keratosis pilaris
- Cardiac abnormalities in 75% of fetuses and 30-40% of those with TS, usually left-sided heart problems including coarctation of the aorta, aortic valve abnormalities, ASD, VSD, hypoplastic left heart conditions, mitral valve prolapse and nonspecific EKG abnormalities
- Hypertension in up to 30-50% of TS
- Congenital renal and urinary tract anomalies in up to 35-40% of TS
- Scoliosis or kyphoscoliosis, Madelung deformity, cubitus valgus, short fourth metacarpal, congenital hip dysplasia
- Short stature and growth deceleration
- Nonverbal learning problems, attention deficit and psychosocial issues including mathematics as well as emotional immaturity, self-esteem, overly dependence personality [11]
- Autoimmune disorders like Hashimoto's thyroiditis, hypothyroidism, celiac disease and other inflammatory bowel disorders (IBD) like Crohn's, ulcerative colitis
- Hypogonadism, lack of pubertal secondary characteristics, lack of menarche, infertility
- Osteopenia and osteoporosis as well as hypovitaminosis D
- Type 2 diabetes, metabolic syndrome

Growth

Specific TS growth charts[25] (free computer download available from www.magicfoundation.org) (see figure 1.1) make such comparisons with the rest of the population rather obvious with significant short stature already present in the preschool years and actual height deceleration already evident by 6-10 years and getting greater and greater 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.[7,9,10,11,26] Higher GH doses work better than lower doses.[27,28,29] Early treatment with growth hormone also improves height velocity and final adult height achievement.[30,31] Oxandrolone, a non-aromatizable anabolic steroid, can be used in combination with growth hormone to augment growth[32,33,34,35] 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.[36] [37] In other studies, however, slightly delaying the initiation of estrogen therapy[38] has shown some benefit in final height achieved.

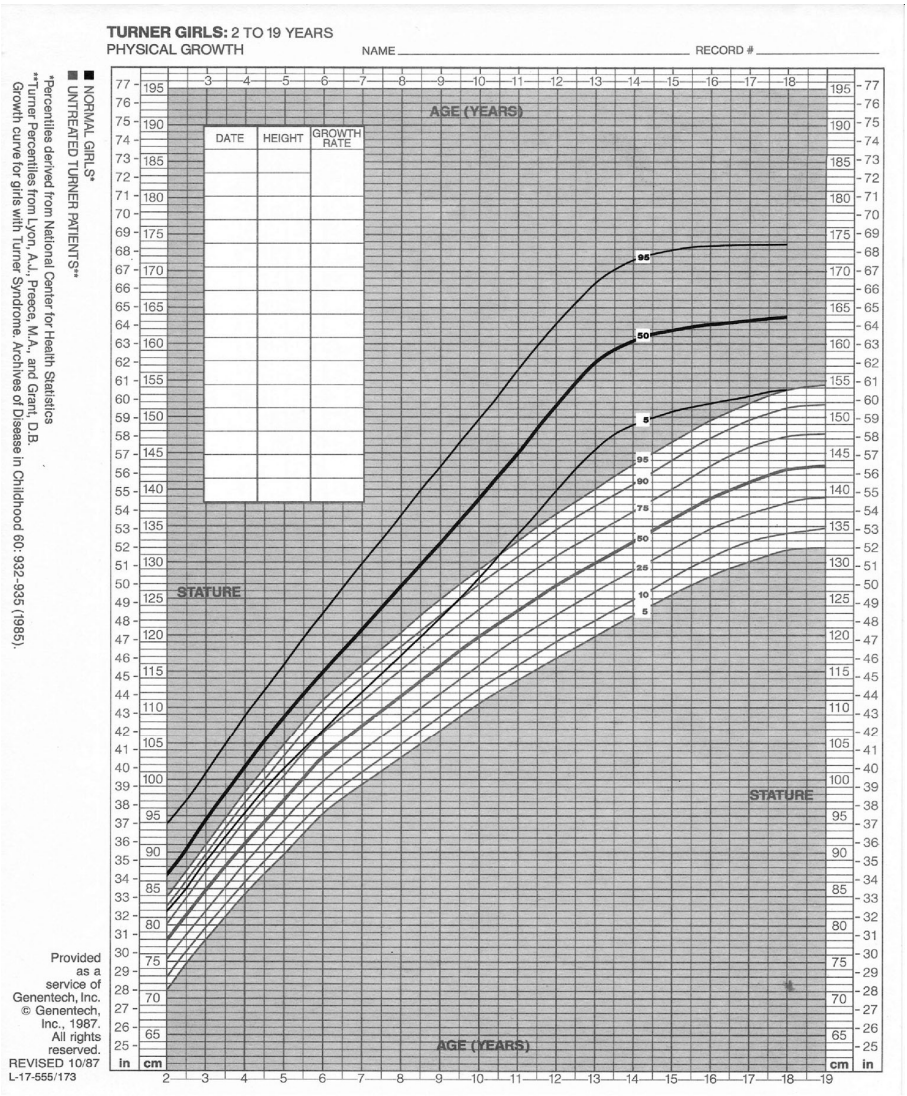

Figure 1.1

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.[39] 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, some authorities suggest that GH treatment should be delayed until a gonadectomy has been performed so even a remote possibility of growth hormone potentiating a gonadoblastoma is eliminated. 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.[10,40]

Figure 1.2 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 and this can occur as early as 2 years of age. GH is administered every night of the week subcutaneously by syringe or pen 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 if a TS girl is significantly short and catch-up growth is desired while titrating the dose later according to IGF-1 levels.[10,11] 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]

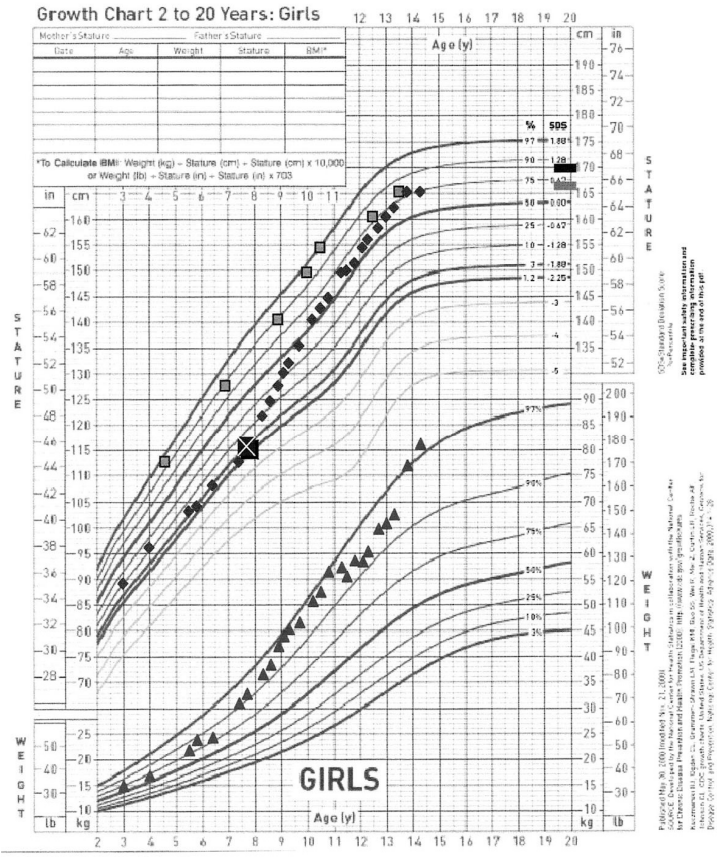

Figure 1.2

Rarely oxandrolone produces clitoromegaly as well as glucose intolerance.

The longer the duration of estrogen-free GH treatment, the taller the final height likely to be reached.[44]

Most experts 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 [41] so some discussion between patient, parents and pediatric endocrinologists should attempt to balance the pros and cons of timing of estrogen initiation. 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.[10,11,42] 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 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 not demonstrating any other risks while documenting growth benefit in TS patient receiving growth hormone according to standard treatment protocols as listed above. Side effects general 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]

TS Co-morbidities include a variety of autoimmune thyroid problems, mostly hypothyroidism but sometimes hyperthyroidism as well as inflammatory bowel disorders like celiac disease, Crohn's disease and ulcerative colitis. None are

increased by use of oxandrolone, growth hormone or estrogen in many studies and exact explanations for such increased autoimmunity risks remains poorly understood. Such co-morbidities can have important effects on growth, of course as well as mineral and vitamin nutrients affecting many aspects of optimizing health and especially increasing risks and worsening osteopenia and osteoporosis vis-à-vis hypovitaminosis D.

Primary ovarian failure and reproductive system in TS

Lack of sexual development[7,9,10,11,43] (breast development, feminine body contours and menstruation) during adolescence is another hallmark of TS with primary gonadal failure (low estrogen and high gonadotropins).

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 or 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. 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 [44] 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 hypoestrogen 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.[45] 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.[46]

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 as much as possible.[7,9,10,11,15,18,47] 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. 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 and 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 [48] 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 [49] 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. 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 risks. Similarly, when and how to add progesterone to estrogen therapy is not optimally known.[10,11] Typical starting estrogen doses 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. Transdermal estradiol [50] can also be used at initiation or after some time with oral treatment if this is desired and as the dose 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]

Spontaneous fertility is rare among patients with TS and more likely in women with mosaicism rather than classical 45XO karyotypes. But there is 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[37,38] and 35% of such offspring may have TS.[51] Appropriate preconception counseling is extremely important. 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.

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 associated with more 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.[52,53] 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 gonadectomy, usually laparoscopically if there is Y chromosomal material or markers prophylactically and postponement of any growth hormone treatment until this is done to minimize any malignant potential.[54]

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.

TS Summary

Turner Syndrome resulting from a complete or partial absence of one X chromes is the most common occurring chromosomal abnormality in females. 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. Psychosocial issues add to the complexities as do eye and ear difficulties and orthopedic problems. 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. The Turner Syndrome support group (www.turnersyndrome.org) has local and national chapters and with the help of the internet can provide support for girls, adolescents, adults and their families around the world.

Klinefelter Syndrome[55,56,57,58,59]

Is usually associated with karyotype 47XXY and occurs in approximately 1 in 700 males (1:500-1:1000)[60]. The most common syndrome of at least two X chromosomes plus one Y chromosome, 47XXY, constitutes the most common cause of male hypogonadism occurring in 70-90% of Klinefelter patients.[4,61] Frequently 47XXY is unrecognized clinically since symptoms and signs can be very subtle.[62,63]

Rarely does the diagnosis get entertained in the pediatric age group although, after the diagnosis is finally made in mid-to-late adolescence or adulthood, it is frequently apparent that it should have and could have been recognized many years earlier. As with Turner Syndrome, late diagnosis or mis-diagnosis is all too common and consideration sometimes does not arise until evaluation for male infertility as an adult because of associated azospermia.

The origin of the additional Y chromosome is generally a result of malsegregation during early paternal meiosis and if there are extra X chromosomes these derive maternally also in early meiosis. Paternal age is not usually advanced but advanced maternal age is associated with an increased incidence of Klinefelter's.

With increasing prenatal chromosome testing/ amniocentesis, in some parts of the world, prenatal diagnosis also may be increasing. About 10% of Klinefelter patients have mosaic Klinefelter syndrome (46XY/47XXY) with have milder features, less gynecomastia and lesser neuropsychological issues. About 15% have mixed karyotypes with more severe breast enlargement, other physical features present and more behavioral and psychological issues.[1-9] Rarer karyotypes of Klinefelter Syndrome also occur associated with polysomy X or Y (ie. 48XXYY, 48XXXY, 48XYYY, 49XXXYY, 49XXXXY) with and without mosaicism (ie. 46XX/47XXY, 46XY/47XXY).

Classical Klinefelter Syndrome was first described by Harry Klinefelter and colleagues in 1942 [64] with varying sexual maturation impairment, smaller than expected penis and smaller testicular volumes in adolescence but generally

normal sized genitalia and gonads prepubertally, decreased secondary sexual hair, eunuchoid body proportions with relatively long legs even in childhood, varying gynecomastia. Nonspecific learning, cognitive and/or behavioral problems usually are recognized but not as part of other genetic syndromes. Jacobs and Strong in 1959 [65] are credited with associating Klinefelter's clinical descriptions with the 47XXY karyotype.

Intelligence per se is usually normal but often is somewhat lower than expected compared to other family members and low-normal compared to the general population at large but with a variety of nonspecific and non-diagnostic learning problems, executive dysfunction and various processing difficulties which can have a large impact on interactions with peers and family members as well as social and learning problems at school. Thyroid disorders, diabetes mellitus and other autoimmune disorders are increased as are breast cancer, testicular cancer, varicose veins, osteopenia and osteoporosis.

Klinefelter is an example of a *primary testicular hypo-functional status with low testosterone production* associated with primary Leydig cell dysfunction. Since testosterone levels normally are quite low postnatally and throughout most of childhood, this explains the difficulties in making such a diagnosis before adolescence and sometimes even not until adulthood. The hypothalamic and/or pituitary response, therefore, at or around adolescence involves increases in GnRH as well as LH and FSH, thus a tertiary hypothalamic GnRH or a secondary pituitary hypergonadotropic hypogonadal biochemical picture on hormone testing associated with concomitant absolute or relatively low testosterone levels. Such testicular disorders and those conditions associated with gynecomastia, a common presenting complaint that may lead to consider Klinefelter Syndrome diagnosis, must be differentiated from primary defects in androgen biosynthesis, post-infectious orchitis (ie. mumps, HIV, leprosy etc), cryptorchidism, anorchia, bilateral post-torsion of the testes, dysgenetic testes, post-testicular

trauma or post-surgical testicular problems, myotonic dystrophies, toxins (such as agent orange (dioxin), kepone, dibromochloroperine), marijuana, alcohol, opiates, fungicides, insecticides, heavy metals, cotton seed oil, marijuana), drugs (such as diethylstilbesterol, medroxyprogesterone acetate fetal exposure, minoxidil, boron, cytotoxin, ketoconazole, cimetidine, spironolactone and perhaps bisphenols as well as other endocrine disruptors) and tricyclic antidepressants as well as systemic illness (such as uremia or liver failure) mostly causing gynecomastia or nonspecific estrogen-like effects. Unintended exposure to estrogens or phytoestrogens (lavender and tea tree oils, estrogen-containing anti-balding creams and cosmetics, licorice, meat and milk from estrogen-treated cows, soy) also are in the differential diagnosis of gynecomastia. However, not all Klinefelter patients have gynecomastia! Purposeful or accidental exposure to high doses of testosterone or its precursors that then may be aromatized such as when athletes or those at gyms take steroids for muscle enhancement also may cause gynecomastia. Autoimmune orchitis is another relatively rare example of a hypergonadotropic hypogonadal state but with positive gonadal antibodies present perhaps also associated with other autoimmune phenomenon (type 1 DM, celiac disease, Hashimoto's thyroiditis, Addison's/adrenalitis and/or pernicious anemia).

Some estimates suggest a range of *1:500-1:1000 men* with Klinefelter with about 10% of individuals diagnosed by karyotype screening prenatally or at birth and overall only 25% diagnosed during life. Thus estimates suggest about 75% of Klinefelter patients are never diagnosed ! [1] and most Klinefelter patients lead presumably normal and uncomplicated lives with few recognizable signs.

However, virtually all with *classical 47XXY karyotype are infertile* because of lack of sperm production. Those with mosaic Klinefelter syndrome have been reported to father children spontaneously.

By around 12-14 years of age, low testosterone and elevated LH and FSH become measurable with differences

compared to the non-Klinefelter population apparent on examination. While there is an unequivocal male phenotype, there is also relative small penis size, small firm testes and eventually decreased male-pattern body habitus (the so called eunichoid habitus) with relative low musculature development, wide hips and thighs and often persistent rather than transient gynecomastia as well as tall stature. Lack of testosterone is associated with lack of aromatized estrogen and thus the epiphyses remain relatively delayed and open contributing to the relative tall stature. There also is lack of the normal development and progression of facial hair, mustache and beard in late adolescence and, of course, since there is less than usual androgen available, the low testosterone levels are associated with little or no acne.

Obesity and varicose veins occur in about 1/3 of Klinefelter patients and there are more reported instances of pulmonary embolism especially in adulthood as well as more weight-related glucose intolerance and type 2 diabetes.

There also is an association of learning disabilities, social maladjustment and dyslexia. When associated with a child, teen (or adult) with tall stature and gynecomastia as well as clinical signs of hypogonadism, such a constellation of findings should raise the possibilities of Klinefelter in its classical genetic form or as one of the mosaic variations.

Association with Hashimoto's thyroiditis and thyroid clinical abnormalities, type 2 diabetes mellitus and apnea may be seen in late adolescent or older Klinefelter patients but positive thyroid antibodies maybe present for some time as a lone marker of thyroid dysfunction even without a goiter or frank abnormal thyroid functions themselves.

Taurodontism (enlarged and elongated teeth shapes with thinning of the tooth surface and hypertrophy of the pulp) may occur in 40% of KS and is associated with increased dental decay.

Mosaic Klinefelter patients generally have a less severe form of the disorder and whereas classical 47XXY Klinefelter patients are virtually all infertile with pathologic hyalinization and fibrosis of the seminiferous tubules on testicular biopsy,

mosaic Klinefelter patients have half as much infertility. Mosaicism may also only be limited to the testes and thus only diagnosed by testicular biopsy chromosome analysis.

Most of the time, testicular biopsies are not needed for diagnosis or to aide therapeutic decisions unless a gonadal mass/suspected malignancy is detected. Palpation of the breast when gynecomastia is present and ongoing surveillance for breast masses and breast malignancy is also important during follow-up because of the increased breast cancer rate especially in adulthood for Klinefelter patients. The differential diagnosis of gynecomastia is important to consider when contemplating other conditions besides Klinefelter's Syndrome since the gynecomastia may be the first and primary complaint of the teenage boy. Any condition associated with relative excess of estrogens or estrogen-like effects as well as primary (gonadal), secondary (pituitary) or tertiary (hypothalamic) hypogonadism may of course cause relative or absolute testosterone deficiency as listed previously. If gynecomastia persists more than 2-3 years, chances are that it will not regress spontaneously.

Testosterone treatment may, in fact, worsen the gynecomastia from nonspecific aromatization of higher levels of circulating testosterone to estrogens. If such gynecomastia does not disappear or is particularly worrisome psychologically, surgical breast reduction is a fairly simple procedure to remove such excess tissue.

Table 1.2: Clinical features associated with Klinefelter Syndrome

- Decreased testicular volume
- Azospermia
- Decreased testosterone with increased gonadotropins
- Decreased facial hair
- Decreased sexual function
- Gynecomastia
- Decreased axillary hair
- Small phallus
- Relative short stature and increased limb length

- Learning difficulties
- Obesity
- Increased risk of breast tumors
- Osteopenia and osteoporosis
- Metabolic syndrome, lipid abnormalities, diabetes
- Thyroid dysfunction

The single most important lab test would therefore be an *elevated FSH and/or LH associated with absent or decreased testosterone level.* FSH elevations are usually more prominent than LH increases in Klinefelter syndrome patients but this too is variable. Most Klinefelter patients transition from prepuberty to puberty without reliance on exogenous androgens. When androgen deficiency and testicular failure are more severe, gradual introduction of testosterone hormone to a full replacement dosage of approximately 200 mg every 2 weeks (if provided by intramuscular testosterone enanthate or propionate) may be helpful and there is new interest in trying to decide if earlier testosterone provision might help with brain function, decreasing obesity and increasing muscle strength and lessening or avoiding osteopenia and osteoporosis. Long term follow-up studies will be needed to answer such questions and there is insufficient data presently available to make definitive recommendations so such decisions would need to occur on a case-by-case basis. The Klinefelter Syndrome support group (www.klinefeltersyndrome.org) is available with local and national chapters and with the help of the internet can provide support for boys, adolescents, adults and their families around the world.

Laboratory evaluation: Testosterone, Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH).

Testosterone levels can be measured at any time but prior to puberty that are not likely to be especially helpful in those with Klinefelter syndrome. However, some boys will already have elevations of LH and/or FSH even before adolescence although this often does not show up until near

or about the teenage years when the hypothalamic and pituitary responsivity changes but the testicles are, of course, unable to produce the testosterone to allow appropriate feedback. Thereafter, one of the ways to try to determine which Klinefelter patients would benefit from testosterone treatment would be to have sequential, ie. annual testosterone, LH and FSH measurements to look for subtle changes in these levels as a guide. If the blood testosterone level is normal and the LH and FSH levels are also normal, then it is unlikely that any testosterone treatment would be required. This recommendation may change if it could be proven that improving muscle strength, body habitus or bone density would be responsive to improved testosterone replacement *a priori*. If the testosterone levels were low, then testosterone treatment should be discussed and the pros and cons of such treatment reviewed.

Genetic testing

Once the diagnosis of Klinefelter is suspected, buccal smear for Barr-body analysis may be less expensive than formal karyotyping but both methods identify the number of X chromosomes present and may also provide information about mosaicism. Any positive findings on Barr body ideally should be confirmed by formal chromosome analysis if possible, available and not excessive costly. XYY patients may be excessively tall and have more severe acne than other subtypes. Those with extra Y chromosomes, in some reports have also had more psychiatric or aggressive criminal behavioral problems reported as well.[66] Genetic counseling for the family when Klinefelter syndrome is identified should include the fact that the risk of Klinefelter in other siblings is rarely increased and there is nothing known that either parent could have done to prevent this type of problem. Specifically verbalizing how common are feelings of parental guilt may also be helpful during such discussions.

Androgen Treatment[1,67,68,69] (*see Table 1.3*).

Preparations of testosterone are the main hormonal treatment for Klinefelter syndrome and include *intramuscular testosterone enanthate and testosterone cypionate* which are depot preparations. These are generically sometimes called *depo-testosterone.* These may be given every month, every 2 weeks or weekly depending upon individual preference and any side effects which may be attributable to large vs smaller peaks and troughs of such schedules.

Usually a starting dose might be 50 mg intramuscularly monthly and then slowly increasing the dosage from 50 to 75 mg, then 100 mg, then 150 mg and then 200 mg monthly taking 1-3 months between dose adjustments. On a weekly schedule rather than a monthly schedule, dose may start at 50 mg and then increase only to 75 or 100 mg weekly; similarly for an every 2 week dose schedule. Peak testosterone values generally occur with such preparation about 3-5 days after each injection with waning thereafter. In some patients, there is a preference for smaller peaks according to how they feel and how they function and somewhat smoother efficacy that is more likely with smaller doses more frequently, thus the weekly or twice-a-month schedules. In others, the hassles of intramuscular injections are more important and they don't have any significant side effects or mood/fatigue swings with the once-a-month schedule. More recently, testosterone creams have been available so that intramuscular injections may be avoided for testosterone replacement treatment. Following testosterone blood levels is also somewhat dependent upon such schedules particularly if one is trying to titrate the dose to reach mid-range normal blood testosterone levels. Interpretation of such results must include knowledge about the timing of the dosage. Relatively new longer lasting depot testosterone preparations such as testosterone undecanoate may be attractive for those desiring the convenience of quarterly injections. The general goal of testosterone therapy in Klinefelter syndrome patients involves starting with a small dosage and gradually increasing the dose to allow normal progression of physical and sexual

development, pubic hair growth, the size of the penis and the scrotum (but not the testes since they would be expected to get smaller, if any change occurred in them), axillary hair growth, beard growth, deepening of the voice and increase in muscle bulk and strength. There may be an improvement in self-image and a more positive outlook on life but coordination itself as well as obesity as well as brain function and emotional function may not change very much and pre-treatment counseling should address such issues to avoid disappointment and frustrations. Caution should be given vis-à-vis changing doses too fast or taking excessive testosterone dosage lest it worsen aggressiveness or depression.

Monitoring of testosterone treatment should include not only testosterone, LH and FSH levels but periodic assessment of glucose metabolism, lipid levels, vitamin D and bone density status as well as monitoring of height, weight and bone age in the school-age and teen-age years. Too rapid elevations of testosterone may results in worsening acne, elevated liver enzymes or too frequent or painful erections.

Some Klinefelter patients may only need to be treated during adolescence to help complete pubertal growth and development while others, especially those whose libido and sexual drive is decreased, may desire continued treatment as adults.

Alternative testosterone preparations instead of intramuscular forms have been available in recent years. These would include *transdermal testosterone* which can be applied to normal skin in a *gel* form or to the scrotal surface itself (Androgel ® packet or pump: 50-100 mg/day; Axiron ® pump: 30-120 mg/day; Fortesta ® pump: 10-70 mg/day; Testim ® gel: 50-100 mg/day) as well as in *patch* format (Androderm ® in 2 and 4 mg patches with some patients needing 2 patches daily to reach sustained therapeutic levels) and this is generally the format for those not desiring intramuscular testosterone injections. These are done daily and similar blood testosterone levels are monitored but the timing of such monitoring is less important for dose titrations with daily dosing than when depot preparations are used.

Patients must be cautioned if they have contact with small children or babies since testosterone on the hands after application may produce virilization effects. Similarly, partners or other family members may also be exposed to such testosterone from skin-to-skin contact.

Buccal testosterone is also available requiring dosing every 12 hours. This sometimes causes some buccal or gingival irritation and some complain of a bitter taste.

Oral testosterone is generally not recommended because of concerns about possible liver toxicity as well as inconsistent potency and efficacy. Implants of testosterone are also theoretically possible but not yet fully available and these would have the theoretical benefit of allowing lower doses and not requiring remembering to apply gels, patches or injection.

In addition to regular evaluation of testosterone, LH and FSH levels to help determine appropriate individualized dosage, periodic assessment of hematocrit, liver functions and, in adults, prostate assessments (physical exams and prostate specific antigen determinations) should occur as well as history and systems review questions aimed at determining fatigue and muscle function, anxiety and depression, aggressiveness, emotional lability, breast changes, erectile function and libido, sleep disturbance/apnea, edema, blood pressure, lipid evaluation and glucose levels usually at 3-4 month intervals for adolescents and at 4-6 month intervals in adults particularly once stable dosage is established clinically and biochemically. Baseline bone density DXA scans to evaluate osteopenia and osteoporosis as well as blood vitamin D levels should be checked and a follow-up assessment schedule determined because the hypoandrogenic state can be associated with more bone mineralization abnormalities.

Klinefelter Syndrome Summary

Klinefelter Syndrome is the most common genetic cause of low testosterone and results in hypergonadotropic hypogonadism since the hypothalamic and pituitary response is otherwise normally responding to the low gonadal output of the testes. 47XXY as well as mosaicisms occur. Adolescents as

well as adults with KS often have small firm testes, sparse body hair, gynecomastia and female body habitus and fat distribution as well as dyslexia and other learning problems. Clinical presentation varies widely and the diagnosis is frequently missed until adult infertility is investigated with only about 25% of KS men diagnosed during life. Treatment with testosterone will lead to masculinization and improvement of symptoms of hypogonadism although infertility is generally irreversible despite hormone replacement therapy.

KALLMAN SYNDROME

Kallman Syndrome is an inherited disorder of neuronal migration characterized by *central hypogonadism* and *anosmia* so an example of *hypogonadotropic hypogonadism* rather than hypergonadotropic hypogonadism of Klinefelter Syndrome. It was first recognized by Kallman, Schoenfeld and Barrera in 1944. [70] It can occur in *both males and females* with an estimated incidence of 1:10,000 in males and 1:50,000 in females with both males and females having delayed or absent pubertal progression associated with absent testosterone or estrogen accordingly.

There are several known genetic abnormalities including ***KAL 1***, an x-linked (Xp22.3) form encoding a protein named anosmin-1. Anosmin-A is also expressed in the cerebellum, meso- and metanephros, oculomotor nucleus and facial mesenchyme explaining some other associated co-morbidities listed below. There also have been described other KAL genes including ***KAL2***, an autosomal dominant form and ***KAL3*** an autosomal recessive form. The brain MRI demonstrates absence or hypoplasia of the olfactory bulb so either absence of smell or markedly decreased sense of smell is common.

Associated defects in the hypothalamic pulsatile release of GnRH occur so there is low LH, low FSH and therefore low testosterone (or estrogen) levels. Nasal embryonic LHRH (NELF) also has been implicated in the pathogenesis of Kallman Syndrome.

In males, neonatal micropenis as well as undescended testes occur as well as obvious absent or delayed puberty, slowed pubertal growth acceleration, gynecomastia and relative eunuchoid body habitus. Other associated co-morbidities have been inconsistently reported including unilateral renal agenesis (up to 50% of boys), ASD, color blindness, synkinesia (mirror hand movements), nystagmus, ataxia, hypovitaminosis D with osteopenia and osteoporosis, metabolic syndrome, weight excess, lipid abnormalities, hypertension and type 2 diabetes. 50% of Kallman Syndrome patients have other midline craniofacial defects such as cleft lip and palate. Positive family history is present in approximately 50% of cases so that detailed family history can aid in thinking about and making the diagnosis of Kallman Syndrome and differentiating Kallman from constitutional pubertal delay. Gynecomastia in Kallman Syndrome is felt to be related to relatively low levels of testosterone and unopposed estrogen effects from the normal conversion of adrenal precursors to estradiol resulting in increased estradiol to testosterone ratio in male Kallman Syndrome patients. Normosmic patients are not diagnosed with Kallman Syndrome but rather as labeled as idiopathic hypogonadotropic hypogonadism (IHH) since they also present with low LH, low FSH and low gonadal hormones and, of course, both Kallman and IHH patients can present with partial or complete forms.

Androgen treatment in males and estrogen treatment in females once the diagnosis of Kallman syndrome is made (and other causes excluded) would be expected to follow similar guidelines as with Turner Syndrome and Klinefelter Syndrome patients.

The Kallman Syndrome support group (www.kallmans.org) is available for supporting those with this diagnosis as well as their families.

PRADER WILLI SYNDROME

Prader-Willi Syndrome (**PWS**) with a prevalence rate of 1:16,000 was the first human disorder attributed to genomic imprinting in which genes are expressed differentially based on the parent of origin. PWS results from the loss of imprinted genomic material from the paternal 15q11.2-13 locus (vs Angelman syndrome which involves loss of maternal genomic material at the same locus). PWS includes deletions in about 70% of patients, uniparental disomy in about 25%, imprinting center defects in<5% and rarely chromosomal translocations in <1%.[72]

PWS is commonly associated with neonatal hypotonia, characteristic facial features including narrow bi-frontal diameter, almond-shaped palpebral fissures, narrow nasal bridge and down-turned mouth and small genitalia which eventually are confirmed in the teenage years as hypogonadism with delayed or absent pubertal progression, low testosterone levels in males and low estrogen levels and lack of menses in females. Some studies have suggested that in males with PWS, primary hypogonadism is a more common cause than hypogonadotropic hypogonadism.

PWS patients also tend to have small hands and feet. Infants are born with intrauterine growth delay and often a history of decreased fetal movement, delayed neurologic development, hypotonia and failure to thrive in addition to poor appetite often being referred for gastrointestinal evaluation in the nursery or soon afterwards; this then changes after about age 2 years to increasing polyphagia and progressive obesity.

Many features of PWS reflect neurohypothalamic dysfunction proved or assumed including learning and behavior problems, hyperphagia, sneaking and stealing food, sleep disorders including apnea, deficient GH secretion (but not documented in all patients studied) and hypogonadotropic hypogonadism as well as primary hypogonadism. Low vitamin D levels and osteopenia or osteoporosis may also be present but is generally asymptomatic unless specifically screened.

Growth rates (height velocity) are borderline normal or diminished in the midst of excessive weight gain in contrast to normal or often accelerated height velocity often seen in non-PWS obese children. Levels of IGF-1 are low-normal and GH response to provocative testing are normal or blunted but usually not seen as classically GH deficient by current norms. PWS short stature, however, is responsive to growth hormone treatment and is an approved indication for GH treatment in some countries because of positive responses documented in numerous national and international studies. [73]

Because of the obesity or associated with the obesity, insulin resistant diabetes mellitus is also part of the clinical presentation and some go on to needing insulin treatment when the polyphagia and obesity cannot be controlled behaviorally or by oral medications or even the newer and more potent type 2 diabetes medications now available.[74]

GH treatment itself often leads to significant catch-up growth velocity comparable to that seen in other children with severe GHD and maintained in similar fashion.[75] Metabolic benefits are also seen with decreased adiposity and increased lean body mass, improved muscle strength, stamina, coordination, pulmonary function and sleep quality but all such parameters still remain below normal compared to non-PWS peers. When PWS patients are continued on GH as adults, some studies report maintenance of improved BMI suggesting potential benefit in continuing GH treatment in PWS patients in an effort to reduce known weight and insulin resistance/diabetes as well as cardiovascular risks[76] but this is not yet definitively proven. Potential side effects also are somewhat increased in PWS children and adolescents treated with GH including glucose intolerance, pseudotumor cerebri, scoliosis and sleep-related deaths so caution is warranted as well as specific ongoing surveillance especially in those with severe obesity or severe respiratory impairment such as sleep apnea.

Gonadal issues with PWS include hypogonadism thought to be present at birth but difficult to diagnose at that age unless, in males, there is micropenis or cryptorchidism.

Sometimes also a relatively small flat scrotum in the boys. Cryptorchidism is present in about 80-90% of boys and in some studies the primary cause of male hypogonadism was felt to be primary testicular failure and not hypothalamic or pituitary abnormalities.[77, 78]

In females, the clitoris and labia minora may be small. For both sexes with PWS associated primary or hypogonadotropic hypogonadism, puberty is often absent or blunted. At puberty in girls, amenorrhea and oligomenorrhea are common and infertility is common in both sexes.[79] Some authors have suggested using human chorionic gonadotropin treatment in males with undescended testes to promote lowering the testes into the scrotal sac or in preparation for orchidopexy to improve surgical outcome. Hormonal treatment with androgens for the males and estrogens for the girls (then cyclical combination estrogen/progesterone) is useful for induction and maintenance of puberty. Some authors have promoted gonadal treatment to help prevent increasing obesity but data are still controversial in this regard.

Because of skin picking behavior in both sexes and aggressive behaviors especially in boys, hormone treatment should be considered and very slowly advanced to help minimize any facilitative abnormal behavior attributable to hormone replacement treatment or a as co-morbidity of the PWS itself.

Standard androgen treatment protocols can be followed to attempt to mimic normal male pubertal progression with the hopes of decreasing obesity, improving glucose intolerance and improving bone mineralization, muscle mass/strength and preventing osteopenia and osteoporosis while paying close attention to behavioral and emotional issues that may be exacerbated.

Standard estrogen treatment protocols in females also can be followed with the same goals in mind and with the same general caveats especially with regard to potential exacerbation of diabetes with estrogen delivery. In those young women who have dysmenorrhea or menometrorrhagia or more severe behavioral problems during specific parts of their

induced menstrual cycles, non-cyclical combination estrogen/progesterone therapy can also be used quite effectively.

Table 1.3: Benefits versus risks of androgen replacement therapy [1, 17, 18]

Benefits

- Produce, increase and/or maintain secondary sexual characteristics
- Increased sexual libido and performance
- Increased muscle mass and strength
- Decreased body fat especially visceral fat
- Decreased bone resorption, increased bone formation and maintain bone mass (less osteopenia and osteoporosis)
- Improve mood parameters
- Improve quality of life
- Improve or prevent deterioration of cognition

Risks

- Increase acne and skin oiliness
- Increased gynecomastia from aromatizable androgens
- Increased hematocrit
- Sleep related breathing disorders, apnea
- Decreased HDL cholesterol and increased cardiovascular risk
- Increased prostatic hypertrophy
- Stimulation of existing prostate cancer
- Worsening aggressiveness and other behaviors [71]

Behavioral difficulties in PWS[80] start often in early childhood including rigid behaviors, temper tantrums, oppositional behavior, odd speech behaviors, skin picking and compulsivity. In adolescence, aggressive behavior as well as overt bipolar disorders including anxiety, depression and even psychoses and food-seeking behaviors can be overwhelming. All these difficulties often need psychiatric intervention and medication(s) that may interfere with thyroid functioning as well as compromise metabolic and gonadal status.

The Prader-Will Syndrome (www.ipwso.org) is available to support and educate about PWS for children, adolescents and their families and also has available free genetic diagnostic testing.

Table 1.4: Benefits versus risks of estrogen replacement therapy

Benefits
• Produce, increase and/or maintain secondary sexual characteristics
• Increased sexual libido and performance
• Decreased bone resorption, increased bone formation and maintain bone mass (less osteopenia and osteoporosis)
• Improve mood parameters, Improve quality of life
• Improve or prevent deterioration of cognition
Risks
• Decreased HDL cholesterol, increased LDL and total cholesterol with increased cardiovascular risks
• Possible breast or uterine malignancy ???
• Increase hypertension
• Vaginal bleeding
• Worsened moodiness or other behaviors

In conclusion

These are examples of many other types of syndromic hypogonadism that may be encountered as listed in *Table 1.5*. The treatment protocols may need to be modified according to other characteristics of these syndromes and especially when concerns over aggressiveness when considering adding androgen therapy occur. For females, the potential benefits of estrogen therapy also need to be weighed and consideration for continuous estrogen treatment rather than cyclical estrogen delivery should be entertained for behavioral and psychosocial reasons.

However, the general principles remain the same:

(1) make the correct and most specific diagnosis as early as possible,

(2) provide appropriate genetic and personal counseling with support services as needed,
(3) identify co-morbidities that need specific intervention and treatment,
(4) consider the most appropriate gonadal treatment for the circumstances with the patient and family.

Table 1.5: Some other syndromes involving hypogonadism

HYPOTHALAMIC:

- X-linked Kallman Syndrome (KAL1)
- Autosomal dominant Kallman Syndrome (FGFR1)
- Other genes causing Kallman Syndrome (PROK2, PROKR2, CHD7, FGF8, WDR11) or other gene mutations that interfere with development and migration of GnRH neurons (NELF, HS6ST1, CHD7, SEMA3A)
- Hypogonadism associated with morbid obesity (Leptin gene and Leptin receptor, Prohormone convertase 1 (PCSK1)
- Adrenal deficiency associated with hypogonadotropic hypogonadism (DAX1)
- Disorders of sexual development associated with hypogonadotropic hypogonadism (SF1)
- Prader-Willi Syndrome (del pat chr 15q11-13)
- Bardet-Biedl syndrome
- Isolated hypogonadotropic hypogonadism (GNRHR, GNRH1, GPR54, TAC3, TACR3)
- Associated with damage secondary to suprasellar/sellar solid tumors (ie. craniopharyngioma), head trauma, brain surgery, cranial irradiation, CNS chemotherapy

PITUITARY:

- Septo-optic dysplasia (HESX1)
- Disorders of pituitary organogenesis (PROP1, LHX3, LHX4, PTX2, SOX3) and congenital hypopituitarism
- Pituitary tumors, apoplexy, head trauma, cranial irradiation, hypophysitis secondary to sarcoidosis, Wegener, histiocytosis X, granulomatous
- Pituitary autoimmune infiltrative disorders (hypophysitis)
- Hemochromatosis and thalassemia major

GONADAL:

- Gonadotropin resistance (FSHR, LH/HCGR)
- Autosomal recessive mutations in steroid enzyme pathway genes
- Disorders of gonadal dysgenesis causing abnormal sexual development (SF1 and others sometimes also associated with adrenal dyshormonogenesis syndromes and adrenal insufficiency)
- Autoimmune polyendocrine syndromes (AIRE) including those associated with lymphocytic infiltration and antibodies of the testes or ovaries
- Galactosemia (GALT)
- Cystic fibrosis (CFTR)
- Myotonic dystrophy (CTG)
- Noonan Syndrome (PTPN11, KRAS, SOS1, RAF1)
- Klinefelter Syndrome (47XXY and variants)
- CHARGE (coloboma, heart defects, choanal atresia, retarded growth and development, gonadal abnormalities, ear abnormalities and sometimes also hypopituitarism)
- IMAGe Syndrome (IUGR, metaphyseal dysplasia, adrenal, gonadal hypoplasia with cryptorchidism and micropenis, hypercalciuria and hypercalcemia)
- Acquired gonadal failure from mumps, coxsackie B virus, cytotoxic drugs, gonadal irradiation and associated with other malignancy related chemotherapeutic regimens. testicular
- Direct trauma or torsion of testes or ovaries as well as surgical resection
- Disorders/Syndromes associated with micropenis or cryptorchidism (Aarskog, Borjeson-Fossman-Lehman, Carevale, Cornelia de Lange, Faciogenitopopliteal, Goldenhar, Holoprosencephaly, Juberg-Marside, Johanson-Blizzard, Lenz-Majewski hyperostosis, Lower, Malpeuch facial clefting, McKusick-Kaufman, Meckel-Gruber, Miller-Dieker, Escobar multiple pterygium, Najjar, Pallister-Hall, Pfiffer, Robinow, Rubinstein-Taybi, Seckel, Shprintzen-Goldbert, Simpson-Golabi-Behmel, Townes-Brocks, Varadi-Papp, VATER, Weaver among others)

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EVOLUTIONARY INSIGHT OF VITAMIN D

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Abstract

Vitamin D is one of the first hormones; it is photosynthesized in all organisms from the phytoplankton to mammals. Exposure to UVB radiation balances the need for vitamin D photosynthesis and degradation of folic acid by UVB radiation. Modern lifestyle limits our exposure to sunlight, which photosynthesizes vitamin D in the skin, and the incidence of nutritional rickets has been resurging. A selective sweep of the promoter of the vitamin D receptor (VDR) happened as soon as Homo sapiens migrated out of Africa; it co-adapted with skin color genes to provide adaptation to latitudes and the levels of exposure to ultraviolet (UV)B radiation along the route out of Africa. Skin color follows a latitude distribution: the darkest populations dwell in the tropical belt; and the fair-skinned populations inhabit the northern countries. Due to their greater need for calcium during their reproductive life, the skin color of women is lighter- than that of men. Vitamin D is essential for mineral homeostasis and has a wide variety of non-skeletal functions, of which the most important for natural selection is a regulatory function in the innate immune system.

In the human fossil record, vitamin D deficiency coincided with bone tuberculosis. About 6000 years ago, a diet which included cow's milk provided Neolithic humans

with twice as much calcium and was more alkaline than that of its Paleolithic predecessors.

Adiposity is negatively associated with the vitamin D status and obese individuals require 2–3 times more vitamin D than non-obese individuals to normalize circulating 25OHD levels. In an era of an obesity epidemic, we need more research to determine whether adiposity should be considered when determining the dietary requirements for vitamin D and calcium and the optimal serum 25OHD levels.

Introduction

Rickets and osteomalacia have always been present in European populations and people from the Middle East. Evidence of juvenile rachitic bones and teeth with dentin defects have been found in various archeological sites. These specimens reveal a high prevalence of mineralization disruption in our human ancestors who lived in the subtropical and temperate climatic zones from over 100,000 years ago to the present era. Although one can only speculate on the exact cause of rickets or osteomalacia in these specimens, vitamin D deficiency may have been the cause.

The first fossil hominin calvaria from Turkey [1] that attributed to *Homo erectus*, preserves unusual findings on the endocranial surface of the frontal bone that is consistent with a diagnosis of *Tuberculosis* (TB). This preserved pathology in a human fossil is the most ancient example of this disease. Kappelman *et al* posited that TB was exacerbated in this dark-skinned individual who lived in the northern latitudes by a vitamin D deficiency because of lower levels of ultraviolet radiation (UVR). The presumption of these paleoanthropologists implies the existence of an association between vitamin D deficiency and TB. This presumption also implies that climate was an adaptive challenge to our ancestors during their migration into the temperate regions of Europe and Asia.

At the start of the industrial revolution, rickets, “the English disease”, was estimated to be present in n 50-80% of

children in industrialized northern Europe[2], and TB was common[3]. In England, the 1840s were known as the hungry forties because many children were literally starving on the streets of London. In 1849, Williams reported the results of administering fish liver oil (vitamin D) to 234 patients with TB [4]. He noted that "*in a few days the appetite, flesh and strength were gradually improved*" and concluded that *"the pure fresh oil from the liver of the cod is more beneficial in the treatment of pulmonary consumption than any agent, medicinal, dietetic, or regiminal, that has yet been employed*".

In contemporary humans, vitamin D deficiency is highly prevalent globally and vitamin D insufficiency affects many people in many countries. Rickets has become an endemic disease of modern civilization and is most prevalent in children with a dark complexion[5]. Modern day vitamin D deficiency/insufficiency is probably due to a mismatch between our genes and our present environment, and an insufficient exposure to sunlight.

The reduction in sun exposure is caused by spending many hours indoors at school and in leisure activities; cultural reasons related to clothing; and public health recommendations to avoid sun exposure so as to prevent skin cancers and skin damage.[6]

The Sunshine Hormone

Most plants and animals that are exposed to sunlight have the capacity to make vitamin D, the sunshine hormone.

Vitamin D is an ancient hormone: it is photosynthesized in many life forms which range from the early life forms the phytoplankton of 750 million years ago (m.y.a) to present-day mammals.[7,8] Although it is understandable why terrestrial animals with a calcified skeleton and lay eggs with a calcified shell need provitamin D for calcium and bone metabolism. The needs and functions of vitamin D in either phytoplankton or zooplankton remain unknown. Holick *et al.* suggested that provitamin D evolved to protect UVR-sensitive macromolecules

from solar UV damage or in regulating membrane permeability to cations, such as calcium.[9]

Fish have the utmost natural content of vitamin D [10] because they consume plankton which is rich in vitamin D and is the basis for the entire marine food web.

Vitamin D3 and its provitamin, have also been identified in the leaves of several species which mostly belong to the *Solanaceae* family of trees, shrubs, and herbs including leaves but not fruit of potato, tomato, eggplant and peppers).[11] Humans routinely consume the fruit and roots but not the leaves of these plants.[12] UVB irradiation of several plants has been shown to induce the production of flavones that promote growth (shoot length and fresh weight), and possibly also promote nodulation in the roots of pea plants.[13]

Vitamin D3, which is synthesized in the skin, requires sequential hydroxylations in the liver and kidney to be converted to its biologically active form, 1*a*,25-dihydroxyvitamin D3 (1,25D3), which binds a unique vitamin D receptor (VDR). 1,25D3 and the VDR are important for calcium absorption, and skeletal development and mineralization, but also for the regulation of proliferation of many cell types. [14,15,16]

Although the VDR, which belongs to the superfamily of nuclear hormones receptors, is well conserved from *Xenopus* to mammals,[17] there has been extensive evolution of the VDR in vertebrates.[18] Using a genomic approach, it has been suggested that VDR might be the original nuclear receptor.[19] VDR polymorphisms are associated with bone size [20] and the risk and incidence of fractures[21]. The widespread abundance of the VDR may be related to a host of recent reports which claim that vitamin D functions as a regulator in many biological processes, which include cell differentiation and proliferation, immunity, muscle strength, and blood pressure control. [22]

Skin color and the impact of climate

Evolutionary pressures due to variation in climate play an important role in determining phenotypic diversity among and within species. In 1833, Gloger published what has become known as *Gloger's rule'* on the coloration of birds.[23] Within a species of endotherms, the heavily pigmented forms tend to be found in equatorial areas of the globe because of the selective pressures of heat, humidity, and UVR.

The original hominines, who inhabited current-day tropical Africa, required minimal substrate and storage of vitamin D in this sun-rich environment because provitamin D is easily photo-isomerized to biologically active isomers under exposure to high UVB radiation. When hominines began to migrate to regions which were either north or south of the equator, dark pigmentation of the skin became a liability because of shorter lengths of daylight and an increase in the number of sunless days. Inbreeding within white-skinned groups, which continually heightened fair skin, made the development of this new human trait possible. Evolutionary analyses indicate that dark skin was the ancestral trait for modern humans, consistent with the evolution of the *homo* lineage in Africa.[24] Hominid migration and the change of latitude heavily impacted the evolution of skin color genes and the VDR. The "vitamin D hypothesis" was refined by Jablonski and Chaplin[25,26] and more recently by Tiosano *et al.*[27] This hypothesis is based on the observation that the skin color of the world's indigenous peoples trails a latitude distribution: the populations with the darkest skin color inhabit the equatorial and tropical belts; the most fair-skinned populations inhabit the northern countries; and those with intermediate pigmentation of their skin inhabit the middle latitudes.[25,27,28]

Exposure to sunlight and UVB radiation have to balance the need for vitamin D photosynthesis and degradation of folic acid by UVR. This balance is maintained by melanism, which determines skin color. Based on reflectance measurements, a comprehensive compilation of skin colors of indigenous

peoples is now available, and this compilation reveals a strong correlation between skin color and the geographical latitude of the habitat.[25] Variation in human skin pigmentation is due to the quantity of melanin, the size of melanin particles, and the distribution of eumelanin (dark melanin) and phaeomelanin (red/yellow melanin) that are generated by the melanocyte. Dark melanin absorbs and scatters the UVB radiation which catalyzes vitamin D3 synthesis. In general, a high amount of dark melanin in the skin slows cutaneous synthesis of vitamin D3. Dark-skinned individuals require a six-time longer exposure to sunlight than fair-skin individuals to achieve the same vitamin D serum levels.

The skin pigmentation of the chimpanzee, which lives in the dark forest, is paler than that of equatorial and Middle Eastern humans. When hominines left the forest for the sun-exposed savannah, they lost their fur, acquired a sweating mechanism, and their skin became pigmented to protect them from the higher levels of UVR. When *H. sapiens* migrated out of Africa, they received significantly less UVB radiation, and their skin depigmented to a degree that permitted UVB-induced synthesis of provitamin D3. This correlation is a compromise on the need for vitamin D and the detrimental effect of UVR on folic acid generation.[29]

Evolution has used the polygenic trait of skin pigmentation as a tool for such balance.

The result of a genome-wide association study of natural hair and skin color identified several genes that are decidedly associated with skin color, which include *TYR, TYRP1, OCA2, SLC45A2, SLC24A5, KITLG*, and *MC1R.*[30,31,32,33] In a recent study of 751 subjects with diverse skin colors from a broad range of latitudes, we investigated possible multilocus correlation variation of skin color genes with the VDR.[27] We discovered two multilocus networks which involved the VDR promoter and skin color genes and show strong latitudinal clines, even though many of their single gene components do not. Considered one by one, the VDR components of these networks show diverse patterns: no cline, a weak declining

latitudinal cline outside of Africa, and a strong in-versus-out of Africa frequency pattern.[27] These results suggested that:

- a selective sweep which favored the VDR promoter haplotype happened as soon as *H. sapiens* migrated out of Africa;
- the VDR promoter haplotype co-adapted with single nucleotide polymorphisms in the skin color genes;
- the main skin color gene that correlates strongly with latitude is the melanoma-associated SLC45A2 gene; and
- the cluster of the VDR promoter with the skin color genes provides a fine-scale adaptation to northern latitudes and decreasing UVB along the route out of Africa.

The strongest determinant of skin color is the cell-surface melanocortin type-1 receptor (MC1R), a G-protein-coupled receptor which is involved in the synthesis of melanin in the melanocyte. Several variant MC1R alleles are associated with the typical Nordic red hair, fair-skin trait.[33,34,35] The common ancestral form of the human *MC1R* gene dates back to 850 k.y.a., and spread out of Africa during the Acheulean expansion which occurred 800 k.y.a.[36] The MC1R gene displays a significant molecular signature of selection within sub-Saharan African populations of purifying selection to maintain its protein structure.[33] The selective sweep for gene variants' frequency in a population that define lighter skin in northern latitudes is among the strongest signals of recent selection in humans, with point estimates of selection of 2–10% per generation.[37]

This rapid pace supports a significant evolutionary advantage of lighter skin as migration advanced to northern latitudes. Although evolution of skin color is partially explained by positive selection of light skin color by mate choice,[37] the main disadvantage is attributed to the vitamin D deficiency in dark-skinned humans who live in areas with low levels of UVR.

Another notable feature of human skin color is that the skin color of women, tends to be lighter than that of men.[38]

This is probably directed by evolutional selection, as the need for calcium is much higher in women due to their periods of pregnancy and lactation, and a fair skin enables women to augment vitamin D generation for a given dose of UVB radiation. Other evolutionary biologists argue that the skin color of women is a result of sexual selection: men prefer women with a light skin color. [39]

Evolutionary of Vitamin D Deficiency

Both calcemic and non-calcemic actions of vitamin D have been proposed as explanations for the strong negative evolutionary selections pressures against individuals with vitamin D deficiency.

Significant deficiency in vitamin D can lead to dysregulation of calcium absorption and bone metabolism.

Severe deficiency can lead to life-threatening hypocalcemia, which in turn may lead to cardiac arrhythmia. Childhood rickets leads to severe skeletal deformities which confer a significant disability with consequent reproductive and survival compromise. In adults, vitamin D deficiency-induced osteomalacia increases the risk of fracture, especially when associated with muscle weakness, another consequence of vitamin D deficiency.[40] The results of several studies of contemporary women and newborns in developing countries have identified vitamin D deficiency as a risk factor for adverse pregnancy outcomes, both maternal (pre-eclampsia, infection, and cesarean section delivery) and neonatal (small size for gestational age, low birth weight, and stunting).[41]

The excess rate of cesarean sections in women with vitamin D insufficiency may possibly be attributed to pelvic inlet deformities caused by osteomalacia or muscle weakness.[42]

The observation that the vitamin D-metabolizing enzymes and the VDR are expressed in cells other than those of the bone, intestine, kidney, and parathyroid gland led to the recognition of the non-calcemic actions of vitamin D. Work done at the turn of the 21st century revealed that vitamin D

has a wide variety of non-calcemic functions,[7,9] of which the most important is probably its regulatory function in the innate immune system.[43,44] Accordingly, it is tempting to speculate that this regulatory function has made vitamin D such an extraordinary highlight of human evolution.[45] The results of previous investigations revealed that (a) monocytes which were incubated with vitamin D (a) induced anti-tuberculosis activity, and (b) monocytes which were incubated with interferon-gamma developed 25-hydroxyvitamin D3-1-hydroxylase activity.[46,47] The innate antimicrobial defense system uses activation of Toll-like receptors (TLR) to generate 25OHD3-1α-hydroxylase for converting an inactive metabolite into active 1,25D3, which in turn triggers the generation of cathelicidin, which kills *Mycobacterium tuberculosis.*[44] It has also been reported that dark-skinned African Americans have lower circulating levels of 25OHD3, they display smaller induction of cathelicidin mRNA, and are more susceptible to *M. tuberculosis* than Caucasian Americans.[48]

In a study of patients with hereditary vitamin D–resistant rickets (HVDRR), who have a defective VDR, Tiosano *et al* reported that cathelicidin expression was lower in monocytes which were collected from individuals with HVDRR than in control monocytes.[49] Tiosano *et al* also reported that 25OHD3 increased significantly the expression of cathelicidin and VDR in the control monocytes but only slightly in HVDRR monocytes and suppressed TLR2 only in the control monocytes.

The results of several randomized controlled trials found that the prevalence of respiratory infections is lowered by vitamin D supplements in children and adults with vitamin D deficiency.[50]

In a cohort of newborns of HIV-positive mothers in Tanzania, the authors reported that a low vitamin D intake was associated with a risk of mother-to-child HIV transmission and a high risk of 2-year infant mortality.[51]

Clear evidence of evolutionary selection by low vitamin D availability through modulation of immune activity can be demonstrated in the enrichment of VDR binding in

lymphoblastoid cell lines from European and Asian populations, but not from an African Yoruba population.[52]

Dietary Calcium

The transition from a lifestyle of hunting and gathering to one of animal and plant domestication 12-8 k.y.a. occurred in parallel or during to the transition from the Pleistocene to the Holocene after the last glacial period (*Figure 2.1*).

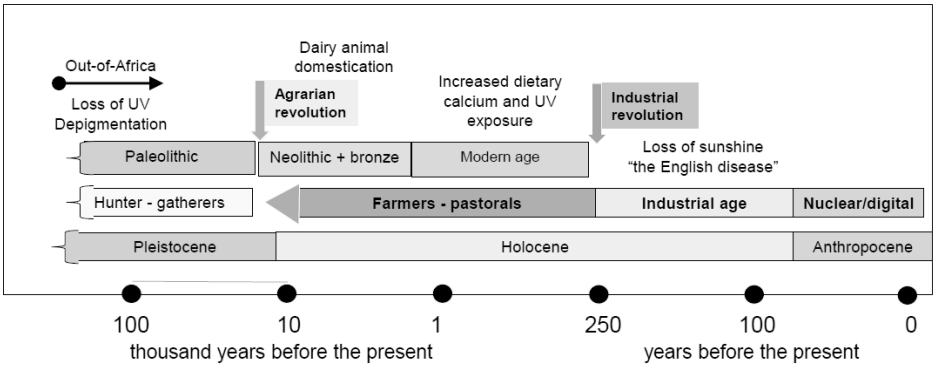

Figure 2.1 – Timeline of geological epochs, archeological periods in human prehistory and history, and the effects of sunshine and dietary calcium. (The migration of man out of Africa to northern and southern latitudes 60-130 k.y.a was associated with depigmentation. Transition from the geological Pleistocene epoch to the Holocene epoch coincided with the 'agrarian revolution' 10-12,000 years ago and from the archeological Paleolithic period (hunter gatherer tool makers) to the Neolithic farmers. The agrarian revolution happened at different times in different parts of the world, and wherever it happened, it was associated with an increase in dietary calcium and crowding of people in cities. The industrial revolution, which began in Europe in the second half of the eighteenth century, was associated with rickets – "the English disease" due to industrial air pollution. The transition to the nuclear age, also called the digital age, define also the transition to the Anthropocene epoch, which is associated with further diminution of UVR exposure in humans.)

GLOSSARY
Pleistocene: A geological epoch, which lasted from about 2,600,000 to 11,000 y.a., between the Pliocene and Holocene epochs. It covers most of the last glacier period.
Holocene: A geological epoch, which started after the last glacial period, 11,000 y.a. and ended recently with advent of the Anthropocene.

Anthropocene: A recently proposed geological epoch from the commencing of significant human impact on the Earth's geology and ecosystems, including anthropogenic climate change.
Paleolithic: Early Stone Age. An archeological period in human prehistory renowned by the earliest development of stone tools some 3.3 million y.a. to the end of the Pleistocene.
Neolithic: Late stone age. An archeological period in human history designated from the domestication of plants and animals 10-12 k.y.a.

The ecological changes associated with this geological transition led to the agrarian revolution of dairy animal and plant domestication and the transition from the Paleolithic to the Neolithic periods. Although post-weaning lactose intolerance occurs in most mammals, at some point in history human populations in northern Europe adapted to permit the consumption of milk beyond childhood and in adulthood by selecting for persistence of intestinal lactase activity beyond infancy.[53] About 6000 years ago, a diet which included cow's milk provided Neolithic humans with twice as much calcium than that of their Paleolithic predecessors.[54]

In Western countries, milk is a major food source for calcium, generally providing 36–70% of the dietary calcium.[55]

The bottleneck of calcium requirements, and apparently a leading evolutionary trigger, is the calcium requirement for skeletal growth in all vertebrate offspring and even more so, in nursing mothers. The calcium that is required for milk production is generated by a dramatic increase in the rates of bone resorption and a decrease in renal calcium excretion.[56]

Whatever the physiological consideration, Thacher *et al* reported the results of a randomized, double-blind, controlled trial study of 123 Nigerian children with rickets.[57] They found that that the intake of calcium in these children was low and that the response to treatment with calcium alone or in combination with vitamin D was better than that to treatment with vitamin D alone. Calcium-deficient rickets shows its presenting signs in toddlers, as compared to vitamin D deficiency which becomes evident in infancy. We have also reported that calcium deficiency is present in severely rachitic toddlers from Egypt.[58] Thacher *et al* published a systematic review of articles that were published in the last 20 years on

the prevalence of nutritional rickets in various geographical regions.[59] They found that calcium deficiency is the major cause of rickets in Africa and some parts of tropical Asia, and the prevalence of rickets is increasing in other parts of the world. [59]

Culture and Technology

Humans have been able to successfully inhabit almost all of the earth's natural environments. Similar to all other organisms, we are still motivated by primordial instincts, among them the drive to propagate our DNA to the limits of possibility.[60] DNA is clearly important for the inheritance of traits, but any process or activity which contributes to parent-offspring resemblance within populations has potential evolutionary relevance. These processes and activities, which include culture, technology, customs, traditions, religion, and governance among others, are bequeathed to future generations and impact strongly on human traits and behavior.

In addition to biological evolution, we are already relying on culture and technology to retain reproductive fitness. For humans, a culture-determined way of life can be (and has been) reinvented, modified, and changed in accordance with the prevailing climatic and environmental changes. For example, domestication of dairy animals liberated humans 6,000 years ago from the constant search for food, including a source of calcium, and this knowledge was passed down to their descendants.

Focusing on vitamin D and rickets, we should also include culture and technology in the evolutionary perspective of rickets and vitamin D.[61] Culture and technology have significant impacts on habitability of northern and southern latitudes, housing, clothing, work practices, diet, and environmental conditions. The lifestyle of the digital age limits our exposure to sunlight, and as a result, there is a resurgence of vitamin D deficiency rickets.

Before the industrial and agrarian revolutions, the technology of clothing and shelter dwelling enabled us to migrate mostly northward 60 k.y.a out of the tropics into regions of low levels of UVR. Whereas biological adaptation through changes in gene frequency in a population or species and modification of population homozygosity takes hundreds of thousand years, cultural adaptation is rapid and depends on the charisma and authority of a few individuals. The more recently a group migrated into an area, the more extensive its cultural, but not biological, adaptation to the area will be. [38]

Although the Sudanese and Saudis live at similar latitudes, Jablonski and Chaplin noted that the Sudanese have a dark skin color and the Saudis have a light skin color.[38] The Sudanese have been dwelling at average latitude 13N ever since *H. sapiens* migrated throughout Africa and their skin color is well adjusted for high-intensity UVB radiation. Dwellers of the Arabian Peninsula have lived at similar latitude only since they arrived there from Europe 2000 years ago. This time frame does not allow for biological adaptation, and they had utilized mostly cultural means to adapt to the same intensity of UVR. Specifically, they wear long protective clothes, they carry their shade with them in the form of tents, and they protected their lighter-skinned women using customary veils and house confinement. Traditional diets in indigenous people in northern latitudes included fatty fish and the blubber from seals and whales which also were sources of vitamin D. Hence, the circulating vitamin D levels in these people are high.[62] Change in eating habits and reduced consumption of these traditional foods in populations living in the Arctic regions has been accompanied by a high prevalence of vitamin D deficiency in the last few decades. [63,64]

Future Perspectives

Sun exposure and skin photosynthesis are the major sources of vitamin D for both children and adults. Sensible sunshine exposure needs to consider all the above-mentioned

benefits, but also the damaging effect of UVR on sunburn, DNA stability, folic acid levels, and skin cancer risk. In individuals with a dark complexion, the risk is small and benefits are great.

In the absence of the sun's UVR, it is difficult to obtain an adequate amount of vitamin D from nutritional sources without supplementation. The current pandemic of rickets can be attributed to our modern lifestyle in which outdoor activities are greatly reduced.[5] This reduction reduces our exposure to the sun's UVB, which is required for the generation of vitamin D in the skin. It is unlikely that an individual who spends his/her entire time indoors would be exposed to the amount of UVR that is required to generate a sufficient amount of vitamin D as a result of human evolution. For contemporary individuals, vitamin D is an essential micronutrient and is no longer the sunshine vitamin because we now need to consume it in our diet. This is easy to prove: we use the plasma 25OHD concentration as a marker of supply, and we use the plasma concentration of parathyroid hormone and serum [65] and urinary phosphorus [66] as markers of function.

An Endocrine Society Clinical Practice Guideline recommends measuring vitamin D in individuals at risk for vitamin D deficiency and that infants and children aged 0–1 year need at least 400 IU/d of vitamin D and children 1 year and older require at least 600 IU/d to maximize bone health.[67] These recommendations are in agreement with those from the European Society for Paediatric Endocrinology.[68] Whether these doses of vitamin D are enough to provide these individuals with all the non-skeletal health benefits to maximize bone health and immune function is not known at this time.[69] While the average serum 25OHD levels in contemporary adult hunter gatherers in East Africa are 46 ng/dL (115 nmol/l),[70] the guidelines recommend a blood level of 25OHD above 30 ng/ml (75 nmol/l), which may require at least 1000 IU/day of vitamin D.[67] We know of no disadvantage to increasing the vitamin D intake in children. Cases of vitamin D intoxication in individuals supplemented with

megadoses of oral vitamin D are rare and usually asymptomatic.

Previous discussions on vitamin D deficiency were usually limited to mineral homeostasis and rachitic bone disease. Evidence is now plentiful that vitamin D has a multiplicity of non-calcemic functions, of which the most important in terms of natural selection is probably the regulation of innate immunity and the prevention of TB and possibly additional serious infections. Future studies need to include the effects on the innate immune system when assessing the outcomes of vitamin D supplementation and when treating nutritional rickets.

Dark pigmented individuals have a higher prevalence of vitamin D deficiency and secondary hyperparathyroidism. Although they have a higher average bone mineral density, are less prone to osteoporotic fractures,[71,72,73] and may be more resistant to the bone-resorbing effect of PTH,[74,75,76] there is no evidence that the optimal vitamin D levels needed for non-skeletal vitamin D functions are affected by skin pigmentation. Many of the benefits of vitamin D sufficiency where demonstrated in dark pigmented individuals and there is no data to support different vitamin D sufficiency cutoff for different populations. Due to their lower skin synthesis of vitamin D, dark skinned individuals in temperate zones most likely need higher doses of vitamin D supplementation all year round.

Obese children and adults require 2–3 times more vitamin D than non-obese children and adults to normalize circulating 25OHD levels. However, we do not know whether non-obese and obese children and adults require similar 25OHD levels for calcium balance and maintaining the integrity of other vitamin D-dependent functions. In an era of an obesity epidemic, we need more research to determine whether adiposity should be taken into account when determining the dietary requirements for vitamin D and calcium and the optimal serum 25OHD levels.

Finally, in an era of data technology, we need

a. to accumulate and analyse data to determine which individuals will get nutritional rickets,
b. to design effective preventive measures that can feasibly reach entire communities, and
c. apply preventive measures which may differ between various affected regions in darkly pigmented individuals living in temperate climes.

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SUBCLINICAL HYPOTHYROIDISM IN CHILDREN

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Definition

Subclinical hypothyroidism (SH) is defined by increased serum TSH values (above the superior limit of the range considered as normal) associated with normal serum FT4 values (within the reference range considered as normal).

SH, also known as isolated *hyperthyrotropinemia, preclinical* or compensated *hypothyroidism*, is a diagnosis based exclusively on biochemical criteria, most of the patients lacking symptoms specific to any thyroid dysfunction.[1]

Epidemiology

In the case of adults, SH is quite common and tends to evolve towards overt clinical hypothyroidism.[1] In the case of children, the prevalence is much lower, the few studies in the specialised literature reporting a prevalence of below 2% in the infant population.[2]

SH in children is a relatively benign condition, which occurs spontaneously and rarely evolves to overt clinical hypothyroidism.[3] The results of studies conducted to date show the beneficial effect of L-Thyroxine replacement therapy in severe forms of SH, but data are insufficient to support the

necessity of therapy in the cases of mild SH.[4] However, it is recommended that minor anomalies of TSH in children be evaluated.[4]

Etiology

From the etiopathogenic point of view, in the case of children, SH is associated with thyroid and non-thyroid disorders. If no responsible reason can be highlighted for the occurrence of SH, it is considered idiopathic.[5]

The most common thyroid causes of SH are: chronic autoimmune thyroiditis (Hashimoto thyroiditis), iodine deficiency and excessively treated Graves disease. Transient neonatal hyperthyrotropinemia or mutations of genes encoding proteins involved in TSH synthesis may also be responsible for the occurrence of SH.[6,7]

The non-thyroid causes of SH include: diabetes mellitus, celiac disease, obesity, genetic syndromes (Down, Turner, Klinefelter syndromes etc), chronic renal failure, medication (carbamazepine, valproic acid, domperidone), X- ray radiotherapy for malignant diseases (of the head and / or neck area).[8,9]

Clinical aspects

In SH, symptoms are usually absent, but there are also situations where specific clinical signs of hypothyroidism are found.

The most common clinical sign in SH is the goiter, whose prevalence - reported in studies - is twice as high as in the general population.[8] In children, SH is commonly associated with overweight and obesity.[7,10,11] Sometimes, the evolution of SH in children describes: impaired linear growth, impairment of psycho-motor and cognitive development, sleepiness, anaemia.[2,12]

Positive diagnosis

The SH diagnosis is exclusively biochemical, confirmed by increased serum value of the TSH associated with a normal value of FT4.

In the healthy individual, the serum level of the TSH exhibits physiological variations (pulsatile secretion) and, on the other hand, it is influenced by a number of factors (stress, iodine, cold etc), especially in children. For this reason, it is recommended to repeat the investigations within the next 3-4 months after the initial assessment, for diagnosis certification.[13,14]

The assessment of the child with SH will consider both significant anamnestic features (family history of thyroid disorders, neonatal screening for hypothyroidism, associated pathology) as well as possible clinical signs suggestive of hypothyroidism.

After the biological confirmation of SH, serological evaluation is recommended: anti-thyroid peroxidase (ATPO) antibodies and, respectively, antithyroglobulin antibodies (antiTg) (*Figure 3.1*).[13]

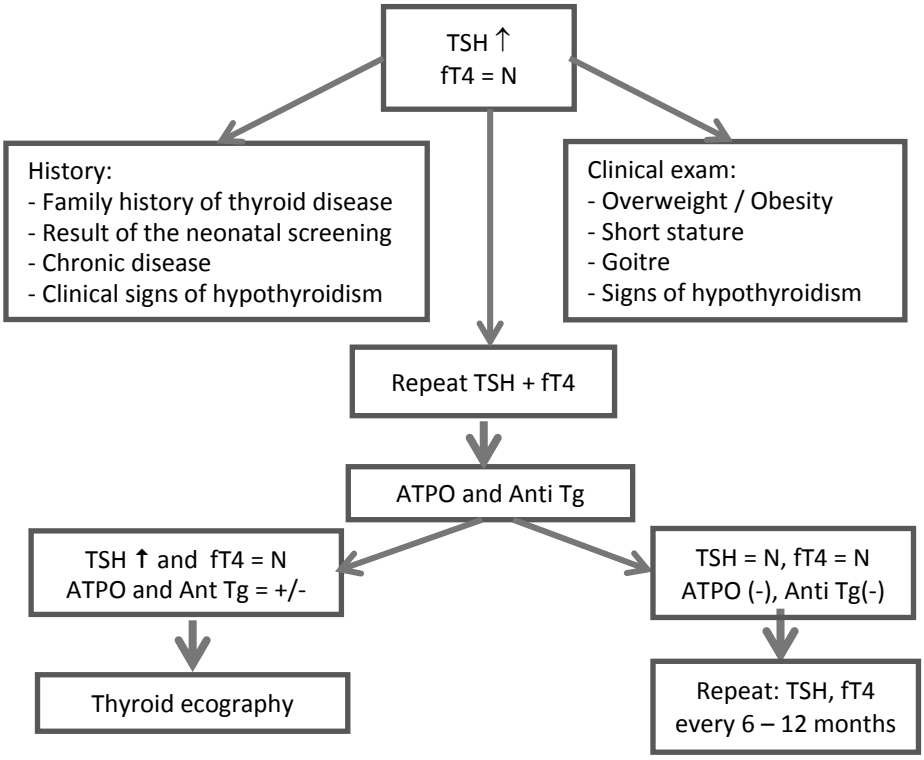

Fig. nr 3.1. Diagnosis algorithm in SH [13]

SH evolution. Factors of predicting SH evolution Complications of the subclinical hypothyroidism

Throughout their evolution, children with SH can return to the state of euthyroidism, or may progress to overt clinical hypothyroidism, while other patients remain in the state of SH. Progression towards hypothyroidism is less common than in the case of adults, data in the specialised literature reports numbers between 0 and 28.8%, most children with SH progressing with a return to the state of euthyroidism or maintenance of SH.

Several studies have attempted to establish predictive factors for the evolution of SH in children. It has been shown that the most important prediction factor for SH evolution is the initial TSH value. It is assumed that some patients require higher serum TSH levels in order to ensure normal thyroid function, so that TSH levels remain high in these patients, for long term.[13,15]

The presence of goiter at onset, positive serology (high titre anti-Tg antibodies and progressive increase of ATPO), association with celiac disease and progressively increasing TSH values represent predictive elements for the evolution of SH to hypothyroidism.[16,17] An important role in SH evolution has its etiology (autoimmune thyroiditis, obesity or idiopathic SH), but also the association with certain genetic syndromes (Turner, Down etc) with susceptibility to autoimmune associations.[18]

There are few studies that trailed the long-term evolution of idiopathic SH in children.

A 5-year retrospective study showed the tendency to normalization of the TSH values depending on its initial value. Initial TSH values >7.5 mIU/l and the female gender were predictive factors for thyroid function deterioration, while age had no predictive value.[15]

Another 5-year prospective study confirmed that the long-term progression of SH is favourable for children; most of the children studied (61.9%) evolved with TSH normalization, only 11.9% developed hypothyroidism.[19] In the same study, in

the case of patients with SH associated with a CAT (chronic autoimmune thyroiditis) the evolution of the majority was by maintaining SH or even the installation of hypothyroidism, only a minority (10.6%) evolving with normalization of TSH values. These results confirm the unfavourable effect of CAT on SH evolution, regardless of other factors.[17,19]

Considering the results of the studies, which show that idiopathic SH is a self-limited process with a tendency to spontaneous remission,[15,17,19,20] the guidelines of the specialised societies consider that the annual evaluation of these patients is sufficient.[21]

In the case of patients with SH associated with CAT, frequent monitoring is recommended, namely, every 6 months.[22] This is due to the fact that in children and adolescents with CAT, the long-term evolution of euthyroid status may be extremely variable.[23-26] This evolution appears to be correlated with the serum TSH value at onset. [23, 27]

Several studies claim that, in CAT, the forms beginning with a biological picture of SH are more likely to evolve toward major thyroid dysfunction in the long term, than the forms with euthyroidism at onset.[28]

A particular evolutionary aspect is found in the SH-CAT association in children with genetic syndromes. Turner syndrome (TS) and Down syndrome (DS) are frequently associated with CAT and, this association negatively influence the evolution of the thyroid dysfunction.

According to recent studies, in children with Turner syndrome and CAT, the evolution of the biological picture is less favourable than in children who do not associate with genetic disorders, especially in the case of patients with SH at onset.

Similarly, the evolution of CAT beginning with a biochemical picture of SH in a child with Down syndrome is generally less favourable than in the case of children without DS; SH is persistent in the long term, or often evolves to manifested clinical hypothyroidism. (19) Furthermore, in children with SH-CAT associated with DS, it is possible to

switch to the clinical-biological picture of Graves disease, respectively hyperthyroidism.[19]

Overweight children often present thyroid dysfunction, especially SH, its degree being correlated with the severity of weight excess.[31,32] The mechanism of SH occuring in the obese is not clear, but some studies claim that leptin plays an important role in regulating hypothalamic secretion of TSH.[33] Weight loss leading to normal weight determines the normalization of serum thyroid hormone levels,[32] which means that SH associated with obesity is a consequence, not a cause of the latter.

The association of SH - obesity increases the metabolic risk in these children. Some studies reveal increased abdominal circumference (AC) and the AC/waist ratio in obese children with SH, compared to euthyroid obese children, the indicators correlating directly with serum TSH levels.[34,35]

Bone maturation and linear growth are not affected in the long-term evolution of a mild, untreated, idiopathic SH.[36,37] Though, growth may be affected by severe forms of SH, with persistent and significantly elevated TSH values (> 50 mIU/l) associated with FT4 values at the lower limit of normal.[37]

Starting from the important role of thyroid hormones in brain maturation and function, several studies followed the influence of untreated SH on cognitive function. The negative influence of SH on the level of attention of these children was revealed, the cognitive function being absolutely normal.[2,12,38]

Other authors analyzed the relationship between SH and the cardiovascular risk factors. The results suggest the involvement of TSH in lipid profile and blood pressure regulation. It can be speculated, though, that these changes could be attributed to some cofactors present (obesity, inflammation). However, it seems that persistent, untreated SH could play an important role in early atherosclerosis, regardless of the presence of other cofactors.[34,39]

In conclusion, idiopathic SH can be considered a minor thyroid dysfunction, which is generally self-limited or with spontaneous remission and has a minimal risk of progression towards overt clinical hypothyroidism.[40] The deterioration, in

time, of the thyroid function can be predicted from onset, in SH associated with celiac disease, goiter or positive titre of the anti-Tg antibodies.[16] The serum TSH level at onset is the most important predictor of long-term evolution of SH.[15,27]

Association with CAT has a negative impact upon the long-term evolution of SH, these patients being more at risk of developing goiter, an effect which can be counteracted by treatment with L-Thyroxine.[17] The unfavourable effect of the SH-CAT association is amplified by its occurence in a child with Turner or Down Syndrome. In the latter case, SH may develop over time with a clinical picture of hyperthyroidism, a particular aspect of this chromosomopathy.[19]

Treatment of SH

The decision whether to treat or not SH in a child is sometimes difficult. This involves the corroboration of anamnestic data (family history etc.) and possible clinical symptoms suggestive of hypothyroidism (constipation, fatigue, bradypsychia etc) with biological investigations for appropriate therapeutic intervention.

Although, according to the specialised literature, SH seems to be a benign condition, with spontaneous remission, the paediatric endocrinologist must also consider the risk of a possible evolution towards overt clinical hypothyroidism with all its consequences, in children. Therefore, the decision whether to treat it or not, should consider, first of all, the age of the child.[41]

There are few data in the literature regarding the consequences of SH and the substitutive treatment of SH on the neuro-intellectual development of children from different age groups, and, especially of those younger than 3 years. For the latter, in the case of persistent high serum TSH levels, guidelines recommend substitutive treatment until the age of 3, period in which the neurological development is dependent on thyroid hormone levels.[21]

An important aspect in the decision whether to initiate therapy is the severity of the thyroid dysfunction.

There is, still, no unanimity regarding the substitution treatment of SH in children, especially in the case of "borderline" TSH values (4.5-10 mIU/l), situation in which substitution is indicated only if it is associated with clinical signs of hypothyroidism, goiter, autoimmune diseases (diabetes mellitus, celiac disease, etc.) or genetic syndromes (Turner, Down).[13,41]

In children with SH, without goiter, with negative specific serology and TSH <10 mIU/l, substitutive therapy is not justified, on the one hand because of the minimal risk of developing overt clinical hypothyroidism and, on the other hand, because they can belong to the low percentage of euthyroid patients with TSH values above the 97.5 percentile.[13]

L-Thyroxine treatment is recommended in children with TSH values above 10 mIU/l or progressively increasing values,[42] in order to avoid hypothyroidism with all its consequences, at this age.

Conclusions

In children, idiopathic SH can be considered a minor thyroid dysfunction, generally self-limited or with spontaneous remission, with a minimal risk of progression towards overt clinical hypothyroidism.

The association of SH with autoimmune chronic thyroiditis, other autoimmune diseases or chromosomopathies has a negative impact upon long-term evolution, thyroid function deterioration being more common in these patients.

In the case of obese children, slightly elevated TSH values are the consequence, not the cause of excess weight.

SH treatment is recommended for children under 3 years of age and for children with TSH values above 10 mUI/L. In children with borderline TSH values (4.5 - 10 mIU/L), substitution is indicated if they associate clinical signs of hypothyroidism, goiter, autoimmune diseases (diabetes mellitus, celiac disease, etc) or genetic syndrome.

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AN EVOLUTIONARY VIEWPOINT ON THE RAMPANT OBESITY

Ze'ev Hochberg

Abstract

An evolutionary approach to obesity involves a genomic-anthropological dimension. For 1,800,000 years, lifestyle of hunter-gatherers comprised excessive physical activity and a high protein-low carbohydrate diet. Genomes of hunter-gatherers were adapted to low insulin sensitivity. When the agrarian epoch began, a new farmer diet high in carbohydrate emerged. Due to famines, the genome may not have adapted; they preserved a hunter-gatherer genome. Ever since the industrial revolution, our genome rapidly adapts to a carbohydrate reach diet. Individuals with preserved of hunter-gatherer genome develop obesity at age 4-8 and need a low carbohydrate diet. In contrast, those with farmers' genome become obese in infancy; they need a low calorie diet. This knowledge fosters exploration of the two genomes and their clinical presentation.

Introduction

We used to believe that *"All obese persons are alike in one fundamental respect; they literally overeat"*. Therefore, *"overweight subjects must eat less and move more in*

order to lose fat". But only a few of our patients lost weight, and we blamed these for the therapeutic failure. Then, an alternative explanation of obesity was proposed: "*all obesity is a hormonal, regulatory defect of insulin sensitivity, and simple obesity is typically associated with insulin resistance.*[1] It is not excess calories that causes obesity, but the quantity and quality of consumed CHOs. This was endorsed, amongst others, by the American Heart Association (AHA).[2] Indeed, excessive consumption of sugars has been shown to be linked with the metabolic syndrome, type-2 diabetes mellitus, and cardiovascular morbidity.[3] Despite the AHA recommending reductions in the intake of added sugars, many obese patients did not lose weight. Therapeutic failure was due to over simplicity, assuming that obesity could be explained by a unitary mechanism.

In 2008, the Obesity Society commissioned a panel of experts to undertake a review of the issue of labelling obesity a disease.[4] The panel unanimously and strongly stated that obesity is a complex condition with many causal contributors, which include many factors that are largely beyond an individual's control.[4] If obesity is a symptom of several diseases, then the first step in treating obesity is to determine its cause(s) in each patient. Obesity may be caused by endocrine and genetic origins, environmental cues (intrauterine and postnatal), an unbalanced diet, feeding behaviours, and sociodemographic influences. Using the urinary steroidal fingerprints of 87 obese children, we proposed that non-syndromic childhood obesity could be classified into five clusters.[5] We found that only a minority of these children had insulin resistance, though they may subsequently develop it.

Evolutionary Reflexion

Obesity is heritable and predisposes to many diseases. Our genome has hundreds of markers linked to obesity and genetic studies of body mass index (BMI) yield insights for 97 loci associated with BMI.[6] These 97 loci account for ~2.7% of

BMI variation, and genome-wide estimates suggest that common variation accounts for >20% of BMI variation. In western societies, the BMI of adoptees in their 40s is strongly correlated with the BMI of their biologic but not of their adoptive parents and their siblings.[7,8]

Type-2 diabetes mellitus, as a consequence of obesity, is practically unheard of in contemporary hunter-gatherers (HGs) [9] as compared to 6 % in developed countries.[10] It is conceivable that both genetic and lifestyle factors are involved the obesity epidemic. In 1962, Neel posited that obesity in modern society has an evolutionary basis and is a trade-off for advantageous 'thrifty' genes.[11] In our early evolutionary history, genes that facilitated voracious appetite, sedentary lifestyle, competent metabolism, and efficient fat deposition were advantageous because they allowed their carriers to survive periods of famine.[11] In modern industrial societies, famine rarely happens, and the same advantageous genes promote fat deposition even when an individual is on a balanced diet. Though our HG ancestors were energetically challenged, periodic famine was a relatively late phenomenon that appeared with the introduction of farming 12,000 years ago (500 generations) *(Figure 4.1.)* [12]

The human genome has remained mostly unchanged during the past 12,000 years,[13] but our diet and lifestyle have diverged substantially from those of our ancestors.

During the 1.8 million years of the Pleistocene epoch, our ancestor's metabolic fuels were high in animal protein and low in CHOs, and we can assume that their genome was adapted to one that required low insulin sensitivity. This Pleistocene protein-rich diet was the rationale for the Palaeolithic diet that became popular in the 1990s and early 2000s [14-16] and its extreme derivation – the Atkins low CHO fad diet.[17]

In 2009, Frassetto and colleagues reported that short-term consumption of the Palaeolithic diet decreases insulin secretion and increases insulin sensitivity in some, but not all, individuals.[18] In another study, O'dea reported marked improvement in CHO and lipid metabolism in diabetic Australian aborigines after temporary reversion to traditional

lifestyle.[19] The unsurprising conclusion is that individuals with a HG genome respond to a HG diet.

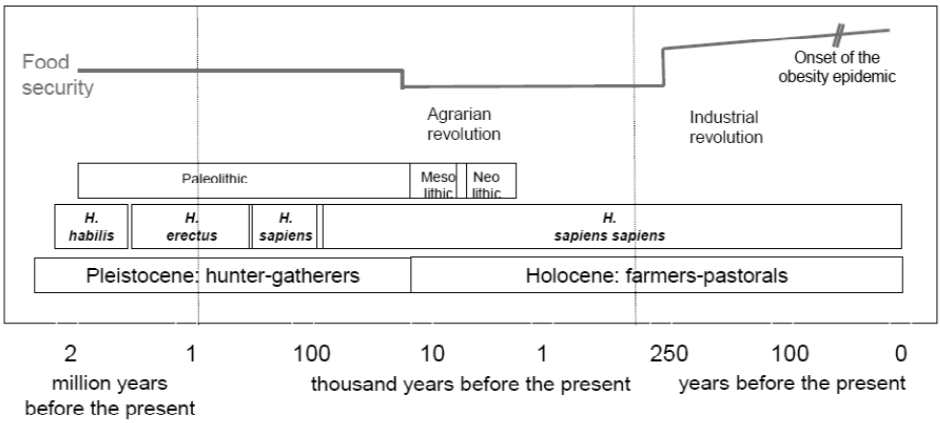

Figure 4.1 - Timeline of geological epochs, hominid evolution, periods in human prehistory, and relative food security.

Legend:
Transition from the Pleistocene geological epoch to the Holocene geological epoch coincided with the 'agrarian revolution' 10-12,000 years ago and from the human prehistory Paleolithic period of human prehistory (hunter gatherer tool makers) to the Mesolithic farmers. Yet, the agrarian revolution happened at different times for different parts of the world, and wherever it happened, it was associated with periodic famines and diminished food security. The next major shift was the security of the food supply and improvements in nutrition that accompanied the industrial revolution, which began in Europe in the second half of the eighteenth century. When obesity is defined using BMI, its prevalence was relatively constant and rose considerably after 1980. Using skinfold thickness as a measure of obesity, the rise in the prevalence of obesity is detectable 10–20 years earlier [57]. In terms of hominid evolution, the transitions from Homo habilis to H. erectus, H. sapiens and H. sapiens-sapiens all happened during the Pleistocene geological epoch.

When the agrarian epoch emerged in the fertile crescent in western Asia, and then expanded into contemporary Europe, China, and Africa, [20] the transition from a HG lifestyle to grain cultivation eventually introduced a high CHO and low protein diet and a sedentary life style. These dietary and life style changes required that the human HG genome adapt to one which required a high insulin sensitivity. Despite the

occurrence of recurring and often severe seasonal food shortages during most of the Holocene Epoch (the last 12,000 years), preserving fertility and reproduction was also crucial for man during this epoch. Hence, the thrifty HG genome was preserved or did not adapt during the Pleistocene-Halocene transition.[21]

The ‘‘escape from hunger’’

The next major shift was the security of the food supply and improvements in nutrition that accompanied the industrial revolution, which began in Europe in the second half of the 18th century - the “escape from hunger”,[22] and this shift has now extended to most of humanity. When considering the evolutionary basis for obesity: the progressive attainment of dietary energy sufficiency after 1800 released human populations from the constraint caused by periodic famines, and a genotype which was better suited to CHO metabolism emerged through the powerful mechanism of fertility selection.[22] The ‘fertility-selecting’ genes, and not the “thrifty” genes, prevailed in the last 200 years, and this genotype is better suited for the modern CHO-based metabolism. I therefore forecast that the increasing rates of diabetes and cardiovascular disease would be tempered by survival-, sexual-, and mostly fertility selection vis-a-vis the conserved HG genome.[23-25] Despite eating a CHO-rich diet, the majority of contemporary children and adults in the industrial world are not overweight or obese. Therefore, one can assume that these individuals do not carry any more the insulin-sensitive “farmer genome” or “industrial genome”, but preserved the insulin-resistant “HG genome”.

Two critical periods for childhood obesity

Two critical periods of development have been recognised for childhood obesity: the period from gestation to early infancy (age 0-6 months) and the period between age 5 and 7 years.[26] These two periods are defined by evolutionary life

history theory as infancy and juvenility, respectively.[27] The odds ratio for obesity at age 35 years increases from 2 early obesity to 5-10 for children whose onset age of obesity occurs during juvenility.[28]

Adult lean BMI is positively associated with rapid increases in BMI between age 2 and 11 years, whereas increasing BMI in infancy does not predict an increased adult obesity.[29] Similar findings were also found in a French cohort, where weight gain during infancy was a poor predictor of adult fat mass.[30] It has also been reported that a rapid gain in BMI during infancy increases adult lean body mass without excess fat accumulation, whereas weight gain in juvenility results in increased adult fat mass.[29]

The transition from childhood to juvenility is characterised by adrenarche and the adiposity rebound [31] and occurs in boys at a mean age of 5.1 years and in girls at a mean age of 4.9 years.[32] The adiposity rebound in overweight children occurs earlier than those who are not overweight [33] and is associated with an increased risk of obesity [34] and developing type-2 diabetes mellitus in adult life.[35]

Classification by Outcome

Outcome can be used to distinguish between obese children who will or will not subsequently develop metabolic syndrome and type 2 diabetes mellitus. [36] Identifying those obese children who will develop the metabolic syndrome and type 2 diabetes mellitus is sometimes possible by determining their insulin sensitivity following glucose loading. However, many of these children will not show an abnormal insulin response during childhood or adolescence. Acanthosis nigricans (AN) is a skin condition that predicts insulin resistance.[37] We have previously characterised the course of AN and obesity in children and adolescents along with their parents and grandparents and found them to follow a detrimental sequence of the metabolic syndrome.[38] Patients with AN have a truncal (android) distribution of fat, their fasting blood glucose level is significantly higher than those

without AN, and they frequently have a parent with the metabolic syndrome and a grandparent with type 2 diabetes mellitus. Interestingly, many of these individuals differed from those without AN in the onset age of obesity according to their parental reports: the onset age of obesity in the patients with AN was much lower than that of the onset age of obesity in the patients without AN.[38]

Table 4.1. - Cross-tabulation of the joint frequency distribution of acanthosis nigricans and onset age of obesity in 23 obese children.

	Age of obesity onset		Total	Chi-square (p)
Acanthosis Nigricans	0-6 m	>3y (7.2 ± 2.7)		
+ -	1 6	13 3	14 9	5.74 (0.0156)
Total	7	16	23	
Chi-square (p)	10.37 (0.0013)			

While the number of subjects is small, the results are unequivocal: children whose onset age of obesity is after the age of 3 years are likely to develop AN (7.2 [mean] ± 2.7 [standard deviation]), and are likely to embark on a trajectory which leads to insulin resistance, the metabolic syndrome, and type-2 diabetes mellitus. In contrast, those children whose onset age of obesity is within the first six months of life are unlikely to develop AN.

Thus, one can posit that an onset of obesity which occurs (a) at the transition to juvenility would be characteristic of children with an insulin-resistant HG genome, and (b) during infancy would be characteristic of children with an insulin-sensitive farmer genome. Whereas we don't have genomic data to support this claim, some indirect evidence implicates several genes in the existence of the HG and farmer genomes.

Genomic Adaptation

Pathway analyses of genome-wide association studies for BMI provide support for a role for central nervous system genes and pathways in overweight and obesity, including those related to synaptic function, glutamate signalling, insulin secretion and action, energy metabolism, lipid biology and adipogenesis.

The sedentary farming economy which spread throughout Europe from the southeast and started ~8400 years ago, catalysed the spread of agriculture, and that genetic admixture eventually shaped the genomic landscape of modern-day Europe.[39] *Lachance et al* sequenced the whole genomes of five individuals in each of three different contemporary African hunter-gatherer populations. They identified 13.4 million variants, and found loci that harbour signatures of local adaptation, which included genes involved in metabolism that would provide for a HG diet.[40]

In terms of obesity-related genes, insulin, the insulin receptor, and post-receptor signalling genes are the centre of the story.[41] There is no fat accumulation without the insulin system, and the genes that encode the insulin system are highly conserved in evolution.[42,43] Indeed, processed, energy-dense foods have been linked to insulin resistance and cardiovascular disease among Australian foragers transitioning to village life.[44]

Frayling et al investigated the association of variation of the obesity gene, *FTO*, with BMI and the risk of being overweight and obese in 10,172 white European children.[45] They found that the *FTO* gene variation is associated with changes in BMI and obesity in children by the age of 7 years, changes that persist into the prepubertal period and beyond. The *FTO* gene is up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain.[46] Indeed, the *FTO* gene is of ancient origin; it is well conserved and found in a single copy in diverse vertebrate species such as fish and chickens. Such findings suggest that this gene's ancestor was present 450 million years ago.[46]

The adipocyte-derived hormone, leptin, exerts profound pleiotropic effects, which satiety, energy expenditure, and neuroendocrine function.[47] From an evolutionary standpoint, it stands out for its bidirectional signalling of metabolic and neuroendocrine physiology to prevent obesity. Thus, leptin resistance is a major mechanism in central obesity. Energy stores are required for survival in periods of famine, and the leptin gene expression may have been selected against during the course of evolution.[47,48] Work with Ache foragers in Paraguay has shown that levels of leptin, critical in fat sequestration, are substantially lower than levels in U.S. adults.[49]

Another gene at the centre of obesity pathophysiology is the appetite hormone, ghrelin, and its complex family of peptides, receptors and modifying enzymes that control multiple pathophysiological processes. Among other, these gene products modulate the activity and synaptic input organisation of midbrain dopamine neurons while promoting appetite.[50] Plasma ghrelin concentrations in patients with simple obesity are lower and higher in patients with anorexia nervosa than those in healthy subjects. [51] Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference.[52] Ghrelin is a hormone that has apparently evaded natural selection during a long evolutionary history in a variety of mammal and non-mammalian vertebrates. In mammals, the amino acid sequences are highly homologous, sharing over 90% identity across the group.[53] *Del Guidice et al* investigated whether ghrelin variants could modulate the obese phenotype during childhood.[54] They found that a Leu72/Met polymorphism in the ghrelin gene was associated with differences in the age at obesity onset; the Met72 allele was associated with a trend towards a lower age of obesity onset.

Booth et al suggested that the physical activity of HGs was extensive, and the sedentary lifestyle that followed the agrarian revolution must have required a sedentary farmer genome.[55] Yet, as mentioned, our current genome is maladapted, and many of us have retained the HG genome,

which can potentially promote overweight and obesity. *Booth et al* also speculated that genes evolved with the expectation of requiring a certain threshold of physical activity, and thus habitual exercise in sedentary cultures may restore perturbed homeostatic mechanisms towards the normal physiological range of the Palaeolithic *H. sapiens.* [55]

Future Perspectives

Focusing specifically on the needs and opportunities in the obesity epidemic, this review offers to include an additional dimension, namely the evolutionary perspective. It appears that the ancient homeostatic systems that were adapted to a HG environment have been pushed beyond their limits in contemporary times and are compromised by altered living conditions. In other words, the HG genome in most modern humans without obesity have finally been substituted and adapt properly to our present-day diet and environment. Possession of the HG or the farmer genome has preventive and therapeutic implications for children which are related to their distinctive insulin sensitivities and the different ages of obesity onset. Excessive weight gain during the first six months of life suggests that their obesity is unlikely to develop into the metabolic syndrome, type-2 diabetes mellitus, and other cardiovascular sequelae.

Accordingly, I propose that obese patients (children and adults) with a farmer genome, which can handle the modern high CHO-rich diet, will probably respond to a low calorie diet. For obese patients, whose onset of obesity occurred at the transition to juvenility at age 4-8 years, suggests that their genome is the thrifty HG genome, and their obesity is adaptive. Accordingly, these individuals need to eat and exercise like the HGs; such patients will probably respond to a low CHO and high protein diet. Chakravarthy and Booth postulate that a crucial mechanism to break the stall of the metabolic processes would be via exercise through the regulation of “physical activity genes” [56] some of which may also be potential candidates for the HG thrifty genome.

This knowledge is fostering exploration of the HG and farmer genomes and their respective clinical presentations. Eventually, this could be a diagnostic and therapeutic tool in the future to help individuals know what types of dietary intake they lifestyle might best fit the unique situation in a personalised approach to childhood and adult obesity.

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SERIOUSLY LOW CARB EATING

Stuart J. Brink

Introduction

A ketogenic diet, very low in total carbohydrate intake has been proposed to assist not only with weight loss but also with management of diabetes and the forerunner of type 2 diabetes, namely insulin resistance and the metabolic syndrome as well as conditions like polycystic ovary syndrome (PCOS) and helping to treat hyperlipidemia, hypertension and prevent future cardiovascular conditions.

The key question is whether or not carbohydrates themselves are "the enemy" that has been failed to be named for many decades in medicine and with proposed restrictions in total calories and especially in saturated fats over these same decades, whether medical and nutritional specialists have not recognized why such an approach has not worked as documented by more obesity, more cardiovascular disease and more diabetes.

Current approaches to restricting carbohydrates now focus on possible scientific rationales, ie. less insulin is required for those who are taking insulin and less insulin is required for those who, while insulin resistant or in a pre-diabetic state associated with their obesity, need less insulin produced by their own pancreas. In so doing, there may be fewer excess calories available for insulin-dependent fat storage and therefore better metabolic profiles ensue. At the

same time, energy to fuel the body's functions can come from protein which slowly gets converted into carbohydrates, thus needing less insulin at any given moment in time as well as some energy directly provided by dietary fat intake. Some of the original work promoted by Dr Robert Atkins and also by Drs Richard and Rachel Heller among many others goes over some of these proposed mechanisms and explanations with many specific recommendations available in articles, books, pamphlets and on the internet listed under titles like low carb eating, ketogenic diets, Atkins Diet/Approach, modified Atkins, modified low-carb restrictions etc. But the debates goes on because long term carb-restricted food plans have not been tested in large, evidence-based fashion or for long periods of time in large numbers of people set up in proper scientific modalities to answer such questions as to efficacy, safety and whether or not there are subsets of the population who may benefit for such an approach. Shorter term studies sometimes offer promise but also sometimes show negative results and selection bias with those recruited for many studies may be very important to ascertain and identify.

Carbohydrate addiction: carboholics

Often our body and our brain feel very good when eating sugary foods. Whether this is simple candy to eat or soda to drink, sugar in our tea and coffee, ice cream, chocolate candy or desserts, bread, pasta, corn, cereal, potatoes or adding sugar to what we are drinking, this is a common sensibility whether at a meal, after a meal or between meals. Many holidays and parties are packed with either large amounts of sugar and carbohydrates or combinations of high fat-carbohydrates, frequently chocolate. Particularly at family or business gatherings, food and snacks as well as drinks are commonly offered with such high carb, high fat content (in addition to large portions).

Studies of television and print media document the commonality of food advertisements for these same categories of high sugar, high carbohydrate, high fat foods with the most

frequent advertising coming from fat food providers, supermarkets, sugar sweetened drinks (soda, juice, coffee, tea), full fat dairy products, chocolate products, alcohol, breakfast cereals, breads and rolls. We recognize the colors of these products, we recognize the cartoon characters and symbols of these products an we are constantly barraged by such advertisements affecting our subconscious brains. We therefore fit in socially with family and friends, eat and drink in this high carboholic craze and continue to respond to our innate carbohydrate addictive choices.

Just blaming lack of willpower is not sufficient to counteract what my be a genetic predisposition in humans for this taste and the good feelings that carbs generate in our mouths and brains. How often might one be in a discussion about sugar, other carbohydrates, total calories, obesity, hypertension, cholesterol and heart disease, diabetes and even anxiety, depression, ADD and sleep disorders only to be stopped by the phrase: "but I love sugar" or …"I love my carbs too much."

As the Hellers have written, carbohydrate "addiction" may not be a matter of willpower but a mater of biology. If you are a carbohydrate addict you may have difficulty stopping once you have started to eat bread, pasta, snack foods or sweets as well as other starches. After you've had a full breakfast with lots of carbohydrate, you may get hungrier way before lunchtime. You may get super tired or super hungry mid-afternoon after another high-carb containing lunch, have a tendency to put on weight around your mid-section ("apple"), binge eat periodically and never be able to maintain sufficiently long caloric restriction or increase exercise related caloric energy use to turn around your weight gain. Certain situations and certain foods may, in fact, be labeled "trigger foods" either because they are high in carbs themselves and thus make the brain and the belly want more carbs or because they binge on carbohydrate cravings, hunger and weight gain: fruits and juices, sugary sodas, yogurt, pita bread, carrots may fall into this group as well as alcohol, artificial sweeteners touted for helping to diminish caloric

intake and the preservative monosodium glutamate. This is particularly so for pizza and pasta, McDonalds and Burger King, Kentucky Fried Chicken, Wendy's, Coca Cola, Pepsi Cola, Gatorade and French Fries in addition to many local varieties around the world. Here too the advertising that goes on for such convenient, relatively inexpensive fast-food restaurants adds to the notion of what we are supposed to desire and plays into our brain's carbohydrate craving centers. Pre-prepared food also may fall into this same general category of high carb, high fat food now conveniently priced and helpful for saving time as well with the main requirement of purchasing these products and just heating them up rather quickly.

When considering the low carb approach in those treated with insulin, key to remember that much less insulin will be needed (self-produced or administered in those with diabetes) and that this may be part of the process for helping to lower glucose levels, lower carb craving and overeating and improving glycemic control in addition to weight loss. In those with diabetes and using multidose insulin algorithms or insulin pumps, basal insulin will still be needed but perhaps 30-50% less than previously needed and correction doses will still be needed in type 1 diabetes. Some bolus doses may be completely eliminated but overall there will be lower glycemic variability, fewer glycemic surges postprandially and less hypoglycemia from mis-matched insulin to food choices.

In those with and without diabetes, hunger will be counteracted by no limit to protein and fat intake so that the stomach is filled up and sending signals to the satiety center that there is sufficient food being provided and visibly confirmed and in the stomach response to the food portions of protein, fat and green veggies itself.

More high fiber, high water containing mostly green vegetables also help produce satiety and so big green salads with mostly low carb dressing or plenty of mixed spices helps prevent common craving feelings. Many times hunger is actually unrecognized thirst so that promoting more water intake, not with sugar sweeteners, can also be extremely

helpful as a strategy. Some studies have suggested that many if not all sugar sweeteners/sugar substitutes may actually help promote the carboholic sensitivities inadvertently although the newest such product, stevia, is sometimes described as the least likely of all the others to produce this subtle effect.

An alternative is either black coffee (no lactose in the milk) or any of the very many flavored and pleasantly smelling teas, again without the sugar sweeteners. Such drinks can either be hot or cold. Seltzers with non-sweetened carbonation can also be had. As such, following such choice guidelines, the classical dietary food pyramid used for so many decades gets turned around rather dramatically as illustrative of these "new" principles.

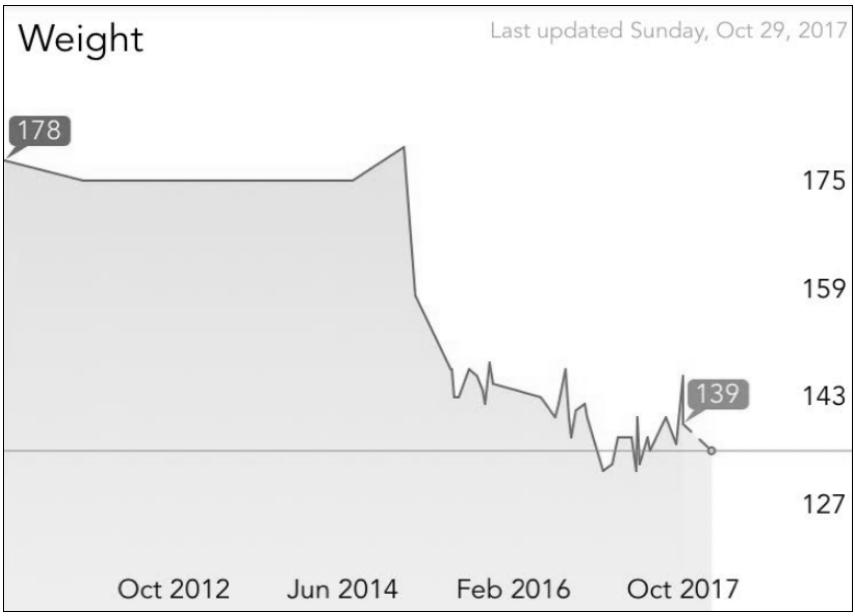
Low carb eating is not a crash diet but requires an initial **psychological** understanding and change of approach … and appears to be sustainable for the long term if continued.

Strategies to learn about and address such a low carb approach to food must start with understanding and teaching about the psychologic situation that often becomes counter-productive with most previous attempts at weight loss or changing food patterns.

Because the health results are long term and not short term, this is quite different from avoiding a certain food because of food allergies, avoiding gluten because of bloating and diarrhea associated with celiac disease or other specific food plans associated with specific symptoms. There is no immediate "reward" for long-term weight loss and cardiovascular or diabetes health so this must be discussed and addressed up front for better adherence. The benefits of this stricter, lower carb intake, however, is often more rapid and easier weight loss even without exercising. With exercise, however, even more weight loss would be expected to occur.

Table 5.1: Proposed metabolic benefits of low carbohydrate eating

- Increase HDL cholesterol associated with decreased heart disease risk
- Decrease heart-risky total cholesterol and LDL cholesterol and change lipid particle sizes to decrease cardiovascular risk
    - Lower lipid medication
- Decrease hypertension to decrease heart, kidney, brain and circulatory problems
    - Lower BP medication
- Lower triglycerides to decrease heart disease
    - Lower lipid medication
- Lower insulin needs, lower fat storage, decrease insulin resistance
    - Lower prandial meal boluses
    - Lower basal insulin
    - Lower other diabetes control medications
- Decrease glycemic variability and surges postprandially
    - Need fewer insulin correction so less chance of post-correction hypoglycemia
    - Lower A1c
- Weight loss and decreased inflammatory markers like CRP
    - Especially decreased abdominal adiposity ("apple" distribution)
    - May be beneficial for insulin resistant states
        - PCOS
        - Endometriosis
- Neurologic function improves
    - Better executive decision making function
    - Improved ADD and ADHD symptoms
    - Better, more restful sleep
    - Some reports of fewer migraine headaches
    - Some reports of less dementia and Alzheimer's disease symptoms
    - Some reports of decrease epileptic seizures in children and adults otherwise unresponsive to anti-epileptic medications
- Better emotional health
    - Feel better because psychologically achieving desired weight and health goals easier
    - Decreased anxiety
    - Decreased depression
    - Decreased anger
- Acne may decrease especially in most resistant, inflammatory type acne



One example of a non-exercise, low-carb dietary approach in an adult male with mild, metformin treated type 2 diabetes and associated hypercholesterolemia, hypertension, hyperuricemia dropped from baseline 181# (82 kg) and mild, central adiposity down to 132-142# (60-64 kg) within 2-3 months and sustained this improvement with only holiday and vacation short periods of weight gain but easily returned to maintenance after each "vacation" and has sustained this effect for now going on five years.

At the same time, blood glucose levels and A1c have improved dramatically, lipid abnormalities have also improved but not fully normalized, mild hypertension has normalized and uric acid elevations have also normalized. Carb cravings still occurred periodically but the initial carboholic cravings lasted almost exactly 14 days when the "brain switch" occurred.

Drinking more and more water reportedly helped enormously.

Eating large portions of green vegetables bigger portions of proteins lasted almost exactly one month and then decreased spontaneously as well, a second "brain switch."

This low carb ketogenic approach is "stricter" than the previous common Atkins-Diet recommendations and aims to get 75% of needed calories from fat, about 20% from protein and less than 5% from sugar and carbohydrates. Total grams of all carbs should aim for <50-70 grams/day in net carb intake from all sources. Has plenty of vitamins and minerals associated with no restriction of the large, green salads.

Seriously Low Carb Guidelines

Stop obvious sugar and restrict most carb intake from sugar, candy, sugary chocolates, sugar containing chewing gum, soda and sweetened drinks (carbonated or not) including fruit juices; restrict bread, toast, rolls, matzo, tortillas, sandwich wraps, buns, cereals (with rice, bran, oats, corn), pasta and noodles, rice; pierogies, wonton, kreplach, empanadas, tortillas, stuffed shells; pizza; breaded anything; fruit and juice; potatoes, french fries, potato chips/crisps; corn, popcorn; rice, beans; cow milk-based drinks and most cheeses, goat milk and almond milk products; French toast, waffles, cookies, pies, crackers, flatbreads, wraps, donuts; sugar-containing peanut butter, nuts with high carbohydrate component; starchy non-green vegetables (yellow, red, orange); sugar containing soda and drinks, sports drinks, drinks or any food with glucose and fructose added; drinks with artificial sweeteners such as acesulfame, aspartame, sugar alcohols, mannitol and stevia; beer, sweet wines, mixed alcoholic drinks; tea and coffee with sweeteners, caloric or non-caloric and milks.

What can one eat: huge, mostly **large green salads**.

The more, the merrier but without sugar-containing salad dressings.

All the typical and unusual spices, salt and peppers can be used without restriction. The more varied the green vegetables including those not usually consumed (ie. not only cucumber, celery, lettuce) but adding green peppers, kale, arugula (rocket), Brussels sprouts, other kinds of lettuce,

mint, asparagus, turnip, squash, artichoke, spinach etc) for variety, taste and texture. A small sprig of a tiny cherry tomato or a bit of green, yellow or orange pepper can be added to provide some interesting color contrast but in small portions because while they have plenty of water and fiber, they also have a bit more carbohydrate including compared to the green vegetables. Note that some of the higher cab content green foods like green peas are not included in this "free" green vegetable garden.

Goal is to fill up your stomach, get the tastes and textures as well as spices with added water, fiber and nutrients. Also not rushing through the meal but slowing down to smell and taste as well as allow the brain to receive the stomach signals that food has been provided.

Ad lib **protein** food **but not breaded**.

As much red meat (beef, pork, duck, lamb, pepperoni, salami, bologna, hotdogs/frankfurters, sausages but not with bread stuffing. Chicken, turkey, liver, veal are fine. Fish including shellfish like lobster, shrimp, crayfish, scallops, oysters. No limit to eggs of any kind.

Saturated fat is also no longer the "enemy." **Eggs** particularly were commonly thought to be verboten because of the yolk continuing pure cholesterol so obviously this was the culprit causing the heart disease and blocking blood vessels with high cholesterol deposition. But, lots of antioxidants in the yolk and no evidence that eating eggs scientifically raises the blood cholesterol count.

Some evidence that what is in the egg yolk may modify the shape of LDL in a way that potentially reduces cardiac risks. And eating eggs whether hard boiled, scrambled, easy over or any other way (but not in French toast, waffles or other high carb products). Perfectly okay to allow initial hunger the first 3-4 weeks on this low carb approach to be compensated for not only by large green salads and large water intake but also by larger protein portions. This will almost always self-decrease at about the 3-4 week in a somewhat "magically" as if some resetting process has

occurred in the brain. Important to state this to yourself or whoever is being guided by these principles since it is so commonly occurring. Nuts and seeds can also be allowed with attention to those nuts that have more carbohydrates than others (ie. cashews and pistachios).

Significant fat and some protein goes with most nuts and most nuts have small amounts of carbohydrates in the 3-5 gram portion of carbohydrates per standard serving portion: almonds, Brazil nuts, macadamia, peanuts, pecans, walnuts as well as chia, flaxseed, pumpkin, sesame and sunflower seeds. Large amounts of nuts, however, can easily produce excess carbohydrate intake even though the carbohydrate effect is rather slow because of the fiber and fat content in the nuts. Some psychological strategies include avoiding nuts and seeds in the first few months of this low-carb approach but than adding them for the maintenance phase for variety as long as the total carb amounts are known and accounted for.

Fats also are not restricted and fatty meats and fish are also fine. Fatty skin also fine as is bacon, butter, margarine, mayonnaise, avocado, olives, coconut oils and other oils. Avocados also have lots of dietary fiber and fat and while containing about 9 grams of carbohydrates, only 2 grams are absorbable as net carbohydrate so perfectly acceptable plus helpful to reduce satiety, high in potassium and lutein as well as numerous vitamins. Important to read labels for salad dressing to choose those without added sugars. As with salads, a large variety of different spices add tastes, smell and textures from salt, pepper and many spices (basil, thyme, cumin, turmeric, chives, garlic, cayenne, saffron, ginger, anise, sichuan, peppers, rosemary, fennel, coriander, tarragon, caraway, cinnamon, oregano, paprika, vanilla, dill, mint… can add to the variety and especially assist psychologically with the carb restrictions so overt to help make the transition more successful and sustained.

Psychologically and mechanistically, important to think about what will be done at family gatherings as well as

restaurants and to strategize in advance of such situations that often can otherwise sabotage efforts to adhere. Being able to ask wait-staff and hosts/hostesses for choices instead of just assuming that one must consume high carbohydrate choices? Can I have a giant salad instead of the potatoes is an okay question to pose? Saying: no bread please or can you remove the bread and rolls from the table. Thanx. Also deciding if one needs a large amount of variety or just some of the times need such variety can help make some choices much easier. For example, the easy of eating mostly pre-prepared pepperoni for breakfast with a large mug of various flavored teas makes a breakfast on the go rather unstressful for some. Similarly, pre-boiling a dozen hard boiled eggs and having spices available at one's desk makes eating and preparing lunches in the office also quite easy with lots of water and no limit to the number of eggs eaten for lunch.

Occasionally making egg salad, shrimp salad, chicken or tuna salad also makes it easy to bring a small container and change the "lunch menu" while dinner time can be as varied as the cook and preparer wishes it to be as long as it is carb restricted following the guidelines presented.

Conclusion

Seriously low carb eating is do-able, safe, health-promoting and can be done for weight loss, to reduce cardiovascular risks and to help in management of type 1 and type 2 diabetes and its variants quite successfully.

A psychological and educational approach should be explored to identify barriers, goals and strategies that can be discussed to promote such an approach and render it more likely to succeed and be sustained. The relative ease of seeing the weight loss so rapidly can be accentuated with exercise but the approach can be accomplished even in those for whom exercise is not wanted or liked.

More science is clearly needed by health care professionals to determine if there are sub-populations where this approach would be more or less beneficial and what

might be attempted in those groups as well as better long term studies of the anti-aging effects, decreased inflammatory benefits, cardiovascular and diabetes/metabolic syndrome benefits an brain and emotional components of previous reports.

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COGNITIVE FUNCTION IN CHILD'S DIABETES

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Introduction

Type 1 Diabetes mellitus (Type 1DM) is one of the most prevalent chronic diseases in children under the age of 18.

Both Type 1 and Type 2 DM are known as causing complications in several systems and organs, including the brain.[1]

In order to achieve the objectives of the treatment (normal psychosomatic growth and development, removing the risk of chronic complications for as long as possible), Type 1 DM should be seen as an ambivalent disease, both acute (every day is fragmented by glycemia dosing, insulin administration, carbohydrate counting, etc.) and chronic from the point of view of the risk of the occurrence of chronic complications overshadowing the future of these children.[2]

The efforts of the medical team aim at ensuring insulin substitution as close as possible to “physiological” in order to obtain and maintain euglycemia. Under these circumstances, it is necessary to accept the risk of cognitive deficits as possible complications in Type 1 DM in children for an individualized therapy and in order to promote educational interventions. [3]

It is difficult to obtain and maintain glycemia control in Type 1 DM, as it is often characterized by significant variability of the glycemia, including prolonged periods of

hyperglycemia and intermittent episodes of hypoglycemia that can be even severe.[4]

Both hypo- and hyperglycemia seem to have a negative impact on the performance of children in both clinic and school assessment situations.

Regardless of the therapeutic scheme employed (multiple injections, insulin pump), the insulin is "supplied" to the patient in a non-physiological manner, at improper times (before eating) and in inappropriate amounts (higher compared to endogenous secretion provided by the pancreas). Due to the current non-physiological nature of the insulin therapy, patients are vulnerable throughout their lifetime and exposed to high blood glucose excursions (either hypoglycemia or hyperglycemia).[5]

The impact of these acute complications on the long term is greater and more difficult to assess if the age of the child is lower at the onset of Type 1 DM because the duration of "exposure" to the disease during periods of vulnerability (growth, puberty, adolescence) is higher.

In childhood and adolescence, the brain is, unfortunately, a target organ of hypo- and hyperglycemia affecting both white matter and gray matter.[6,7] It should not be forgotten that the child's brain "needs" increased energy and is continuously changing. Consumption of glucose in the brain increases and reaches adult rates until the age of 2 almost doubling the rate of adults around the age of 5 (*Figure* 6.1).

Gradual reduction of glucose needs towards adult levels occurs in the next decade. [8,9] These unique properties have led to the idea that in a child with DM, the brain, being in development, is particularly vulnerable to glycemic extremes.[10,11] Therefore, exposure to glycemic extremes during childhood may modify normal brain development trajectories depending on the age of the child and the severity of the fight against these extremes.[5]

Meta-analyses in the specialized literature bring into focus the fact that both children and young people with Type 1 DM appear to have a slight impairment of the intellectual function compared to control groups without DM, the executive functions, learning and memory, as well as reaction time/ processing speed being particularly affected.[12,13]

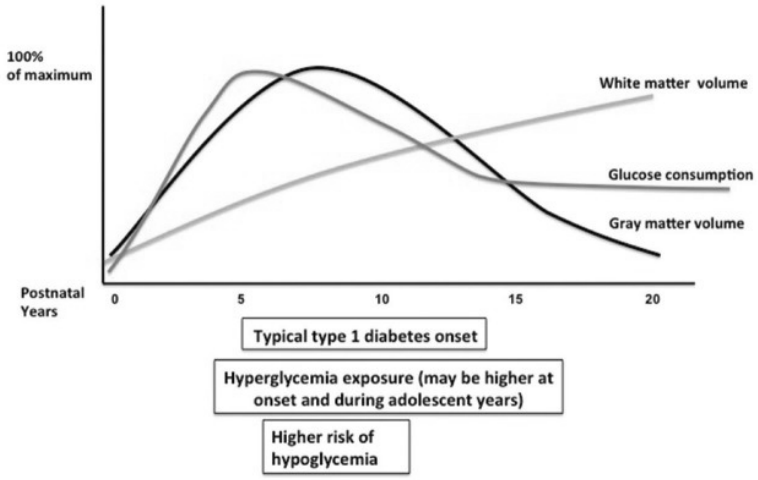

Fig. no. 6.1 – Glucose need at the level of the brain throughout childhood.[5]

However, the "magnitude" of the impairment resulting from glycemic variability does not appear to be alarmingly high, patients with certain risk factors being excepted. Once identified, they can be removed or therapeutic measures can be taken in order to reduce their impact.

Age of the child at the onset of DM

Recent epidemiology data show an increase in the number of cases of Type 1 DM with an onset at younger and younger ages (the age group below 5), which increases the risk of hypoglycemia both in number and severity with the possibility of perturbation in the neurocognitive function of these children. Arguments in this regard are provided by several studies presenting the decrease in IQ test scores [14], the decrease of memorization and learning capacity [12,15] as well as the decrease of the information processing speed.[16]

He J. et al. [17] in a meta-analysis of 19 studies involving 1355 patients with Type 1 DM revealed the fact that children with Type 1 DM, as compared to 696 control subjects, witness the decrease of: cognitive performance (g = - 0.46), attention (g = -0.60), psychomotor speed (g = - 0.46) as well as specific

deficits in overall intelligence (g = -1.06). Glycemic extremes were associated with a weaker overall knowledge (g = -0.18), as well as a slightly lower performance of the memory (g = -0.27).

The impact of hypoglycemia on the brain

Severe hypoglycemic episodes during childhood may lead to the impairment of neurocognitive functions. It is well-known that brain activity is based on a continuous supply of blood glucose. There are studies that bring into focus the ability of the brain to use lactate, alanine and ketone bodies as energy sources, without any evidence that this mechanism also works for patients with DM.

During hypoglycemia, cerebral blood flow increases very little in children. It is unlikely that this mechanism could explain alone the maintenance of glucose use. [18]

Inadequate glucose supply of the brain during childhood (when brain is still developing) due to recurrent episodes of severe hypoglycemia may lead over time to a neurocognitive disorder (mild to mental retardation), memory deficits predisposing these children to new episodes of hypoglycemia and ignoring hypoglycemia, epilepsy, microcephaly or even hemiparesis or aphasia. [19]

Severe hypoglycemia

There are significant individual differences in vulnerability to hypoglycemia. Adaptive responses to hypoglycemia may vary depending on the degree and frequency of previous hypoglycemia and the presence of structural changes in the brain induced by chronic hyperglycemia.[18] Severe recurrent hypoglycemia may cause long-term cognitive impairment in children with Type 1 diabetes mellitus.

Acute hypoglycemia affects brain function, and available data show that cognitive performance is impaired in the case of healthy subjects at a glycemic level of 2.6-3.0 mmol / l. The onset of hypoglycemic cognitive dysfunction is immediate but recovery may be significantly delayed.

There is evidence that bring into focus the ability to adapt to hypoglycemia, partly due to the increased absorption ability of glucose in the brain, although other mechanisms seem to exist as well. Patients exposed to chronic or recurrent hypoglycemia become remarkably tolerant to health status, but this is not enough to prevent severe hypoglycemia with neuroglycopenic decompensation, probably due to the fact that symptomatic and counterregulatory responses adapt even more.

During experimental hypoglycemia, the administration of non-glucose cerebral "fuels" maintains the cognitive function. However, in clinical practice, little progress has been recorded in protecting cognitive function during hypoglycemia.

The chronic effects of recurrent hypoglycemia still remain controversial. There are several cases of post-hypoglycemic brain damage and cognitive impairment attributed to repeated severe hypoglycemia. Major prospective studies, including DM control and complications, did not report cognitive decline in intensively treated patients, probably due to the unrepresentative study group and/ or the duration of the study which was too short to detect such effects.

Structural and functional changes of the brain are not only associated with recurrent severe hypoglycemia, but also with hyperglycemia and early onset of the disease, and appear to be partly due to the hyperglycemic microvascular disease.[20]

Effects of acute hypoglycemia on short-term memory

Sommerfield A.J. et all., conducted an experimental study on 16 adults with Type 1 DM where the arterialized glycemia level was maintained at 4.5 mmol / l for euglycemia and 2.5 mmol / L for hypoglycemia. The participants completed the (immediate and delayed) verbal memory tests, (immediate and delayed) visual memory and working memory during each experimental condition. They were also subject to two other mental tests, the Trail Making B test and the digit symbol test.

The analysis of the tests revealed significant impairment of the performance of immediate visual memory. The effect of hypoglycemia on the working memory and delayed memory was more profound.

Performances in non-memory tests ("Trail Making B" test and Digit Symbol Test) were also affected during hypoglycemia.[21]

The effect of acute hypoglycemia on cognitive motivation and interference

In a similar study carried out on adults with Type 1 DM, McAulay V. et al., used the same limits for euglycemia (4.5 mmol/ L) and hypoglycemia (2.6 mmol/ L), and the Dundee Stress State Questionnaire (DSSQ) had been administered before and after the cognitive function tests. The analysis of the questionnaires revealed that hypoglycemia reduced interference (P = 0.03) and irrelevant tasks (P = 0.02). Careful focusing was much higher after hypoglycemia than under conditions of euglycemia (P = 0.02). During hypoglycemia, the motivation of the participants to the study (P = 0.07) declined, negative state of mind with a significant decrease in energy levels (P = 0.03) occurred as well as simultaneous increase in anxiety level (P = 0.05). The subjective perception of concentration was not affected during hypoglycemia (P = 0.14), while control and confidence scores did not decrease (P = 0.19).

All these results have led to the idea that in people with Type 1 DM, hypoglycemia causes a state of awareness and distraction during active mental activity, which could leave fewer processing resources available to enable the accomplishment of cognitive tasks. In other words, acute hypoglycemia induces a state of significant state of worry and anxiety that can affect social, personal and work activities of patients with DM. [22]

In another study, Allen K.V. et al., using the same thresholds for euglycemia and hypoglycemia on a group of 20 adults with type 1 DM compared to 20 healthy volunteers, applied **linguistic tests** in order to assess the effects of hypoglycemia on the relationship between working memory

and language (reading interval), grammar decoding (reading at their own pace) and grammar coding (subject-verb agreement). The results showed that in the cases with hypoglycemia a significant decline of the reading interval (P <0.001, n (2) = 0.37, Cohen d = 0.65), a decrease in the number of correct responses (P = 0.005) occurred and the reading time increased (P = 0.039; n (2) = 0.11; Cohen d = 0.25). Nevertheless, hypoglycemia did not significantly affect the number of errors in sentence comprehension or the time required to answer the questions. Hypoglycemia also caused a decline in the subject-verb understanding (correct answers: P = 0,011; η (2) = 0,159; Cohen d = 0.31).

Assessment of the neurocognitive function (WISC-R and NEPSY method)

Hannonen R. et al. assessed through the WISC-R (Wechsler Intelligence Scale for Children-Revised) and NEPSY (for attention, motosensory functions, visual-spatial processing, learning and memory) method 31 children out of which 11 with Type 1 DM and antecedents of severe hypoglycemia, 10 with Type 1 DM without a history of severe hypoglycemia and a control group of 10 healthy children. The age of the children was between $5^{6}/_{12}$ years old – $11\ ^{11}/_{12}$ years old.

In the end, patients with a history of severe hypoglycemia presented more neuropsychological disorders, more learning difficulties (reported by parents), and needed more specialized education as compared to the ones in the other two groups. Significant differences were found in short-term verbal memory and phonological processing. The results suggest that severe hypoglycemia is a risk factor for learning because of deficits in the auditory-verbal function.[24]

Global memory during acute hypoglycemia

In order to assess whether moderate hypoglycemia disrupted learning, Warren RE et al used a new potential memory test that could better reflect the role of memory in everyday life than conventional tests. The study involved 36

subjects with Type 1 DM, 20 with normal hypoglycemia awareness (NHA) and 16 with cardiac insufficiency (IHA) participated in the study. All the patients were subjected to a hypoglycemic clamp with a target value of 2.5 mmol/ l. Before hypoglycemia, the subjects completed the learning and immediate recall steps of three conventional memory tasks (word recall, story recall, visual recall).

Hypoglycemia affected the potential memory task ($p = 0.004$) as well as the immediate and delayed recall of word and story recall tasks ($p < 0.01$ in each case). There was no significant decline in the performance of the visual memory task. The effect of hypoglycemia did not differ significantly between the subjects with NHA and IHA.

Depreciated performance upon the potential memory task during hypoglycemia proves that recall is interrupted by hypoglycemia and the poor performance of conventional memory tasks proves that learning is also disturbed by hypoglycemia.[25]

The duration of DM evolution[26]

In children diagnosed with DM before the age of 10, the focus is also on the duration of diabetes mellitus among the category of risk factors.

In the Woodcock-Johnson Psycho-Educational Medicine Center, 55 out of 62 eligible patients, with an average age of 7.9 +/- 1.6 years old, underwent the Beery developmental test of visual motor integration, touching with toes, tests to assess: memory/ attention, visual-perceptual, broad cognitive functions, academic achievements and speed/ fine coordination of the motor. Twenty-seven subjects had less than 5 years from the onset of DM, the average diagnosis age being 4.5 +/- 2.1 years old and the average duration of DM of 2.6 +/- 2.0 years. 18 patients had a history of severe hypoglycemia, eight of whom had hypoglycemic convulsions. In the year before the tests, the average HbA1c was of 7.8 +/- 1.1%. The results of the study showed that the average global scores for the extensive neurocognitive battery were in the

normal range and were comparable to the screening scores corresponding to the age. The age of diagnosis and duration of diabetes did not refer to the results of neurocognitive tests. The average HbA1c had a negative association with certain memory/ attention tests ($p<0.03$-0.04) and academic achievements ($p <0.005$-0.03), while the number of hypoglycemia had a positive association with memory/ attention ($p <0.004$-0.04), verbal comprehension ($p <0.03$) and academic achievement ($p <0.018$-0.05).

There was no association of neurocognitive scores with severe hypoglycemia, but subjects with a history of hypoglycemic convulsions had a decline in scores in memory skill assessment tests ($p <0.03$) including short-term memory and word memory. These data suggest that overall scores of neurocognitive tests were in the normal range and comparable to those in the control group. Nevertheless, specific aspects of neurocognitive function may be adversely affected by the fact that they had a hypoglycemic convulsion, but not by episodes of severe hypoglycemia without convulsions.

Lower HbA1c and increase in glycemia levels by values of <70 mg / dl (subtle hypoglycemia), associated with higher scores in certain areas of academic achievement and memory suggests that stable glycemia can influence cognitive abilities.

Under these conditions, strategies to reduce the risk of convulsions with hypoglycemia should be investigated.[26]

Identifying predictors for modifying the neuropsychological profiles of children with Type 1 DM in the ***first 2 years of the disease***

Children aged between 3-14 years old were assessed shortly after diagnosis and re-assessed 2 years later in order to examine relationships between disease variables, such as onset age and metabolic check history and neuropsychological modifications in the first 2 years of Type 1 DM.

The disease variables were significant predictors of modifications in neuropsychological test scores within 2 years from the onset of Type 1 diabetes. The age at the onset of Type

1 DM predicted an impairment of the intelligence performance, while both recurrent severe hypoglycemia and chronic hyperglycemia were associated with a reduced memory and learning ability.

These results suggest that the relationship between metabolic control and neuropsychological risk is non-linear. Children with recurrent severe hypoglycemia or chronic hyperglycemia exhibit negative modifications in their neuropsychological profiles. Very early onset of Type 1 DM adds another dimension of risk, which particularly affects the acquisition of visual-spatial skills.

90 children with Type 1 DM aged between 6 and 17 years old, who were assessed after diagnosis and 2 years later, were re-assessed 6 years after the onset of the disease. Their neuropsychological profiles were compared to those of a community control group (n = 84), which were assessed at similar intervals.

Six years from the onset, the children with Type 1 DM had lower scores than the control subjects regarding the measurements of intelligence, attention, processing speed, long-term memory, and executive abilities.[28] Attention, processing speed and executive abilities were particularly affected in children with onset before the age of 4 years old, while severe hypoglycemia was associated with verbal scores and full scale of information.

The neuropsychological profiles of children with Type 1 DM 6 years after the occurrence of the disease are consistent with the subtle impairment of anterior and medial temporal brain regions. Severe hypoglycemia, especially in very young children, is the most plausible explanation for neuropsychological deficits, but the contribution of chronic hyperglycemia justifies further exploration.[28]

Lin A. et al expanded the exploration of neuropsychological profiles of a cohort of 106 young people with Type 1 DM ***12 years after the diagnosis*** compared to a control group consisting of 75 healthy individuals. There were no significant differences between groups regarding the large-scale IQ assessed at study entry in the past 12 years, the

socio-economic status, the gender distribution or the age. Neuropsychological tests evaluated eight cognitive domains: verbal skills, perceptual judgment, new learning, working memory, non-verbal processing speed, mental efficiency, divided attention and sustained attention. Episodes of severe hypoglycemia and HbA (1c) levels were recorded in the diagnosis.

Young people with Type 1 DM had poorer results than the controls regarding the working memory ($p < .05$). Hypoglycemia has been found to negatively affect verbal capacities, working memory and non-verbal processing speed (all $p < .05$). Poorer working memory has been associated with hyperglycemia ($p < .05$). Young people with any combination of two or three risk factors for the disease (early onset of DM, hypo-/ hyperglycemia) had poorer results than the controls and young people without risk in the assessment of verbal abilities, working memory and mental efficiency.

Results suggest that early onset of DM and hypoglycemia has an impact upon developing SNC as compared to hyperglycemia, which has a lower role.[29]

A different category assessed is represented by patients with type 1 DM with ***impaired awareness of hypoglycemia.***[30]

Out of a total of 68 adults with Type 1 DM, 33 were affected and 35 had normal awareness of hypoglycemia, confirmed by official tests. The groups were homogeneous regarding age, sex and duration of DM. Cognitive tests of verbal memory, object memory, pattern separation, executive function, working memory and processing speed were performed.

Depreciated awareness of hypoglycemia has been associated with decreased learning, memory, and pattern separation. These cognitive tasks depend on the hippocampus, which is vulnerable to neuroglycopenia.

The results suggest that hypoglycemia contributes to the correlation found between hypoglycemia awareness and cognitive impairment.[30]

Relation between Type 1 DM – brain development

Over a decade ago, Ho M.S. et al., analyzed the effects of severe hypoglycemia on the developing brain in children with early-onset type 1 DM (onset age <6 years old).

Those with severe hypoglycemia (coma/ convulsions) were compared to the elderly without such events in the past using MRI exploration. From the diagnosis, in all the patients the following were monitored: HbA1c, diabetic ketoacidosis episodes and clinical variables.

MRI exploration detected a high prevalence of structural anomalies in the central nervous system (CNS) (29%), and mesial temporal sclerosis (MTS) was detected in 16% of the total number of patients.[31] The conclusion is that the early age of the onset of Type 1 DM is associated with a high incidence of CNS anomalies, especially MTS, suggesting hippocampal lesions. Severe hypoglycemia with early onset may have an impact on the volume of gray matter. [31]

However, the impact of Type 1 DM on the development of the central nervous system is not yet well understood. Transversal, retrospective studies suggest that exposure to glycemic extremes during development is detrimental to brain structure in young people with Type 1 DM. However, it is still not possible to identify regions of the brain that differentially modify over time depending on the degree of exposure to glycemic extremes.

The impact of Type 1 DM on the development of the central nervous system is not yet well understood. Transversal, retrospective studies suggest that exposure to glycemic extremes during development is detrimental to brain structure in children and young people with Type 1 DM. However, it is still not possible to identify regions of the brain that differentially modify over time depending on the degree of exposure to glycemic extremes.

Perantie DC et al. [32] conducted a longitudinal, prospective, neuroimaging study on 75 children with type 1 DM (average age = 12.5 years old) who were compared to their non-diabetic (n = 25, average age = 12.5 years) siblings. Each participant was scanned twice at an interval of 2 years.

HbA1c, glycemias and severe hypoglycemia reports were stored during the 2-year follow-up. Sophisticated image capture algorithms were performed, followed by full brain statistical analysis regarding the modification of the gray and white matter volume, based on age, gender and age at the onset of Type 1 DM.

At the end of the study, participants of the group with type 1 DM with more hyperglycemia presented a greater decrease in the gray brain content overall, as compared to those with lower hyperglycemia ($P < 0.05$). Participants who experienced severe hypoglycemia showed a greater decrease in the occipital/ parietal volume of white matter as compared to those without severe hypoglycemia ($P < 0.05$) and compared to the group without Type 1 DM ($P < 0.05$). These results suggest that within Type 1 DM, exposure to severe hyperglycemia and hypoglycemia may cause subtle deviations from the normal development trajectories of the brain.[32]

Guàrdia-Olmos J et all.,[33] analyzed 15 young people with Type 1 DM with sustained clinical metabolic stability and a control group similar in age, gender and educational level. The participants in the two groups performed 2 visual-spatial work memory tasks using a block design within an MRI scanner. Compared to the control group, the group of patients with Type 1 DM presented a significant reduction in brain activity in the two estimated networks. The actual connection patterns have highlighted a larger brain connection in the case of healthy people, as well as a more complex network. Patients with Type 1 DM have shown a connection pattern involving mainly the cerebellum and the red nucleus, which probably suggests a compensatory mechanism to meet the task requirements.[33]

Hippocampal neurons in adult animals and humans are vulnerable to both severe hypoglycemia and hyperglycemia. The effects are supposed to be exacerbated throughout development, but existing studies regarding the development of the human brain are limited.

In this regard, Hershey T. et al. analyzed the MRI images of 95 young people with Type 1 DM and 49 female control subjects aged between 7 and 17 years old. Young people with Type 1 DM were classified according to the number of hypoglycemic episodes: 0 (n = 37), 1 - 2 (n = 41), 3 or more (3+, n = 17) severe hypoglycemic episodes before exploration. Exposure to hyperglycemia was estimated from the value of HbA1c, weighted for the duration of diabetes. Stereological measurements of hippocampal volumes were performed in the space recorded in the atlas in order to correct the entire brain volume.

Exposure analysis has revealed that greater exposure of patients to severe hypoglycemia was associated with large volumes of hippocampus, while exposure to hyperglycemia was not associated with the volume of the hippocampus.

These data suggest that the extension of the hippocampus denotes a pathological reaction throughout brain development in hypoglycemia such as gliosis, reactive neurogenesis or disruption in normal brain development.

Neuropsychological function during the periods of experimentally induced hyperglycemia and hypoglycemia[35]

The study was conducted on 20 men and 22 women aged between 18 and 44 years old, with Type 1 DM for 3 to 14 years and with HbA1c values ranging from 5.8% to 18.0 %. A controlled experimental setting was employed, using tests for sensory perception, simple motor skills, attention, learning and memory, language and spatial and constructive skills at plasma glucose levels of 2.2, 5.6, 8.9, 14.4 and 21.1 mmol / L. Tests used in each glycemia level included response time (simple and choice), numerical vigilance, word recovery, digital sequence learning, and verbal fluency.

Cognitive function in patients with Type 1 DM was generally well preserved, even at high blood glucose levels. Deficits in all relevant areas of cognitive function occurred during hypoglycemia (2.2 mmol / L) regardless of the previous glycemic control, and women with Type 1 DM were less cognitively impaired than men with Type 1 DM during hypoglycemia.[35]

The effects of hyperglycemia on the neurocognitive function

There is considerable evidence that post-prandial hyperglycemia is associated with higher risks of macro-vascular disease.

Recent studies suggest that acute hyperglycemia affects knowledge and other performance indicators, equivalent to visual disturbances noticed during hypoglycemia. There is evidence to suggest that acute hyperglycemia may lead to poor cognitive performance and productivity, but the relationship between these effects and daily activities remains insufficiently understood (36). Further research is required in order to improve understanding of acute hyperglycemia in everyday life.

There is increasing evidence that transient hyperglycemia has similar negative effects.

At relatively light levels of extreme blood glucose - either hypoglycemia or hyperglycemia - cognitive efficiency may decrease by one-third. The impact of this effect will depend on the task the patient is facing at that time. If the person engages in a relatively dangerous task, such as driving a vehicle, there may be significant consequences.[37]

Both hypoglycemia and hyperglycemia have proved not only acute but also chronic effects in patients with Type 1 DM.

Although **HbA1C** is accepted as an important marker in the risk assessment of DM complications, the increasing use of continuous glycemic monitoring (CGMS) to facilitate efficient and effective management of diabetes, it is important to understand the value of reports obtained in therapeutic decisions.

Several patients and clinicians believe that HbA1c is a useful educational tool, but others are often confused or even frustrated if eA1C (estimated through CGMS) and A1C measured in the laboratory do not agree.

In the U.S.A., the Food and Drug Administration has established that the eA1C nomenclature should be changed. This led the authors to work on renaming eA1C as *glucose management indicator* (GMI) and generating a new formula for

the conversion of the average glucose derived from CGMS to GMI based on recent clinical studies using the most accurate CGMS systems available. [38]

Ensuring a smooth transition from the former eA1C to the new GMI provides new analyzes and explanations for CGMS in order to understand the way GMI should be interpreted and used most effectively in the clinical practice as an instrument in the education or management of diabetes.

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ENDOCRINE DISORDERS IN CYSTIC FIBROSIS IN CHILDREN

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Abstract

Cystic fibrosis (CF) is caractherized by exocrine pancreatic insufficiency and a progressive lung disease. In addition, people with CF have different endocrine abnormalities including diabetes, bone disease, pubertal disorders, poor linear growth and adrenal function alterations.

Cystic fibrosis-related diabetes (CFRD) is an entity distinct from diabetes mellitus type 1 and 2, but with symptoms characteristic to both.

The prevalence of diabetes increased in association with a severe decline of the lung function and a poorer nutritional status. Specific CF factors determining fluctuations in the glucose metabolism are: lung infection and inflammation, increase of energy consumption, malnutrition, glucagon deficit, gastro-intestinal anomalies, liver disease. Insulin-therapy represents the only recommended medication therapy. Osteo-articular disorder in CF is considered a common complication. The causes of CF related bone disease are multi-factorial: deficiencies of vitamin D, K and calcium, glucocorticoid use, sex steroid deficiency, an altered growth hormone axis, inflammation and the mutation of the CFTR gene. Poor linear growth and inadequate weight gain are very

common problems in CF children. The most important factors involved in growth failure are undernutrition, chronic inflammation, lung disease, and corticosteroid treatment. Early diagnosis is essential to ensure better nutritional status and growth, potentially associated with better respiratory function and prognosis. Delayed puberty and menstrual irregularities were noticed especially in those with poor nutritional status, abnormal OGTT and diminished lung function. The use of systemic or inhaled corticosteroid in conjunction with azole-derivates or macrolides can lead patients with CF to develop iatrogenic Cushing's syndrome.

Conclusions.

It is essential to detect and treat endocrine complications as part of high-quality medical care for people with CF. The addition of an endocrine specialist to the CF care team is important to continue to improve health outcomes in CF.

Key words. *Cystic fibrosis, children, endocrine disorders.*

Introduction

Cystic fibrosis (CF) is the most frequent monogenic autosomal recessive disorder found in Caucasian populations, characterized by exocrine pancreatic insufficiency and a progressive lung disease, with chronic evolution, potentially lethal.[1] In addition, people with CF have different endocrine abnormalities including diabetes, bone disease, puberty disorders, poor linear growth and adrenal function alterations.

Cystic fibrosis-related diabetes (CFRD)

First described in 1955, CFRD is an entity distinct from diabetes mellitus type 1 and 2, but with symptoms characteristic to both.[2,3,4]

Specific CF factors determining fluctuations in the glucose metabolism are: lung infection and inflammation; increase in the energetic consumption, malnutrition, glucagon deficit; gastro-intestinal anomalies (malabsorption, gastric emptying disorders) and liver disease.[5,6,7,8] The risk factors involved in the CFRD are: age, female gender, exocrine

pancreatic insufficiency, altered exocrine pancreatic function and organ transplant. For example, the risk of CFRD is about 5×higher in people with CFTR genotypes that cause exocrine pancreatic insufficiency.[4] The discovery of vitamin D receptor presence outside the skeletal system led to the conclusion that vitamin D is responsible not only for mineral economy, but also for CFRD course and immunological processes, respiratory status and intestinal microflora.[5]

The prevalence of diabetes increase in association with a severe decline of the lung function and a poorer nutritional status. CFRD prevalence also increases with age: 9% between 5-9 years, 26% between 10-20 years, 40% between 20-30 years and 50% over 30 years. The European Epidemiologic Registry of Cystic Fibrosis (ERCF) reported a prevalence of 5% between 10 to 14 years old and 13% at group 15-19 years of age.[3] The disease is found in 20% of the patients with CFRD mutations class I-III and only in 1.5% of the patients with CFRD mutations class IV-V.[5,6,7,8]

The prevalence of CFRD is higher in patients with liver disease.[3]

From the pathophysiological point of view, CFRD is characterized by reduced or delayed insulin secretion with generally normal sensitivity to insulin action.[4]

The onset of the disease is insidious, patients being asymptomatic for many years (at least 4 years). Presentation with diabetic ketoacidosis (DKA) is rare.[3] The average age of the onset is 18-21 years, being rarely under 10 years old. In girls, the age of the onset is smaller by 5-7 years in comparison to boys, probably because of the earlier debut of puberty and the association of the increased insulin resistance at this age.

The onset is more frequent in circumstances in which insulin resistance is higher:

–acute lung infections;

–severe chronic lung disease;

–treatment with glucocorticoids;

–carbohydrate supplements (oral, percutaneous, intravenous, gastrostoma);

–post-transplant immunosuppressive treatments.[8,10,11]

The clinical picture includes classic symptoms for diabetes (polyuria, polydipsia, weight loss) as well as other symptoms: fatigue, alteration of the lung function without a direct connection to the exacerbation of the lung infection, late puberty.[8,12,13]

The diagnosis of CFRD can be made in CF patients with acute illness when fasting, plasma glucose (FPG) levels are higher than 126 mg/dL (7.0 mmol/L) or 2-hour postprandial plasma glucose levels more than 200 mg/dL (11.1 mmol/L) persist for more than 48 hours. Annual screening for CFRD should begin at least by age 10 years in all CF patients who do not have CFRD. The standard oral glucose tolerance test (OGTT) is at present the only accepted screening test.

The dietary recommendations for persons with CFRD are the same as in cystic fibrosis patients without diabetes (high-calorie, high-fat diet). Insulin therapy is the only recommended medical treatment. Oral diabetes agents are not recommended in CFRD.[3]

Patients with CFRD who are on insulin should perform self-monitoring of blood glucose at least three times a day. HbA1c cannot be used as a screening test for CFRD, but HbA1c measurement is recommended quarterly for these patients to guide insulin therapy.

Continuous glucose monitoring is useful in patients with insulin-treated CFRD and is also a useful tool for insulin dosage adjustment and to alert the patient to hypoglycemia. Monitoring for complications of CFRD is similar to that for other forms of diabetes.[14]

The microvascular complications of diabetes must be monitored annually, beginning 5 years after the diagnosis of CFRD or, if the exact time of diagnosis is not known, at the time that fasting hyperglycemia is first diagnosed.[3]

CF Bone Disease

Patients with CF are at risk of developing low bone mineral density (BMD) and fractures. Low BMD is due to the imbalance in bone remodeling with decreased bone formation

and increased bone resorption, especially during pulmonary exacerbations.

The risk factors for CF bone disease include:

–vitamin D insufficiency,
–poor nutritional status,
–lung infection,
–vitamin K insufficiency,
–negative calcium balance,
–abnormal fatty acid status,
–hypogonadism, delayed puberty,
–CF related diabetes,
–glucocorticoid treatment,
–reduced levels of weight bearing activity,
–the effect of CFTR dysfunction on bone cells.[14]

According with Tangpricha V. and all., "*vitamin D deficiency in CF is associated with decreased bone mass in children, failure to achieve expected peak bone mass in young adults, and osteoporosis in mature adults, and it may impact other co-morbidities common in CF*".[15]

Dual energy X-ray absorptiometry (DXA) is currently the gold standard method for measuring bone mineral content (BMC) and BMD in people with CF. Osteodensitometry must be performed for all the patients with CF starting at the age of 10, once a year or every 1-3 years, but at least one test until 18 years.[16,17,18]

Serum 25-hydroxyvitamin D is the best biochemical marker of vitamin D status and CF Foundation recommends its measurement annually at the end of winter. It is also recommended the routine measurement of parathyroid hormone (PTH), osteocalcin, alkaline phosphatase or other indirect markers to assess vitamin D status in all individuals with CF.[15]

The current guidelines of European Cystic Fibrosis Society (ECFS) and Lawson Wilkins Pediatric Endocrine Society suggest a threshold level of 20 ng/ml (50 nmol/l) to prevent deficiency, but North American CF Society recommends a minimum 25-hydroxyvitamin D concentration

of 30ng/ml. They consider that higher 25-hydroxyvitamin D concentrations may offer extra skeletal benefits (enhanced immune function, reduced risk of developing diabetes, cancer and cardiovascular disease).[19]

The patients with vitamin D deficiency and insufficiency should receive vitamin D supplements, starting dose for infants being 1000–2000 IU/day of vitamin D2 or D3 (preferably vitamin D3) and 1000-5000 IU/day for children above one year old and adults.[19]

Regarding the recommendations for calcium supplementation**,** daily calcium intakes recommended by the Food and Nutrition Board, for each age group, are 210 mg for 0–6 months, 270 mg for 7–12 months, 500 mg for1–3 years, 800 mg for 4–8 years, 1300 mg for 9–18 years, 1000 mg for 19–50 years and 1200 mg for adults more than 50 years old.[19]

In order to correct vitamin K deficiency, the starting dose recommended is 0.5 mg - 2 mg/day in infants and 1-10 mg/day in children above one year of age and adults with CF.[19]

For the patients with CF who take continuous systemic oral glucocorticoids for more than three months and with a bone mineral density Z/T-score of −1.5 or less (adults) or bone mineral density Z-score of −2 or less (children), it is recommended treatment with Bisphosphonate.

To assess response, bone densitometry should be repeated after 12 months of bisphosphonate treatment in adults, respectively after 6 months of bisphosphonate treatment in children. [19]

Puberty disorders

Adolescents with CF may start puberty later than the adolescents without CF. The age at onset of menarche is most delayed in girls with the F508del/ F508del genotype.

The principal cause of delayed puberty in CF is malnutrition. The physical signs of puberty may be delayed in patients with CF who have severe lung disease, too. The mutated CFTR gene may cause disturbances in the secretion of gonadotropin-releasing hormone (GnRH) and thus delay

puberty. Hormonal imbalances determine irregular menstrual cycles and lead to the development of Polycystic Ovary Syndrome (PCOS).[20]

Delayed puberty and other features of CF that mark the patient as "different".

Girls that are severely underweight may begin their periods later or their periods are irregular, but late menarche was also observed in girls with normal nutritional status. Lack of pubertal delay was a major issue for over 60% of adolescent girls.[21,22]

The children who don't show any signs of puberty by age 14 for boys and age 13 for girls must contact the pediatrician and pediatric endocrinologist. [21]

Growth failure

Inadequate weight gain and poor linear growth are frequent problems in CF children. According to the CF Foundation Patient Registry Report, 23% of children with CF are below the 10th percentile for weight based on age and gender.[23] The causes of growth failure are undernutrition, chronic inflammation, lung disease, and corticosteroid treatment.[24] In his study, Powers demonstrated that only 11% of infants and toddlers with CF receive recommended energy input and that lipid intake does not account for 40% of the energy supply.[25]

Energy deficiencies may occur as a consequence of increased energy expenditure due to inflammation and pulmonary infection and increased respiratory effort, increased gastrointestinal loss and low oral intake.[26]

Respiratory infections and inflammation can induce anorexia, creating a vicious cycle between malnutrition and worsening lung disease. The patient may enter into a catabolic state with perpetuation of failure to gain weight and poor linear growth.[26] Insulin-like growth factor I (IGF-I) signaling is mediated by CFTR and CFTR dysfunction may directly affect linear growth.[28]

Acute pulmonary infection is accompanied by complex nutritional and metabolic responses, while chronic pulmonary

infection can contribute to weight loss by increasing energy expenditure rates and decreasing protein synthesis.

Insulin deficiency can increase malnutrition with clinical consequences in patients with CF due to the fact that protein and lipid catabolism is accelerated in chronic infections.[29]

Deficient nutritional status is an independent risk factor for inadequate survival in CF and is associated with the occurrence of disease complications. Malnutrition could contribute to a poor clinical outcome. Undernutrition affects respiratory muscle function, decreases exercise tolerance, and leads to immunological impairment.[30] Thus early diagnosis is necessary for a better nutritional status and growth, potentially associated with better respiratory function and prognosis.

Growth hormone (GH) treatment may be associated with improvement in both height velocity and weight gain in children with CF.[31] Ivacaftor treatment in prepubescent children may help to amelioration of short height and of altered growth velocity in children with CF.[32]

Adrenal function alterations

In CF the glucocorticoids (inhaled or systemic) are frequent use and may induce the long-term suppression of the hypothalamic-pituitary-adrenal axis and lead to insufficient cortisol production in response to stress. But, the prevalence of adrenal insufficiency in CF is unknown. After the review of the clinical records of 385 CF patients, Preville-Ratelle S. and all. suggested that the prevalence of adrenal insufficiency in CF population is between 8% and 9%. Dysglycemia and colonization with *Aspergillus* also appear as potential risk factors for adrenal insufficiency. Chronic inflammatory response associated with CF could also explain the presence of adrenal insufficiency in patients who did not take corticosteroids.[32] The use of systemic or inhaled corticosteroid in conjunction with azole-derivates or macrolides can lead patients with CF to develop iatrogenic Cushing's syndrome. [33]

Conclusions

It is essential to detect and treat endocrine complications as part of high-quality medical care for people with CF.

The addition of an endocrine specialist to the CF care team is important to continue to improve health outcomes in CF.

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GENETIC TESTING IN MEDULLARY THYROID CARCINOMA

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Introduction

Medullary thyroid carcinoma (MTC) derives from the parafollicular C cells of the thyroid. These cells are included in the APUD (**A**mine **P**recursor **U**ptake **D**ecarboxylase) system and originate embriologically in the neural crest. C cells secrete the hormone calcitonin.

In the condition of MTC, calcitonin becomes a tumoral marker specific for this cancer. Therefore, the clinical pre-operative diagnostic of MTC is based on increased serum calcitonin levels in the presence of a thyroid nodule (or multinodular goiter). Serum calcitonin is measured in basal condition and/or after stimulation with pentagastrin or calcium.

Treatment of MTC is surgical – total thyroidectomy with central compartment cervical lymph nodes dissection. Nowadays, new systemic therapies, like tyrosine kinase inhibitors, are available for progressive disease. Even so, extensive and meticulous surgical resection is the only method that could provide cure of the disease, if the patient is diagnosed in an earlier localised stage.

Aproximately 75-80% of all MTCs are **sporadic**, but almost a quarter (20-25%) of them appear in **hereditary**

conditions – respectively in the **multiple endocrine neoplasia syndrome type 2 (MEN2)**.

MEN2 is an autosomal dominant transmited hereditary neoplastic syndrome that affects tissues derived from neural ectoderm. The predisposition for this syndrome occurs as a result of germline point-mutations in the *RET* proto-oncogene, situated on chromosome 10q11.2. The product of this gene, protein RET (REarranged during Transfection), is a transmembrane tyrosine kinase receptor expressed inSi normal tissues (sympathetic ganglia, adrenal medulla, C-cells of thyroid, kidneys) and in human tumors (neuroblastomas, pheochromocytomas, medullary thyroid carcinomas) of neural crest origin.

Homodimerization and abnormal activation of oncoprotein RET resulted from mutated *RET* proto-oncogene determine downstream activation of extracellular signal-regulated kinase AKT and Jun N-terminal kinase (JNK). A constitutive increase in these effectors leads to the increased proliferation, decreased apoptosis, increased cell migration and altered cell adhesion characteristic of malignancy.[1]

There are **three clinicallly distinct forms of MEN2 syndrome**: MEN2A, MEN2B and familial medullary thyroid carcinoma (FMTC). **MTC** is **the common element of all**.

MEN2A syndrome includes the classic triad: MTC (up to 100% of cases), pheochromocytoma (in over 50%) and primary hyperparathyroidism (HPT), in 15-30% of the patients. MEN2A is the most common subtype of MEN2 (over 90% of cases).

MEN2B is responsable for 5% of all MEN2 cases. It is the most aggressive form of MEN2, with the highest rates of morbidity and mortality. The onset of the disease is 10 years earlier than in MEN2A.

MEN2B is characterized by the main neoplasms of MEN2A (MTC and pheochromocytoma), plus a marfanoid habitus, mucosal and intestinal ganglioneuromatosis, but not HPT.

FMTC comprises families with MTC as their only disease phenotype.

RET phenomic data accumulated over the past years have led many to suggest that FMTC is not in fact a distinct clinical entity, but it rather represents cases of MEN 2A with low penetrance.[2]

In hereditary MTC, hyperplasia of the C cells precedes the development of carcinoma. Characteristic for hereditary MTC is the multicentricity of the tumor, virtually in both lobes of the gland.

Since the discovery of the *RET* oncogene, over 100 mutations, duplications, insertions or deletions involving *RET* have been identified in patients with hereditary MTC.[3]

Regarding the most frequently mutated codons of the *RET* gene, in MEN2B a single point-mutation in exon 16, M918T is responsible of 95% of cases. Codon 634 mutations are the most common in MEN2A, in almost 87% of cases.

Many reports on the genotype-phenotype correlations in hereditary MTC proved that the aggressiveness of MTC correlates with MEN2 variant and with the mutated codon of the *RET* gene.

The aggressiveness of MTC was characterized by the age of onset and the age and progression speed of metastatic disease.

The most common *RET* germline mutations causing MEN2A and MEN2B and the clinical aggressiveness of the mutations are shown in the *table 8.1.*

Revised ATA Guideline for the management of MTC (2015) classified mutations by the MTC risk level in: highest risk of aggressive MTC, high risk and moderate risk.[3]

Importantly, 1%-7% of patients with presumed sporadic MTC actually have hereditary disease.[3]

The first and most simple method of raising the suspicion of MEN2 syndrome in a case with a MTC is, in the first place, a thoroughly registered personal pathologic and family history.

Table 8.1 - Relationship of Common RET Mutations to Risk of Aggressive MTC in MEN2A and MEN2B [3]

MTC risk level	Exon	RET mutation
Highest	16	M918T
High	11	C634F/G/R/S/W/Y
	15	A883F
Moderate	8	G533C
	10	C609F/G/R/S/Y
	10	C611F/G/S/Y/W
	10	C618F/R/S
	10	C620F/R/S
	11	C630R/Y
	11	D631Y
	11	K666E
	13	E768D
	13	L790F
	14	V804L
	15	S891A
	16	R912P

Currently, genetic screening for *RET* germline mutations became the standard method for identification of subjects at risk for MEN2 syndrome. The practical benefit of the identification of asymptomatic carriers of a hereditary MTC predisposing *RET* mutation is the prophylactic total thyroidectomy at an early age, in purpose to avoid the metastatic disease.

Prophylactic thyroidectomy should be recommended only to mutation carriers who were identified by DNA-based analysis.

According to 2015 ATA guideline, the recommended method of initial testing for MEN2 is either a single or multi-tiered analysis to detect *RET* mutations in exon 10 (codons 609, 611, 618 and 620), exon 11 (codons 630 and 634) and exons 8, 13, 14, 15 and 16. Sequencing of the entire coding region should be reserved for situations in which no *RET* mutation is identified or there is a discrepancy between the MEN2 phenotype and the expected genotype.

Even patients with presumed sporadic MTC should have genetic testing to detect a germline *RET* mutation.

Genetic counseling and genetic testing for *RET* germline mutations should be offered to:

a. first-degree relatives of patients with proven hereditary MTC;
b. parents whose infants or young children have the classic phenotype of MEN2B; and
c. infants or young children with Hirschsprung disease (HD) and exon 10 *RET* mutations, and adults with MEN2A and exon 10 mutations who have symptoms suggestive of HD.

Our experience proves that in our country, DNA-analysis for screening persons „at-risk" for MEN syndromes is only sporadic available, rather by individual efforts than by an organized infrastructure.[4,5]

Information and support for mutation carriers are necessary, as well as the multidisciplinary collaboration for developing of uniform guidelines of diagnostic, treatment and follow-up.

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