

Hypothalamic Amenorrhea-an Update on Aetiopathogenesis, Endocrine Profile and Management

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Abstract

Hypothalamic Amenorrhea is one of the most common causes of amenorrhea in women of reproductive age, accounting for almost 50% cases of secondary amenorrhea, while the genetic causes account for a lower percentage of cases of HA presenting as primary amenorrhea. HA is a heterogeneous condition with main characteristics being reduced pulsatile GnRH secretion which is evidenced as reduced circulating LH levels along with decreased frequency and amplitude of LH plasticity. It has been observed in 10% of female athletes. Besides that, affected patients may have more than one etiological factor like low body weight, excessive exercise as well as stress. Other important causes such as heterozygous mutations in genes associated with GnRH migration or GnRH secretion maybe responsible for congenital GnRH deficiency whose presentation is also as HA. Thus pulsatile s/c administration remains the treatment of choice in women desiring pregnancy. Priming with LH followed by FSH initiation may be effective in ovulation induction as well. But since infusion pumps requiring GnRH administration intermittently are not available in all countries Kp54/kp10 offers a better alternative once its efficacy and full therapeutic window has been accurately worked out, besides being very effective in Kp/GPR54 mutations as well as neurokin deficiency as it acts downstream of Kp. Emphasis on weight increase and correcting anetiological factors remains the mainstay in patients presenting with functional HA as secondary amenorrhea and factors which will increase intake as well as reduce energy expenditure will go a long way in correcting the GnRH and thus LH pulsatility. Only if these patients require immediate fertility is hormonal therapy indicated along with ovulation induction.

Keywords: Hypothalamic amenorrhea; Anorexia nervosa; Bulimia nervosa; Exercise; Stress; GnRH mutations; Idiopathic hypogonadotropic hypogonadism; Pulsatile GnRH therapy; Kisspeptin 54; Estrogen supplementation; Weight control

Introduction

Secondary amenorrhea defined as the absence of menses for three consecutive cycles, affects 3-4% of women of reproductive age. Over 50% of cases of secondary amenorrhea result from perturbations in the hypothalamo-pituitary-adrenal (HPA) axis [1]. Hypothalamic Amenorrhea (HA) patients have a deficiency in pulsatile GnRH secretion. These diagnoses are made by excluding pituitary lesions and are the commonest in the category of hypogonadotropic amenorrhea, where a functional suppression of reproduction is often a response to life psychobiological events [2]. Many a times there is an association with some kind of stressful situation, be it at work or school. Also a large number of women are underweight with a previous history of menstrual irregularity. Besides that, many women with HA show endocrine, behavioral, metabolic and psychological consequences of subclinical eating disorder [3,4]. A process of exclusion is needed before any hormonal therapy is prescribed or an attempt is done in induction of ovulation.

The clinical presentation depends on the degree of GnRH suppression. Just mild suppression, may be associated with a marginal effect on reproduction, like luteal phase inadequacy. With moderate suppression, anovulation along with menstrual irregularities is the presentation; severe suppression ultimately results in HA. Patients with HA present with low or normal gonadotropins, normal prolactin, normal sella tursica imaging along with failure to demonstrate a progesterational withdrawal bleed. Follow up is needed annually. Reason being that in a long term follow up of two large studies, it was shown that amenorrhea associated with psychological stress or weight loss demonstrated a spontaneous recovery after 6yrs in 72% of the women in Scandinavia and after 8yrs in 71% in Italy [5,6]. Another study revealed 83% patients resuming menses on follow up, once the precipitating cause of amenorrhea like stress, weight loss or eating disorders were taken care of [7]. Still a large number of women require long term surveillance. In patients having eating disorders, gain in bodyweight is associated with clinical improvement in the basic condition, thus weight gain may be an obvious marker [8].

Hormonal Mediators of HA

Women with HA, and no obvious cause have an increase in H-P-Adrenal activity. CRH inhibits gonadotropin secretion as shown by experimental means;

possibly by augmenting endogenous opioid secretion [9]. This pathway is the possible pathway by which stress interrupts reproductive function. Patients with idiopathic HA have decreased secretion of FSH, LH and prolactin but have increased levels of cortisol [10-13]. Also some evidence is there that patients with HA have a dopaminergic inhibition of GnRH pulse frequency [14]. These suppression of GnRH pulsatile secretion maybe because of an increase in both endogenous opioids as well as dopamine. CRH induced hypercortisolism, returning to normal, precedes the return of the normal ovarian function. Which emphasizes the primary role for a stress induced increase in CRH secretion [15].

When physical, nutritional or extreme emotional stress occurs the HPA axis gets activated and inhibits the HPO axis at multiple levels. At the hypothalamic level, CRH suppresses GnRH secretion. In women who are cycling normally, an infusion of CRH suppresses FSH/LH secretion but this gets prevented by GnRH administration, which suggests that CRH acts by inhibiting GnRH secretion [16].

i) At pituitary level, ACTH is shown to have negative reproductive effects in mice. In mice that have been adrenalectomized and maintained on physiological levels of glucocorticoids, ACTH administration x10days results in absence of corpus lutea [17], which demonstrates that ACTH has reproductive effects independent of adrenal steroid production.

ii) Also females with FHA have greater 24h mean plasma cortisol levels in contrast to controls [18], and cortisol which is the end product of the HPA Axis, suppresses reproductive function at the hypothalamic, pituitary, and uterine levels. i) In rhesus monkeys supraphysiological doses of hydrocortisone suppresses gonadotropin secretion, which is almost completely reversed by intermittent GnRH infusion, which suggests that cortisol suppresses GnRH secretion [19]. ii) Similarly women with Cushing's disease and women on long term supraphysiological prednisolone therapy have a reduced LH response to GnRH [20,21], which suggests that glucocorticoids also suppress pituitary responsiveness of pituitary gonadotropins to hypothalamic input iii) Glucocorticoids also inhibit the effect of E2 on the uterus. Dexamethasone co administered with E2 attenuates the expected increase in uterine weight seen with E2 alone, at least partly by reducing estrogen receptor concentrations [22]. Therefore, crosstalk between the HPA and HPO axis

promotes the development of amenorrhea as a functional adaptation to stress.

Other Hormonal mediators of FHA

Leptin: Leptin levels are low in women with FHA, which is a hormone secreted by the adipocytes and this maybe a mediator of amenorrhea [23,24]. Two studies investigated the effects of recombinant methionyl human leptin (rhleptin). In FHA women- in the first open label study, it was found that LH frequency increased 2 weeks post treatment with rhleptin and in second, which was a randomized placebo controlled study, a significant number of women who were treated with rhleptin got a menstrual bleed, out of which half were associated with anovulation [25,26]. Though the exact mechanism of action by which a decrease in leptin cause anovulation is unknown, low leptin levels may suppress GnRH through a kisspeptin (kp)-mediated pathway [27].

Insulin: Body weight changes are be it weight gain or loss can affect fertility. Marked weight loss secondary to chronic under nutrition can cause FHA and is associated with low insulin levels, while appreciable weight gain may cause obesity associated with insulin resistance i.e. a state of functional hypoinsulinemia. In animals, insulin is shown to modulate HPO axis, and therefore low or functionally low insulin levels may mediate infertility. Disruption of insulin receptors in a mouse model is associated with significantly reduced antral follicle count due to 90% reduction in circulating LH [28].

Fibroblast growth factor-21(FGF21): FGF21 is a liver derived hormone, whose production is up regulated in response to starvation [29,30]. FGF21 has been shown to be responsible for starvation induced amenorrhea [31]. FGF21 transgene mice are anovulatory, with low LH levels, while administration of GnRH, but not gonadotrophins, elicit an ovulatory LH surge, which demonstrates that FGF21 acts at the level of hypothalamus to disrupt ovulation [31]. FGF21 transgenic mice have decreased kiss-1 gene expression in the antral periventricular nuclei of the hypothalamus, and the product of Kiss-1, Kisspeptin is a potent stimulator of GnRH secretion, which suggests a mechanism by which FSH disrupts ovulation [31]. It is not clear whether FGF21 is a mediator of FHA in humans.

Although the patient may not be interested in pregnancy currently, assurance is important regarding when needed induction of ovulation and pregnancy can be achieved, since concerns' regarding potential fertility

remains an unspoken fear; although this is to be done only for the purpose of achieving pregnancy. There is no evidence that cyclic hormonal administration or induction of ovulation stimulates the return of normal function.

Weight Loss, Anorexia, Bulimia: Although obesity can be associated with amenorrhea, amenorrhea is usually due to anovulation in obesity, and a hypogonadotropic state is not encountered unless the patient also has a severe emotional disorder. But conversely, acute weight loss in some unknown way can lead to a hypogonadotropic state. A pituitary tumour must be excluded, with the diagnosis of HA made by exclusion. Clinically anorexia may present as a spectrum ranging from limited period of amenorrhea associated with crash diet, to the severely ill patient with the life threatening condition of anorexia nervosa (AN). Usually the clinician maybe the first to diagnose anorexia nervosa in a patient who presents with complaints of amenorrhea. Also sometimes not uncommonly a physician may treat infertility due to hypogonadism, without being aware of the developing anorexia. Since mortality rate is high with the condition, around 6% [32], it warrants close attention. While some studies have shown that most patients recover and there is no increase in mortality [33,34]. This reported differences reflects the populations being studied; but clinicians should keep it in mind that some cases of anorexia may die.

Diagnosis of AN is based on the following criteria:

- i. Onset around 10-30
- ii. Weight loss of 25% or weight 25% below normal for age and height
- iii. Special attitudes a) denial b) distorted body image c) Unusual hoarding or holding of food
- iv. At least one of the following a) lanugo b) bradycardia c) over activity d) episodes of overeating(bulimia) e) vomiting, which maybe self-induced.
- v. amenorrhea
- vi. no known medical illness vii) no other psychiatric disorder
- vii. Other characteristics a) constipation b) low blood pressure c) hypercarotenemia d) diabetes insipidus.

AN has been initially reported to occur in young white middle or upper class females below 25, but now it is clear that it involves all socioeconomic levels in about 0.5-1% of young women [35]. Families of anorectics are success-achievement-appearance oriented. There may be serious problems in the family but parents try to make all

efforts to maintain an apparent marital harmony, denying conflicts. According to one psychiatric evaluation, each parent in secret dissatisfaction with the other expects affection from their perfect child. Anorexia starts when the role of a perfect child becomes too difficult. Initially the pattern starts with a voluntary diet to control weight. A sense of power and accomplishment is felt which is followed by a fear that weight cannot be controlled if discipline is allowed to relax. Thus a view is to consider anorexia as a mechanism which identifies a generally disturbed family [36]. The symptoms are the expression of various psychological, familial and cultural factors involved.

At puberty, they may interpret the normal weight gain as excessive, which may trip this teenager into true anorexia. Excessive physical activity can be the early signs of incipient anorexia nervosa. These children are characteristically over achievers and strivers. They do not give any trouble but are judgmental and demand others to live up to their rigid value system, which leads them to social isolation. They usually demonstrate delayed psychosexual development, which is marked by sexual experiences occurring later in life [37]. Since culturally society demand thinness, that plays an important part in the development of the eating disorder. Occupational and recreational environments with stress on slimness put women at greater risk of anorexia nervosa and bulimia. Basically eating disorder is a method being utilized to solve a psychological dilemma.

Besides amenorrhea, constipation is a common symptom and is often severe and pain accompanies it. Preoccupation with food may, manifest itself by large intake of lettuce, raw vegetables and low calorie foods. Hypotension, hypothermia, rough dry skin, soft lanugo hair on the back and buttocks, bradycardia and edema are the commonest encountered signs. Long term laxative and diuretic abuse can produce significant hypokalaemia. Hypokalaemic alkalosis can cause fatal cardiac arrhythmias. An increase in serum carotene is not always associated with a large intake of yellow vegetables, which suggests that a defect in vitamin A utilization is present. The yellowish coloration of skin is usually seen on the palms. Hypercarotenemia should be considered as a metabolic marker, although not every woman with hypercarotenemia will be amenorrheic or anovulatory [38].

Bulimia is a syndrome marked by episodic and binge eating followed by self-induced vomiting, fasting or use of laxatives and diuretics, even enemas [39,40]. It appears to

be a growing problem among young women; however careful studies indicate that although bulimic behaviors may be fairly common, clinically significant bulimia is not (1% of female students and 0.1% of male students in a college sample) and the overall prevalence of eating disorders may be declining [41-43]. Erosion of dental enamel produces unattractive teeth which can be a diagnostic tip off. Bulimic behavior is frequently seen in patients with anorexia (about half), but not in all. Patients with bulimia often have depressive symptoms or anxiety disorders, and a problem with shoplifting (usually food) [44]. Both anorexia nervosa and bulimia persist as a long term problem, at least in 50% of cases [45]. Patients with bulimia can be divided into bulimic anorectics or those who fast and exercise excessively. Bulimic anorectics are older; more depressed, less isolated socially, and have a higher incidence of family problems. Body weight in bulimic fluctuates, but does not fall to the low levels seen in anorectics. Patients who overcome the problem of bulimia have normal fertility [46].

The serious case of anorectic is usually seen by an internist. But the borderline anorectic frequently presents to a gynaecologist, paediatrician or a family physician as a teenager who has low body weight, amenorrhea and hyperactivity (excellent grades and many extracurricular activities). Amenorrhea can proceed, follow, or appear coincidentally with the weight loss.

The various problems associated with anorexia nervosa represent dysfunction of the body mechanisms regulated by hypothalamus: appetite, thirst and water conservation, temperature, sleep, autonomic balance, and endocrine secretion [47]. Endocrine studies are as follows; low FSH and LH levels, elevated cortisol, normal prolactin, normal TSH, normal thyroxine (T4), but the 3,5,3'triiodothyronine is low but reverse T3 is high. Many of the symptoms can be explained by relative hypothyroidism (constipation, cold intolerance, bradycardia, dry skin, low metabolic rates, hypercarotenemia). There is a compensation to the state of undernourishment, with diversion from the formation of the active T3 to the inactive metabolite, reverse T3. With weight gain all metabolic changes revert to normal. Even though abnormal gonadotropins secretions maybe restored by weight gain, 30% of patients remain amenorrheic, this is a good size of ongoing psychological conflict [42].

The central origin for the amenorrhea is suggested by the demonstration that the response to GnRH is regained at approximately 15% below the ideal weight, and this

normal responsiveness occurs before the resumption of menses [48]. A patient has persistent low gonadotropins similar to prepubertal children. Once weight gain occurs, sleep associated episodic secretions of LH appear just like in prepubertal children. After full recovery the 24-h pattern is similar to that of an adult marked by fluctuating peaks. This sequence of changes with increasing and decreasing weight is explained by increasing and decreasing pulsatile secretion of GnRH. Neuropeptide Y (NPY) maybe a link between this control of food intake and GnRH secretion [49]. NPY cell bodies are located in the arcuate nucleus of the hypothalamus. It stimulates both feeding behavior and inhibits gonadotropin secretion (probably by suppressing GnRH pulses, although a direct action on pituitary is also possible). In response to food deprivation, the endogenous levels of NPY increase and elevated NPY levels can be measured in the intracerebral fluid of anorectic women. This is consistent with the known actions of leptin. This is one of the rare conditions where gonadotropins maybe undetectable, besides large pituitary tumours and genetic deficiencies. High plasma cortisol can differentiate, if necessary, the condition from pituitary insufficiency. But extensive lab testing is not necessary in these patients. A careful and gentle revelation to the patient regarding the relationship between amenorrhea and low body weight, is often all that is necessary to stimulate the patient to return to normal weight as well as normal menstrual function. Occasionally it is essential to see the patient more frequently and monitor calories, with a minimum intake of 2600 calories, to break the patients established eating habits. In case of slow progress hormone therapy should be initiated. In adult weighing <100lbs a psychiatric consultation is required. Although some say all such patients require a psychiatric evaluation [50].

Going away to school or the development of relationship with a male friend are the turning points in many young patients with mild to moderate anorexia. A failure to respond to these life changes can be ominous, which predicts a severe problem with a retracted course. It is very disappointing that despite so many studies on AN, no impressive therapy or specific therapy is available. Hence it is important to recognize it early before the syndrome reaches its full severity. Cognitive –behavioral therapy to help patients cope with their feelings has been shown to be effective in randomized controlled trials. A team work with a combination of a psychiatrist, primary clinician and a nutritionist is most effective. In addition, treatment with antidepressants is worthwhile. Both clinicians and parents should give special attention to both weight and diet in young women with amenorrhea.

Even in young amenorrheic adolescents of normal and above normal body weights, disordered eating patterns (fasting and purging) are often present, which is a sign of an underlying disorder [51].

Exercise and Amenorrhea

The awareness that competitive female athletes, along with women involved in strenuous recreational exercises and women involved in other forms of demanding activity such as ballet and modern dance have a significant incidence of menstrual irregularity and amenorrhea in a pattern known as hypothalamic suppression. The incidence has been underestimated because of lack of significance given to anovulatory cycles. As much as 2/3rd of recreational runners who have normal menstrual periods has short luteal phases or is anovulatory [52]. Also recreational runners who have normal menstrual periods show greater variability from cycle to cycle, often with decreased hormonal function [53]. If training starts before menarche, menarche can get delayed by as much as 3 years, with high subsequent incidence of menstrual irregularity. While in some women secondary amenorrhea is associated with delayed menarche, although training did not start until after menarche. There has been a suggestion that such girls with these characteristics may be socially influenced to pursue athletic training. Conversely exercise has little effect on the timing of puberty in boys. Although changes in testicular function can be shown in males, the changes are much subtler and less meaningful clinically [54].

There are two major influences, one a critical level of body fat and the effect of stress itself. Young women who weigh less than 110 lbs and lose more than 10 lbs while exercising are the women most likely to develop the problem an association which supports the critical weight hypothesis of Frisch [55,56]. The critical weight hypothesis states that the onset and regulation of menstrual function, necessitates maintaining weight above a critical level, and therefore above a critical amount of body fat. Dealing with the patients it is good to use the normogram which Frisch derived, based on the calculation of the amount of total body water as a percent of body weight. This relates to the percent of body fat, and therefore an index of fatness. The 10th percentile at age 16 is equivalent to about 22% body fat, which is the minimal weight for height necessary for sustaining menstruation, and the 10th percentile at age 13 is around 17% body fat, the minimum for initiating menarche. A loss of weight in the range of 10-15% of normal weight for height represents a loss of approximately one third of the total body fat which results in a drop below the 22% line and

may result in abnormal menstrual function [57]. Though the normogram is used to show the relationships to patients, there are individual variations, so that the normogram cannot be used to predict without fail the return of menses for any Individual patient. The accuracy of this normogram has been challenged by some [58]. These fat criteria were derived from the individual estimation of body fat from predicted total water, with a regression equation that employs height and weight. The most reliable as well as accurate method of identifying body fatness is hydrostatic weighing of body density, although dual energy x-ray absorptiometry (DEXA) is also excellent. One can hardly maintain a small pool for this purpose in a clinical outpatient office. Although it is agreed that the normogram and specifically the 22% body fat criterion are not absolutely accurate; this concept is still useful, and the normogram remains helpful to illustrate the concept to the patients.

The competitive female athlete has about 50% less body fat than the non-competitor, who is very much under the 10th percentile of secondary amenorrhea (the 22% body fatline). This change in body fat can occur with no discernible change in total body weight, because fat is converted to lean muscle mass [59]. Looking analytically at the critical weight hypothesis, it argues that there is no cause and effect relationship between body fat and menstrual function, but only a correlation [60,61]. Because of this considerable variation is seen with many examples of normal and abnormal menstrual function at all levels of body fat content. Still some correlation does exist and body fat content and body weight are useful guidelines for the relationship between menstrual function at all levels of body fat. The leptin story has restored credibility to the critical weight hypothesis. There had always been a mystery how total body fat can talk to the brain. This got resolved with the answer that leptin communicates to the brain regarding the fat status and thus controls reproduction.

Besides the role of fat, independent roles are played by stress and energy expenditure. Dancers are known to get return of menses during periods of rest despite no change in body fat [62]. Thus high energy output and stress each can act independently as well as additively, with low body fat to suppress reproductive function. Negative energy balance either due to low energy intake or increased energy expenditure leads to an inhibition of ovulatory menstrual function. Leptin levels are even lower in exercising amenorrheic women (which maybe even lower than expected due to lower body fat) [63]. Lower leptin levels can be triggered by both a reduction in body fat and

an increase in negative energy balance, which leads to suppression of reproduction along with thyroid function while simultaneously increasing brain adrenal activity. Running in the dark gets even more risky. Ovarian activity has been shown to be affected by strenuous activity as well as seasonal variations [64]. In autumn decreased ovarian activity could be related to a longer dark photoperiod, associated with increased pineal secretion of melatonin. Conception rates in women living in northern Scandinavia is higher during summer as compared to winter. Thus serious runners can expect to encounter more menstrual problems with autumn and winter.

This menstrual dysfunction is similar to the hypothalamic dysfunction which is more marked in the classic cases of AN. Acute exercise decreases gonadotropins, increases prolactin, growth hormone, testosterone, ACTH, the adrenal steroids and endorphins because of both increased secretion and reduced clearance [65]. The increase in prolactin is in contrast to no changes in undernourished women. The prolactin increases are variable, small in amplitude and very short in duration. Thus it is unlikely that increased prolactin is responsible for suppression of the menstrual cycle. Also insignificant differences are observed when amenorrheic runners are compared with eumenorrheic runners/nonrunners [66]. Also women athletes have daytime increase in melatonin levels, and amenorrheic athletes have an exaggerated nocturnal secretion of melatonin [67]. The nocturnal increase in melatonin is also seen in women with hypothalamic amenorrhea, and therefore appear to reflect the suppression of pulsatile GnRH secretion [68]. The other difference from undernourished women is in thyroid axis. Athletic women have relatively lowT4 levels, but amenorrheic athletes have a total suppression of all circulating thyroid hormones, which includes reverse T3 [69].

One suggestion given is that insufficient or suboptimal body fat adversely affects estrogen metabolism, leading to an increased conversion of biologically active estrogens to relatively inactive catechol estrogens [70]. The conversion of estradiol to catechol estrogens rapidly yield 2-hydroxy estrone and 4-hydroxy estrone, which are relatively inactive metabolites which are further metabolized to 2-methoxy estrogen and 4-methoxy estrogen by methylation. These products and this pathway is increased by physical exercise [71,72]. The extent of 2-hydroxylation correlates inversely with body fat, increasing with decreasing adiposity [59,73]. This could be a mechanism which interferes with the negative

feedback and local roles of estradiol in pituitary-ovarian interactions.

In runners the feeling of high or exhilaration and euphoria occurs after competition and an exhaustive workout. It is still not clear whether this is due to certain psychological factors or an increase in endogenous opiates or endorphins. The site from where GnRH is released, namely arcuate nucleus in the hypothalamus is rich in opioid receptors and endorphin production. Several evidences are there to prove that opioids inhibit GnRH secretion and thus subsequent gonadotrophins. Women who have been studied during a high endurance conditioning showed that there is a steadily increasing endorphin output after exercise [74-77]. Naltrexone a long acting opioid receptor blocker restores menstrual function, when administered long term to women with amenorrhea associated with weight loss, which indicates a key role of endorphins in stress related hypothalamic amenorrhea [78]. The measurements of circulating β - endorphin levels may not reflect the central mechanism as; both eumenorrheic as well as amenorrheic athletes have exercise induced increases in the blood levels of β -endorphin [77].

CRH directly inhibits hypothalamic GnRH secretion, possibly by augmenting endogenous opioid secretion [79,80]. Women with hypothalamic amenorrhea (both due to eating disorders or athletes) have hypercortisolism, both due to increased CRH and ACTH, which suggests this is the pathway by which stress interrupts reproductive function [11,81-82]. Amenorrheic athletes who have cortisol levels, which return to normal range, regain menstrual function within 6months, as compared to athletes who maintained their elevated cortisol levels and continue to be amenorrheic [83].

Amenorrheic athletes remain in a state of negative energy balance, further distinguished by elevated levels of insulin like growth factor binding protein(IGFBP1), increased insulin sensitivity, decreased insulin levels, IGF1 deficiency, and increased GH level [84,85]. Increase in IGFBP1 can limit IGF activity in the hypothalamus and thus provide another mechanism for suppression of GnRH secretion. A unifying hypothesis focuses on energy balance [61,63,85-88]. If available energy is excessively diverted, as in exercise or when insufficient, as in eating disorders, reproduction gets suspended to support essential metabolism for survival. Thus reproduction is not directly affected by the level of body fat; body fat is just a marker for the energy state. The circulating levels of reverse T3 are even a better marker of the energy state

than body weight, which demonstrates a correlation with the presence and absence of menstrual function [86].

The effect of leptin on reproduction can be seen as an additional factor for maintaining responses to stress. Weight loss is associated with increased adrenal response, decreased thyroid function; these changes occur, along with suppression of estrus cycle, occur in the fasted mice and get reversed by treatment with leptin [89]. The leptin levels in anorectics and bulimic individuals are markedly suppressed and although response to eating and fasting are blunted, leptin still serves as a signal to the brain, indicating the energy states available [90,91].

One question remains why is CRH elevated in stress amenorrhea specially that associated with weight loss as compared to fasting in normal and obese individuals. One reason given is that the decrease in leptin and increase in NPY associated with stress related weight loss is the expected response, but it is inadequate to suppress the stress induced increase in CRH. The blunted patterns in amenorrheic athletes support this. The increase in CRH and hypercortisolism resulting therein, further increases metabolism and weight loss. Athletes with cyclic, menses show a normal diurnal rhythm and leptin levels. But amenorrheic athletes do not have a diurnal pattern [92]. Both cyclic athletes and amenorrheic athletes have a low leptin level (3fold decrease) which correlates with reduced body fat, but the levels are further lowered by hyperinsulinemia and hypercortisolism. Additionally, amenorrheic athletes have a blunted leptin response to the increase in insulin following meals. An increase in menstrual irregularity and amenorrhea is correlated with a decrease in body fat below 15% of body weight and leptin levels that are <3ng/ml [93].

As there are high levels of leptin present in overweight people; the purpose of leptin may be limited to an effect at low levels. A low level of circulating leptin may serve as a signal, that stores are not sufficient for growth and reproduction. Thus these low levels would ordinarily stimulate hyperphagia, reduce energy expenditure, and suppress gonadotropin secretion and reproduction. The high levels of leptin and the apparent leptin resistance, which is associated with excess body weight and fat may then reflect not resistance, but a lack of physiological effect.

Regarding reproduction, the final pathway is suppression of GnRH, a response to multiple inputs indicating the availability of metabolic fuels [94]. Even in runners with regular menstrual patterns, LH pulsatile

frequency and amplitude are significantly reduced [95,96]. A central inhibition of GnRH can be discerned even before there is perceptible evidence of menstrual irregularity. The clinical presentation which includes inadequate luteal phase, anovulation or amenorrhea, depends on the degree of GnRH suppression. Insufficient estrogen secretion at a critical time of growth can impede the growth spurt and yield short stature.

The characteristics of women in this subculture of exercise and amenorrhea give a striking reminder of anorexia nervosa patients; significant physical exercise and necessity for control of body, striving for artistic and technical proficiency, and the consequent preoccupation with the body, combined with the stressful pressures of performing and competition [97,98]. Individuals with this lifestyle profile are prone to develop anorectic reactions [99]. Fries have described four stages of dieting behavior that can be a continuum [100]. i) Dieting for cosmetic reasons ii) Dieting due to neurotic fixation on food intake and weight iii) The anorectic reaction iv) True anorexia nervosa.

There are several important differences between the anorectic reaction and true AN. Psychologically patients with true AN has a misperception of reality and a lack of insight into the disease and her problem. She does not consider herself underweight and displays a lack of concern over her dreadful physical condition and appearance. Patients relationship with her clinician is difficult, with no visible emotional involvement along with a great deal of mistrust. While patient with the anorectic reaction have the capacity of self-criticism. They can see the problem and describe it with an insight and an absence of denial. The exercising woman or a competing athlete or a dancer can develop an anorectic reaction. The anorectic reaction develops consciously and voluntarily just as in AN, as the exercising woman deliberately makes an effort to reduce body weight. The clinician may be the first to be aware of the problem, having had encountered the patient because of the presenting complaint either of amenorrhea or now uncontrolled weight loss. Early recognition, concentrated counseling, and confidential support can intercept and prevent a progressive problem. The high prevalence of eating disorders in female athletes indicates that the threat of progression should not be underrated [98]. The prevalence in general population, not only athletes, of the early or partial disorder is twice that of the full syndrome, and progression from Pathological dieting to the full syndrome can occur in some individuals in as short a period as 1-2yrs [101].

The prognosis is excellent with early treatment and simple weight gain can reverse the state of amenorrhea. The degree of reversibility is unknown, although general experience indicates that the majority of women regain ovulation when stress or exercise diminish or cease [102,103]. Elite gymnasts who have delayed their pubertal growth spurt achieve their genetically determined height by undergoing a late acceleration of growth [104]. However, these patients are unwilling to change their routines of exercise and a sensitive clinician can perceive that the exercise is an important means of coping with the daily life. Hormonal therapy is therefore encouraged for these hypo estrogenic patients to protect against the loss of bone and cardiovascular changes. However, in a patient with eating disorder, restoration of normal hormonal levels is not sufficient to return bone density to normal; resumption of an adequate diet and weight gain are essential [105-107]. When pregnancy is desired, a reduction in the amount of exercise and a gain in weight is recommended or induction of ovulation must be pursued.

Genetic Defects

Hypothalamic idiopathic hypogonadotropic hypogonadism (IHH): IHH comprises of a group of disorders with patients presenting with delayed or absent pubertal development, either due to a GnRH mutation or a GnRH deficiency, in which hypothalamic/pituitary imaging is normal. Usually it affects males, but can present in women as a cause of primary amenorrhea occasionally. The most common phenotypic association is anosmia in Kallmann's syndrome (KS). Normally there is a shared embryonic development of GnRH neurons and olfactory neurons. Causes of IHH, may be due to mutation in KAL-1 gene [108], the FGF1 receptor [109], FGF8 [110], the CHD7 gene [111], gene encoding semaphorin 3A [112], semaphorin 7 [113], besides the genes encoding the prokineticin-2 and its receptor that is prokineticin receptor-2 [108-114, reviewed in 115]. Also genetic defects in GnRH secretion as well as function can be associated with normosmia, in IHH cases. Kisspeptin being a known regulator of GnRH secretion, mutations of genes like KISS1 and KISS-1R, which encode kp and its receptor, lead to hypogonadism [116,117]. Similarly, mutations of leptin, its receptor along with mutations in the prohormone convertase 1 gene lead to severe hypogonadotropic hypogonadism along with severe obesity [118-120]. Other causes like GnRH receptor mutations, lead to HH, [121,122]. Additionally, mutations in genes like TAC3 and its receptor TAC3R which code for neurokinin cause IHH [123]. In addition, genes like Axl

[124, HESX3 [125], TSHZ1 [126] are newer additions as the causative factors of IHH]. The identification of zinc finger homeodomain factor teashirt finger family member 1 (TSHZ1), a key regulator of mammalian olfactory bulb (OB) development not only in mice but also in humans as revealed by Ragancokoya et al [126] has given some answers to a lot of unanswered questions regarding role of PROK2 ligands acting through their G protein coupled receptors PROKR2 regarding how their mutations cause both KS and IHH [115,127-129]. Although HESX3 has been another gene its mutations have currently been seen only in male cases of KS. Axl is another gene found to be involved in the migration of GnRH neurons.

In a study of 55 women with FHA, 13% were found to be having heterozygous mutations, in genes associated with IHH, as compared to no mutations in 422 controls [127]. Thus FHA results from an interplay between environmental as well as genetic factors, where inherited defects in GnRH biology may lower the threshold at which external stressors suppress the HPO axis [130]. Pituitary Mutations in genes encoding transcription factors involved in the cellular proliferation and differentiation of the pituitary gland e.g. HESX1 [131]/GLI2 [132] and SOX3 [133] mutations, have been shown to be associated with hypopituitarism, while mutations in SOX2 [134], LHX3 [135], LHX4 [136] and PROP1 [137], may be responsible for gonadotropin deficiency. Other associated factors with the mutations may present with other clinical features helping in the diagnosis. An e.g. of this is SOX2 mutations which may result in anophthalmia, while mutations in LHX3 have been known to be associated with a rotationally limited cervical spine [135].

Shaw et al retrospectively studied 248 female patients of IHH from 1980 to 2010 seen in Massachusetts general hospital. The clinical presentation varied from primary amenorrhea and absence of any sexual characteristics to spontaneous breast development and occasional menses. In this cohort rare sequence variants were present in all known genes associated with GnRH deficiency, including novel identification of GnRH deficient women with KAL 1 variants. They concluded that the pathogenic mechanism through which KAL 1 variant disrupts female reproductive development requires further investigation [138].

Management of HA

IHH in women (as in men) is treated with sex hormones. Initially low dose estradiol (1mg) is begun in

order to develop sexual characteristics and then it is gradually increased in doses [139-142]. From the second year of treatment estrogen supplementation with chlormadinone acetate is done [140-142]. Desire for pregnancy warrants pulsatile GnRH therapy to stimulate production of serum FSH and LH [143,144]. Further the importance of GnRH administration should be intermittent and pulsatile to be able to restore activity of the reproductive axis in patients with HA and other disorder of GnRH deficiency was emphasized by Knobil et al [145]. Tonic exposure of GnRH inhibited pituitary gonadotropin secretion paradoxically [146]. Although a chance of success is good, where GnRH is not available, LH or FSH can be administered alternatively [147].

Other causes like GnRH receptor mutations, lead to HH, which typically do not respond to usual doses of pulsatile GnRH administration, though successful conception with high dose pulsatile GnRH has been reported [148].

Just like in male subjects Abel et al repeated the study in 37 IHH women treated with i/v pulsatile GnRH therapy (75ng/kg/bolus) (retrospective study (1980-2012), all patients over 16yrs with 46% anosmic and tested for all 14 genes and found that during first cycle 60% (22/37) recreated normal cycles, 30% (12/37) demonstrated altered gonadotropin response indicating pituitary resistance and 10% (3/37) an exaggerated FSH response consistent with ovarian resistance. Mutations in CHD7, FGFR1, KAL1, TAC3, TACR3 were documented in IHH women with normal cycles, whereas mutations were identified in GNRHR, PROKR2 and FGFR1 in those with pituitary resistance. Women with ovarian resistance were mutation negative. Thus they concluded that although physiological replacement with GnRH recreates normal menstrual dynamics in most IHH ladies [149], Hypogonadotropic responses in first week of treatment identify a subset of women with pituitary dysfunction, only some of whom have GNRHR mutations. IHH women with hyper gonadotropic responses to GnRH replacement, consistent with an additional ovarian defect did not have mutations in genes known to cause IHH similar to their findings in men with evidence of an additional testicular defect. Hence they hypothesized that identification of women with abnormal responses to physiological GnRH replacement would give greater insight into the pathogenesis of HH. Hyper/hypogonadotropic responses would implicate an ovarian/pituitary defect, respectively. Such findings would suggest that genes involved in gonadotropin or ovarian development and function that are also expressed in the hypothalamus should be given greater consideration in the search for new IHH genes.

High dose kisspeptin 54(kp-54) acutely stimulates LH secretion in women of hypothalamic amenorrhea (HA) but chronic administration is associated with desensitization [150,151, reviewed in ref 152]. Since GnRH has paradoxical effects on reproductive activity Jayasena et al hypothesized that there maybe a dose dependent therapeutic window with in which kp treatment restores the GnRH/LH pulsatility in a woman with HA. They examined 5 patients with HA, with each having 6 assessments of LH pulsatility. Single blinded continuous iv infusion of vehicle or kp54 (0.01,0.03,0.1,0.3,1nmol/kg/h) was administered. LH pulses were monitored subsequently.

Kp restored LH pulsatility in all patients with HA, with peak responses observed at different doses in each patient. The mean peak number of pulses during kp 54 infusion was 3 fold higher as compared to vehicle (number of LH pulses/8h; 1.6 ± 0.4 , vehicle; and 5 ± 0.5 with kp54, $p < 0.01$ vs vehicle). The mean peak LH pulse secretory mass during kp54 was 6-fold higher LH as compared to vehicle (pulse secretory mass in $\mu\text{g}/13.92 \pm 2.31$ for vehicle; and 23.44 ± 12.59 with kp54 $p < 0.05$ vs vehicle). Hence they concluded that kp54 infusion temporarily increase LH pulsatility in women with HA. They also determined the dose range within which kp54 treatment increases basal and pulsatile LH secretion in women having HA. Thus this forms the basis for studying the potential of kp based therapies to treat women with HA [153]. Since a distinct combination of participants such as leptin deficiency, weight loss, exercise, stress and mutations of GnRH associated genes cause HA, in each of the 5 participants, etiological differences may have accounted for heterogeneity of individual response to kp54 administration [153].

Although TAC3/TAC3R mutations also are associated with IHH administration of NKB was not accompanied by increase in serum LH and testosterone levels, hence role of NKB doesn't appear to be useful in treating these patients presenting with TAC3/TACR3 mutations [154] however since KISS1 appears to be downstream of NKB signaling, pulsatile gonadotropin secretion is restored by kisspeptin administration to patients with HH secondary to NKB and or its receptor [155,156].

Conclusion

Hypothalamic Amenorrhea is one of the most common causes of amenorrhea in women of reproductive age, accounting for almost 50% cases of secondary amenorrhea, while the genetic causes account for a lower

percentage of cases of HA presenting as primary amenorrhea. HA is a heterogeneous condition with main characteristics being reduced pulsatile GnRH secretion which is evidenced as reduced circulating LH levels along with decreased frequency and amplitude of LH pulsatility. It has been observed in 10% of female athletes. Besides that, affected patients may have more than one etiological factor like low body weight, excessive exercise as well as stress. Other important causes such as heterozygous mutations in genes associated with GnRH migration or GnRH secretion maybe responsible for congenital GnRH deficiency whose presentation is also as HA. Estrogen supplementation provides relief which is just symptomatic, protecting the patients from osteoporosis as well as develops the secondary sex character in patients presenting as primary amenorrhea. For fertility restoration, hypothalamo-pituitary function needs to be restored. Because of poor functional estrogen status these patients do not respond with a progesterone withdrawal bleed and respond badly to clomiphene citrate, which decreases the estradiol mediated negative feedback [157]. IVF involves a lot of cost and these patients respond to recombinant FSH with inadequate E2 production [158]. Thus pulsatile s/c administration remains the treatment of choice in women desiring pregnancy. But since infusion pumps requiring GnRH administration intermittently are not available in all countries Kp offers a better alternative once its efficacy and full therapeutic window has been accurately worked out, besides being very effective in Kp/GPR54 mutations as well as neurokinin deficiency as it acts downstream of Kp. Emphasis on weight increase and correcting the aetiological factors remains the mainstay in patients presenting with functional HA as secondary amenorrhea and factors which will increase intake as well as reduce energy expenditure will go a long way in correcting the GnRH and thus LH pulsatility. Only if these patients require immediate fertility is hormonal therapy indicated along with ovulation induction.

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