

Neuroendocrine Causes of Amenorrhea—An Update

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Context: Secondary amenorrhea—the absence of menses for three consecutive cycles—affects approximately 3–4% of reproductive age women, and infertility—the failure to conceive after 12 months of regular intercourse—affects approximately 6–10%. Neuroendocrine causes of amenorrhea and infertility, including functional hypothalamic amenorrhea and hyperprolactinemia, constitute a majority of these cases.

Objective: In this review, we discuss the physiologic, pathologic, and iatrogenic causes of amenorrhea and infertility arising from perturbations in the hypothalamic-pituitary-adrenal axis, including potential genetic causes. We focus extensively on the hormonal mechanisms involved in disrupting the hypothalamic-pituitary-ovarian axis.

Conclusions: A thorough understanding of the neuroendocrine causes of amenorrhea and infertility is critical for properly assessing patients presenting with these complaints. Prompt evaluation and treatment are essential to prevent loss of bone mass due to hypoestrogenemia and/or to achieve the time-sensitive treatment goal of conception. (*J Clin Endocrinol Metab* 100: 812–824, 2015)

Secondary amenorrhea (the absence of menses for three consecutive cycles) and infertility (the inability to conceive after 12 months of regular intercourse) are common complaints evaluated by primary care physicians, obstetrician/gynecologists, and endocrinologists. Over 50% of cases of secondary amenorrhea result from perturbations in the hypothalamic-pituitary-adrenal (HPA) axis (1). An understanding of the neuroendocrine causes of amenorrhea and infertility is therefore critical when evaluating patients presenting with these complaints in order to implement the most appropriate treatment regimen. We review the physiological, pathological, and iatrogenic causes of neuroendocrine-associated amenorrhea and infertility, including genetic causes, and the hormonal mechanisms responsible for the perturbations in the hypothalamic-pituitary-ovarian (HPO) axis (Figure 1).

Physiological Causes of Amenorrhea

Functional hypothalamic amenorrhea (FHA)

FHA, or stress-induced anovulation, is one of the most common causes of secondary amenorrhea (1), and it accounts for the reproductive dysfunction seen in undernutrition, excessive exercise, severe emotional stress, and chronic disease. From a teleological standpoint, in the face of nutritional or physical stress, it is adaptive for an organism to allocate energy resources for its own survival rather than the costly process of reproduction, and therefore FHA is a physiological response to environmental and physical stressors.

Hormonal mediators of FHA

HPA axis. During periods of physical, nutritional, or extreme emotional stress, the HPA axis is activated and inhibits the HPO axis at multiple levels (Figure 2). At the

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Abbreviations: CAH, congenital adrenal hyperplasia; CTLA-4, cytotoxic T-lymphocyte antigen 4; FGF-21, fibroblast growth factor-21; FHA, functional hypothalamic amenorrhea; HPA, hypothalamic-pituitary-adrenal; HPO, hypothalamic-pituitary-ovarian; IHH, idiopathic hypogonadotropic hypogonadism; NC21OHD, nonclassic 21-OH deficiency; 21-OH, 21-hydroxylase.

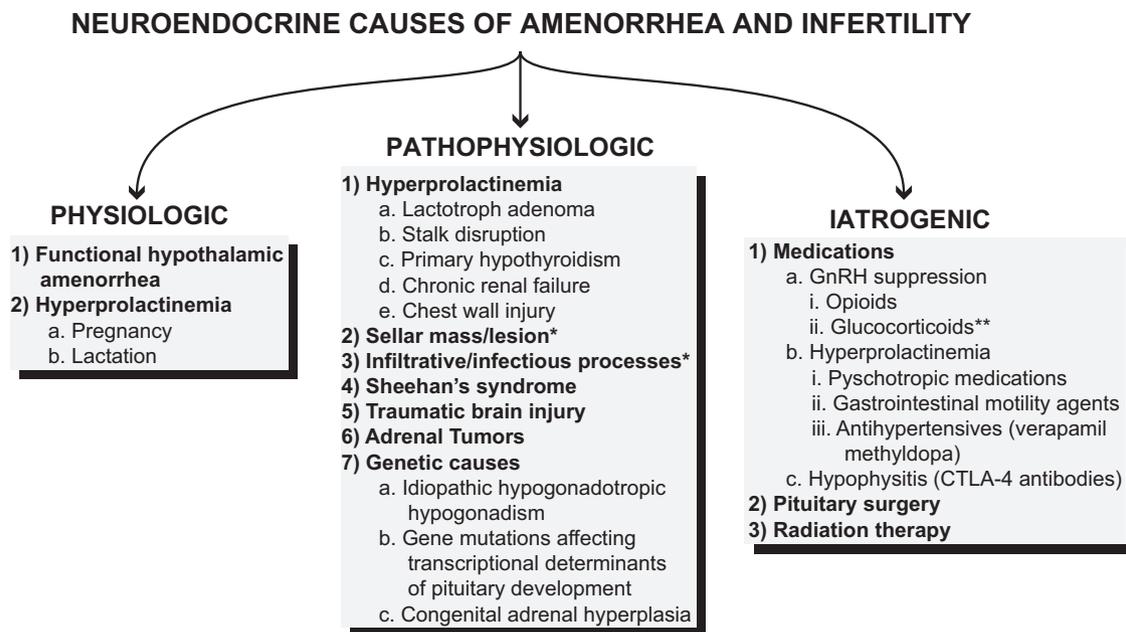


Figure 1. An overview of neuroendocrine causes of amenorrhea and infertility, including physiological, pathophysiological, and iatrogenic etiologies. *, Possible causes of sellar masses and lesions, including infiltrative and infectious processes, are listed in Table 1. **, In humans, high-dose glucocorticoids may also decrease the responsiveness of the pituitary to GnRH stimulation (7).

level of the hypothalamus, CRH suppresses GnRH secretion. In normally cycling women, an infusion of CRH markedly decreases FSH/LH secretion, but GnRH administration prevents this decrease, suggesting that CRH acts by inhibiting GnRH secretion (2). At the level of the pituitary, ACTH has been shown to have negative reproductive effects in mice. In adrenalectomized mice maintained on physiological doses of glucocorticoid, ACTH administration for 10 days results in an absence of corpora lutea (3), demonstrating that ACTH has reproductive effects independent of adrenal steroid production.

Women with FHA also have higher 24-hour mean plasma cortisol levels compared to controls (4), and cortisol, the end-product of the HPA axis, suppresses reproductive function at the hypothalamic, pituitary, and uterine levels. In rhesus monkeys, supraphysiological doses of hydrocortisone suppress gonadotropin secretion, but this suppression is almost completely reversed by intermittent GnRH infusion, suggesting that cortisol suppresses GnRH secretion (5). Women with Cushing's disease and women on long-term supraphysiological prednisolone therapy have a reduced LH response to GnRH (6, 7), suggesting that glucocorticoids also suppress the responsiveness of pituitary gonadotrophs to hypothalamic input. Glucocorticoids also inhibit the effects of estradiol on the uterus. Dexamethasone coadministered with estradiol attenuates the expected increase in uterine weight seen with estradiol alone, at least in part by reducing estrogen receptor concentra-

tion (8). Therefore, cross talk between the HPA and HPO axes promotes the development of amenorrhea as a functional adaptation to stress.

Leptin. Levels of leptin, a hormone secreted by adipocytes, are low in women with FHA (9, 10), which may be a mediator of amenorrhea. Two studies have investigated the effects of recombinant methionyl human leptin (rh-leptin) in women with FHA. An open-label study found that LH-pulse frequency increased after 2 weeks of treatment with rhleptin (11). In a randomized placebo-controlled study, significantly more women treated with rhleptin had a menstrual bleed, half of which were associated with ovulation (12). Although the exact mechanism by which decreased leptin levels cause anovulation is not known, low leptin levels may suppress GnRH through a kisspeptin-mediated pathway (13).

Insulin. Changes in body weight—both weight loss and weight gain—may affect fertility. Significant weight loss due to chronic undernutrition is a cause of FHA and is characterized by low insulin levels, whereas significant weight gain may result in obesity and insulin resistance—a state of functional hypoinsulinemia. Importantly, in animal models, insulin has been shown to modulate the HPO axis, and therefore low or functionally low insulin levels may be a mediator of infertility. In a mouse model, females with neuron-specific disruption of the insulin receptor had a significantly reduced antral follicle count due to a 90%

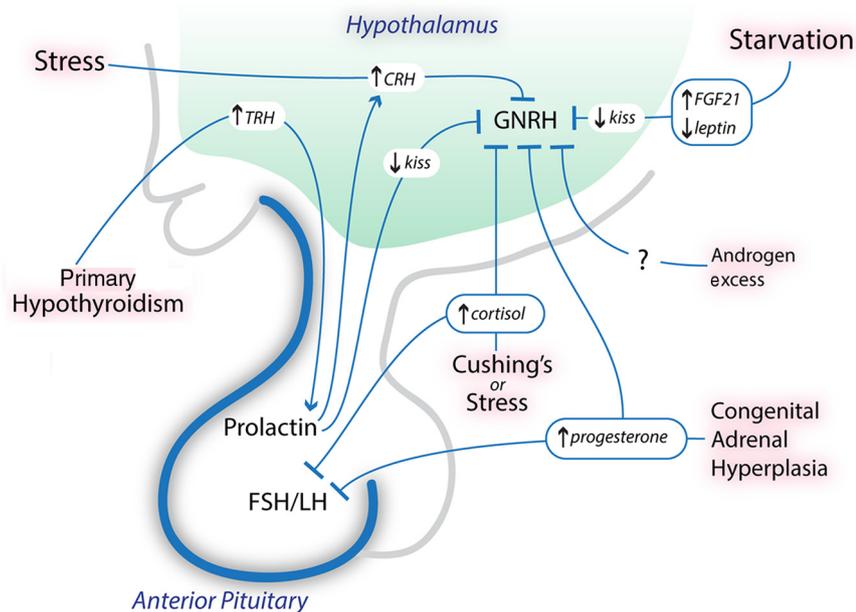


Figure 2. Potential hormonal mediators of amenorrhea and infertility. Physical, emotional, or nutritional stress may increase CRH levels, which suppress GnRH release. Cortisol levels, which increase in the setting of stress and Cushing's syndrome, also suppress GnRH release and likely decrease LH responsiveness to GnRH. Starvation results in low leptin levels and increased FGF-21 levels in animal models; the low leptin levels and increased FGF-21 levels likely suppress GnRH release by decreasing kisspeptin expression. In primary hypothyroidism, TRH levels are increased and stimulate prolactin release. Hyperprolactinemia suppresses GnRH release by decreasing kisspeptin expression, and possibly through a CRH-mediated pathway. High androgen and progesterone levels may be seen in CAH, and these hormones likely suppress GnRH release. kiss, kisspeptin.

reduction of circulating LH (14), demonstrating the importance of insulin signaling in fertility.

Fibroblast growth factor-21 (FGF-21). FGF-21 is a liver-derived hormone whose production is up-regulated in response to starvation (15, 16). Recently, FGF-21 was shown to be a potential mediator of starvation-induced amenorrhea (17). FGF-21 transgenic mice are anovulatory, with low LH levels, and administration of GnRH, but not exogenous gonadotropins, elicits an ovulatory LH surge, demonstrating that FGF-21 acts at the level of the hypothalamus to disrupt ovulation (17). FGF-21 transgenic mice have decreased *Kiss-1* gene expression in the anteroventral periventricular nuclei of the hypothalamus, and the product of *Kiss-1*, kisspeptin, is a potent stimulator of GnRH secretion, suggesting a mechanism by which FGF-21 disrupts ovulation (17). Whether FGF-21 is a mediator of FHA in humans is unknown.

Hyperprolactinemia

Hyperprolactinemia—the most common cause of pituitary-associated amenorrhea—may be due to physiologic or pathophysiologic causes. The exact mechanism by which hyperprolactinemia causes hypogonadotropic hy-

pogonadism is not known, but hyperprolactinemic women have reduced LH-pulse frequency and reduced LH responsiveness to estrogen (18), suggesting that GnRH suppression may be a key factor. This is supported by the fact that in hyperprolactinemic, amenorrheic women, treatment with pulsatile GnRH results in follicular maturation and ovulation when coupled with a human chorionic gonadotropin trigger (18). CRH (19) and kisspeptin may be important mediators of prolactin-induced GnRH suppression. In rodent models, prolactin-receptor mRNA has been localized to kisspeptin neurons in the hypothalamus (20), and kisspeptin administration to hyperprolactinemic female mice increases circulating gonadotropin levels and restores ovulation (21). In vitro, prolactin inhibits granulosa cell function (22), and therefore prolactin may also act by directly disrupting ovarian function.

Although most women with hyperprolactinemia are amenorrheic, some have ovulatory menstrual cycles. These women may still suffer from infertility due to a short luteal-phase defect (23), and therefore treating hyperprolactinemia may be important even in regularly menstruating women. Importantly, a number of physiological causes of hyperprolactinemia and amenorrhea—including pregnancy—do not warrant treatment and therefore should be excluded before initiation of treatment.

Physiological causes of hyperprolactinemia

Pregnancy. Pregnancy is the most common cause of hyperprolactinemia. Prolactin levels increase during pregnancy and peak at delivery, with levels reaching as high as 600 ng/mL (24). In women who do not nurse, prolactin levels usually decrease during the first 72 hours postpartum (24, 25) and normalize by 3 weeks (25).

Lactation. In nursing women, suckling increases prolactin levels, although this usually subsides by 12 weeks due to the postpartum drop in estradiol levels resulting in decreased lactotroph hyperplasia (24). Lactational amenorrhea is the result of a number of factors, including maternal nutritional status relative to the frequency/intensity of

nursing (26) and hyperprolactinemia. Prolactin levels, their response to suckling, and prolactin bioactivity are all associated with duration of amenorrhea (27–29), and like prolactin-induced amenorrhea, lactational amenorrhea is associated with reduced LH-pulse amplitude and frequency (30) and reduced LH responsiveness to estrogen (31). Moreover, administration of pulsatile GnRH to women with lactational amenorrhea stimulates follicle maturation, ovulation, and luteal-phase function (30), suggesting that GnRH suppression may be a causative factor.

Macroprolactinemia. Macroprolactinemia is another cause of physiological hyperprolactinemia. The predominant form of prolactin is a 23-kD form, but some individuals may have a significant percentage of circulating prolactin consisting of a higher molecular mass form called macroprolactin. Macroprolactin is thought to be either a complex consisting of the 23-kD form and an immunoglobulin (32) or a complex consisting of glycosylated prolactin aggregates (33). Macroprolactin is cleared from the circulation more slowly than the 23-kD form (34), thereby causing hyperprolactinemia, but it is also less bioactive, supporting the observation that macroprolactinemia is usually asymptomatic (35). Importantly, up to 25% of individuals with hyperprolactinemia may have macroprolactinemia (36, 37), and therefore evaluation for macroprolactinemia in asymptomatic individuals may be warranted to prevent unnecessary treatment. Macroprolactin can be detected through gel-filtration chromatography or polyethylene glycol precipitation (38).

Pathophysiological Causes of Amenorrhea

Pathophysiological causes of hyperprolactinemia

As mentioned above, there are both physiologic and pathophysiologic causes of hyperprolactinemia. Pathophysiologic causes of hyperprolactinemia typically result in amenorrhea and/or infertility in premenopausal women, and therefore an elevated prolactin level requires prompt evaluation (Figure 3). Pathophysiologic causes of hyperprolactinemia include lactotroph adenomas, stalk disruption, primary hypothyroidism, renal failure, and chest wall injury.

Lactotroph adenomas

Prolactin-secreting adenomas (lactotroph adenomas) are the most common subtype of secretory pituitary adenoma. These tumors are usually benign, and prolactin levels typically correlate with tumor size. Individuals with large adenomas can have prolactin levels on the order of 10^4 ng/mL

(39), yet with poorly differentiated or cystic lesions, prolactin levels will be lower than expected based on size.

Stalk disruption

Dopamine is produced by neurons in the arcuate nucleus of the hypothalamus and tonically suppresses pituitary prolactin production. Any disruption of the stalk connecting the hypothalamus to the pituitary can prevent the flow of dopamine into the pituitary gland and result in hyperprolactinemia and amenorrhea. Large sellar masses (Table 1) or traumatic injuries are common causes of stalk disruption.

Primary hypothyroidism

TRH, secreted by the hypothalamus, not only stimulates release of TSH by the pituitary but also stimulates prolactin release (40). Individuals with primary hypothyroidism have a heightened prolactin response to TRH, resulting in greater prolactin secretion in response to TRH stimulation (41); therefore hyperprolactinemia and resultant amenorrhea can be a consequence of primary hypothyroidism (42). Primary hypothyroidism may also lead to significant enlargement of the pituitary gland due to thyrotroph hyperplasia and likely lactotroph hyperplasia, but treating the hypothyroidism should result in regression of the hyperplasia (43) and normalization of the prolactin level (44).

Chronic renal failure

Chronic renal failure may result in hyperprolactinemia due to both decreased renal clearance and increased secretion of prolactin. The latter may be the result of reduced lactotroph responsiveness to dopamine suppression (45). Although liver disease was once posited as a common cause of hyperprolactinemia (46), recent reports suggest that it is a very rare cause of hyperprolactinemia (47).

Chest wall injury

Traumatic chest wall injury is a rare potential cause of hyperprolactinemia (48). In a patient with hyperprolactinemia after a severe burn to the chest wall, intercostal nerve blockade resulted in normalization of her serum prolactin level (48), suggesting that a neurogenic stimulus at the site of injury was responsible.

Non-lactotroph adenomas and sellar masses

Any sellar mass or lesion (Table 1) can cause amenorrhea. Mechanisms of amenorrhea include hyperprolactinemia due to stalk disruption and/or compression of the pituitary gonadotrophs, particularly when the lesion is ≥ 1 cm. In the case of functioning adenomas, hormonal effects may also play a role in the development of amenorrhea.

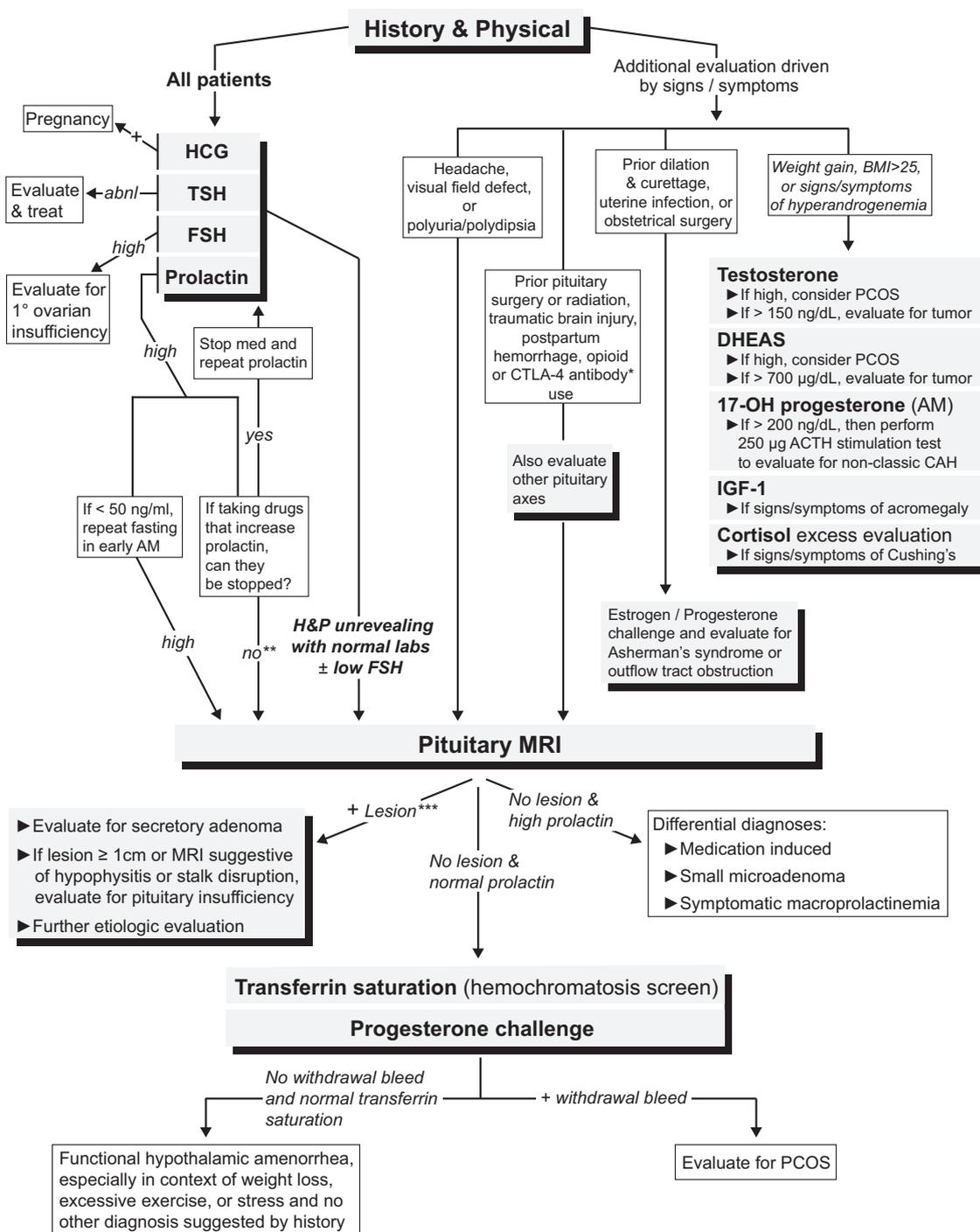


Figure 3. A suggested diagnostic algorithm for evaluating patients with secondary amenorrhea, including non-neuroendocrine causes. Importantly, all patients presenting with secondary amenorrhea should have a history and physical examination performed and a basic laboratory evaluation consisting of serum human chorionic gonadotropin, TSH, FSH, and prolactin levels. *, Individuals who have been prescribed CTLA-4 antibody therapies (ipilimumab or tremelimumab) should have an MRI with pituitary cuts to evaluate for hypophysitis. **, Unless the cause of the hyperprolactinemia can be definitively attributed to the medication (for example, by stopping the medication and repeating the prolactin level), it is difficult to exclude the possibility of a prolactinoma or stalk disruption (due to a sellar lesion) causing the hyperprolactinemia. ***, If the lesion is small (<1 cm) and is found to be nonsecretory, we would recommend following the “No lesion and normal prolactin” pathway because this lesion is unlikely to be the cause of the amenorrhea. Abbreviations: abnl, abnormal; BMI, body mass index; DHEAS, dehydroepiandrosterone sulfate; PCOS, polycystic ovary syndrome; 17-OH progesterone, 17-hydroxyprogesterone; H&P, history and physical.

Cushing’s disease

Amenorrhea is a common finding in women with ACTH-secreting adenomas (Cushing’s disease). In a series of 45 women with Cushing’s disease, 33% were amenor-

rheic, and amenorrhea was associated with higher mean serum cortisol levels and lower estradiol and SHBG levels (49). Although adrenal androgen levels are typically elevated in Cushing’s disease, amenorrhea was not associated

Table 1. Sellar Masses and Lesions (128, 129)**Benign and malignant neoplastic processes**

Pituitary adenoma
 Craniopharyngioma
 Chordoma
 Metastatic disease
 Meningioma
 Germ cell tumor
 Ependymoma
 Granular cell tumor
 Hemangioma
 Primary lymphoma
 Sarcoma
 Chondrosarcoma
 Osteosarcoma
 Fibrosarcoma
 Carcinoma of sinus origin
 Pituicytoma
 Papilloma
 Schwannoma

Cystic lesions

Rathke's cleft cyst
 Arachnoid cyst
 Dermoid cyst
 Epidermoid cyst
 Suprasellar cyst
 Mucocele

Vascular lesions

Aneurysm
 Arteriovenous fistula of cavernous sinus

Infiltrative and infectious processes

Abscess
 Hypophysitis
 Lymphocytic
 Granulomatous
 Xanthomatous
 IgG4-related disease
 CTLA-4 antibody-related
 Sarcoidosis
 Fibrous dysplasia
 Hemochromatosis
 Langerhans cell histiocytosis
 Pituitary hyperplasia
 Sphenoid sinusitis
 Tuberculosis
 Cysticercosis

with serum androgen levels or free androgen index (49). Therefore, amenorrhea in Cushing's disease is more likely mediated by suppression of GnRH by cortisol (49) rather than hyperandrogenemia.

Acromegaly

Amenorrhea and infertility are also common findings in women with GH-secreting adenomas (acromegaly). In a study of 47 women with acromegaly, 62% had amenorrhea, which was associated with higher GH levels and lower LH and estradiol levels (50). As with any sellar mass, compression of pituitary gonadotrophs, or hyperprolactinemia—either due to cosecretion of prolactin by the tumor or stalk disruption—may be important in the development of amenorrhea, but another potential cause

may be increased androgen bioavailability due to decreased SHBG levels (50). In normally cycling women, T administration reduces LH-pulse frequency, suggesting a mechanism by which increased androgen activity may mediate amenorrhea (51). In men, GH excess may also have a direct effect on gonadal function, suggested by the fact that men with acromegaly have significantly lower semen parameters compared to controls, and post-treatment decreases in IGF-1 levels correlate with increases in total sperm motility (52); therefore, a direct gonadal effect may potentially play a role in the development of amenorrhea as well.

Thyrotroph adenomas

Amenorrhea or oligomenorrhea may also be a presenting finding in women with TSH-secreting adenomas (53), and hyperthyroidism may be a contributing factor. Compared to euthyroid controls, hyperthyroid women with amenorrhea have higher SHBG, FSH, LH, and estradiol levels (54) but do not have a midcycle LH peak, suggesting that amenorrhea results from anovulation due to the failure of estrogen to stimulate LH release (55).

Infiltrative processes

Any inflammatory or infectious disease can infiltrate the hypothalamic-pituitary axis, resulting in anterior pituitary dysfunction and hypogonadotropic hypogonadism. Metastases—most commonly from primary tumors of the breast or lung—may also invade the hypothalamic-pituitary axis and cause amenorrhea (56).

Hypophysitis

An inflammatory infiltrate in the pituitary gland (hypophysitis) typically results in hypopituitarism due to destruction of cells in the anterior pituitary. Hypophysitis may also cause hyperprolactinemia due to stalk disruption, and therefore there are multiple mechanisms by which amenorrhea may occur. The two most common types of hypophysitis are lymphocytic and granulomatous hypophysitis. Lymphocytic hypophysitis, occurring primarily in females, is commonly diagnosed during pregnancy or postpartum, and a majority of patients develop anterior pituitary dysfunction (57). Granulomatous hypophysitis—characterized by granulomas formed by multinucleated giant cells—may occur in the setting of systemic diseases, including Wegener's granulomatosis (58).

IgG4-related disease is a more recently described cause of hypophysitis characterized by an infiltrate consisting of IgG4-positive plasma cells (59). This entity has been associated with hypogonadotropic hypogonadism (59), although it predominantly affects males and individuals >50 years old and therefore is a rare cause of amenorrhea.

Granulomatous diseases

Sarcoidosis and tuberculosis may also cause amenorrhea. In sarcoidosis, hypogonadotropic hypogonadism is usually due to granulomatous infiltration of the hypothalamus, although granulomas may also invade the pituitary and infiltrate gonadotroph cells (60). In addition, both intrasellar tuberculomas and tuberculous meningitis have been associated with amenorrhea (61–63). A study of 49 patients with a history of tuberculous meningitis in childhood found that 10% developed gonadotropin deficiency, likely due to progressive hypothalamic scarring (63).

Hemochromatosis

Although hereditary hemochromatosis is an important cause of hypogonadism in men due to iron deposition in the pituitary gonadotrophs and/or testes, it rarely causes hypogonadism in women (64), likely because menstrual bleeding protects against iron deposition.

Langerhans cell histiocytosis

Langerhans cell histiocytosis is characterized by the inappropriate proliferation and infiltration of a type of dendritic cell into various regions of the body including the hypothalamic-pituitary axis. Although typically associated with diabetes insipidus, hypogonadotropic hypogonadism has been reported (65).

Sheehan's syndrome

During pregnancy, the pituitary gland enlarges due to estrogen stimulation of lactotroph cells (66). This enlarging tissue may compress the superior hypophyseal artery—a major source of blood for the anterior pituitary—making the gland vulnerable to changes in blood supply. Women with significant peripartum blood loss may develop ischemic necrosis of the pituitary gland, resulting in hypopituitarism. This form of hypopituitarism, referred to as Sheehan's syndrome, is usually seen in cases of significant hemorrhage, although rare cases have been reported in individuals with minimal postpartum bleeding (67), and therefore pituitary autoimmunity has been hypothesized to play a role in the development of anterior pituitary dysfunction (68).

Sheehan's syndrome is now less commonly seen in developed countries due to improvements in the management of peripartum hemodynamic complications; however, recent reports suggest that its incidence in the developed world may be greater than previously thought (69). A retrospective study from France demonstrated a mean delay of 9 years before diagnosis (70), suggesting that the diagnosis is often overlooked in developed countries.

Traumatic brain injury

Amenorrhea may also present after traumatic brain injury or subarachnoid hemorrhage. Importantly, even mild head injuries—those not requiring hospitalization—can result in hypogonadism (71). Loss of pituitary function may be delayed and can present 12 months after the initial insult (72).

Adrenal tumors

Amenorrhea is frequently observed in women with glucocorticoid and/or androgen-secreting adrenal neoplasms. As mentioned above, the elevated cortisol levels produced by glucocorticoid-secreting tumors likely suppress GnRH secretion, thereby causing amenorrhea (5). Neoplasms producing androgens alone are exceedingly rare, and approximately 50% are malignant (73). Commonly presenting symptoms include hirsutism, acne, and oligomenorrhea (73). In women with normal menstrual cycles, T reduces LH-pulse frequency (51), suggesting a mechanism by which hyperandrogenemia may cause oligomenorrhea.

Genetic causes of neuroendocrine-associated amenorrhea

Hypothalamus: idiopathic hypogonadotropic hypogonadism (IHH)

IHH is a heterogeneous group of disorders in which individuals have delayed or absent pubertal development—typically due to GnRH deficiency or a GnRH receptor mutation—coupled with normal anatomic findings on hypothalamic/pituitary imaging. Although IHH predominantly affects males, it is a cause of infertility in women, typically presenting as primary amenorrhea.

There are a number of associated phenotypic findings in individuals with IHH, the most common of which is anosmia (Kallman's syndrome). Genetic mutations that impair neuronal migration commonly result in concurrent hypogonadism and anosmia due to the shared embryonic development of GnRH and olfactory neurons. In recent years, a number of genetic mutations that cause Kallman's syndrome have been discovered. These include mutations in the *KAL1* gene (74), the FGF-1 receptor (75), FGF-8 (76), the gene encoding semaphorin-3A (77), the *CHD7* gene (78), and the genes encoding prokineticin-2 and prokineticin receptor-2 (79).

Genetic defects in GnRH secretion and function have also been identified in cases of IHH with normosmia. Kisspeptin is a potent regulator of GnRH secretion, and mutations in *KISS1* and *KISS1R*, the genes encoding kisspeptin and its receptor, cause hypogonadism. Mutations of the gene encoding leptin (80), its receptor (81), and mutations in the prohormone convertase 1 gene (82) have all

been associated with both hypogonadotropic hypogonadism and severe obesity. GnRH receptor mutations also result in hypogonadotropic hypogonadism (83). Unlike most patients with IHH, individuals with GnRH receptor mutations typically do not respond to conventional doses of pulsatile GnRH administration, although successful conception with high-dose pulsatile GnRH has been reported (84).

Although IHH represents only a small subset of infertility, the underlying mutations may confer susceptibility to the development of FHA. In a study of 55 women with FHA, 13% were found to have heterozygous mutations in genes associated with IHH, as compared to no mutations found in 422 controls (85). Therefore, FHA may stem from interplay between environmental and genetic factors, whereby inherited defects in GnRH biology may lower the threshold at which external stressors suppress the HPO axis (85).

Pituitary

Primary amenorrhea may also result from mutations in genes encoding transcription factors involved in the cellular proliferation and differentiation of the pituitary gland. *HESX1* (86), *GLI2* (87), and *SOX3* (88) mutations have been linked to hypopituitarism, and mutations in *SOX2* (89), *LHX3* (90), *LHX4* (91), and *PROT1* (92) may cause gonadotropin deficiency. Alterations in these genes may present with other clinical findings, which may help with the diagnosis. For example, mutations in *SOX2* may result in anophthalmia, and mutations in *LHX3* have been associated with a rotationally limited cervical spine (90).

Adrenal: congenital adrenal hyperplasia (CAH)

Reduction in the activity of adrenal enzymes responsible for cortisol production may also result in infertility. CAH is a family of inherited disorders characterized by defects in cortisol production, resulting in increased ACTH production due to reduced negative feedback and consequent adrenal gland proliferation. Although there are rare forms of CAH that result in infertility, including 11 β -hydroxylase deficiency (93), 17 α -hydroxylase deficiency, and 3 β -hydroxysteroid dehydrogenase deficiency (94), CAH most commonly arises from autosomal recessive mutations in the gene that encodes 21-hydroxylase (21-OH), *CYP21A2*, which result in shunting of steroid precursors toward androgen biosynthesis. There is considerable variability in disease phenotype, depending on the severity of the mutation. Classic 21-OH deficiency is typically diagnosed at birth and can present with severe salt wasting and/or prenatal virilization of external female genitalia, whereas nonclassic CAH (nonclassic 21-OH de-

ficiency [NC21OHD]) presents postnatally with signs of hyperandrogenism including hirsutism, acne, frontal balding, and menstrual irregularities (95).

The oligomenorrhea in women with 21-OH deficiency is likely due to elevated androgen and/or progesterone levels. Patients with classic 21-OH deficiency with irregular menstrual cycles have elevated progesterone and androstenedione levels that are associated with reduced LH-pulse amplitude and frequency (96). In vitro, androgens increase GnRH-pulse frequency (97), supporting the observation of elevated LH levels in women with NC21OHD (98); yet in normally cycling women, high-dose T infusion results in reduced LH-pulse frequency, even when blocking estrogen aromatization (51). Therefore, although the exact mechanism by which androgens act to induce oligomenorrhea is unknown, hyperandrogenemia may be a contributing factor in CAH. Elevated levels of progesterone, a substrate of 21-OH, also likely contribute to menstrual irregularity. Administration of progesterone to normally cycling women reduces LH-pulse frequency, and the progesterone receptor has been identified on gonadotroph cells in the pituitary, suggesting that progesterone's gonadotropin-suppressing effects may be mediated by both the hypothalamus and pituitary (99).

Although classic CAH is relatively rare, NC21OHD is one of the most common autosomal recessive diseases known, occurring in 1 in 100 individuals in a mixed ethnic population (100) and at a much higher frequency (1 in 27) in the Ashkenazi Jewish population (101). Given its unique treatment implications, it is important to distinguish NC21OHD from other causes of hyperandrogenism and oligomenorrhea, such as polycystic ovary syndrome. In women with evidence of hyperandrogenism and/or oligomenorrhea, the prevalence of NC21OHD has been reported to range from 1.2–13.8% (102–107). Not detected on newborn screening tests, the most definitive hormonally based diagnostic test for NC21OHD is an ACTH stimulation test measuring serum concentrations of 17-hydroxyprogesterone, a substrate of 21-OH, after ACTH administration (108). Patients with NC21OHD have stimulated 17-hydroxyprogesterone levels that are intermediate between normal subjects and those with classic 21-OH deficiency (108).

Iatrogenic Causes of Amenorrhea

Medication-induced amenorrhea

Various drugs, including hormonal contraceptives and leuprolide, interfere purposefully with normal menses by acting directly on the hypothalamus or pituitary. A number of other medications cause infertility or amenorrhea as

a side effect of their use. These medications act by: 1) inhibiting GnRH release; 2) inducing hyperprolactinemia; or 3) inducing hypophysitis.

Inhibition of GnRH release

Opioids. Opioids are an under-recognized but common cause of hypogonadism (109). The effect of opioids on the gonadal axis has been extensively studied in men, and hypogonadotropic hypogonadism may be a more common side effect in men compared to women (110). In vitro and human studies suggest that opioids act by directly inhibiting GnRH release (111, 112), although they may also act by inducing hyperprolactinemia (113).

Medication-induced hyperprolactinemia

Psychotropic medications. A number of medications cause hyperprolactinemia by interfering with dopamine's inhibitory effect on prolactin secretion. The medications that most commonly cause hyperprolactinemia are neuroleptics, which act by blocking dopamine receptors. Due to their greater affinity for the D2 receptor (114), typical antipsychotics have a more robust effect on elevating prolactin levels compared to atypical antipsychotics, with the exception of risperidone (115). Data on selective-serotonin reuptake inhibitors are conflicting, but a large open-label study of depressed individuals demonstrated the development of hyperprolactinemia in 22% of women after 12 weeks of fluoxetine use (116). Clomipramine, a tricyclic antidepressant that inhibits serotonin uptake, also increases prolactin levels (117). These serotonin uptake inhibitors may increase prolactin levels by increasing serotonergic inhibition of dopaminergic neurons.

Other medications. Through a similar antidopaminergic mode of action, certain gastrointestinal motility agents and antihypertensives have also been associated with hyperprolactinemia. Serum prolactin levels increase 6-fold after oral or iv metoclopramide (118), and domperidone also stimulates prolactin release (119). Methyl dopa, an α -adrenergic agonist, and verapamil, a calcium-channel blocker, both increase prolactin levels, likely by decreasing dopamine synthesis (120, 121).

Medication-induced hypophysitis

Cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibodies. Ipilimumab and tremelimumab are human monoclonal antibodies targeting CTLA-4, thereby promoting an immune-mediated response against cancer. Ipilimumab has been approved by the Food and Drug Administration to treat advanced malignant melanoma and is undergoing trials to explore its use in other malignancies. Given its immunostimulatory properties, ipilimumab has been as-

sociated with autoimmune reactions including hypophysitis resulting in hypopituitarism. A large randomized controlled trial studying the effects of ipilimumab in metastatic melanoma found the incidence of hypopituitarism to be 2.3% (122).

Pituitary surgery

Although reports in the literature suggest that surgical treatment of a pituitary lesion is rarely associated with hypogonadism (123), this is likely an underestimate of the true prevalence. This is due to the fact that surgeons with higher surgical volumes have fewer postsurgical complications (124), and most of these reports reflect rates of hypogonadism of dedicated pituitary surgeons.

Radiation treatment

Radiation therapy directed at the hypothalamus or pituitary is a well-known cause of hypopituitarism (125). Importantly, individuals who have radiation therapy for nonpituitary brain tumors are also at significant risk of developing hypogonadism (126). The development of hypogonadism and hyperprolactinemia in these individuals is associated with the dose of radiation therapy administered (127).

Conclusion

In conclusion, perturbations of the HPA axis due to physiological, pathological, and iatrogenic causes constitute a majority of cases of amenorrhea and infertility. FHA, a physiologic response to physical, emotional, or nutritional stress is the most common cause of neuroendocrine amenorrhea, and identifying this entity is essential in order to diagnose and treat the underlying disorder. Given the consequences of not treating amenorrhea, namely loss of bone mass, as well as the age constraints in treating female patients with infertility, prompt diagnosis and institution of treatment are critical.

Acknowledgments

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