NEW ASPECTS IN HYPOGONADISM

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NEW ASPECTS IN HYPOGONADISM

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EDITORIAL
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Editorial: New Aspects in Hypogonadism

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Editorial on the Research Topic

New Aspects in Hypogonadism

Testosterone and estradiol play critical, wide-ranging roles during development, before and beyond the traditional notion of a reproductive lifespan. In fetal life, testosterone is crucial for sexual differentiation and the proper development of male external genitalia (1). During late pregnancy and the first 6 months of neonatal life (i.e., minipuberty), testosterone and follicle-stimulating hormone play a major role in priming the male reproductive axis for fertility potential in later life (2). During puberty, rising sex steroids induce the development of secondary sexual characteristics—reaching adult normal levels necessary for full reproductive capacity. Later in life, bone and metabolic health are affected by falling estrogen levels of menopause and progressive declines in circulating testosterone levels in men (3, 4).

Hypogonadism describes the deficiency of sex steroids and accompanying symptoms. Classically, hypogonadism has been divided into primary (gonadal failure) and secondary (neuroendocrine defects). Given the role of sex steroids in development, hypogonadism may have far-reaching consequences on health and well-being including absent minipuberty, incomplete sexual maturation, altered sexual behavior, infertility, and disrupted metabolism. There is a broad array of unanswered questions about hypogonadism. For example, Klinefelter syndrome has a highly varied phenotype, presumably due to differential silencing of the supernumerary X, yet we know very little about the causality. Further, questions remain regarding the optimal timing of introducing testosterone replacement and fertility preservation to improve metabolic health in adulthood and improved quality of life. Similarly, the molecular basis of secondary hypogonadism (e.g., congenital hypogonadotropic hypogonadism/Kallmann syndrome) has been charted and absent minipuberty and cryptorchidism are known to be an early life determinants of fertility potential. Currently, it remains unclear if neonatal gonadotropin therapy in these contexts may improve future fertility, although evidence that it may reduce the need for surgical orchidopexy is reasonably compelling. Despite the availability of effective treatment for decades, there is yet consensus on the optimal sex steroid replacement regimen and approach for treating adolescent males and females with hypogonadism. Late onset hypogonadism has gained increasing interest, yet the etiology remains to be fully elucidated, the degree of testosterone deficiency is often quite modest and longer term clinical consequences remain uncertain. Moreover, the definition itself may need to be revised in line with more recent data, so as to specifically reference aging-related primary hypogonadism. The nine articles in this Research Topic shed light on diverse, neglected, but nevertheless important and illuminating aspects of hypogonadism.

Two articles address key—but often overlooked—principles and applications of sex steroid treatment in hypogonadal men and women. Al-Sharefi et al. concisely summarize the evidence on androgens and anemia, highlighting the clinical relevance of testosterone for men with chronic kidney disease, or with apparently unexplained anemia-of-aging. A second article examines the evidence relating to what best constitutes safe and adequate estrogen replacement for transgender

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women and young women with premature ovarian failure. Authors draw lessons that deserve wider application to the care of hypogonadal women. Specifically, they identify the advantages of using native 17β-estradiol (compared to ethinylestradiol or conjugated equine estrogen), and the logic of adjusting treatment doses according to serum estradiol levels as well as patient symptoms and bone densitometry (Swee et al.).

Two further articles examine the early life impact of hypogonadism. Kuiri-Hänninen et al. review activation of the hypothalamic-pituitary-testicular axis in relation to testicular descent. Authors draw attention to minipuberty for providing a window of opportunity to examine the axis in early infancy (Kuiri-Hänninen et al.). The second article provides a complementary perspective underscoring that minipuberty is an unique opportunity for early diagnosis of hypogonadism and timely initiation of treatment to improve health, fertility and well-being (Swee and Quinton). Two review articles provide comprehensive pictures of the psychosocial impact of primary and secondary hypogonadism in males. Hanna et al. present a narrative review combining quantitative and qualitative studies on Klinefelter syndrome to provide a holistic view of how primary hypogonadism can affect psychological and emotional well-being. The complementary article on psychological aspects of secondary hypogonadism is co-authored by patient leader, facilitator and educator, Neil Smith. This article provides a comprehensive review of the state of the science in the area, supplemented by patient perspectives that are too-often neglected in the medical literature (Dwyer et al.).

A controversial topic of debate concerns late-onset hypogonadism—sometimes called andropause or low T syndrome by enthusiast clinicians. Swee and Gan review evidence from recent population-based and intervention studies providing a balanced overview of the diagnosis, pathophysiology, and management approaches. Finally, two articles provide insights into the mechanisms underlying the metabolic effects of hypogonadism and patho-mechanisms contributing to decreased testosterone production with aging. Dimakopoulou et al. summarize evidence from animal models to identify the links between metabolism and reproduction—with particular attention to diabetes mellitus as a predisposition to male hypogonadism. Lee et al. present mouse data on the role of nitroso-redox imbalance in decreased testosterone production. Such mechanistic studies deepen our understanding of strong association between hypogonadism and diabetes and patho-mechanisms underlying late-onset hypogonadism. It is striking how the ∼3-fold greater risk of having Type 2 diabetes mellitus observed in Klinefelter syndrome also seems to be common to other forms of primary gonadal insufficiency in males, including the age-related form.

The articles included in this Research Topic fill important gaps in current understanding of hypogonadism. Articles cover the human lifespan, from late intrauterine life and minipuberty through to old age. Contributions include perspectives from basic scientists, clinicians, psychologists, sociologists, and patients advocates. We hope the diverse articles included in this Research Topic will spark reflection on hypogonadism and a deeper appreciation for some of these neglected areas of inquiry.

AUTHOR CONTRIBUTIONS

AD made substantial contributions to the design of the work, drafted the editorial and approved the final version. RQ made substantial contributions to the design of the work, edited the manuscript, and approved the final version. All authors contributed to the article and approved the submitted version.

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REVIEW
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Congenital Hypogonadotrophic Hypogonadism: Minipuberty and the Case for Neonatal Diagnosis

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Congenital hypogonadotrophic hypogonadism (CHH) is a rare but important etiology of pubertal failure and infertility, resulting from impaired gonadotrophin-releasing hormone secretion or action. Despite the availability of effective hormonal therapies, the majority of men with CHH experience unsatisfactory outcomes, including chronic psychosocial and reproductive sequelae. Early detection and timely interventions are crucial to address the gaps in medical care and improve the outlook for these patients. In this paper, we review the clinical implications of missing minipuberty in CHH and therapeutic strategies that can modify the course of disease, as well as explore a targeted approach to identifying affected male infants by integrating clinical and biochemical data in the early postnatal months.

Keywords: congenital hypogonadotropic hypogonadism, kallmann syndrome, puberty delay, minipuberty of infancy, infertility–male, cryptorchidism, gonadotrophin releasing hormone deficiency

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INTRODUCTION

Congenital hypogonadotrophic hypogonadism (CHH) is a rare genetic condition characterized by reproductive disorder due to deficiency in secretion or action of gonadotrophin-releasing hormone (GnRH). It is traditionally considered to be a male-predominant condition, with a male: female gender ratio 3.6: 1 that remains unexplained (1). The major clinical consequences of CHH are pubertal failure and infertility.

Although generally considered a rare disorder, accurate determination of the prevalence of CHH has not been possible because of scarce literature. Based on a French study of potential military conscripts who attended medical examination (2) and, more recently, a retrospective study of nationwide hospital records in Finland (3), (both of which were methodologically prone to under-ascertainment), male CHH prevalence of 1 in 4,415–15,000 is currently estimated.

The genetic defects underpinning CHH broadly fall into two principle groups, comprising (a) those causing neurodevelopmental defects of GnRH neuron migration frequently associated with non-reproductive defects, particularly anosmia/hyposmia from olfactory axon misrouting (i.e., Kallmann syndrome–KS), and (b) those causing pure neuroendocrine impairment of GnRH secretion or action (normosmic CHH). Belying this apparently simple dichotomy is the huge diversity of genetic mutations, with over 30 gene loci implicated to date, despite nearly half of CHH cases remaining unaccounted for. Moreover, some genes are implicated in both normosmic CHH and KS (4). This complexity is also reflected in the various modes of transmission possible, including oligogenicity as well as all forms of classical Mendelian inheritance (4, 5).

Furthermore, CHH is phenotypically heterogeneous. Besides the variable association with non-reproductive features, such as deafness, synkinesis (mirror movements), renal agenesis, digital and dental anomalies, and clefting, reproductive manifestation range from absent puberty, pubertal arrest, to even spontaneous reversal of hypogonadism in a small minority (5). CHH males frequently present with cryptorchidism and/or micropenis, which are important features of severe fetal-infancy GnRH deficiency (absent minipuberty) (6). However, patient experiences indicate that these early presentations only rarely signpost timely disease recognition and treatment-initiation in later life.

Despite medical advances in genetics, diagnostics and treatment, health outcomes of CHH men remain disappointing, with a significant proportion bearing the long-term consequences of suboptimal care (7). In this chapter, we will review the factors contributing to poor outcomes of men with CHH, and the strategies that can improve the quality of life and fertility potential, with special focus on harnessing the window of minipuberty for early diagnosis, and intervention.

DELAYED DIAGNOSIS AND TREATMENT

Delayed puberty is the main mode of presentation in CHH males. Approximately 2/3 of CHH males adolescents do not show any sign of spontaneous puberty at >17 years of age (testis volume <4 mL), with the remainder exhibiting arrested early puberty (8).

Unfortunately, CHH is biochemically indistinguishable from constitutional delay in growth and puberty (CDGP), the latter accounting for up to 65% of delayed puberty in younger teenage boys (9), but obviously declining precipitously with advancing age at presentation. Both conditions are characterized by low sex steroids in association with low or inappropriately normal follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels. In terms of their height development, the baseline height SD scores and growth velocities do not appear to differ significantly between CDGP and CHH adolescents, unlike in functional hypogonadotrophic hypogonadism where there is a tendency for lower height SD scores and reduced growth velocity of <3 cm/year (9, 10). It is however worth noting that progressive reduction in height SDs has been observed in a proportion of CDGP boys during the pre-pubertal years, with final attained height falling short of their genetic potential (11). On the other hand, preservation of height relative to parental height in CHH patients has been suggested (12). Hence, careful interrogation of the growth history can yield useful information.

Although a variety of stimulation tests (such as GnRH stimulation test and hCG test) and, recently, inhibin B (I_B) concentrations (marker of Sertoli cell function), have been proposed, there is still lack of consensus on the "gold standard" test to reliably distinguish them (13). Hence, making a diagnosis of CHH remains a challenging task and clinicians frequently default to the classic dogma of expectant management, i.e., allowing adolescents enough time for those with CDGP to undergo spontaneous initiation of puberty, as a means to identify those with CHH (14). It is noteworthy that in a recent large single center retrospective series, the combination of testicular volume and basal I_B level–both of which are obtainable with relative ease without the involvement of complicated dynamic tests–demonstrated utility in discriminating between CHH and CDGP, and therefore could have the potential for wider application(10).

However, there seems to be consistent misapplication of "wait & see & reassure" guidance (intended for individuals with undifferentiated pubertal delay) to those with red flag markers of CHH who should, instead, be presumed hypogonadal until proven otherwise and receive sex hormone replacement from mean age of pubertal-onset in that population i.e., 12 years in boys (6, 15), whereas treatment should not be unduly withheld in those without CHH features but have turned 14 years old (16).

As a result, the diagnosis of CHH and initiation of clinically-meaningful treatment to induce puberty is typically unduly delayed until late adolescence or early adulthood. Despite different survey techniques across different European countries, data on the mean ages of diagnosis and initiation of clinically-meaningful[1] treatment for CHH men have been remarkably consistent, with Dwyer et al (web-based, pan-European; $n = 101$) finding these to be 18 ± 6 and 19 ± 5 years, respectively (7); Raivio et al (nationwide Finnish cohort study) finding the median age of starting treatment to be 18.3 years (range, 11–34 years) (17), and Quinton (case notes based survey; $n = 200$) finding it to be 18.9 ± 9 years (18).

A surprisingly common pitfall is the failure to recognize the significance of cryptorchidism in an adolescent with pubertal delay. In retrospective CHH series, between 30 and 50% of CHH males have a history of cryptorchidism, of whom up to 2/3 have bilateral undescended testes (8, 19–21). By contrast, cryptorchidism is very rare in CDGP. In a large series of boys seen at a specialized center referred from primary care for delayed puberty, only 2% of CDGP boys had a history of cryptorchidism, compared with 36% of those with CHH (10). Hence, a history of cryptorchidism should alert clinicians to presume the diagnosis of CHH until proven otherwise (22). A concomitant family history of cryptorchidism, micropenis, infertility and/or non-reproductive features such as anosmia, renal agenesis and cleft palate/lip could provide valuable clues to an underlying diagnosis of CHH, though it is absent in majority of cases, in part due to variable disease penetrance and phenotypic expression, as well as oligogenic inheritance with unaffected parents (4).

Likewise, the significance of bilateral undescended testes in neonates as a possible indicator of CHH is typically underappreciated. Persistent bilateral cryptorchidism is found in a quarter of CHH infants, as compared to <0.7% among infants in the general population (**Table 1**) In a large single-center retrospective series, only one-third of CHH males with history of bilateral orchidopexy in childhood were referred to pediatric endocrinology for evaluation, with most cases re-presenting much later in life with absent puberty (19). Current clinical guidelines have largely focused on evaluation for possible congenital adrenal hyperplasia (CAH) and disorder

[1]For instance, 50mg intramuscular testosterone monthly would not remotely constitute appropriate or meaningful replacement therapy for a 20 year-old, 1.7m and 70kg prepubertal male with Kallmann syndrome.

TABLE 1 | "Red Flag" clinical markers for congenital GnRH deficiency*.

INDICATORS OF ABSENT MALE MINIPUBERTY
All apparent at or shortly after birth
- Cryptorchidism (38% compared with general population prevalence 3.68–6.9% at birth, 1.0–2.4% at 3 months) (23–26)
- Bilateral cryptorchidism (25% compared with general population 1.66–4.54% at birth, 0.09–0.66% at 3–12 months) (23–26)
- Microphallus (9% compared with general population birth prevalence 0.015–0.35%) (27, 28)
- Absent erections on nappy change
NON-REPRODUCTIVE PHENOTYPES STRONGLY ASSOCIATED WITH CHH
Apparent at or shortly after birth
- Cleft lip and/or palate (5% compared with general population prevalence 0.1%) (29–31)
- Hearing impairment *via automated optoacoustic emissions test* (6% compared with UK birth prevalence 0.12%) (32)
Usually apparent by 6–8 years of age
- Anosmia or hyposmia [43% compared with general population prevalence 1.4–19.1% (comprising both congenital & acquired)] (33–35)
FAMILY HISTORY OF CHH
Including offspring of CHH patients from ovulation- or spermatogenesis-induction (risk apparent even preconception)

*Composite data including 4 published studies (8, 19–21).

of sex development (DSD), and neglected to provide sufficient guidance on the investigations necessary in cryptorchid boys to rule out CHH (36, 37), resulting in missed opportunity for early recognition.

As a result of late diagnosis and delayed treatment, the optimal standard of care is not delivered with patients with CHH, leading to significant psychosocial and reproductive sequelae.

UNMET HEALTH NEEDS

Poor Psychological Well-Being

Psychological morbidities and antidepressant usage are highly prevalent among CHH men (17). In an international study comprising of participants from North and South America, Europe and Australasia, nearly two-thirds suffered from depression, with majority of them exhibiting moderate to severe symptoms (38). An important consequence arising from chronic affective disorders is poor adherence to long-term hormone replacement. It is concerning that more than one-third of the survey respondents reported long gaps (>1 year) in treatment, which could potentially exacerbate affective symptoms in turn as part of a vicious cycle.

The psychosexual impact of the disease is tremendous. A significant number of patients suffer from anxiety and low self-esteem, resulting in inability to form intimate relationships and social isolation. Consequently, in the aforementioned study cohort (38), approximately 50% of the men were not in a stable relationship at the time of survey, and many never had a sexual partner.

On the positive side, the majority of men surveyed had received tertiary level education and were in gainful employment, reflecting reasonable socioeconomic circumstances, although this did not negate the psychological effects of their disorder. However, CHH men with adopted or biological children were less likely to report depressive symptoms, suggesting the positive effect of family companionship, and the vicious circle that socially-withdrawn CHH men may be trapped in.

A major factor that contributes to poor mental health and impaired quality of life is the delay in diagnosis and the failure to administer age-appropriate induction of secondary sexual characteristic in many CHH males. As alluded to earlier, most patients only start to receive meaningful treatment in late adolescence (median age 18–19 years) typically after a prolonged and frustrating diagnostic odyssey (frequently shared by their female counterparts too) (1). Consequently, they are at risk of developing a multitude of psychosocial problems associated with pubertal delay, including low self-esteem, social withdrawal, poor school performance and even higher rates of substance use disorder (16, 39, 40). Furthermore, inadequately treated cryptorchidism and/or micropenis can lead to long-lasting adverse impact on their sexuality (17).

Compromised Fertility Potential

In young adult males with CHH, gonadotrophin treatment achieves a significantly greater positive impact than direct testosterone (T) replacement on health-related quality of life (41). While both treatments are effective in improving physical function and general health, patients receiving gonadotrophins perform better in psychological domains, including emotional and mental health, particularly if sperms are found in ejaculate. This strongly suggests that patients psyches are deeply affected by their perceived chances of achieving paternity.

The infertility in CHH patients is due to spermatogenic failure, which is potentially amenable to GnRH or gonadotrophin treatment. Unfortunately, classic spermatogenic treatment–human chorionic gonadotrophin (hCG) monotherapy or combined gonadotropin treatment (hCG+FSH)–is much less successful in men with severe CHH (testes <4 mL), particularly those with history of bilateral cryptorchidism, than in men with HH of postpubertal-onset, e.g., due to acquired pituitary disease (42). Nonetheless, in centers experienced in the care of CHH patients, up to three-quarters can achieve spermatogenesis during hormonal induction treatment (6, 43), and pregnancy rates can be further enhanced with assisted reproductive techniques (6, 44).

Patients with rare medical disorders, defined by a prevalence of <5 in 10,000 in the population, often face challenges due to the lack of knowledge of their healthcare providers (45, 46). As such, specialized centers with expertise in diagnosis and interdisciplinary treatment of rare diseases are vital in the provision of care to these patients (47). Similarly, patients with CHH should ideally receive tertiary-level care to avoid gaps in treatment, and benefit from the latest technologies and advances in the research field. Early diagnosis would provide the opportunity for patients to receive appropriate and consistent care and support at specialized centers without delay. Treatment can also be tailored according to the needs and goals in different stages of life (6, 48).

But in real-life setting, according to one survey, it appears that only a minority of CHH men seeking fertility are able to achieve desired outcomes on fertility-inducing treatment (7). It is unclear how many of these men were treated at centers with the necessary expertise, but given that only half of the whole study cohort being followed-up at specialized/academic centers, access to such resources is likely to be correspondingly restricted.

Elevated Risk for Low Bone Mineral Density

Chronic sex steroid deficiency is a major risk factor for osteoporosis and fragility fractures that affects both genders. As men with CHH have early-onset of T insufficiency, a delay in and/or lack of adequate androgen replacement would result in poor bone mass accretion and accelerated bone loss (49). Indeed, patients who are initiated on T replacement at older age appear to accrue less bone mineral compared to younger age, further supporting the importance of timely treatment (50). Nonetheless, even for those who are diagnosed and started on treatment only later in life, encouraging improvement in bone mineral density, particularly at trabecular-rich lumber spine, has been observed (51).

MINIPUBERTY–CRITICAL PERIOD OF GENITALIA DEVELOPMENT AND THE WINDOW TO EARLY DIAGNOSIS

High incidence of cryptorchidism and microphallus is observed in CHH males because of the absence of minipuberty, which is a critical period in the ontogenesis of the male reproductive tract, characterized by activation of the GnRH axis in the initial months postnatal. During this developmental phase, serum T and gonadotropin levels rise rapidly and peak at age 3 months–remarkably approaching adult male levels–before to mid-childhood quiescence by about 6 months of age (52, 53).

This robust hormonal activity is necessary to complete the processes of inguinoscrotal testicular descent and anchoring in the scrotum as well as penile growth, which had begun earlier *in utero* during the third trimester. Specifically, LH-stimulated secretion of T and INSL3 peptide by Leydig cells are the key factors involved in driving these physical changes (54, 55). There is a concurrent exponential increase in FSH-stimulated I_B and anti-Müllerian hormone (AMH) secretion, signifying active proliferation of Sertoli cells. Expansion of Sertoli and germ cells and seminiferous tubule formation are key determinants of future fertility potential and are responsible for 90% of testicular volume (56). Despite the brisk gonadotrophic activity and T secretion during this proliferative phase, germ cell maturation and spermatogenesis do not occur, because androgen receptors are not expressed on Sertoli cells until 5 years of age (57).

Following minipuberty, the hypothalamic-pituitary-testicular (HPT) axis retreats into quiescence for the rest of childhood. Serum T, LH, and FSH levels decline to low levels until reactivation of gonadal axis occurs in early adolescence, heralding the onset of puberty and marked by testicular enlargement ($\geq$4 mL), followed by penile and pubic hair growth. At this stage, Sertoli cell maturation occurs, evidenced by a rise in I_B and decline in AMH levels, and spermatogenesis is achieved by the concerted actions of FSH and intra-testicular T (58). Therefore, pulsatile GnRH secretion in the neonatal period appears to be paramount for the normal development of male genitalia, with a far-reaching impact on male reproductive phenotype and fertility potential later in adult life.

Another important clinical implication of minipuberty is that it potentially provides a window-of-opportunity to facilitate detection of children with congenital GnRH deficiency, who would demonstrate abnormally low FSH, LH, and T levels if measured, thereby offering the advantage of a definitive prepubertal diagnosis and sign-posting them to pre-planned pubertal-induction with sex hormones at median age of pubertal-onset, rather than expectative management.

Early Diagnosis for Avoiding Delay in Pubertal Induction

Diagnosis of CHH in early life facilitates the structuring of long-term surveillance and treatment plans, as well as ensuring that counseling and psychological support to patients and families are made available. When patients reach early adolescence, age-appropriate pubertal induction treatment will ensure that secondary sexual characteristics develop in tandem with peers (15), thereby avoiding the delay that has been traditionally experienced by most CHH men. As such, uncertainties are minimized and anxieties can be allayed.

Potential Benefit of Early Diagnosis in Improving Prospect of Fertility

Early identification of boys with CHH could also plausibly provide the opportunity for intervention to optimize fertility potential. Although GnRH or gonadotrophin combination treatment are effective for most CHH men in spermatogenesis-induction, sperm outcomes are generally suboptimal (43, 59, 60). Moreover, nearly one-third with severe CHH remain azoospermic even with prolonged combined gonadotropin therapy, and hCG-monotherapy has proved to be despairingly ineffective.

Clinical features (consistent with long-standing severe GnRH deficiency minipuberty) that are predictive of poor treatment response, include: complete absence of puberty at presentation, cryptorchidism (especially if bilateral), low serum I_B concentrations (indicating depleted Sertoli cells) and prepubertal testicular volume (indicating depletion of Sertoli and germ cells and seminiferous tubules) (43, 59). On the other hand, CHH men with partial GnRH deficiency (testicular volume $\geq$4 mL) respond much better to combined gonadotrophin treatment, with around 80% achieving sperm in the ejaculate (61).

Therefore, in men with complete CHH, it would be theoretically advantageous to first maximize proliferation of Sertoli and germ cell and growth of seminiferous tubules by administering FSH-monotherapy prior to the introduction of hCG, so as to prevent premature maturation of a depleted pool of Sertoli cells under the influence of intra-testicular T.

Pathfinder studies of FSH-monotherapy in children and adolescents with HH of prepubertal-onset demonstrated its efficacy in promoting testicular growth and circulating I_B concentrations (62, 63). In particular, there was an encouraging spermatogenesis response in a subgroup of CHH adolescents, in whom FSH-priming before the combination with hCG successfully induced spermatogenesis in 3 out of 4 patients (63).

The potential benefit of unopposed FSH treatment was further studied in a randomized, open-label trial of 13 treatment-naive adult CHH men with prepubertal testes (<4 mL) (64). Seven men were randomized to recombinant FSH-pretreatment for 4 months before embarking on a 24-month GnRH treatment protocol. During the FSH-only phase, testicular volume doubled and I_B levels rose to adult levels, and all subjects in this arm subsequently developed sperm in ejaculate on GnRH therapy, compared to 4 of 6 men in the 24-month GnRH-only arm. There were also trends to larger testicular volume, higher maximal sperm counts and shorter time to first appearance of sperm in the ejaculate in the FSH-pretreated group.

Therefore, the findings of the benefits of FSH-priming and the potential deleterious effect of premature hCG therapy would be important to inform clinicians on the choice of treatment in adolescents with CHH, which would be greatly facilitated by timely diagnosis.

Potential of Neonatal Gonadotrophin Treatment to Further Optimize Outcomes

Recognizing the critical role of minipuberty in the development of external genitalia and Sertoli cell proliferation and its subsequent influence on future reproductive function, the feasibility and benefits of recreating the physiological hormonal milieu in male CHH infants has been studied.

In the first published report of a boy with CHH and micropenis who received short-term recombinant human LH and FSH from age 7.9–13.7 months, the penile length successfully increased by 50% and the testicular volume nearly tripled by the end of treatment (65). In another report of 2 male infants, one case each of combined pituitary hormone deficiency (CPHD) and CHH, 6-month gonadotrophin combination therapy via subcutaneous pump infusion initiated at age 8 and 20 weeks, respectively, led to 4-fold increase in both penile length and testicular volume (66). More recently, 3–6 months of continuous subcutaneous infusion of recombinant human gonadotrophins in 5 male infants [4 CHH, 1 CPHD] produced several-fold increase in I_B concentrations, testicular volume, and T secretion (67).

Besides these encouraging responses to combined gonadotrophin treatment during infancy, it could also aid in the management of undescended testes. Cryptorchidism is present in approximately one-half of boys with severe CHH (19), and it is an independent predictor of infertility. Delay in orchidopexy has been associated with dramatic decline in germ cells (68), and as such it is generally recommended that surgical treatment take place by about 1 year of age (37, 69). However, small testes render surgical manipulation technically challenging and would result in excess risk of testicular trauma and tissue loss (70). By administering a period of presurgical gonadotrophin therapy, it allows the enlargement of testicular volume to facilitate the procedure.

Indeed, there are emerging evidence that spontaneous testicular descent could be successfully induced by gonadotrophin treatment in infants with central hypogonadism, and thus obviate the need for surgery. In a cohort of eight infants with maldescended testes due to underlying hypogonadotrophic hypogonadism (5 CHH, 3 CPHD), gonadotrophin infusion induced full testicular descent in 6 boys and partial descent in 2 boys, such that only 1 had to undergo orchidopexy nearly a year later because of re-ascent of both testes (71). Another study showed that combined recombinant FSH and LH in the first 6 months of life successfully induced spontaneous testicular descent in 2 of 4 bilateral-cryptorchid CHH/CPHD boys (67).

Therefore, short-term neonatal gonadotrophin treatment in ascertained cases of hypogonadotrophic hypogonadism appears to be effective in replicating the effects of minipuberty, by correcting micropenis, promoting testicular growth due to Sertoli cell expansion, and inducing spontaneous descent of malpositioned testes. Importantly, treatment was well-tolerated in all reported cases. Although definitive evidence is currently lacking, the prospect of early childhood hormonal intervention in CHH boys in augmenting sexual and reproductive function in adult life is worth serious consideration, and hopefully will provide impetus for larger clinical trials.

A PROPOSED STRATEGY TO IMPROVE DETECTION OF MALE INFANTS WITH SEVERE CHH

The phase of male minipuberty provides an extraordinary useful diagnostic window to confirm (or refute) the diagnosis of congenital hypogonadism with a relatively straightforward biochemical hormone profiling without the need for complex dynamic testing. In a cohort of CPHD male infants (predominantly presenting with hypoglycaemia), findings of low circulating FSH, LH, and T concentrations reliably identified concomitant hypogonadotrophic hypogonadism in 14 of 15 infants with genitalia anomalies, whereas all other boys with normal genitalia demonstrated intact pituitary-gonadal axis function (72). This contrasts starkly with the conundrum of differentiating CHH from CDGP in adolescence.

However, unlike CPHD, neonates with CHH without cryptorchidism do not necessarily have clinical manifestations that would trigger referral to pediatric endocrinologists for pituitary hormonal evaluation. In addition, there is a lack of awareness and clinical guidance to consider CHH as a differential diagnosis of male infants with cryptorchidism, particularly if bilateral or associated with micropenis, so that appropriate evaluation can be undertaken (22, 37, 69). Indeed, the presence of "red flag" markers (**Table 1**) should warrant further investigations to exclude such a possibility.

Another challenge would be the interpretation of less robust serum gonadotrophin and testosterone results in the male

infants. Of relevance, normative data of several important reproductive hormone values during minipuberty–including LH, FSH, T (by both radioimmunoassay and tandem mass spectrometry), AMH and I_B-have recently been derived from a large cohort of healthy Danish infants, with cut-off values discriminating between sexes determined (53). By extension, values above the cut-off levels–generally lower than adult ranges–could be regarded as intactness of HPT axis function, whereas equivocal biochemical results that lie below the cut-off values may warrant repeat testing. Furthermore, expanding screening panel to include AMH and I_B could improve diagnostic confidence. Crucially, this study also demonstrated that the various sex hormones peak just before 3.5 months of age, suggesting that diagnostic performance would be most optimal when evaluation is undertaken around this time.

Bilateral Cryptorchidism as Potential Screening Criterion

While routine screening for minipuberty is impractical, a targeted approach to evaluate male infants with suspicious signs of CHH could be cost-effective. Among the associated clinical features, bilateral cryptorchidism is particularly important because of its high prevalence (13.9–34.5%) among CHH males and the association with severe GnRH deficiency that has both prognostic and therapeutic implications (8, 19, 21).

Although cryptorchidism is a common congenital urogenital abnormality in newborns, most would undergo spontaneous descent in the absence of hypogonadism or other organic disorders. Data from prospective studies shows that the prevalence of bilateral cryptorchidism decreases substantially from 1.66 to 4.54% at time of birth to 0.09–0.66% by 3–12 months of age (23–26). In addition, spontaneous descent is unlikely to occur beyond 3 months postnatal (73), which coincides closely with the expected peak of minipuberty, making it an ideal time point to undertake testing of reproductive hormones in boys with persistent bilateral cryptorchidism. Moreover, although non-CHH cryptorchid boys may also exhibit hormonal abnormalities, they tend to have higher FSH, similar T and slightly lower I_B values compared to healthy infants, which are different from the biochemical pattern expected of in CHH male infants (74).

Therefore, testing male infants with bilateral cryptorchidism with or without micropenis at 3 months of age for absent minipuberty could potentially be a feasible approach to facilitate the early diagnosis of CHH. The diagnostic yield of such a selective screening strategy is explored here, using Britain's birth data as an example for mathematical illustration:

- With an average male live birth rate of 390,070 per annum in Britain (75), between 26 and 88 boys born each year could be affected by CHH (based on estimated prevalence of 1 in 4,415–15,000).
- On the other hand, bilateral cryptorchidism could affect between 6,475 and 17,709 (1.66–4.54%) of all boys at birth, regardless of the presence of CHH. Among them, between 4 and 30 infants could have underlying CHH (as bilateral cryptorchidism affects 13.9–34.5% of CHH population), thus representing 0.02–0.46% of all bilateral-cryptorchid boys at birth.
- By 3 months of age, following spontaneous testicular descent expected in majority of non-CHH infants, the total number of infants with persistent bilateral cryptorchidism would be expected to reduce substantially to between 351 and 2,574 (0.09–0.66%).
- As spontaneous testicular descent is not expected in CHH-affected infants, they now account for greater proportion of all persistently bilateral-cryptorchid boys: 0.16–8.55% (in contrast to 0.02–0.46% at birth). Hence, screening at 3 months of age appears to be most cost-effective by avoiding investigations in vast majority of infants without persistent bilateral cryptorchidism.

Based on this hypothesis, possibly up to 1 in 11–12 male infants with persistent bilateral cryptorchidism at 3 months of age could have underlying CHH. Interestingly, this estimate is consistent with the findings from an historical surgical series of patients who had been treated with orchidopexy. In this study, of the 98 patients evaluated for possible underlying endocrine cause of cryptorchidism, 2 were found to have CHH in adult life and both of them had bilateral undescended testes (76). That represents 6.1% (2/33) of all individuals with bilateral cryptorchidism in the series.

However, it is worth reiterating that, at present, there is no literature to suggest that CHH screening in infancy has been systematically studied by any research group, and thus is a working concept that remains in early exploratory phase. Further long-term multicentre research is necessary to validate the utility of any screening strategy that seeks to establish early diagnosis of this rare condition, as well as the effectiveness and safety of hormonal treatment in CHH infants. Protocols should also be developed collaboratively by pediatric and adult endocrinologists to ensure that these children are placed in a structured follow-up and transition programme, thereby ensuring that no one falls through the crack in the health system.

A Short Note on Pre-pubertal Acquired Hypogonadotrophic Hypogonadism

Pubertal failure in male adolescents with known history of acquired hypopituitarism generally avoids the diagnostic conundrum of CHH, hence allowing pubertal-induction therapy to be planned for at around 12 years of age, with gradual increase in testosterone dose before reaching adult replacement dose in about 3 years (15). Importantly, because of preserved minipuberty, normal Sertoli cell proliferation is expected in early childhood, and testicular maldescent would be less likely to occur (63). Therefore, these individuals tend to have a more optimistic fertility outlook, with greater spermatogenic response to gonadotrophin treatment anticipated (77), unless testicular tissue has been compromised e.g., prior gonadotoxic chemotherapy treatment.

CONCLUSION

Early detection of CHH through detection of absent minipuberty can potentially modify patients' experience by facilitating timely interventions at different stages of life. This is achievable with a systematic approach in place to identify male infants with "red flag" markers of CHH, particularly bilateral cryptorchidism, so that biochemical testing can be undertaken within the narrow diagnostic window.

Neonatal gonadotrophin treatment appears to be beneficial in correcting micropenis and even cryptorchidism. In adolescents, age-appropriate pubertal induction is the main goal, and a brief course of FSH monotherapy should be considered to optimize future fertility potential. Close collaborations between pediatric care providers and adult endocrinologists would ensure that these patients transit smoothly to adulthood, during which the aims of treatment would shift to fertility-induction and long-term androgen replacement. Equally important are the psychological support and genetic counseling that should be provided along the way to empower patients, and families, so that they can cope with their conditions confidently.

FUTURE DIRECTIONS

Proactive screening of male infants with bilateral cryptorchidism (and/or micropenis) is anticipated to have a direct impact on the quality of care delivered to affected children by establishing early diagnosis. However, this issue has not been addressed by major clinical guidelines developed for the management of infants with abnormal genitalia, thereby leading to missed opportunities for patients and clinicians alike in accessing the most appropriate treatment possible. To overcome this barrier, it is imperative that collaborative research by key stakeholder centers seek to clarify the feasibility of such an approach systematically, as well as expanding the work on neonatal gonadotrophin treatment, so as to advance the agenda for wider adoption among endocrinologists, pediatricians, and pediatric surgeons.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Age Induced Nitroso-Redox Imbalance Leads to Subclinical Hypogonadism in Male Mice

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Objective: The cause of age-related changes in testosterone remains unclear. We hypothesized that increased nitroso-redox imbalance with aging could affect testosterone production.

Materials and Methods: We measured several markers of nitroso-redox imbalance (4-HNE, 3-NT, and NT) in serum of S-nitrosoglutathione reductase knock out (GSNOR KO) mice that have increased nitroso-redox imbalance and compared these to wild-type (WT) mice. We evaluated the impact of age-induced nitroso-redox imbalance on serum luteinizing hormone (LH) and testosterone (T) in WT young (<2 months), middle-aged (2–6 months), and aged (>12 months) mice. Finally, to elucidate the susceptibility of testes to nitroso-redox imbalance, we measured 4-HNE protein levels in the testes of WT and KO mice.

Results: We identified 4-HNE as a reliable marker of nitroso-redox imbalance, as evidenced by increased protein levels in serum of GSNOR KO mice compared with WT mice. We demonstrated that 4-HNE serum protein levels increase in WT mice with age but do not accumulate in the testes. We also found that T levels were similar in all age groups. Interestingly, we found that serum LH levels in aged and middle-aged mice were increased when compared to young mice ($n = 5$) consistent with the phenotype of subclinical hypogonadism.

Conclusions: Increased serum 4-HNE and LH levels without changes in T with age suggest that nitroso-redox imbalance is associated with subclinical hypogonadism in aged mice. Recognizing the relationship and etiology of a currently poorly understood classification of hypogonadism could be a paradigm shift in how age-related testosterone change is diagnosed and treated.

Keywords: subclinical hypogonadism, compensated hypogonadism, aging, nitroso-redox imbalance, nitrosative stress, 4-hydroxynonenal, S-nitrosoglutathione reductase

INTRODUCTION

Age-related reproductive and sexual decline is a ubiquitous process. Recently, interest in identifying sources of age-related decline in sexual function in males has grown due to an increasing proportion of older people in the population (1). The hypothalamic-pituitary-gonadal (HPG) axis, which regulates sexual function in males, is responsible for the control of production of luteinizing hormone (LH) and testosterone (T). Serum levels of T change with age (2, 3), and these changes can be due to a combination of testicular (primary hypogonadism, high LH, and low testosterone) and pituitary or hypothalamic (secondary or tertiary hypogonadism, low, or normal LH with low testosterone) failure. Although it has been suggested that hypogonadism effects as much as 23.3% of men aged 40–79 years, the pathophysiology of age-related hypogonadism and its specific classifications have not been well-defined. In one study, in addition to those with low T, an additional 9.5% of men in the study had compensated or subclinical hypogonadism (increased LH and normal T) (4). The mechanism of this type of hypogonadism remains unknown.

Currently, the free radical theory of aging is the most accepted hypothesis to explain factors that contribute to functional decline associated with aging. (5, 6) This theory of aging proposes that reactive oxygen species (ROS), such as superoxide ($O2^-$) and hydrogen peroxide (H_2O_2) and reactive nitrogen species (RNS) including nitrogen dioxide (NO_2) and peroxynitrite ($ONOO^-$) play key roles by inhibiting cellular function, growth, and apoptosis. (7–13) When ROS and RNS are produced in excess, a situation of oxidative stress, or nitrosative stress ensues, respectively. However, instead of implicating one or the other in biological systems, many have reasoned that the balance between both types of reactive species is responsible for biological effects (14). A disarray in this balance is better known as nitroso-redox imbalance; it has been associated with several degenerative diseases and negative cellular impacts and have further been shown to increase as a function of age (14, 15). We have previously demonstrated that young adult S-nitrosoglutathione reductase (GSNOR) knock-out (KO) mice have decreased circulating T and LH levels compared to wild-type (WT) controls (16). In the absence of GSNOR, these mice develop excess RNS (17), and their lower levels of T and LH suggest that the nitroso-redox imbalance may disrupt hormone production.

What is unknown is whether age-related changes in T are associated with increasing RNS and ROS (nitroso-redox imbalance). We hypothesized that increased nitroso-redox imbalance can occur with aging and be associated with changes in T production. To investigate this relationship, we confirmed that 4-hydroxynonenal (4-HNE), a sensitive marker for oxidative damage (18) can also reliably be used to measure nitroso-redox imbalance. We then evaluated 4-HNE levels in the serum of young and aged WT mice and compared them to young and aged KO mice. We compared the levels of 4-HNE to changes in LH and T levels that could occur with aging in WT mice. Finally, in an effort to identify the mechanism of the effects of nitroso-redox imbalance we measured 4-HNE levels in the testes of WT mice.

Abbreviations: WT, wild-type; GSNOR, S-nitrosoglutathione reductase; LH, luteinizing hormone; HPG, hypothalamic-pituitary-gonadal; ROS, reactive oxygen species; RNS, reactive nitrogen species; KO, knock-out; 4-HNE, 4-hydroxynonenal; 3-NT, 3-Nitrotyrosine; NT, Nitrotyrosine.

MATERIALS AND METHODS

Mice

Male WT mice (C57/BL6) (Jackson Laboratories, Bar Harbor, ME, USA) and mice lacking GSNOR (KO) (The University of Miami Miller School of Medicine, Miami, FL, USA) were used. Pups were genotyped and sacrificed at the indicated ages. Mice were kept in a barrier-protected animal facility with 12-h light-dark cycles. All experiments were carried out before 10 am to reduce the impact of diurnal variation of LH and T levels. Blood was obtained from WT mice by cardiac puncture followed by euthanasia for the young (<2 months), middle aged (6 month), and aged (12–15 months) time points. We obtained blood via saphenous vein draw for the 2, 3, and 4 month time points. Saphenous vein blood collections were done on the same mice over time as they aged. All saphenous vein and cardiac puncture blood collections were done under isoflurane anesthesia. Euthanasia was performed by cervical dislocation under isoflurane anesthesia. All methods were performed in accordance with the approved institutional animal care and use committee protocol at our institution. All methods were performed with the approval of the University of Miami Institutional Animal Care and Use Committee (IACUC) protocol at our institution (Protocol # 15167).

Western Blotting

Serum proteins were isolated from total blood and processed with RIPA lysis buffer. Lysates were mixed with 5× sample buffer, heated to 100°C for 5 min, and separated by 10% odium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Resolved proteins were transferred onto a 0.45-μm polyvinylidene fluoride (PVDF) membrane. Proteins were studied by exposing the membranes to antibodies against 4-HNE (Alpha Diagnostic International Intl. Inc., HNE11-S), Transferrin (Abcam, ab84036), and Albumin (Abcam, ab19195), 3-Nitrotyrosine (Abcam, ab61392), and Nitrotyrosine (Abcam, ab42789). Immunoreactive bands were visualized using the Thermo Scientific Chemiluminescent Pico Kit.

Testes Protein Isolation

Testes from mice were dissected, and the tunica albuginea was removed and collected in chilled RIPA buffer (150 mM NaCl. 0.1% SDS, 1% NP-40, 50 mM Tris pH 7.5, 0.5% Sodium-Deoxycholate) for preparation of whole testis protein lysates. Tissues were disrupted mechanically and incubated on ice for 30 min and lysates were cleared from residual cell debris by centrifugation for 10 min at 10,000 × g. The supernatant was collected, and protein concentrations were determined by Bradford colorimetric assay.

Testosterone and LH Assay

Serum total testosterone and LH levels were measured using the Ligand Assay & Analysis Core of the Center for Research in Reproduction at the University of Virginia (Charlottesville, VA, USA). Techniques for measurements of each hormone are described in detail at https://med.virginia.edu/research-in-reproduction/ligand-assay-analysis-core/assay-methods/.

For serum testosterone, the mean intra-assay variation was 12.8% and the intraassay variation was 9.3%. Serum LH was measured using an ultrasensitive enzyme-linked immunosorbent assay. In brief, 6 μL of whole blood was collected in 54 μL of assay buffer for analysis. Intraassay coefficients of variation were 7.3% (low quality control [QC]; 0.13 ng/mL), 5.0% (medium QC; 0.8 ng/mL), and 6.5% (high QC; 2.3 ng/mL). All experiments were performed in the morning to minimize the impact of diurnal variation of hormones.

Epididymal Sperm Concentration

After the mice were euthanized, epididymides were collected and used for semen analysis. Fresh epididymis was cut into small pieces and dispersed in F12 medium 200 μL (Invitrogen, Waltham, MA, USA) containing 0.1% bovine serum albumin (Invitrogen) pre-warmed to 37°C and incubated for 15 min to facilitate the transmigration of sperm from the epididymis. Each epididymal sperm suspension was subjected to sperm counting and sperm motility analyses by a computer-aided semen analysis (CASA) system (Microptic SL, Barcelona, Spain).

Statistical Analysis

Data were analyzed for significance using analysis of variance with the Student *t*-test, as indicated. All analyses were performed using GraphPad Prism 4.03 (GraphPad, South San Francisco, CA, USA), and a $P < 0.05$ was considered significant. All data are presented as mean ± standard error.

RESULTS

Serum 4-HNE Levels Are a Reliable Marker of Nitroso-Redox Imbalance

We evaluated markers of nitroso-redox imbalance such as 4-HNE, 3-Nitrotyrosine (3-NT), and Nitrotyrosine (NT) in the serum of WT and GSNOR KO mice. We identified 4-HNE as a reliable marker of nitroso stress as evidenced by higher serum protein levels in GSNOR KO mice compared to WT mice (**Figure 1**). The other markers, 3-NT and NT, did not demonstrate significant differences in serum protein levels between GSNOR KO and WT mice (data not shown).

Age Is Associated With Increased Nitroso-Redox Imbalance

Nitroso-redox imbalance is associated with increasing age. We investigated whether 4-HNE protein levels increase with age. We evaluated the levels of 4-HNE in serum samples from young (<2 months) and aged (>12 months) mice using Western blot. We found that 4-HNE levels were higher in older mice when compared to young mice (**Figure 2**). This result suggests that nitroso-redox imbalance increases with age.

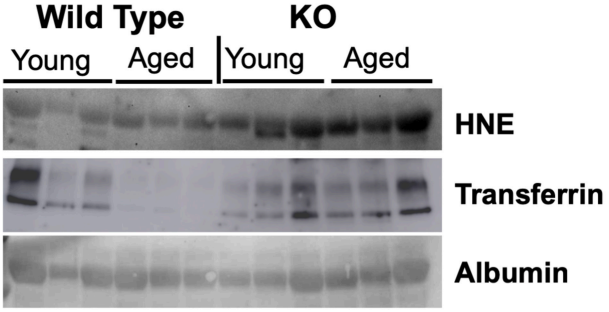

FIGURE 1 | 4-HNE is a reliable marker of nitroso-redox imbalance. To identify a reliable marker of nitroso-redox imbalance in serum, we evaluated several markers such as 4-HNE, 3-NT, and NT. Western blot indicated that 4-HNE protein levels are a reliable marker of nitroso-redox imbalance, as evidenced by increased expression in GSNOR KO mice compared to WT mice.

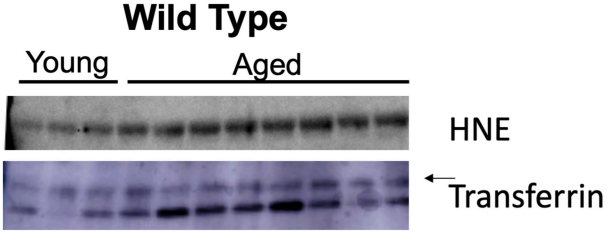

FIGURE 2 | Age is associated with increased nitroso-redox imbalance. To validate that nitroso-redox imbalance is increased with age, we compared 4-HNE serum protein levels in young (<2 months) and aged mice (>12 months). Western blot demonstrated 4-HNE serum protein levels are increased with age.

Aging Affects Circulating LH, Testosterone, and Sperm Concentration

We have previously demonstrated that nitroso-redox imbalance in GSNOR KO mice decreased serum T and LH levels in addition to impairing sperm parameters, compared to WT controls (16). We examined if age-associated nitroso-redox imbalance impacts LH, T, and epididymal sperm concentration. For these experiments, we measured T, LH, and epididymal sperm concentration in young (<2 months), middle-aged (2–6 months), and aged mice (>12 months) over time. We found that LH levels in aged ($n = 8$) and middle-aged ($n = 5$) were increased when compared to young mice ($n = 5$) (**Figure 3A**). T levels were not significantly different between any of the age groups ($n = 5$) (**Figure 3B**). We also did not identify any changes in epididymal sperm concentration (**Figure 3C**). Collectively, LH concentration increased with age, while T and sperm concentration were not significantly impacted (**Supplementary Figure 1**). Taken together, these results suggest that age-induced increase in nitroso-redox imbalance is associated with subclinical hypogonadism.

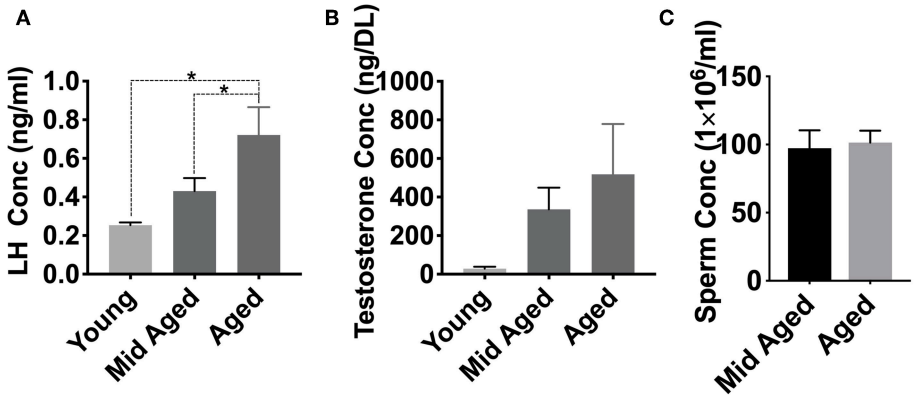

FIGURE 3 | Aging effects on LH and testosterone levels. We examined if age-associated nitroso-redox imbalance impacts serum LH, Testosterone (T), and epididymal sperm concentration. **(A)** LH levels are higher in aged (>12 months) mice than in young (<2 months) or middle-aged (2–6 months) mice (*$p < 0.05$) **(B)** T concentrations show no significant differences with age **(C)** Epididymal sperm concentrations are not different between middle-aged and aged mice.

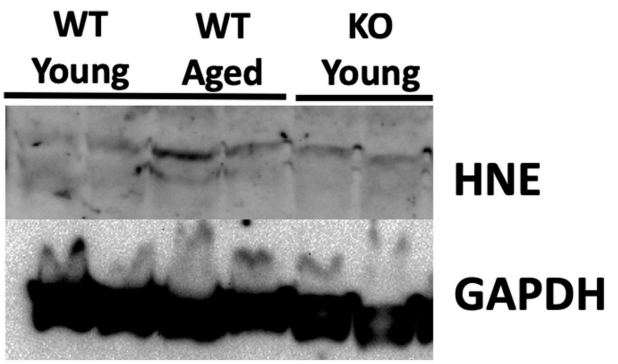

FIGURE 4 | 4-HNE protein expression does not accumulate in the testis with age. We measured protein levels of 4-HNE in the testes of young WT, middle-aged WT, and young KO. Protein levels were similar in all of the groups suggesting that 4-HNE protein levels in the testes may not be a reliable indicator of nitroso-redox imbalance.

4-HNE Protein Levels Are Not Accumulated in the Testes With Aging

Previous studies have demonstrated that testicular tissue is particularly susceptible to nitroso-redox imbalance (19). To examine this, we measured 4-HNE protein levels in the testes of young WT, middle-aged WT, and young KO mice. Our results demonstrated that testicular 4-HNE protein levels were similar in all groups (**Figure 4**). This result suggests that the testis does not accumulate nitroso-redox imbalance with age, or that and that testicular 4-HNE protein levels may not be a reliable indicator of nitroso-redox imbalance.

DISCUSSION

The mechanism of age-associated changes in testosterone production remains unclear. Accumulating free radicals with nitrosative and oxidative stress can lead to cellular aging. We investigated whether increasing nitroso-redox imbalance with aging can explain changes in T. We identified that age is not only associated with increasing nitroso-redox imbalance as reflected by serum 4-HNE protein levels but is also associated with increased LH with unchanged T levels, suggesting a profile of compensated hypogonadism. As this imbalance increases with age, T production can decrease, resulting in a reduction of the negative feedback inhibiting LH production. The result is an increase in LH that is enough to stimulate steroidogenesis to produce T levels in the normal range; increased LH "compensates" for the decreased T production. As a result, T levels remain within normal reference ranges concurrently with elevated LH levels. While the mechanism remains uncertain, our results indicate that Leydig cells capacity for T production is not diminished since T levels remain in normal range. However, the mechanism may lie in the HPG axis in the Leydig cells ability to respond to LH and stimulate T production. Thus, we suggest that nitroso-redox imbalance may lead to compensated hypogonadism through effects of the HPG axis. Our results suggest an association between nitroso-redox imbalance and subclinical hypogonadism, but the cause must still be elucidated.

Compensated hypogonadism, also referred to as subclinical hypogonadism, has been suggested to be its own clinical condition (20, 21). The overall clinical and physiological significance of compensated hypogonadism is poorly understood, but it has been found to be associated with both aging and increased physical symptoms of T deficiency (22). This association, combined with its surprising prevalence in men [9.5% of all men aged 40–79 (4)], raises the question of whether this classification of hypogonadism should be diagnosed or potentially even treated. Men, and especially older men, may benefit from having LH included in the initial screening for hypogonadism in order to diagnose subclinical hypogonadism. It also should be investigated whether treating subclinical hypogonadism, much like treating subclinical hypothyroidism, can be of benefit. In this study, we show that increased levels of nitroso-redox imbalance occur with age suggesting a possible etiology for compensated hypogonadism in older men. This discovery provides an aim for future studies to evaluate if

measuring nitroso-redox imbalance in older men may be of diagnostic benefit by identifying a potential therapeutic target in the aging process. Novel therapeutic strategies such as anti-oxidant therapy, may have a significant impact on treating subclinical hypogonadism. Since subclinical hypogonadism represents a form of testicular failure future studies should continue to investigate the mechanism of nitroso-redox imbalance at the level of Leydig cell steroidogenesis and whether treating the nitroso-redox imbalance could reverse these HPG axis changes and potentially prevent a progression of testicular failure resulting in primary hypogonadism.

Our study has strengths and limitations. A strength of this study is that it is the first study to show that 4-HNE can be a reliable marker for nitroso-redox imbalance. This study also demonstrates that nitroso-redox imbalance increases with age. Limitations of the study include the small sample size (limited by the breeding capabilities of GSNOR KO mice) and variability in serum LH and T levels in mice. Mice are also known to have variable T levels due to lack of circulating sex hormone binding globulin (23). We accounted for this variability by consistently drawing the blood in the morning (before 10 a.m.).

CONCLUSION

Aging is associated with increased nitroso-redox imbalance as reflected by serum 4-HNE protein levels and subclinical hypogonadism. Recognizing the relationship and etiology of a currently poorly understood classification of hypogonadism could be a paradigm shift in how we diagnose and treat age-related hypogonadism. Future studies can focus on identifying markers of nitroso-redox imbalance in testis and the mechanisms of how they impact steroidogenesis.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

JL, MK, HA, SK, TM, JH, UK, and RR designed the research. JL, MK, HA, and EG performed the research. JL, MK, HA, SK, and RR analyzed the data. JL, MK, and RR wrote the paper.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2019.00190/full#supplementary-material

Figure S1 | LH and testosterone concentrations further broken down by age show the linear increase in LH concentration as the mice aged.

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Conflict of Interest Statement: JH discloses a relationship with Vestion, Inc., that includes equity, board membership, and consulting; is the chief scientific officer, a compensated consultant and advisory board member for Longeveron, and holds equity in Longeveron; and is the co-inventor of intellectual property licensed to Longeveron.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPINION
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Late-Onset Hypogonadism as Primary Testicular Failure

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Keywords: late-onset-hypogonadism, hypogonadism, andropause, obesity, testosterone, aging

INTRODUCTION

Testosterone (T) therapy has garnered widespread public enthusiasm and media attention due to its potential role in age-related T decline in men, commonly known as late-onset hypogonadism (LOH), andropause, or low T syndrome. The serum T concentration gradually declines across the lifespan and the symptoms between aging and hypogonadism overlap. These have led to the speculation that a causal relationship might exists between age-related reduction in serum T concentration and symptoms commonly seen in aging. However, it remains uncertain if T therapy could ameliorate symptoms associated with LOH, without significant risks. Despite the lack of clinical evidence and long term safety data, prescribing rates of T therapy have skyrocketed in many countries (1, 2), leading to efforts by regulatory authorities to limit such inappropriate prescribing practice (3).

Importantly, the fundamental question of what constitutes clinically significant LOH was largely unaddressed until recently. Heterogeneity in definitions of LOH and the use of specificity-limited immunoassays for T measurements in many previous epidemiological and interventional studies have precluded robust comparisons across studies (4). Due to the expanding aging population, LOH is becoming an increasingly important topic. We reviewed the evidence from recent population-based studies and intervention trials to provide better understanding of the diagnosis, pathophysiology, and management for LOH.

PATHOPHYSIOLOGY OF T DECLINE IN AGING

The testicular function undergoes natural decline with age. Compared to younger men, healthy older men has 40% less Leydig cell mass and a corresponding rise in luteinizing hormone (LH) concentration (5). Decreased testicular T production was also observed in aged Leydig cells, following diminished LH-stimulated cAMP production, and reduced downstream steroidogenic enzymatic activity (6). On the other hand, aging is associated with changes in LH secretory pattern. A reduced T production and frequent, small irregular LH pulses was observed in healthy older men (7), despite preservation of pituitary gonadotrophs' response to exogenous gonadotropin-releasing hormone (GnRH) (8).This suggests age or factors associated with aging reduced negative feedback inhibition by T. An ensemble-based analysis also predicted a >30% fall in GnRH output in healthy older men (9). However, a recent study has demonstrated that healthy older men without late-onset hypo-gonadism (LOH) have preserved hypothalamic response to kisspeptin-54 and pituitary response to GnRH, with impaired testicular response as compared to younger men (10). This suggests that primary testicular failure accounts principally for the normal aging-related decline in T production. In majority of healthy older men, the compensatory increase in gonadotrophins serves to maintain T levels within eugonadal ranges (11).

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The pathophysiology of LOH is complicated by comorbidities associated with aging. The development of chronic illnesses, including diabetes, cardiovascular disease and inflammatory disorders, is associated with a contemporaneous accelerated rate of aging-related T decline, ranging between 1.5- and 3.6-fold compared to men who remain disease-free (12, 13). Furthermore, excess adiposity exerts potent suppressive effects on the HPT axis. Individuals with BMI $\geq$30 kg/m^2 are at 13-fold increased risk of LOH compared to those with BMI $<$25 kg/m^2 (14). Overall, men with comorbidity and/or obesity failed to exhibit compensatory rise in LH levels which would otherwise expected in healthy non-obese men suggesting a significant disruption at the hypothalamic-pituitary level which compromises T production (15).

Consonant with that, obesity has been shown to be the most common factor associated with the development of low T in middle-aged and older men (11). The pathogenic role of excess adiposity has been postulated to be linked to several adipose tissue-derived factors, including pro-inflammatory cytokines and leptin, and altered insulin-signaling, which act in concert to produce central inhibitory effects on the HPT axis, leading to secondary hypogonadism (16–18). Interesting, obesity also increase oxidative/nitrosative stress leading to nitroso-redox imbalance and male sexual dysfunction (19). The potential mechanisms underpin the development of LOH is depicted in **Figure 1**.

AGING-RELATED DECLINE IN T CONCENTRATIONS

Serum total T concentrations were historically thought to decline at a rate of 1–2% per annum from 4 to 5th decade onwards (20, 21). One of the population-based studies demonstrated that $>$50% of men aged $\geq$80 years had T level in hypogonadal range, as defined by $<$2.5th percentile for young men ($<$11.3 nmol/l) (22).

However, accumulating evidence from newer studies suggest that age-related fall in serum total T is closer to 0.5% per year (12, 15, 23), and healthy older men actually experience minimal changes in T levels. A community-based longitudinal study from South Australia showed that the rate of decline of total T concentrations in a subset of men without chronic illnesses was a non-significant 0.27% per year (12). In another study, no appreciable change in serum T up to 8th decade of life was observed among men with self-reported very good to excellent health (24). In European Male Aging Study (EMAS), 2,736 men aged $\geq$40 years were followed up for an average of 4.4 years, and $>$80% of men in their 7–8th decade continued to have normal T values (11). Therefore, LOH is less prevalent than previously thought, and low T in older men is mostly related to co-existing medical conditions and obesity.

THE CHALLENGES IN DIAGNOSING LOH

LOH has conventionally been defined as low serum T in older men, irrespective of the luteinizing hormone (LH) levels. This has led to a prevalence as high as 50% been quoted in some studies. However, the European Male Aging Study (EMAS) has demonstrated two distinct groups of older men with low total T (14). The majority of older men were found to have low T associated with low-normal luteinizing hormone. This is not independently associated with aging *per se* but is mediated indirectly via age-related non-gonadal co-morbidities, including obesity, and increased visceral adiposity. Only a small number of older men (2.1%) had low T with high LH, in keeping with primary testicular insufficiency. This specific primary hypogonadism profile has been directly associated with both aging and metrics of ill health.

On the other hand, it is imperative that the diagnostic evaluation of male hypogonadism be corroborated with signs and symptoms (25). However, there is substantial overlap between symptoms arising from chronic diseases and hypogonadism, posing significant challenge to determining clinically relevant LOH (14). Indeed, men reported hypogonadal symptoms frequently have T concentrations in the eugondal ranges (26). Moreover, the clinical significance of borderline or modestly low T levels typically seen in LOH is often hard to ascertain.

To address some of these gaps, EMAS investigators established a set of minimum criteria (14). In this study, 32 candidate symptoms were shortlisted, and after reductive analysis, only the co-occurrence of three sexual symptoms (decreased morning erection, poor libido, erectile dysfunction) and low T level (total T $<$ 11 nmol/L and free T $<$ 220 pmol/L) had consistent syndromic association. With that, the overall prevalence of LOH in EMAS population was determined to be 2.1%, widely believed to be the most accurate estimate hitherto, lower than previous studies using less stringent criteria (26). Stratifying by age groups, $<$1% of men aged $<$60 years, 3.2% of men aged 60–69 years, and 5.1% of men aged 70–79 years met the proposed criteria.

THE CLASSIFICATION OF LOH ACCORDING TO LH LEVEL AND ASSOCIATED RISK FACTORS

The hypothalamus-pituitary- testicular (HPT) axis is tightly regulated in an interdependent fashion to maintain hormonal homeostasis. In hypogonadism, the gonadotropins can either be elevated (primary hypogonadism) or low/normal (secondary hypogonadism). In EMAS, subjects are classified into primary hypogonadism (LH $>$ 9.4 u/L, T $<$ 10.5 nmol/L), secondary hypogonadism (LH $\leq$ 9.4 u/L, T $<$ 10.5 nmol/L) or compensated (primary) hypogonadism (LH $>$ 9.4 u/L, T $\geq$ 10.5 nmol/L) (11). Through this approach, unique clinical characteristics and risk factors were identified in each subgroup.

Primary hypogonadism was found to be uncommon in the study. It affected only 2% of the entire cohort and had a low annual incidence of 0.2% (27). At-risk men had poorer baseline physical function, and suffered from deterioration in erectile function, vigor and hemoglobin as they progressed to hypogonadism. Advanced age ($>$70 years) and comorbidities were strongly associated with increased risk of primary hypogonadism, with an odds ratio of 12.5 and 4.24, respectively. The serum T concentrations continued to decline with time

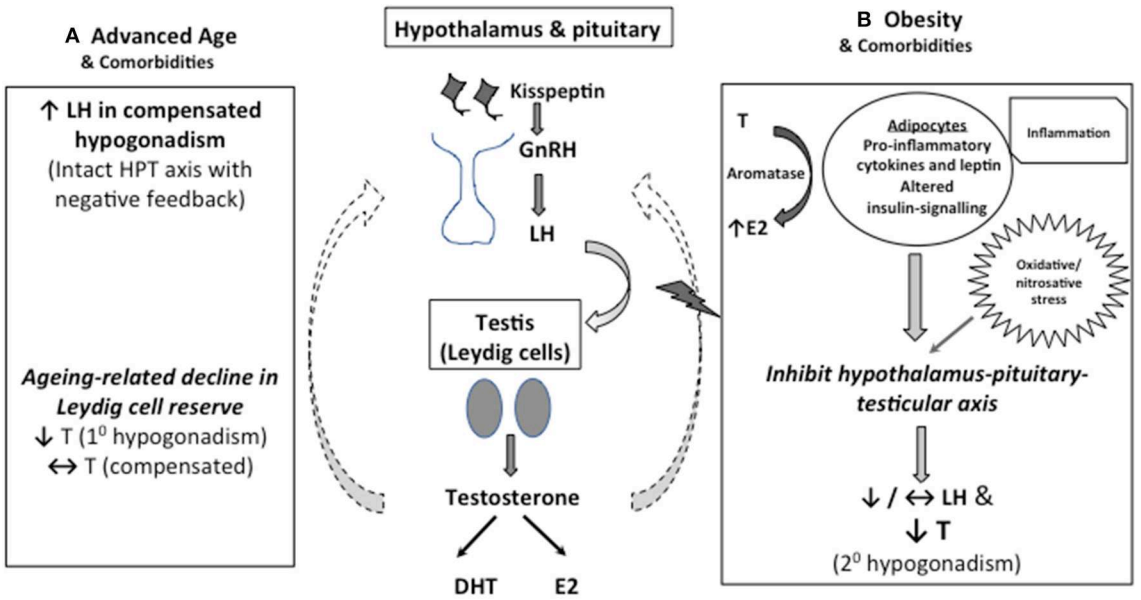

FIGURE 1 | Mechanistic explanation for low serum T in middle-aged and older men. **(A)** As Leydig cell reserve decline with aging, compensatory rise in luteinising hormone (LH) occurs to maintain circulating testosterone (T) concentrations (compensated hypogonadism). In more advanced state, elevated LH can no longer overcome the diminished testicular function, leading to overtly low T levels (primary hypogonadism). **(B)** Obesity is the predominant cause of functional suppression of hypothalamic-pituitary-testicular (HPT) axis in middle-aged and older men, manifesting as failure of LH response to low T (secondary hypogonadism). Multimorbidity is also associated with both primary and secondary hypogonadism, albeit to a lesser degree. Excess adiposity has been linked to altered insulin signaling, oxidative stress and increased pro-inflammatory cytokines and leptin levels, which act in concert to suppress the central HPT axis. Adipose tissues also express aromatase which convert testosterone to estradiol, especially in the inflammed state, exerting inhibitory effects on the HPT axis.

with little sign of recovery. For the minority whom T levels returned to eugonadal range, the mean LH levels remained persistently elevated to the same degree, indicating persistent Leydig cell failure.

Secondary hypogonadism accounted for majority (85.5%) of older men with low T, with an annual incidence of 1.6% (11). The mean LH level was not different from that of eugonadal men, indicating a failure in the compensatory hypothalamic-pituitary axis. Unlike primary hypogonadism, there was no significant relationship between the prevalence of secondary hypogonadism and aging. Instead, obesity emerged to be the most potent risk factor (14, 15), with a lesser contribution by comorbidities. Therefore, secondary hypogonadism represents a state of functional HPT suppression driven principally by obesity and poor health, rather than chronological aging.

The third classification was compensated hypogonadism, present in close to 10% of the study cohort. This group of men had normal circulating total T concentration and raised LH level. They exhibited some clinical features in keeping with primary hypogonadism (27), making it a clinically relevant entity. Despite being relatively common, progression to hypogonadism range of T concentration was very infrequent, suggesting that most men in this group could retain the capacity to sustain adequate T levels.

MANAGEMENT OF LOH

Subtyping LOH according to both T and LH levels provides useful clinical information in elucidating the underlying etiology, and allows management to be tailored accordingly. For LOH due to testicular failure (primary hypogoadism), T treatment could be used to improve anemia, sexual activity and libido in older men (28–34). However, T therapy was found to have no significant impact on energy level, physical function, weight, or cognitive function among older men with LOH (28, 35–40). Despite the reassuring data from majority of interventional trials with regards to short term safety (41–44), a meta-analysis of 27 placebo-controlled trials has concluded that T therapy was associated with an increased cardiovascular risk, with an odds ratio of 1.54 (95% confidence interval, 1.09 to 2.18) (45). Furthermore, T therapy is associated with increased hematocrit, serum concentrations of prostate-specific antigen (PSA) and prostate volume, as well as gynecomastia and secondary infertility. Hence, T therapy should only be considered after careful consideration of the risks and benefits, while bearing in mind that the cardiovascular safety profile of T therapy in this population has yet to be fully established. Ongoing surveillance of hematocrit and prostate specific antigen is also required whilst on T treatment (24).

On the other hand, human chorionic gonadotropin (HCG) may have a therapeutic role in LOH (46, 47). HCG therapy is known to increase serum testosterone concentration and preserve global activity of the testis (e.g., fertility and insulin-like factor 3 production) (48, 49). A clinical trial comparing 6-months HCG vs. T therapy in LOH has demonstrated higher 25-OH-vitamin D and lower serum estradiol concentrations in men treated with HCG (47). The prostate volume and hematocrit

level were also significantly lower compared to the groups treated with T (47). The Leydig cells have been shown to contribute to the 25-hydroxylation of vitamin D and a higher 25-OH-vitamin D level may reflect improved Leydig cell function following HCG treatment (50). Hence, HCG therapy may have a favorable profile in LOH but larger safety and efficacy trials would be required to determine if HCG could be used as a long-term therapy in LOH.

It should be emphasized that obesity and co-morbidities underlies most cases of low T in older men with secondray hypogonadism, and thus, lifestyle intervention and cardiometabolic risk reduction should be the first line treatment for this cohort of patients. Notably, the potential for reversal to eugonadism in secondary hypogonadism is promising for obese men; nearly half of the men recovered their T levels over a period of ∼4 years, predicted by attainment of healthier weight (51).

CONCLUSION

Establishing the diagnosis of LOH remains a conundrum in clinical practice because of imprecise criteria and confounding factors relating to health alterations in old age. Nonetheless, if we define LOH as age-related primary testicular failure, only a minority of men appears to be affected. While studies have demonstrated some positive effects of T therapy, the clinical meaningfulness of these findings remains debatable. Moreover, the absence of long-term cardiovascular safety data continues to be an area of concern and controversy.

Hence, we suggest that future interventional trials for LOH should aim at older men with primary testicular failure, or classify the study cohorts according to LH levels so that a more clinically meaningful risk-benefit stratification can be elicited. This will clarify the safety and benefit profile of T therapy or other treatments in LOH and inform decision of the most appropriate management for LOH in men.

AUTHOR CONTRIBUTIONS

DS wrote the first draft of the paper. EG amended and rewrote the paper so that it matches the opinion style (paper was first submitted as review). EG produced the figure.

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Psychological Aspects of Congenital Hypogonadotropic Hypogonadism

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Congenital hypogonadotropic hypogonadism/Kallmann syndrome (CHH/KS) is a rare, treatable form of infertility. Like other rare disease patients, individuals with CHH/KS frequently experience feelings of isolation, shame, and alienation. Unlike many rare diseases, CHH/KS is not life threatening and effective treatments are available. Nevertheless, it remains a profoundly life-altering condition with psychosocial distress on a par with untreatable or life-limiting disease. Patients with CHH/KS frequently express lasting adverse psychological, emotional, social, and psychosexual effects resulting from disrupted puberty. They also frequently experience a "diagnostic odyssey," characterized by distressing and convoluted medical referral pathways, lack-of-information, misinformation, and sometimes-incorrect diagnoses. Unnecessary delays in diagnosis and treatment-initiation can significantly contribute to poor body image and self-esteem. Such experiences can erode confidence and trust in medical professionals as well as undermine long-term adherence to treatment–with negative sequelae on health and wellbeing. This review provides a summary of the psychological aspects of CHH/KS and outlines an approach to comprehensive care that spans medical management as well as appropriate attention, care and referrals to peer-to-peer support and mental health services to ameliorate the psychological aspects of CHH/KS.

Keywords: coping, hypogonadotropic hypogonadism, kallmann syndrome, patient activation, patient centered care, patient experience, transitional care

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INTRODUCTION

Congenital hypogonadotropic hypogonadism (CHH) is a rare genetic endocrine disorder causes by the insufficient secretion or action of gonadotropin-releasing hormone (GnRH). Biochemically it is defined by very low sex steroid levels (testosterone, estradiol) in the setting of low or inappropriately normal serum levels of gonadotropins (luteinizing hormone, follicle stimulating hormone) (1). Clinically, the condition manifests as absent or incomplete puberty with infertility. Thus, without exogenous hormonal therapy, individuals remain in a state of arrested pubertal development. Individuals typically exhibit some scant signs of androgenization (i.e., scant/Tanner II axillary and pubic hair) arising from secretion of weak androgens by the adrenal glands. In males, the absence of normal sex steroid levels is evidenced by lack of virilization, i.e., poor muscle development, gynoid habitus, sparse body hair, high-pitched voice, and undeveloped genitalia. In females, there are little to no secondary sexual characteristics (i.e., breast development, pubic hair) and absent menses (amenorrhea).

Additionally, clinical presentation may be accompanied by a variety of highly variable non-reproductive phenotypes (**Box 1**) (1, 2). Notably, when patients exhibit a diminished/altered

Box 1 | Signs of CHH/KS and associated phenotypes.

Hallmark signs of absent/incomplete puberty

Males	Females
• High-pitched voice	• Absent/limited breast development
• No beard development	
• Lack of muscle mass	• Undeveloped feminine figure
• Scant body/pubic hair	• Scant pubic hair
• Underdeveloped genitals	• Primary amenorrhea

ASSOCIATED PHENOTYPES

Reproductive
- Maldescended testes (cryptorchidism)
- Micropenis

Sensory and Neurologic
- Defective sense of smell (hyposmia/anosmia)
- Sensorineural hearing loss
- Ocular and oculomotor defects (coloboma, micropthalmia)
- Mirror movement (synkinesia)
- Ataxia (Gordon-Holmes syndrome)

Musculo-Skeletal
- Eunuchoidal proportions
- Scoliosis, osteopenia/osteoporosis
- High arched palate
- Cleft lip/palate
- Dental agenesis
- Digit anomalies (syndactyly, clinodactyly, split hand-foot)

Dermatologic
- Pigmentation defects (achromic patches)
- Ichthyosis

Internal Organs
- Renal agenesis (unilateral)
- Heart defects

Box 2 | Red flags pointing to CHH/KS diagnosis.

Positive family history—including offspring of CHH patients secondary to fertility-inducing treatment

Signs of absent mini puberty (first 6-months of life)
- Maldescended testes (unilateral or bilateral cryptorchidism)
- Micropenis
- No erections noted during diaper changes

Absent sense of smell (anosmia)—typically not evident until age 6–8 years

Presence of midline or skeletal defects
- Cleft lip and/or palate
- Syndactyly (webbing) or other anomaly of digits

sense of smell (hyposmia/anosmia) it is termed Kallmann syndrome (KS). Associated phenotypes occur at highly variable rates. Thus, patients present on a spectrum ranging from relatively milder forms (e.g., CHH with normal sense of smell and partial puberty) to more severe, syndromic forms of CHH (e.g., Kallmann syndrome with complete absence of puberty, unilateral renal agenesis and cleft lip/palate) (3).

THE CHALLENGE OF DIAGNOSIS

In parallel to the clinical heterogeneity of CHH, the molecular basis is likewise diverse and complex (4). Inheritance patterns include X-linked, autosomal recessive, autosomal dominant, as well as digenic and oligogenic forms (5). Since the early 1990's, more than 30 genetic loci have been identified to underlie CHH/KS. Significant advances have been made in understanding the molecular basis of CHH/KS yet the known genes only account for ∼50% of cases (1). As such, genetic testing may be informative in helping to confirm a diagnosis in less than half of cases, with the mainstay of diagnosis remains based on clinical ascertainment and biochemical measurement of serum hormones.

Importantly, CHH can be a difficult diagnosis to make. The hallmark signs of CHH include failure to initiate spontaneous puberty or inability to maintain progressive pubertal development. In the general population, pubertal onset is highly variable. One may view a photograph of a middle school class picture and quite easily see that some students have yet to begin puberty (e.g., short stature and Tanner I) while other classmates have begun or are well into puberty (e.g., growth spurt, acne, facial hair development in boys and breast development in girls). Indeed, delayed puberty statistically defined by the bell-shaped curve of puberty (6). Constitutional delay of growth and puberty (CDGP) occurs in 2.5% of the population and represents those individuals at the far tail of the distribution who will undergo spontaneous puberty—yet will do so significantly later than their peers.

Currently, there is no gold-standard test to differentiate CDGP from CHH. A number of serum biomarkers have been identified and dynamic tests have been developed and evaluated. To date, all these approaches lack appropriate sensitivity and specificity to accurately tease apart delayed spontaneous puberty and abiding absent puberty (7). In some cases, clinical "red flags" may point to a diagnosis (1, 8–10) (**Box 2**). Unfortunately, such clinical signs often go unrecognized (or their significance is not appreciated) and a "watchful waiting" approach is taken (11). Making a diagnosis is complicated and difficult because CHH/KS is a diagnosis of exclusion and other potential causes (i.e., functional, iatrogenic and tumors) must be ruled-out (1, 12).

Although a detailed three-generation family pedigree provides important genetic insights to a case, it may not always be informative for CHH/KS diagnostics. Studies demonstrate that pedigrees of patients with CHH/KS are enriched with family members with a history of delayed puberty (13, 14). Thus, clinicians may incorrectly assume that the individual is genetically programmed for late puberty. This can result in a "watchful waiting" approach and a missed opportunity for earlier diagnosis resulting from a more active investigation. Being labeled a "late developer" or "late bloomer" may be difficult for teens to accept and they may not feel healthcare professionals are taking them seriously. Such feelings may further inhibit patients discussing their puberty and seeking help for what may be a highly sensitive and embarrassing condition. Guidelines and review articles on delayed puberty are invariably directed at the evaluation and treatment of individuals for whom the cause of pubertal delay may not be initially obvious. However, a "watchful waiting" lacks a logical basis for those individuals with "red flag"

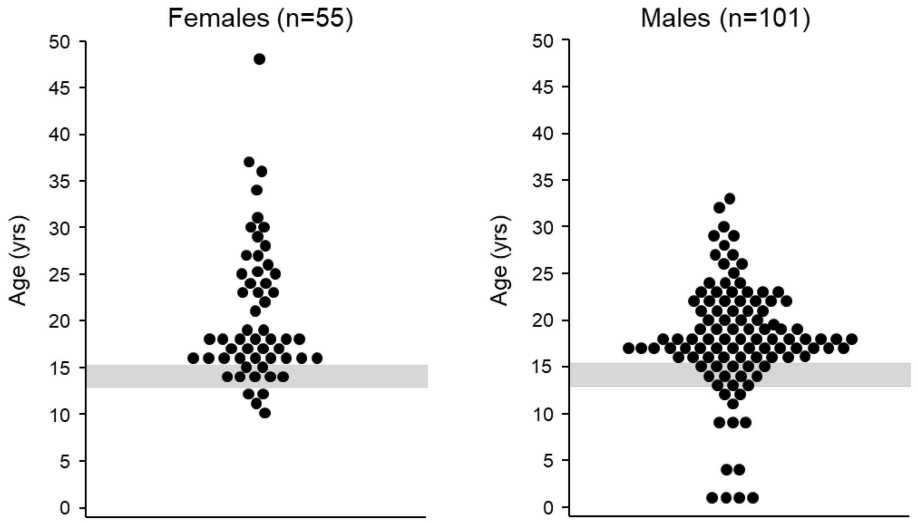

FIGURE 1 | Age at diagnosis. **Left** age at CHH/KS diagnosis for female patients ($n = 55$), median age at diagnosis = 18 years (mean: 21 ± 7 years). The gray shaded region depicts the mean age of menarche plus two standard deviations (15). Figure adapted from Dzemaili et al. (16). **Right** age at CHH/KS diagnosis for male patients ($n = 101$), median age at diagnosis = 18 years (mean: 18 ± 6 years, The gray shaded region depicts mean age of genital stage 3 plus two standard deviations (17). Figure adapted from Dwyer et al. (18).

features (**Box 2**) indicating high pre-test probability of CHH/KS. In such cases, sex hormone replacement therapy should not be delayed beyond median age of pubertal onset. Cumulatively, all these factors often contribute to a late diagnosis (**Figure 1**).

Moreover, patients with rare diseases often experience a "diagnostic odyssey," including incorrect diagnoses, incomplete information, delays in finding expert care and accurate diagnosis, and misleading or frankly incorrect advice along the way from non-specialists. Such experiences can significantly erode patient confidence in healthcare providers and health systems and affect quality of life (19). For patients with CHH/KS, there is all too often an unacceptable and inexplicable delay between the age of presentation (e.g., at 3-months of age with bilateral cryptorchidism and micropenis) and the age at diagnosis (e.g., at age 50 years with spontaneous vertebral fracture).

MISCONCEPTIONS REGARDING TREATMENT

Unlike many rare diseases, there are simple, effective, affordable treatments available for CHH/KS. Exogenous sex steroids can safely and effectively induce development of secondary sexual characteristics in males and females [reviewed in: (1, 20, 21)]. Similarly, exogenous gonadotropins (human chorionic gonadotropin ± recombinant FSH or pulsatile GnRH) can make fertility possible in roughly 75–80% of male patients [reviewed in: (1, 22–25)]. For female patients, ovulation induction can be achieved using exogenous gonadotropins (FSH for follicle development followed by hCG to induce ovulation) yet pulsatile GnRH is the preferred treatment for fertility induction due to decreased risk for multiples (26). If gonadotropins are used for ovulation induction, the risk of multiples can be mitigated by careful serial ultrasound monitoring to ensure that only one single dominant follicle is ovulated following hCG administration. Unfortunately, many patients are incorrectly labeled as "sterile" and remain unaware that fertility is likely possible with specialized regimens. For decades, using low dose testosterone esters (in males) and low dose estradiol (in females) has been the standard treatment to induce secondary sexual characteristics. However, to date, there is no standardized regimen to guide the induction of secondary sexual characteristics, particularly in older adolescents or those of adult age.

An important principle is starting with low dose replacement and gradually escalating dosage in order to maximize growth and attain normal breast contour in females. Importantly, females started directly on full-dose estrogen and progesterone (i.e., hormone replacement therapy, combined oral contraceptive pills) typically achieve markedly suboptimal breast development (1). It remains unclear if optimal breast development results from the progressive, incremental dose increase in estrogen, or an indirect effect of delaying the introduction of progesterone as long as possible. The classical approach is to introduce progestin when girls begin to experience significant vaginal spotting/bleeding on unopposed estrogen. Perhaps a more logical approach might be to monitor endometrial thickness by ultrasound to guide the adjustment of estrogen dose, and thereby prolong the introduction of progesterone until breast development is deemed appropriate. This point is relevant because breast size and appearance can be a source of significant anxiety and may impair body image for many eugonadal women–let alone those women with CHH/KS. Once cyclical estradiol and progesterone therapy has been

initiated, some women will incorrectly presume that their regular withdrawal bleeds will be associated with natural ovulation. Hence, anticipatory guidance regarding fertility is important. Additionally, monthly menses may be bothersome to many women with CHH/KS—and potentially undermine adherence to avoid menses. Patients should be informed they can safely have a few withdrawal bleeds per year–rather than menses being a monthly event.

In males, testosterone therapy will induce secondary sexual characteristics, but will not stimulate testicular growth (either gonadotropin therapy or pulsatile GnRH are required for this). Patients are typically not informed of this fact. Accordingly, male patients often have incorrect assumptions that testosterone will induce puberty and normal appearing testes. Lack of appropriate anticipatory guidance and patient education can result in frustration and may erode a therapeutic relationship between the patient and provider—and undermine adherence. Further contributing to frustration and loss-of trust, reviews and guidelines for pubertal-induction emphasize starting with low-dose treatment aiming to complete pubertal maturation over 2–3 years "in line with peer group." For many patients, this may seem agonizingly slow. For patients diagnosed at an adult-age treatment regimen is both safe and appropriate (27, 28). Once normal sex steroid levels in the serum have been attained, lifelong treatment is required with at least annual monitoring.

Recent studies have revealed that despite the presumed availability of safe and effective treatment, there are major gaps in both anticipatory guidance when initiating treatment as well as significant challenges for long-term adherence to treatment (16, 29, 30). The majority of patients with CHH/KS are diagnosed late (**Figure 1**) and thus, their physical appearance is much younger than their chronological age. Patients have a strong desire to "catch up" in pubertal development. Patients' desire to more closely resemble peers creates a temporal conflict with the "slow and low" approach to dose increase. Patients may become frustrated without appropriate anticipatory guidance on when changes can be expected. Feelings of dissatisfaction may undermine compliance with treatment (see Targets for Improving Care). CHH/KS is a chronic condition and long-term medication adherence is required for sexual function, bone health, preventing potential metabolic disease and overall well-being (1).

Like many chronic diseases, more than half of patients with CHH/KS struggle with adherence and 48% of women and 38% of men have treatment gaps of more than 1 year (16, 29). Major drivers of adherence include patient beliefs and concerns (31). While patients may be able to find clinicians who are knowledgeable about CHH/KS, evidence suggests that the understanding of the emotional and psychological aspects of care are underappreciated and neglected. A quote from a 1964 article reflects this perspective: "There is a tendency among physicians to assume that a corrigible pathological condition ought to be corrected and that the emotional well-being of the patient will improve concomitantly with his physical condition" (32). The quote was published more than 50 years ago, yet recent data suggest that this view persists. Sixty seven percent of patients believe their provider understands the medical aspects of CHH/KS. However, significantly fewer patients (38%, $p < 0.001$) perceive their provider as understanding the emotional impact of living with CHH/KS (30).

CHALLENGES FACED BY PATIENTS

Rare genetic diseases are often associated with psychological burden and negative emotional and psychosocial effects (33). Some have put forth the notion that challenges and inequities faced by rare disease patients put them in the realm of health disparities (34). Patients with CHH/KS may experience physical, cognitive and psychosocial consequences (**Box 3**). The lack of sex steroids due to CHH/KS can affect patients physically and cognitively. Physically, sex steroids are critical for bone health—both in formation and maintaining bone density. Because patients with CHH are hypogonadal without treatment, periods without treatment put them at increased risk for compromised bone health (35, 36). Indeed, a Finnish study of 26 patients with CHH found that long periods of non-adherence to treatment

Box 3 | Patient identified unmet needs*.

Knowledge of the CHH/KS

Patients often have limited understanding of the:
- Clinical difficulty in making a diagnosis
- Possibility of early (neonatal) identification
- Range and severity of signs and symptoms
- Symptoms that may or may not be associated (e.g., fatigue, cognition/learning/attention problems—including autism spectrum).

Access to Expert Care

Patients frequently have difficulty:
- Finding clinicians with experience in diagnosing CHH/KS
- Locating endocrinologists who know how to treat CHH/KS (including fertility-inducing regimens)

Genetic Testing

Patients have a poor understanding of:
- The complex genetics of CHH/KS
- What can and can not be achieved through genetic testing (i.e., treatment and potential risk of passing CHH/KS to offspring)
- How to communicate possible risk to family members

Treatment and Care

Patients are often unaware of:
- Types of physical/emotional changes that occur with treatment initiation (including timing of changes and what will/will not occur on treatment)
- Necessity of long-term treatment
- Consequences of poor adherence on health and wellbeing
- Treatment options (including dosage and timing intervals for testosterone injections)

Psychological and Psychosocial Consequences

Patients may not perceive:
- The importance of identifying and treating psychological issues related to CHH/KS (including those patients diagnosed early and those who are married with children)
- The life-changing opportunities available through peer-to-peer support (including body image concerns, self-esteem and sexuality)

**common concerns and issues raised by patients derived from online peer discussions (personal communication–N. Smith).*

was associated with worse bone density (37). Thus, consistent long-term adherence is essential for mitigating the risk for osteopenia and osteoporosis.

In terms of cognition, sex steroids are known to have activational and organizational effects on the brain and neural circuits. Research findings indicate that periods of rising circulating sex steroids (i.e., during the first 6-months of life in the so-called "mini puberty" and during puberty) are important developmental windows in which testosterone and estradiol have sex-specific effects on brain and behavioral development (38). While cognitive impairment is not a hallmark of CHH/KS and most patients have normal IQ, there are data indicating lasting effects on spatial abilities. A study comparing spatial abilities of men with CHH and acquired HH found that deficits observed in patients with CHH were not ameliorated by testosterone treatment. These observations suggest that androgens exert a permanent organizing influence on the brain (39). More recently a study of 34 Lithuanian CHH males at diagnosis (prior to sex steroid treatment) identified significantly lower executive function, attention, visual scanning and psychomotor speed compared to age-matched healthy controls (40). Notably, after 2 years of treatment, scored in these domains were improved—yet without significant changes in either emotional state of quality of life (41).

Approximately half to two-thirds of patients with CHH have diminished/absent sense of smell (i.e., Kallmann syndrome) (1, 42). Patients with defective olfactory function may be prone to eating food/drinks that have spoiled and often have concerns about not being able to detect body odor- contributing to feelings of self-consciousness and insecurity in social situations. Similarly, studies of patients with isolated anosmia reveal associations with increased social insecurity and depressive symptoms (43). One's olfactory acuity is indiscernible to others but absent pubertal development and looking younger than one's age is outwardly evident. Indeed, the disparity between chronological age and appearance can pose a significant barrier for dating and intimate relationships. Puberty is a biologic process that includes physiologic, psychosocial, and emotional changes and adolescence is a period of developing self-concept. Thus, disruption of puberty can carry a psychological burden (6).

Studies in late maturing 14–16 year-old boys identify body image concerns, low self-esteem, social isolation and experiences of teasing and bullying (44, 45)—common risks for depression in adolescents (46). For patients with CHH/KS, experiences are strikingly similar, if not magnified. Survey data indicate 56% of females and 72% of males experience teasing and victimization related to their condition. Body image concerns (e.g., body shame) are reported in 93% of males and 80% of females with CHH/KS. Supplementing these quantitative data, focus groups discussions reveal that concerns about low self-esteem, shame and social isolation are pervasive (16, 18, 30). These experiences often have lasting effects and perhaps not surprisingly, significant impact of intimate relationships and psychosexual development. Prior studies of late maturing boys have revealed they are more dissatisfied with their body image and less sexually active compared to peers who underwent normal pubertal timing (47). Studies in patients with CHH/KS corroborate these findings.

There are anecdotal reports in the literature mentioning low self-esteem and poor body image among patients with CHH/KS (32, 48, 49). Some have posited that pubertal failure and underdeveloped genitalia (i.e., prepubertal testicular size and small penis—both secondary to absent mini puberty) may pose barriers to engaging in sexual activity (50). Online patient discussions are filled with stories highlighting concerns about genital development conveying the impact such concerns have on seeking/initiating intimate sexual relationships. Studies examining quality of life and sexuality in CHH have been sparse with only a few small, anecdotal reports (32, 48, 49). More recently, this topic has gained increased attention. Aydogan et al. reported on 39 Turkish boys with CHH initiating testosterone treatment. Prior to treatment, the young men exhibited increased anxiety, depression, and worse quality of life (using the SF-36) compared to age-matched controls (51). Notably, 6-months of testosterone therapy improved physical function and vitality yet increased anxiety and significant emotional difficulties persisted as they adapted to life as a sexual adult (51). Longer duration of treatment up to 2 years in a cohort of 19 Lithuanian boys also failed to show significant improvements in emotional state and quality of life (40, 41). A Finish study found decreased quality of life in 30 males with CHH who exhibited high levels of distress and depression (52). In 2014, Shiraishi et al. studied six patients undergoing fertility-inducing gonadotropin treatment over 2 years (53). In contrast to testosterone replacement (51), gonadotropin therapy stimulates testicular growth. The investigators observed significant improvement in SF-36 measures including amelioration of the emotional difficulties not seen with testosterone treatment. The authors posited that the genital development might have had a role in improving the emotional and body image concerns of the six patients.

Subsequently, Dwyer et al. reported on the largest cohorts studies to date (101 males, 55 females) providing robust evidence of the psychosexual impact of CHH/KS pervasive (16, 18). Patients found intimate relationships as "very difficult" (68% of males, 59% of females). Females were more likely to have ever been sexually active (89%) compared to male counterparts (74%). Notably, more than a quarter of men with CHH have never been sexually active. This is five-times the rate observed in similarly aged men drawn from a population-based sample (26% vs. 5.4%, $p < 0.001$). In parallel, qualitative focus groups explored the impact of disrupted sexual maturation on psychosexual development and intimate relationships in detail. Patients reported feeling isolated and "left behind" as peers advanced through puberty and started dating and taking on roles that are more adult. Fear and anxiety about being exposed was common and many tried to hide their lack of sexual development and sometimes avoided social interactions—creating a reinforcing and cyclic pattern that lasted well into adulthood (see Targets for Improving Care) (18). Similarly, a Finnish study demonstrated that despite long-term treatment, men with absent mini-puberty (i.e., cryptorchidism with/without micropenis) had the lowest scores on dimensions of sexual activity (52). These data support the notion of persisting body shame and low

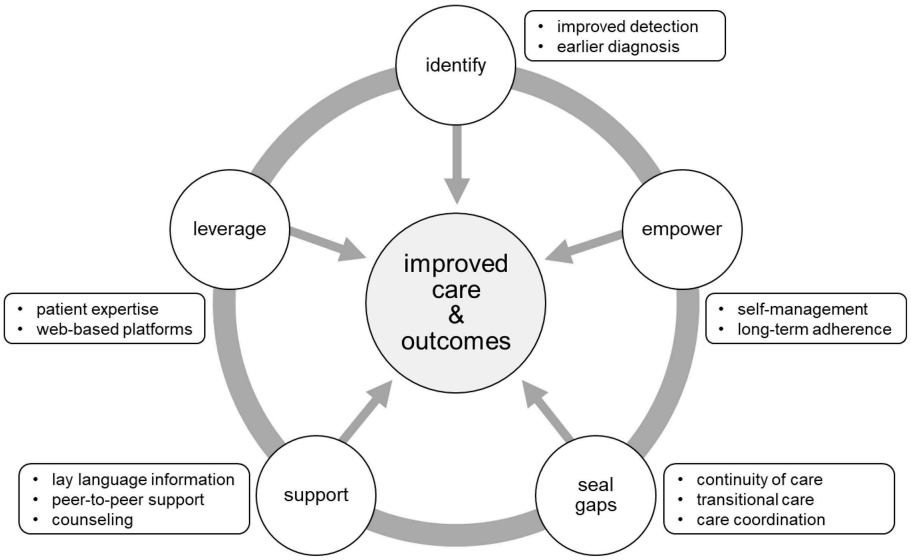

FIGURE 2 | Targets for improving care and outcomes. Five main targets were identified from the literature and feedback from the patient community. Opportunities to improve care and outcomes include: (1) better detection and earlier diagnosis, (2) activating and empowering patients for enhanced chronic disease self-management, (3) promoting continuity of care through care coordination and structured transition from pediatric to adult-oriented care, (4) providing information, enhanced mental health services and access to peer-to-peer support, (5) leveraging technology to extend the reach of care to geographically dispersed patients.

self-esteem despite long-term treatment with a lasting impact on psychosexual functioning.

For patients with CHH/KS, the pervasive negative illness perceptions provide insights into the burden many patients face (16, 29). Notably, clinicians often underappreciate the psychosocial impact of CHH/KS. Recent research indicates that 34% of patients with CHH/KS exhibit moderate-severe symptoms of depression (16, 29)—yet barely one quarter of patients report ever having a provider discuss psychological support or services. Patients with CHH/KS face a number of challenges that range from feelings of isolation and alienation related to living with a rare disease, possible physical and cognitive issues as well as a constellation of emotional, psychological, and psychosexual problems. It is worthwhile to note that just as the clinical presentation and genetics of CHH/KS are heterogeneous, so too are the coping responses of patients. Some patients struggle with some or many of these challenges yet others effectively cope with these difficulties. The following section outlines areas for improving the care of CHH/KS and avenues for supporting patient empowerment and more patient-centered approaches to care.

TARGETS FOR IMPROVING CARE

As stated previously, CHH/KS is a difficult diagnosis to make. A major challenge for clinicians and patients alike is the problem of late diagnosis. Healthcare professionals can be frustrated by the genetic heterogeneity of CHH/KS as well as the current lack of plasma/serum biomarkers or sensitive and specific dynamic test to differentiate CDGP and CHH/KS. For patients, the psychosocial ramifications of late diagnosis can have lasting effects. Thus, one of the main targets for improving care and outcomes relates directly to enhanced detection and earlier diagnosis (**Figure 2**). It is critical to raise clinician awareness of red flags (**Box 2**). Defective sense of smell (anosmia) is a strong clue for making a KS diagnosis. In male infants, signs of absent mini puberty (e.g., cryptorchidism with/without micropenis) during the neonatal window (first 6-months of life) represent the earliest opportunity to make a diagnosis.

Other opportunities for improving the care and outcomes for patients relate to chronic disease management. Patients with CHH/KS must be activated and empowered for self-care. Indeed, effective, long-term adherence to hormonal treatments are critical for mitigating metabolic risks (e.g., metabolic syndrome, type 2 diabetes) (54) and maintaining bone health (37), sexual function, and well-being. Much has been written on the topic of adherence and the subject is multifaceted and complex yet adopting a patient-centered approach to care is associated with better adherence. Briefly, the Picker Principles of Patient-Centered Care (55) are integral parts of providing high-quality healthcare and include: (1) respect for patients' values, preferences and expressed needs, (2) coordination and integration of care, (3) information, communication and education, (4) physical comfort, (5) emotional support and alleviation of fear and anxiety, (6) involvement of family and friends, (7) continuity and transition, and (8) access to care. Thus, effective patient-provider communication, therapeutic education (including anticipatory guidance) and shared decision-making are key elements for supporting adherence and patient self-management. Patients with CHH/KS often have long gaps in care (16, 30, 37). As with other chronic health conditions, continuity of care is an important factor in reducing complications and improving outcomes. Thus, co-ordinated care and structured transition programs to effectively move young adults form

Box 4 | Resources and links for patients.

Medical information and finding experts
https://rarediseases.info.nih.gov/diseases/10771/kallmann-syndrome
https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=GB&Expert=478
https://www.chuv.ch/en/hhn/hhn-home/

Lay Information
https://www.youtube.com/watch?v=yKfThHq9Vjs
https://globalgenes.org/raredaily/the-24-year-old-late-bloomer-kallmann-syndrome/
https://en.wikipedia.org/wiki/Kallmann_syndrome
https://www.chuv.ch/en/hhn/hhn-home/

Patient Perspectives and Peer-to-Peer Support
Facebook:
– Kallmann Syndrome Links and Help (OPEN group)
– Kallmann Syndromers (CLOSED group)
– Kallmann Syndrome & Hypogonadotropic Hypogonadism (SECRET group)
https://www.news-medical.net/health/Kallmann-Syndrome.aspx
https://www.rareconnect.org/en/community/kallmann-syndrome
https://www.youtube.com/watch?v=eitQYgCqA-0

General Rare Disease Resources
https://rarediseases.org/
https://www.eurordis.org/
http://www.agsa-geneticsupport.org.au/

**Working links as of August 2018.*

pediatric to adult-oriented care are critical for closing gaps in care (56, 57).

Patients frequently express the desire for more information (16, 30) (**Boxes 3**, **4**). Importantly, healthcare professionals and patients have complementary knowledge and expertise. Providers understand the genetics, pathophysiology and treatment while patients understand what it is like to live with a rare disease. This presents opportunities for co-creating solutions—as recently demonstrated in a project pairing expert clinicians and patients to create patient educational materials in lay language (and translated into 20 languages) (58). Moreover, peer-to-peer support is a means to pierce the veil of isolation many patients feel and offer opportunities for patients to connect and crowdsource solutions (30). There is a need to raise provider sensitivity to the psychosocial aspects of CHH/KS to increase screening for symptoms of anxiety and depression. Further, many patients may benefit from mental health services to address self-esteem and psychosexual issues.

Rare disease patients are dispersed geographically. This makes is difficult for patients to reach expert centers and clinicians who are experienced in using specialized fertility-inducing regimens. Therefore, leveraging technology to reach dispersed patients and connecting patients with specialists is a key part of improving care for CHH/KS. As demonstrated in a recent needs assessment (30), patient partnerships combined with a web-based approach is a highly effective. Patients with rare diseases are internet "power-users" who go online to learn about their condition, access care, and connect with other patients (59). An international network of CHH/KS clinicians and researchers partnered with patients and advocates to respond to the unmet needs identified in the needs assessment. Together, they collaboratively developed a virtual toolkit to help patients learn about their condition, find clinical centers, access genetic testing services and join peer-to-peer support groups (58). This example highlights how respectful and trusting patient-provider partnerships can use co-creation to translate research into improved clinical care. Importantly, for long-term sustainability of such platforms, ongoing patient engagement and participation will be essential.

FUTURE DIRECTIONS

There are a number of unanswered questions related to CHH/KS. Studying rare diseases and developing an evidence base to guide best practices is challenging as publications typically come from single centers and have relatively small populations. One opportunity is for broader, international collaboration with harmonized definitions and measures. Patient registries are particularly helpful for rare disease research (60). Such tools can be used to conduct natural history studies and better understand long-term health outcomes and impact on quality of life.

For CHH/KS, such a natural history study could be extremely useful for exploring the phenomenon of reversible CHH (61). Data suggest that ∼10% of patients recover function of their hypothalamic-pituitary-gonadal axis and can sustain normal sex steroid levels and fertility following discontinuation of hormonal treatment (62). Interestingly, reversal is not always lasting and these patients appear susceptible to relapse and a subsequent "crash" of their reproductive axis (63). Long-term studies of such cases could potentially help identify biomarkers and predictors for reversal and potentially open new avenues for developing novel treatments. Registries and natural history studies can also help examine how CHH/KS evolves over time and the impact on quality of life. These data can be used to identify patient-reported outcome measures (PROMs) (64, 65). Subsequently, identified PROMs could be used as outcomes and secondary endpoints for clinical trials (66). Indeed, further work is needed to clarify the optimal treatment(s) for CHH/KS and the best timing for treatment initiation (1). Thus, a patient registry and natural history study could help advance the field.

Additional future directions include developing tools and identifying biomarkers to facilitate early diagnosis. Genetic testing can be informative in approximately half of cases. However, an unmet need is access to decisional support for genetic testing. Online discussions and unpublished data indicate that patients may struggle with genetic testing decisions—particularly related to the complex genetics of CHH/KS (5). Patients could benefit from decisional support, interventions promoting active coping strategies and approaches supporting effective family communication of risk. Currently, expertise in specialized fertility-inducing treatment is limited and dispersed. Future directions may include international multicenter trials to determine optimal treatment regimens and using web-based "e-consulting" to share this dispersed, specialized expertise. More work is needed to develop and test effective both face-to-face and web-based interventions for activating and empowering patients for long-term adherence and self-management. Additionally, transitional care has only recently gained attention. As such, there is a limited evidence base for supporting best practices (or exemplar models) for effectively transitioning patients from pediatric to adult oriented care.

CONCLUSIONS

Congenital hypogonadotropic hypogonadism and Kallmann syndrome (CHH/KS) is a rare, treatable form of infertility. Like other rare disease patients, individuals with CHH/KS frequently experience feelings of isolation and alienation. Effective hormonal treatments are readily available for inducing secondary sexual characteristics and fertility (in the vast majority of cases). Indeed, CHH/KS is not life threatening, but it is a severely life-altering condition. Disrupted puberty can have lasting psychological, emotional, and sexual effects. As part of comprehensive care, clinicians should give appropriate attention, care and referrals (e.g., peer-to-peer support, mental health services) as appropriate to ameliorate the psychological aspects of CHH/KS.

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MINI REVIEW
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Postnatal Testicular Activity in Healthy Boys and Boys With Cryptorchidism

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Cryptorchidism, or undescended testis, is a well-known risk factor for testicular cancer and impaired semen quality in adulthood, conditions which have their origins in early fetal and postnatal life. In human pregnancy, the interplay of testicular and placental hormones as well as local regulatory factors and control by the hypothalamic-pituitary (HP) axis, lead to testicular descent by term. The normal masculine development may be disrupted by environmental factors or genetic defects and result in undescended testes. Minipuberty refers to the postnatal re-activation of the HP-testicular (T) axis after birth. During the first weeks of life, gonadotropin levels increase, followed by activation and proliferation of testicular Leydig, Sertoli and germ cells. Consequent rise in testosterone levels results in penile growth during the first months of life. Testicular size increases and testicular descent continues until three to five months of age. Insufficient HPT axis activation (e.g., hypogonadotropic hypogonadism) is often associated with undescended testis and therefore minipuberty is considered an important phase in the normal male reproductive development. Minipuberty provides a unique window of opportunity for the early evaluation of HPT axis function during early infancy. For cryptorchid boys, hormonal evaluation during minipuberty may give a hint of the underlying etiology and aid in the evaluation of the later risk of HPT axis dysfunction and impaired fertility. The aim of this review is to summarize the current knowledge of the role of minipuberty in testicular development and descent.

Keywords: HPG axis, cryptorchidism, minipuberty of infancy, testicular descent, gonadotropin (FSH and LH)

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INTRODUCTION

Testicular descent initiates during early fetal development and finalizes during the first postnatal months. This complex process is regulated by multiple genetic, anatomical and hormonal factors and environmental factors may influence its course. Failed testicular descent (i.e., cryptorchidism) is one of the most common congenital anomalies with prevalence between 2 and 9% (1, 2). However, the etiology of isolated cryptorchidism often remains unknown.

Definitions related to congenital cryptorchidism vary. In his landmark paper, Scorer considered testes that were within the 4 cm distance from the pubic bone as undescended among term boys (3), which roughly corresponds with the 2.5th centile at birth (4). However, most cohort studies have used criteria developed by John Radcliffe Hospital Study group, in which the testicular position is classified in reference to anatomical landmarks as "non-palpable," "inguinal," "suprascrotal," and "high scrotal," whereas testes that lie in the bottom of the scrotum (scrotal) are considered normal (**Figure 1**) (5). Testes that can be manipulated to the bottom of the scrotum, and stay there at least for a while are termed retractile and are often considered normal (7). However, data on long-term fertility and testicular cancer morbidity outcomes within this subgroup of cryptorchidism remain scarce. Some authors have questioned whether high scrotal testes indeed should be considered abnormal (8). On the other hand, there seems to be some evidence of reduced testicular growth among boys with retractile testes (9).

Complete testicular descent requires adequate and timely function of the fetal Leydig cells from the first to the third trimester of pregnancy. Factors that interfere with Leydig cell function may predispose infants to cryptorchidism. During the first trimester, placental hCG regulates Leydig cell function. During the second trimester, the fetal hypothalamic-pituitary (HP) unit takes control. Besides the critical intrauterine phases of testicular development, the early postnatal period has emerged as an important period in male reproductive development. The HPT axis is transiently activated during the first months of life (10–12) and this activation is associated with penile and testicular growth as well as continued testicular descent (4, 12, 13). The minipuberty of infancy is transient since HPT axis activity decreases toward the 6 month of postnatal life (12) (**Figure 2**).

In milder cases of cryptorchidism, spontaneous testicular descent is common during minipuberty, but rarely seen thereafter (14, 15). There are reports of infants with congenital hypogonadotropic hypogonadism (CHH) and absent minipuberty who present with testicular ascent and involution of the penis and scrotum during the first year of life (16). Moreover, two small studies indicated that among boys with cryptorchidism due to hypogonadotropic hypogonadism, gonadotropin therapy induces testicular descent and surgical orchidopexy may be avoided (17, 18). However, CHH is a very rare cause of cryptorchidism and treatment with human chorionic gonadotropic hormone (hCG) or GnRH analogs is no longer recommended, mainly because of poor efficacy and possible adverse effects (19, 20).

In this review, we evaluate and summarize the current knowledge of the role of the HPT axis in pre- and postnatal testicular descent in humans and compare the differences in minipuberty between healthy and cryptorchid boys.

OVERVIEW AND ONTOGENY OF THE HPT AXIS

Specific gonadotropin-releasing hormone (GnRH) secreting neurons in the hypothalamus are the major controllers of the reproductive function. During early embryogenesis, GnRH neurons migrate from the nasal placode to their final location in the anterior hypothalamus and complete their journey by 15 weeks gestation (21, 22). The function of the mature GnRH neurons is modulated by several other hypothalamic hormones (23), such as glutamate and γ-aminobutyric acid (GABA), and many neuropeptides, including neuropeptide Y, galanin-like peptide, opioid peptides, and orexins. However, the most important upstream regulator of the GnRH neurons is a neuromodulatory peptide kisspeptin, encoded by the *KISS1* gene and acting via a G protein-coupled receptor GPR54 that is now termed KISS1R (24). Kisspeptin neurons can co-express neurokinin B and dynorphin, termed kisspeptin-neurokinin B-dynorphin (KNDy) neurons (25). Currently, the ontogenesis of this complex neuronal network in humans is not fully understood.

Pulsatile release of GnRH results in pituitary secretion of two gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH). Both LH and FSH are detectable in the fetal pituitary and peripheral circulation by 12–14 weeks gestation (26–28). LH and FSH bind to their specific receptors in the testicular Leydig and Sertoli cells, respectively. Androgens secreted by the Leydig cells and inhibin B secreted by the Sertoli cells regulate the activity of the HP unit by negative feedback. The negative feedback effects of testosterone are largely mediated by estradiol after local aromatization of testosterone to estradiol in the brain. Inhibin B specifically regulates pituitary FSH secretion, whereas the negative feedback by estradiol suppresses the secretion of both LH and FSH (29).

During the first trimester of pregnancy, testicular hormone secretion is not dependent on the integrity of the HP unit of the fetus. Instead, Leydig cells are stimulated by placental hCG which acts through the LH/hCG receptor. Leydig cell activity is critical for fetal masculinization programming during a discrete window between 8 and 14 weeks gestation (30). Notably, defects in HP function do not affect masculinization as the axis is still immature at this time. In addition to the canonical steroidogenic pathway in the testis, there is an alternative backdoor pathway of androgen production in the fetus that uses the placenta and liver in androgen synthesis (31). Disruption of either pathways lead to problems in masculinization (32).

The fetal HP unit is functional during the second trimester and testicular testosterone and inhibin B are major regulators. The activity of the fetal HPT axis peaks during the late first and early second trimester when testosterone levels reach levels observed in adult men (33). At this time, fetal gonadotropin levels markedly differ between sexes as LH and FSH levels are higher in female fetuses (27, 28). This sex difference is probably due to the negative feedback effects on the fetal HP unit mediated by the testicular testosterone and inhibin B. In males, inhibin B levels are higher compared to levels observed in female fetuses at midgestation and levels remain elevated until term (34). The activity of the HPT axis decreases toward the end of gestation and is low at term. This shift is possibly due to maturing negative feedback mechanisms and suppression mediated by increased placental hormone levels, especially estrogens (34, 35).

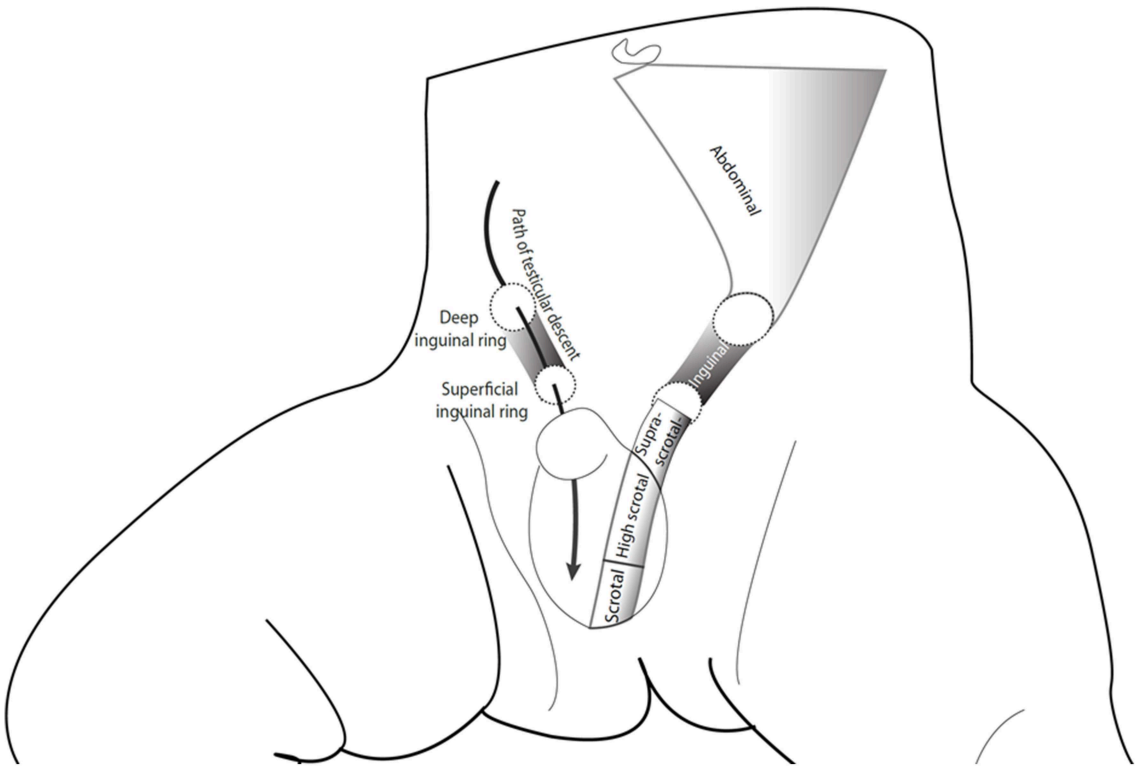

FIGURE 1 | The path of testicular descent and classification of testicular position according to the John Radcliffe Hospital Cryptorchidism Study Group (5). The figure is reproduced from Koskenniemi (6) with the permission of the copyright holder.

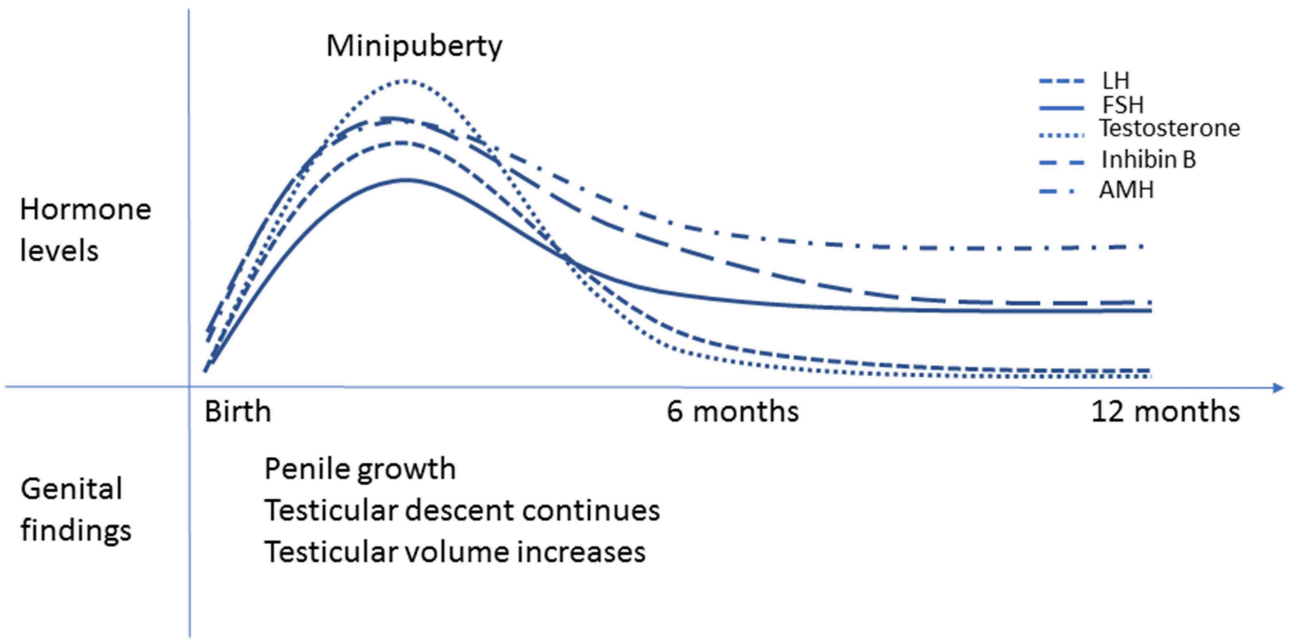

FIGURE 2 | Minipuberty of infancy. Schematic presentation of the changes in reproductive hormone levels during the first year of life in healthy boys. The peak hormone levels are observed between 1-3 months of age. LH and testosterone levels decrease by 6 months of age, but FSH and inhibin B levels remain elevated longer. AMH levels increase from birth to 3 months of age, then slightly decrease but remain higher than in adults until puberty. Penile length and testicular volume increase and testicular descent continues during minipuberty.

The Role of Fetal HPT Axis Activity in Intrauterine Testicular Development and Descent

By 6 weeks of gestation, the bipotential gonad differentiates into the testes and by week 8, Leydig cells produce testosterone and Sertoli cells produce anti-Müllerian hormone (AMH). This early hormonal activity guides the normal development of the internal and external male reproductive organs (36, 37). Initial testicular development occurs proximal to the posterior abdominal wall. The abdominal phase of the testicular descent begins between the 7th cervical and 8th thoracic vertebra with the initial formation of the gonadal streak (38). From that position, the testes descend to the 9th thoracic to 3rd lumbar vertebra during the first months post-conception. This initial descent is caused by the descent of the primordium of the diaphragm and the regression of the mesonephros. A ligament called *Gubernaculum Hunteri* anchors the testis to the abdominal wall at developing opening of the inguinal canal. Swelling of the gubernaculum during gestational week 20 dilates the inguinal canal and the gubernaculum migrates down the inguinal canal (39). This transinguinal testicular descent usually occurs between gestational weeks 23–30 (39, 40).

Leydig cell product, insulin-like 3 (INSL3) and its receptor RXFP2 play a central role in the transabdominal phase of testicular descent—which is usually completed by 15 weeks of gestation (41, 42). In mice with *Insl3* inactivation, swelling of the gubernaculum does not occur, and testicular descent is terminated high in the abdomen (43). In humans, mutations in *INSL3* and *RXFP2* are rare, but have been associated with cryptorchidism (44). Human fetal INSL3 secretion is regulated by hCG and LH *in vitro* (45). Unexpectedly, a study found that cryptorchid cases appeared to have increased INSL3 levels in the amniotic fluid compared to controls between gestational weeks 13–16 (43). INLS3 seemed lower in cryptorchid fetuses vs. controls during gestational weeks 17–22, suggesting altered Leydig cell activity later when the testicular descent continues, although the difference was below the level of statistical significance, possibly due to larger variability in INSL3 concentrations (46).

The role of androgens in the first phase of testicular descent is not completely understood. However, inguinoscrotal testicular descent is generally thought to be androgen dependent (42, 47). Complete lack of androgen activity in 46, XY fetuses with inactivating mutations of the androgen receptor (AR) results in phenotypic female development and abdominal/inguinal testis. Milder forms result in a range of phenotypes including micropenis, cryptorchidism, bifid scrotum and/or hypospadias (48). Similar phenotypes may result from mutations in the LH/hCG receptor (*LHR*), which block androgen synthesis in the testis (49). Fetal Leydig cell control transitions from placental hCG to pituitary LH somewhere during late first to early second trimester of pregnancy. Low circulating hCG levels have been observed during gestational weeks 12–16 in mothers of boys with cryptorchidism at birth (50). During the inguinoscrotal phase of testicular descent, hCG levels decline and pituitary LH is important for stimulating Leydig cell androgen production. In fetuses with CHH and very low LH levels, testosterone levels during the second and third trimester are low, often resulting in micropenis and cryptorchidism (51, 52).

In addition to INSL3 and androgens, AMH may play a role in testicular descent. Indeed, there is a high prevalence of cryptorchidism among subjects with mutations in *AMH* or *AMHR2* (i.e., persistent Müllerian duct syndrome) (53). However, the exact mechanism is unclear as *Amh* knock-out mice have normally descended testes (54).

INSL3 levels in the cord blood of cryptorchid boys are reduced compared with boys with normally descended testes (55, 56). Cryptorchid boys with a non-palpable testis have lower cord INSL3 levels compared to boys with palpable testes (56). Notably, levels of testosterone, LH, FSH, AMH, sex hormone binding globulin (SHBG) and inhibin B in cord blood do not differ between cryptorchid boys and controls (56).

Postnatal Activation of the HPT Axis, Minipuberty

Near term, activity of the fetal HPT axis is low. Cord blood testosterone and inhibin B levels are higher in boys than in girls indicating that the testes are not completely inactive (34, 57). hCG levels remain relatively high in the cord blood, but hCG, in addition to the high placenta-derived estrogen levels, are cleared from the newborn's circulation during the first week of life. Removal of the suppressive effects of placental hormones after birth results in re-activation of the newborn's HP unit as demonstrated by increased LH and FSH levels during the first weeks of life (12, 58–60). This gonadotropin surge results in testicular activation and consequent increase in testosterone (11, 12, 61), inhibin B (11, 62), and AMH levels (60, 63).

Gonadotrope (LH)-Leydig Cell Axis

Testosterone levels increase concomitantly with LH levels until 1–3 months of life. Serum levels reach the pubertal range and then decline toward the age of 6 months (11, 61, 62). In boys, LH levels exceed FSH levels during the first postnatal months. As pituitary activity wanes, LH levels fall below FSH levels around 3 months of age (62). By 6–9 months of age, LH and testosterone levels decline and remain at low levels for the duration of the childhood (11, 12). INSL3 levels correlate positively with both LH and testosterone in boys at the age of 3 months, suggesting that postnatal LH also stimulates INSL3 secretion (55). INSL3 levels are higher in infancy than later in childhood (55) and increase again at puberty (64).

The biological activity of testosterone during minipuberty has been questioned as SHBG levels increase simultaneously—leading to low levels of free testosterone (65). However, observations from longitudinal studies suggest the opposite effect. The testosterone surge in minipuberty is associated with simultaneous biological effects in androgen target tissues as evidenced by penile growth, transient secretion of prostate specific antigen and sebaceous gland activity (12, 13, 66). Surging testosterone levels are also associated with accelerated growth velocity during minipuberty and probably explain why boys grow faster than girls in early infancy (67). Moreover, in boys who lack minipuberty because of CHH, linear growth in infancy is slower compared to healthy boys

(68). Postnatal testosterone levels potentially modulate the masculine neurobehavioral development, as testosterone levels during minipuberty have been associated with male-typical play behavior at 14 months of age (69). In another study, penile growth during minipuberty was associated with sex-typed play behavior at 3–4 years of age (70). Testosterone levels in minipuberty have been associated with early language development (71).

Gonadotrope (FSH)-Sertoli Cell Axis

In newborn boys, both inhibin B and AMH levels increase and peak near the age of 3 months. Inhibin B levels at this time exceed adult levels and AMH levels reach their highest level of the entire lifespan (11, 62, 63, 72). Inhibin B levels at 3 months of age correlate negatively with FSH levels (62, 73). Unlike LH levels, FSH levels often remain measurable during childhood (11). Inhibin B levels decline at approximately 15 months of age falling to the prepubertal range then increase again during puberty (11, 72). AMH levels decline toward 1 year of age, but remain high during childhood until increased testosterone levels in puberty cause AMH level to decline (63).

Notably, testicular volume increases during minipuberty (12, 73). This growth most likely represents the proliferation of the Sertoli cells in the seminiferous compartment (74, 75). Furthermore, the number of Leydig and germ cells increase during the first postnatal months (74–76). Testicular volume and inhibin B correlate positively at 3 months of age (73). The AR is not present in Sertoli cells in fetal or newborn testis. This observation likely explains why high intratesticular testosterone levels do not activate spermatogenesis and why AMH levels remain high (77, 78). Following the minipubertal gonadotropin surge, testicular volume appears to slightly decrease during infancy (12, 79). In addition to testicular growth, testicular descent continues after birth. Recent data from a large prospective Danish-Finnish birth cohort study suggest that the testes clearly migrate toward the bottom of the scrotum between birth and 3 months of age (4). Testicular position is associated with indices of both Leydig and Sertoli cell function (i.e., testosterone:LH and inhibin B:FSH ratios) as well as IGF-I levels (4). Furthermore, in cryptorchid and non-cryptorchid boys, a small but significant testicular ascent is observed between 3 and 18 months of age (4). This fits well with the observations that spontaneous testicular descent is unlikely after minipuberty (15, 80).

In preterm boys born before 37 weeks of gestation, cryptorchidism is a common finding. However, spontaneous testicular descent usually occurs by term or during the first months of postnatal life. Indeed, testicular descent after 3 months of age is more common in preterm than in full-term boys (14). The prolonged period of spontaneous testicular descent probably reflects the longer period of postnatal HPT axis activity in preterm boys (12). However, when corrected for the age of prematurity, HPT activity wanes at a developmental age similar to full-term boys (12). Consequently, the duration of minipuberty seems to be programmed according to the child's developmental age.

Germ Cells

There are limited human data on the early development of germ cells and related regulatory factors (81). In a post-mortem study of testicular pathology samples from 48 boys aged <3 years, the number of germ cells was higher in boys between the ages of 50–150 days (∼2–5months-old) compared to either younger or older boys (74). In another post-mortem study, germ cell apoptotic index (i.e., percentage of apoptotic cells/total cell number) increased and proliferation index decreased after the first month of life (n = 18) whereas no further change was observed between boys 1 and 6 months of age (n = 13) and 1–6 years of age (n = 13) (82). Gonocytes, the fetal stem cells, mature during the first year of life into adult dark (Ad) spermatogonia, that are presumed to be the stem cells for later spermatogenesis (83). This maturation coincides with minipuberty. Accordingly, a causal role for hormonal activity during minipuberty has been suggested (84). However, the role of gonadotropins and testicular hormones in this process has yet to be clarified and existing data on the role of androgens are controversial (85). The maturation of spermatogonia continues after minipuberty and some primary spermatocytes can be observed around 3–4 years of age (86).

Consequently, minipuberty is important for male reproductive development as it seems to finalize and stabilize the genital development of boys. It is clear that testicular hormones exert negative feedback on the HP unit in infancy. However, as agonadal children exhibit a biphasic pattern of gonadotropin levels (87), the factors that silence HP unit activity during childhood do not appear to be of gonadal origin.

Minipuberty in Cryptorchid Boys

Available data on reproductive hormone levels in cryptorchid boys during minipuberty are limited and interpreting the existing data is challenging due to the small number of patients in most studies, variation in the age at sampling and classification of testicular position and the phenotypes (e.g., unilateral/bilateral, palpable/non-palpable, etc). As hormone levels during the first months of life undergo developmental changes (described above), interpreting values observed in cryptorchid infants must be done with caution. In contrast, boys with anorchia present a typical hormonal profile during minipuberty (and childhood) with very high LH and FSH levels and low or undetectable levels of testosterone, inhibin B and AMH—regarded as the most specific marker of existing testicular tissue (87). Studies on association of reproductive hormone levels and testicular position in infancy are summarized in **Table 1**.

Gonadotrope (LH)-Leydig Cell Axis in Cryptorchidism

Higher minipuberty LH levels in cryptorchid boys compared to boys with normally descended testes have been reported in some (92), but not all studies (89, 91, 93). Gendrel et al. reported higher LH and T levels in cryptorchid boys with spontaneous testicular descent compared to boys who remained cryptorchid during minipuberty (88).

Despite the central role of androgens in testicular descent, circulating concentrations of testosterone seem to be comparable between congenitally cryptorchid and non-cryptorchid boys at birth (56). In addition, comparing testosterone levels in

TABLE 1 | Studies reporting the association between reproductive hormone levels and testicular position in infant boys.

References	Participants	Age at hormonal measurements	Outcome measures	Results
Gendrel et al. (88)	27 transiently cryptorchid, 30 persistently cryptorchid	1–4 months	LH, FSH, T	Lower LH and T levels in persistently cryptorchid vs. transiently cryptorchid boys.
De Muinck Keizer-Schrama et al. (89)	160 control, 19 transiently cryptorchid, 29 persistently cryptorchid	1) 3, 6, 12 months 2) 12 months	1) basal and LHRH-stimulated LH and FSH, basal T 2) hCG-stimulated T	Higher basal LH levels in transiently cryptorchid than in controls. No difference in T levels between the groups.
Raivio et al. (90)	35 control, 45 cryptorchid	3 months	Serum androgen bioactivity	Quantifiable serum androgen bioactivity in 46% of boys with scrotal/high scrotal testicular position, but not in any of the boys with suprascrotal or higher testicular position.
Barthold et al. (91)	26 control, 20 cryptorchid	2 months (plasma), serial urine samples up to 4 months	Plasma LH, FSH, T, E2, inhibin B, leptin, SHBG; urine LH, FSH, T, E2	No differences between controls vs. cryptorchid boys.
Suomi et al. (92)	Finnish boys: 300 control, 88 cryptorchid Danish boys: 399 control, 34 cryptorchid	3 months	LH, FSH, T, inhibin B	Higher FSH levels in cryptorchid than in control boys. Lower Inhibin B and higher LH in Finnish (not in Danish) cryptorchid boys vs. controls. No difference between groups in T levels.
Bay et al. (55)	100 control, 28 transiently cryptorchid, 51 persistently cryptorchid	Birth, 3 months	INSL3, LH, T	Cord blood INSL3 reduced in persistently vs. transiently cryptorchid boys and controls. LH to INSL3 ratio higher in persistently vs. transiently cryptorchid boys at 3 months of age.
Pierik et al. (93)	113 control, 43 cryptorchid	1-6 months	LH, FSH, T, AMH, inhibin B, SHBG	Lower T and non-SHBG-bound T in cryptorchid than in control boys
Fenichel et al. (56)	128 control, 52 cryptorchid	Birth	INSL3, T, LH, FSH, AMH, hCG, SHBG	Lower INSL3 levels in cryptorchid vs. control boys. No difference in other hormone levels.
Koskenniemi et al. (4)	2,545 boys Blood tests: Finnish 362, Danish 680	3 months	Testicular distance to pubic bone at birth, 3 and 18 months; LH, FSH, T, INSL3, inhibin B	Testosterone/LH-ratio and inhibin B/FSH-ratio were positively associated with lower testicular position.
Grinspon et al. (94)	1) 24 control, 19 cryptorchid (7 bilateral) 2) 26 control, 80 cryptorchid (52 bilateral)	1) 1–5.9 months 2) 6 months- 1.9 years	LH, FSH, T, AMH	1) Lower T in unilaterally cryptorchid vs. control boys. 2) Lower AMH levels in bilaterally vs. unilaterally cryptorchid boys and controls.

cryptorchid and non-cryptorchid boys at the age of two to 3 months does not reveal significant differences between the groups (91, 92).

However, testicular position at 3 months (or the change in testicular position between birth and 3 months) may be related to reproductive hormone levels during minipuberty. A small Finnish study noted the differences in circulating testosterone levels between cryptorchid and healthy boys at 3 months (90). Furthermore, Raivio et al. (90) found measurable androgen bioactivity at the age of 3 months in 26 of 64 (41%) of boys with scrotal or high scrotal testes, whereas androgen bioactivity was undetectable in all 16 boys with suprascrotal/inguinal/non-palpable testes.

In a Finnish-Danish cohort study, higher testosterone levels were observed among boys who were cryptorchid at birth yet underwent spontaneous testicular descent at 3 months compared to healthy controls (92). Boys with mild cryptorchidism (i.e., high scrotal testis) at the age of 3 months also exhibited higher testosterone levels than those with severe cryptorchidism (suprascrotal, inguinal or non-palpable) (92). A similar difference in testosterone levels between cryptorchid boys with spontaneous descent and those who remained cryptorchid was noted in an Egyptian study (80). Likewise, Job et al. found higher testosterone levels during minipuberty in boys with spontaneous descent than those who remained cryptorchid (88, 95). Finally, a Dutch study reported an overall difference in the testosterone levels between cryptorchid and healthy boys and revealed that a higher proportion of cryptorchid boys had undetectable testosterone levels compared to controls at 100+ days postnatal (93). These observations are in contrast to older data from a Dutch study of similar gonadotropins (i.e., basal and stimulated) and testosterone levels in cryptorchid compared to control boys at 3, 6, or 12 months of age (89).

In the longitudinal Finnish-Danish cohort study, the testosterone:LH-ratio correlated positively with improved testicular position from birth to 3 months in cryptorchid and non-cryptorchid boys alike (4). These observations suggest that persisting cryptorchidism through minipuberty might reflect a deficiency in androgen action. Indeed, reduced Leydig cell numbers have been reported in testicular biopsies of cryptorchid

boys compared with those with normally descended testes during the first months of life (83).

At 3 months of age, a higher LH to INSL3 ratio has been reported among persistently cryptorchid boys compared to healthy boys. Further, INSL3 correlated with LH, testosterone and inhibin B in healthy boys yet no correlations were found in cryptorchid boys (55).

Data on biological effects of testosterone in cryptorchid boys in infancy are scarce. In one study at birth, cryptorchidism was associated with shorter penile length yet penile length was not adjusted for gestational age—which was lower among the cryptorchid boys. At 3-months follow up, no differences were observed (15).

Gonadotrope (FSH)-Sertoli Cell Axis in Cryptorchidism

Higher FSH levels have been reported in cryptorchid 3-month-olds compared to healthy boys (92). In several smaller studies, no differences were observed (88, 89, 91, 93).

Lower inhibin B levels have been reported in a cohort of Finnish cryptorchid boys than in healthy boys at 3 months of age whereas among Danish boys, no significant difference was observed (92). Similarly, Pierik et al. found no differences in inhibin B or AMH levels during minipuberty when comparing cryptorchid boys to controls (93).

While gonadotropin and testosterone levels decline to very low levels by 6 months of age, inhibin B and AMH levels remain measurable after minipuberty. At 2 years of age, inhibin B and AMH levels were lower in cryptorchid boys prior to treatment ($n = 27$) compared to controls ($n = 27$) (96). Further, boys with bilateral cryptorchidism have even lower levels of inhibin B and AMH compared to unilateral cases. In boys aged 6 months to 8.9 years, Grinspon et al. reported lower AMH levels in boys with bilateral cryptorchidism ($n = 186$) vs. controls ($n = 179$). However, AMH levels did not differ between unilateral cases ($n = 124$) and controls (94). In principle, these results may partially reflect the germ cell/Sertoli cell damage as a consequence of abnormal testicular location.

Testicular volume in cryptorchid boys reflects the number of Sertoli and germ cells (97). In unilateral cryptorchidism, the maldescended testis is smaller after birth and grows slower during the first 6 months of life compared to the contralateral scrotal testis (98). In a randomized controlled trial, early surgery at 9 months of age (vs. later orchidopexy) resulted in larger testicular volume at 3 years of age (97, 99). Neither gonadotropin, testosterone nor inhibin B levels measured during minipuberty (at 2 months of age) predicted the number of Sertoli or germ cells (97).

Germ Cell Development in Cryptorchidism

After 1–2 years of age, the number of germ cells in cryptorchid testes is lower than in normally descended testis (97, 100, 101). These observations form the rationale for the early surgical treatment between 6-12 months. Early maturation of germ cells has been shown to be diminished and delayed in cryptorchid testis (84). In boys with unilateral cryptorchidism who are younger than 1 year old, disappearance of gonocytes is delayed and the number of Ad spermatogonia is diminished in the undescended testis compared to the contralateral descended (scrotal) testis (100). The number of Ad spermatogonia in pre-operative testicular biopsy (prior to 9 months of age) seems to be associated with future sperm count as all boys with Ad spermatogonia at orchidopexy subsequently have normal sperm counts—in contrast to abnormally low sperm counts in those boys with no Ad spermatogonia (102).

Changes in Testicular Position After Minipuberty

A longitudinal study in England revealed that 4% of 12-month-old boys had acquired cryptorchidism (i.e., testes that ascended from the scrotum to suprascrotal, inguinal or abdominal position during infancy) (15). Later during childhood, the prevalence decreased (0.6–1.3%) (103). A cross-sectional Dutch study suggests that the prevalence of acquired cryptorchidism is approximately 1.1–2.2% in childhood (104). Further, a small but significant testicular ascent of approximately 5 mm was observed between the age of three and 18 months in a prospective longitudinal study (4).

The prognosis of acquired cryptorchidism is not well-characterized. In the majority of cases, testes descend during mid-puberty (105, 106). Despite the tendency for spontaneous descent, semen quality is reduced compared to controls—and comparable to levels observed in congenital cryptorchidism (107). Some authors speculate that the risk of malignancy might be lower in acquired than in congenital cryptorchidism (53). However, this has not yet been definitively proven. Most clinical guidelines advocate operative treatment (i.e., orchidopexy) in the absence of randomized controlled studies to indicate otherwise (19, 20, 108).

Little is known about the etiology of the testicular ascent. A British study showed that up to 40 percent of boys with congenital cryptorchidism and spontaneous testicular descent during minipuberty were noted to have a cryptorchid testis again at the age of 12 months (5). Thus, it has been proposed that slight abnormalities in prenatal testicular descent (i.e., borderline cases of congenital cryptorchidism) might later manifest as acquired cryptorchidism during growth in childhood (109). Recently published data indicate that in contrast to overall body growth, the testes physiologically descend during and subsequently ascend after minipuberty (4). Interestingly, the lack of sufficient Leydig cell action during minipuberty may be associated with acquired cryptorchidism. Compared to controls, slower penile growth has been noted during minipuberty among boys who later exhibited acquired cryptorchidism (15). In addition, two retrospective studies observed an association between acquired cryptorchidism and hypospadias (110, 111)—which has been thought to be caused by reduced androgen action.

Thus, we regard it plausible that altered minipuberty might be associated with the pathogenesis of acquired cryptorchidism.

Management of Cryptorchidism

Cryptorchidism is associated with impaired fertility and an increased risk of developing germ cell cancer (112, 113) and

hypogonadism (114). Early surgical treatment has been shown to improve fertility, but the effect on cancer risk remains controversial (115, 116). The amount of the lost germ cells in testicular biopsies at the time of orchidopexy has been associated with the timing of the surgical treatment (97, 117) and recent guidelines advise early treatment between 6 and 12 months, and at the latest, by 18 months of age. However, surgery is often delayed beyond this age. In a survey conducted in Germany and Switzerland between 2009 and 2015, 81% of boys with congenital cryptorchidism underwent orchidopexy after 1 year and 54% after the age of 2 years often because of a late referral (118).

Despite the early surgical repair, some cryptorchid boys become infertile. Bilateral disease and non-palpable position are associated with worse fertility outcomes. Hormonal treatment with hCG injections (or intranasal GnRH) is no longer recommended due to the lack of evidence of long-term efficacy and possible adverse effects (19, 20). Currently, there are ongoing studies examining the effectiveness of adjuvant GnRH analog treatment with orchidopexy with the intention of maturing spermatogenic stem cells as maturation is presumed (but not proven) to be associated with HPT axis activity during normal minipuberty (119, 120).

Hormonal Treatment of Cryptorchidism in Congenital Hypogonadotropic Hypogonadism (CHH)

But for cases of CHH, hormonal treatment of cryptorchidism is not recommended (19, 20). The efficacy of hormonal treatment for cryptorchidism is poor and has potential side effects as interstitial bleeding, inflammation, and germ cell apoptosis (121–123).

Approximately 30% of boys with CHH have undescended testis. Cases are equally distributed between unilateral and bilateral cryptorchidism (51, 52). Cryptorchidism is more common than micropenis (29 vs. 15%). In a Finnish-Danish cohort of 36 boys with CHH, history of cryptorchidism and/or micropenis was reported in half of the patients (68). Acquired cryptorchidism has also been described in CHH. Main et al. reported three cases of hypogonadotropic hypogonadism and early acquired cryptorchidism in association with penile involution (16). All patients responded to testosterone therapy.

Lambert and Bougneres reported effects of combined recombinant LH and FSH treatment to mimic minipuberty in boys < 12 months old with CHH (isolated $n = 5$, combined $n = 3$) (17). At treatment onset, five boys had non-palpable testes and three had high scrotal testes. Duration of treatment was ∼6 months and cost ∼$18,000 per child. All boys had testicular descent during the treatment and only one boy had re-ascent (at 11 months of age, requiring orchiopexy). Very recently, Papadimitriou et al. reported results of three-month replacement therapy with subcutaneous recombinant LH/FSH for 10 boys with CHH and associated micropenis and cryptorchidism beginning from the median age of 0.35 years (18). The treatment resulted in high normal and supranormal LH and FSH levels, respectively, and normal testosterone and inhibin B levels for age. Penile length increased and testicular descent to scrotal position took place in all boys, however two required orchidopexy later. During the follow-up of 3–10 years scrotal testicular position has been maintained for all cases. At the moment, there are no data on long-term effects of this treatment on later fertility.

CONCLUSIONS

Minipuberty appears to be an important phase in finalizing and stabilizing of the normal male genital development. Minipuberty is associated with penile and testicular growth as well as testicular descent. Moreover, proliferation and maturation of Sertoli and germ cells during minipuberty are seemingly important for later fertility potential. Some boys with cryptorchidism undergo spontaneous testicular descent during minipuberty but are at increased risk of later re-ascent, and a careful follow-up is warranted. Notably, spontaneous descent is rare after the first 3–6 months of age.

Despite the central role of HPT axis in testicular descent, reproductive hormone levels in cryptorchid infant boys are often in the normal range for age. This might reflect the multifactorial, yet poorly understood, etiology of cryptorchidism. In certain pathologic situations, hormone levels in cryptorchid boys during minipuberty may present a recognizable pattern. These include anorchia together with very high gonadotropin levels and low/undetectable testosterone, AMH and inhibin B levels, or CHH presenting with cryptorchidism and small, but normally formed penis (i.e., micropenis) in the setting of very low gonadotropin and testosterone levels. Based on available data, higher gonadotropin and lower inhibin B levels during minipuberty in some cryptorchid boys may reflect a primary testicular defect/dysfunction. However, it is not known whether these changes are the cause or merely a consequence of the testicular maldescent.

Based on the evidence documented in this review, the following are presented as guidelines for management of cases of cryptorchidism before the age of 6 months and referral for orchidopexy. Infants with normal penile size and anatomy and no family history of reproductive disorders do not need hormonal evaluation for isolated unilateral cryptorchidism. However, boys with bilateral non-palpable testes, palpable testes but other signs of a possible disorders/differences of sex development (DSD) (i.e., severe hypospadias, bifid scrotum) and boys with associated micropenis should immediately be referred for a pediatric endocrine consultation. Despite careful investigation, the etiology remains unknown in most cases of cryptorchidism. However, in boys with bilateral cryptorchidism or micro-orchidism, hormonal evaluation during minipuberty can be useful. Evaluation should include LH, FSH, and testosterone measurement at 1–2 months of age while AMH and inhibin B level may be informative later. Hormonal treatment of cryptorchidism should be considered by a pediatric endocrinologist only in those cases with

an identified hormonal defect such as in CHH or partial androgen insensitivity.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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OPINION
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Animal Models of Diabetes-Related Male Hypogonadism

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INTRODUCTION

Hypogonadism is the clinical syndrome associated with low testosterone secretion in men. Hypogonadism affects ∼37–57% men with diabetes mellitus (1). Male reproduction is orchestrated by the hypothalamo-pituitary-gonadal (HPG) axis, which regulates the biosynthesis of testosterone from the testes. Diabetes may cause hypogonadism through multiple mechanisms including suppression of hypothalamic gonadotrophin-releasing hormone (GnRH) secretion, or direct disruption of spermatogenesis (2). Clinical stigmata of hypogonadism include reduced libido, erectile dysfunction (ED) and reduced physical strength. This article will summarize the evidence from animal models including how diabetes affects male reproductive endocrine function and predisposes to hypogonadism.

EPIDEMIOLOGY OF HYPOGONADISM IN DIABETES MELLITUS

Fifty percent of men with diabetes reportedly have a reproductive disorder, including hypogonadism, defective spermatogenesis, psychosexual dysfunction due to depression associated with chronic illness, ejaculatory disorders and ED (3). The most characterized and studied manifestation of reproductive dysfunction affecting men with diabetes is erectile dysfunction. However, changes in endocrine function (4) and the central nervous system control of sexual arousal (5) also play a crucial role in the development of hypogonadism and infertility in these men.

A recent study observed that 37% (397/1089) men with type 2 diabetes mellitus (T2DM) had a serum total testosterone (TT) level <10.4 nmol/L (3 ng/ml). Among T2DM patients with low serum TT, 16.9% had primary hypogonadism (defined as LH >10 mIU/ml with TT <3.0 ng/ml). The remaining 83.1% has secondary (hypogonadotrophic) hypogonadism (defined as LH <2mIU/ml with TT <3.0 ng/ml) (1). Both primary and secondary hypogonadism are followed by rapid decline in serum testosterone in men with diabetes (6). Although, very few studies have quantified the magnitude of hypogonadism in men with type 1 diabetes (T1DM) and T2DM, a common finding between studies is the significant reduction in serum total and free testosterone in men with diabetes compared to men without diabetes (7).

The global prevalence of hypogonadism in men ranges between 10 and 40% (8), with levels declining as rapidly as 0.4–2% annually after 30 years of age (9). Diabetes mellitus affects an estimated 285 million people worldwide and this number is expected to double by 2030 (1). Consequently, hypogonadism will affect an even larger proportion of the male population in the future.

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ANIMAL MODELS OF DIABETES-INDUCED HYPOGONADISM

Evidence of Hypothalamo-Pituitary Dysfunction Caused by Diabetes Mellitus

A number of studies on rodent models of T1DM have observed hypogonadism. Streptozotocin (STZ)—induced destruction of pancreatic β-cells results in the rodent model of diabetes, which is useful for the additional study of reproductive function (10). STZ-injection markedly reduces serum testosterone (T) as well as plasma luteinizing hormone (LH) (11). According to previous studies, STZ—induced diabetic rodents exhibit significant catabolic effects of insulin deficiency, undergo substantial weight loss and HH by impairing hypothalamic function (10, 11).

Insulin administration can affect hypothalamo-pituitary function. It has been observed that insulin replacement acutely increases pulsatile LH secretion in rodent diabetes models (10, 12). Steger and Kienast (11) studied comprehensively the endocrine and sexual function in adult male Sprague-Dawley rats on twice-daily insulin replacement after sham or STZ injection. STZ injection reduced plasma testosterone four-fold when compared with plasma testosterone of sham-injected rats. However, twice-daily insulin replacement fully normalized testosterone levels in male STZ-injected rats. The same authors randomized STZ-injected male rats to twice-daily insulin replacement starting either 1 day after STZ injection or 4 weeks after STZ injection. Ejaculatory function as measured by latency to ejaculation with stimulus, was fully restored to normal when insulin therapy was given without delay, but only partially restored when insulin therapy was given 4 weeks following STZ injection (11). This data suggests that insulin signaling plays a crucial role in supporting hypothalamic reproductive function. Furthermore, prolonged insulin deprivation may dampen the effectiveness of insulin treatment to restore sexual function.

Gonadotropin releasing hormone (GnRH) is secreted from the median eminence of hypothalamus to the hypophyseal portal circulation in pulses every 1–2 h, triggering corresponding pulsatile LH secretion from the pituitary gland. Since GnRH is not secreted into the peripheral circulation, pulsatile LH secretion i.e., every 5–10 min over several hours, is considered the gold-standard measure of GnRH secretion (12). When studying the effects of DM on hypothalamic GnRH function, it is important to study castrate animals to remove peripheral testosterone feedback as a confounding factor. Intriguingly, pulsatile LH secretion is significantly lower in castrate Wistar rats with STZ-induced DM when compared with castrate rats without STZ-induced DM. Pituitary sensitivity to exogenous GnRH has also been observed to be reduced by 67% ($P = 0.001$) in castrate rats with diabetes when compared with controls (12). This suggests that pituitary gonadotroph dysfunction might co-exist in diabetes-induced hypogonadism.

Altered insulin signaling in diabetes may play a detrimental role in GnRH signaling. There is evidence to suggest that central hypothalamic insulin signaling in a mouse model of diet-induced obesity (DIO) resulting in T2DM is associated with infertility (13). Knock-out of insulin receptors on GnRH-neurones led to a significant improvement in fertility rate ($P < 0.05$) and a GnRH pulsatility profile similar to that of lean mice. Control female DIO mice had an average fertility rate of 0.25 whilst the knock out of insulin receptors DIO mice had an average fertility rate of 0.67 (13). Interestingly, GnRH secretion and mean pulse amplitude in knock-out DIO mice was significantly lower compared to control mice. This was also accompanied by higher basal LH levels, determined in the morning of diestrus (13). However, this observation did not confer any improvement in fecundity. Knock-out DIO mice had a GnRH and LH profile similar to that of lean mice, suggesting that hypothalamic insulin receptor signaling may be playing an influential role in governing the reproductive axis in mice (13). No male mouse studies with DIO with or without knock out of insulin receptors have been performed so far, and remains important to determine if these would have shown similar results.

A separate study analyzed the effect of selective deletion of leptin and insulin receptors on hypothalamic pro-opiomelanocortin neurones (POMC) using a Cre-Lox recombination method to allow DNA modification. Female mice lacking leptin and insulin receptors showed reduced hepatic glucose production, decreased body weight and features suggestive of polycystic ovarian syndrome. Additionally, mice with POMC double leptin and insulin receptor knockout showed a four-fold increase in serum testosterone levels suggesting that hyperinsulinemia may be the primary factor driving increased ovarian androgen production (14). However, as this study was on female mice without diabetes, further study is required to comprehensively establish the link between insulin signaling in male with diabetes and central neuroendocrine reproductive function.

Noradrenergic signaling within the hypothalamus is implicated in the regulation of reproductive behavior (15). Hypothalamic regions associated with reproduction include the median eminence (ME), medial basal hypothalamus (MBH) and anterior hypothalamus (AH). Norepinephrine (NE) turnover is significantly reduced following STZ administration in the hypothalamic regions associated with reproductive function in adult male Sprague-Dawley rats (11). Furthermore, insulin replacement has been observed to restore NE turnover in the ME, MBH and AH following STZ treatment (11).

Hypothalamic GnRH function in T1DM may be regulated by insulin deficiency and weight loss, which is known to reduce leptin signaling required for GnRH activation (16). Studies have therefore been performed in animal models with STZ-induced diabetes (17). STZ-induced diabetic rats have significantly reduced testosterone levels when compared with controls rats subjected to caloric restriction in order to the weight loss in the STZ-injected rats ($P < 0.05$) (17). This suggests that hypogonadism associated with the STZ-induced diabetic rodent model is not merely explained by weight loss.

Targeted tissue specific insulin receptor knock-out murine models of T2DM show a spectrum of dysfunctional glucose metabolism. Liver-specific insulin receptor knock-out models are severely glucose intolerant and insulin resistant (18). Skeletal muscle specific knock-out models have increased fat mass, serum triglycerides and free fatty acids, despite having normal blood glucose and glucose tolerance (19). Adipose tissue specific knock-out models are lean, have improved glucose sensitivity and are

resistant to diet-induced obesity (20). It is probable that future study of reproductive hormones in such tissue specific insulin receptor knock-out models could advance our knowledge on reproductive dysfunction in men with diabetes.

Evidence of Testicular Dysfunction Caused by Diabetes Mellitus

In addition to impairment of hypothalamic function, diabetes may directly impair testicular endocrine function. The administration of high doses (100–200 mg/kg) of STZ to male rats induces a decrease in testosterone production in the testes (21). STZ-induced diabetes is accompanied by hypogonadism due to a reduced number of functioning Leydig cells in the testes, and impaired androgen biosynthesis within remaining functional Leydig cells (22). Insulin is expressed in the testes and regulates normal Leydig cell function by promoting DNA synthesis and steroidogenesis during puberty. In addition, insulin is crucial to Sertoli cell function, as it mediates glucose transport and lactate synthesis which is an important substrate for germ cells. Therefore, diabetes-related effects on testicular function may be a consequence of reduced insulin signaling and defective energy metabolism, although direct effects of hyperglycaemia cannot be excluded (23). In another study, the number and function of Leydig cells was markedly reduced in rats with STZ-induced diabetes when compared with controls. Reductions in testicular insulin-like growth factor I (IGF-I), androgen receptors and overall tyrosine phosphorylation were observed following STZ injection when compared with control injection, but these differences were not statistically significant (24). Further studies are needed to investigate how insulin signaling regulates testicular cell function and androgen synthesis.

Another model of T1DM is the biobreeding (BB) rat, which spontaneously develops autoimmune diabetes between 50 and 90 days (25). A study looking at gonadal dysfunction in infertile male BB Wistar rats reported a decrease in serum testosterone alongside increased Leydig cell lipid accumulation. This was also accompanied by morphological abnormalities in the Leydig cells, which had increased lipid accumulation. In addition the seminiferous tubules demonstrated increased tubular thickness, germ cell depletion and Sertoli-cell vacuolisation (26).

Recently a study investigating the effects of oral antidiabetic drugs on rodents with STZ-induced diabetes suggested a significant improvement in plasma testosterone levels following metformin treatment (27). The metformin-treated group restored testicular weight and increased serum testosterone to values similar to the control group. In contrast, pioglitazone- and sitagliptin-treated rats showed significantly lower testosterone levels compared to the control as well as the non-treated group with STZ-induced diabetes. Interestingly, the testis, epididymis and seminal vesicles of the pioglitazone- and sitagliptin-treated rats demonstrated adverse histopathological changes due to lipid peroxidation. This data suggests that metformin may be a better antidiabetic treatment option for young adults with T2DM when compared with pioglitazone- and sitagliptin. Exercise training may be another possible treatment option to restore testosterone levels, erectile function and improve insulin sensitivity in animal models with the metabolic syndrome. Rabbits fed a high fat diet exhibit glucose intolerance and decline in testosterone levels similarly to humans. After a 12 period of running on a treadmill specifically designed to accommodate rabbits, testosterone levels were found to be negatively associated with glucose levels and positively associated with the running distance (28).

Limitations of Animal Models

The validity of the STZ rodent model of DM has recently been subject to question, as current evidence suggests that STZ can itself have cytotoxic effects on Sertoli cells, cause oxidative stress and DNA damage (29). These cytotoxic effects as well as adverse reno-hepatic effects of STZ have previously been reported (24). Furthermore, chronic experimental DM may be associated with structural damage in the hypothalamus (30). Further work, possibly using non-chemically induced diabetic rodent models such as the biobreeding (BB) rat or the non-obese mouse with diabetes may help to clarify the reproductive phenotype of diabetes.

SUMMARY

Diabetes causes hypogonadism through multiple mechanisms. The pathogenesis of low testosterone in animal models with diabetes includes impaired hypothalamic signaling and hypogonadotophic hypogonadism, as well as reduced testosterone production by testicular Leydig cells. Insulin replacement restores hypothalamic signaling at early stages of diabetes. Furthermore, oral antidiabetes agents such as metformin, may improve plasma testosterone levels in animal models. Further mechanistic information may help identify novel therapeutic targets for treating or even preventing diabetes-associated hypogonadism.

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception and design of the work. CJ, NO, WD, MA, and SM conceptualized the study. AD, UR, CJ, and MA performed the literature search. All authors contributed to writing the manuscript. All authors are in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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OPINION
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Estrogen Replacement in Young Hypogonadal Women—Transferrable Lessons From the Literature Related to the Care of Young Women With Premature Ovarian Failure and Transgender Women

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INTRODUCTION

Nearly two decades have passed since the release of Women's Health Initiative (WHI) postmenopausal hormone therapy trial findings, yet the medical community, and general public remain unsettled by ongoing debate over the benefits and safety of sex hormone replacement therapy (HRT). Among the contentious issues is the elevated risk of venous thromboembolism (VTE) and stroke observed in HRT users (1). While major guidelines rightly recommend the use of transdermal estradiol in women with risk factors, little attention has been given to the potential impact of the type of estrogen molecule. This review aims to highlight the importance of selecting appropriate estrogen therapy to enhance safety.

MISINTERPRETATION OF WHI DATA COMPROMISES CARE OF HYPOGONADAL WOMEN

Hypogonadism in women of pre-menopausal age group is more frequent than is commonly anticipated; spontaneous or autoimmune primary ovarian insufficiency affects ∼1% of the female population, and an estimated 5% experience early menopause prior to age 45 (2). Other important causes of premature estrogen deficiency include congenital conditions such as Turner syndrome and Kallmann syndrome, as well as surgical oophorectomy. For these patients, HRT is a well-established endocrine treatment aimed to replace estrogen physiologically until at least the average age of menopause. Untreated individuals are at substantial risk of sexual dysfunction, genitourinary symptoms, accelerated bone loss, vasomotor symptoms, and coronary heart disease (CHD) (3).

Similarly, in the management of postmenopausal women suffering from climacteric syndrome, estrogen is unequivocally more efficacious compared to non-estrogen-based pharmacological treatments, and plays a crucial role in holistic menopause management particularly in those with impaired quality of life from persistent vasomotor symptoms.

Unfortunately, many healthcare providers and patients became resistant to the use of HRT in the aftermath of WHI. Not only are menopausal women in their 50s and those with vasomotor

symptoms unnecessarily deprived of HRT (4), there are also worrying signs of under-treatment among young hypogonadal women; a recent Swedish report on women with central hypogonadism found that at least half of the cohort failed to receive adequate replacement during their estrogen-deficient premenopausal years, placing them at heightened risk of complications in the ensuing years (5).

Prior to the landmark WHI trial, several large-scale observational studies were actually in favor of HRT's protective effects, as treated women were found to be at lower risk of CHD and mortality (6). To substantiate these observations, WHI postmenopausal hormone trials set out to investigate HRT in women aged 50–79 years; participants randomized to intervention arms received either conjugated equine estrogen (CEE) 0.625 mg alone (absent uterus) or with cyclical medroxyprogesterone acetate (MPA) 2.5 mg (intact uterus). Not only did WHI unexpectedly fail to demonstrate cardiovascular benefits, a disconcerting increase in incidence of breast cancer, stroke and VTE in treatment arms led to the premature closure of study after a median follow-up of 5.6 years (7).

Around the same time, the Heart and Estrogen/Progestin Replacement Study (HERS) trial also reported neutral effect of HRT (CEE+MPA vs. placebo) on CHD risk along with increased VTE events (8). Since then, the "timing hypothesis" has been widely proposed to explain the discordance in observational and trial findings because, unlike in typical clinical settings where most patients considered for HRT are early post-menopausal, the average age at which WHI subjects were initiated on HRT was 63.3 years (9). Indeed, *post-hoc* analyses showed better outcomes including reduction in CHD risk in subgroups of age <60 or <10 years from the time of menopause (10), with corroborative CT evidence of lower coronary calcified-plaque burden compared to placebo arm (11).

Aside from age factor, the type of estrogen therapy should also be carefully considered in HRT decision-making. It is imperative that estrogen products with the greatest safety margins be selected. However, this is an aspect that has often been overlooked in HRT guidance, with results of WHI/HERS often being inappropriately applied to all estrogen formulations. As will be elaborated further, non-physiological estrogenic compounds—by virtue of having greater propensity in inducing prothrombogenic state across ages—should be avoided in patients prescribed HRT.

CHOICE OF ESTROGEN FORMULATION INFLUENCES TREATMENT RISKS AND BENEFITS

There are three main types of estrogen formulations available for therapeutic purposes, namely 17β-estradiol (E_2), ethinylestradiol (EE) and CEE. The former (available in oral and transdermal formulations) is the predominant endogenous human estrogen. To overcome its poor oral bioavailability (<10%), E_2 is typically esterified or micronized; pro-drug esters, such as estradiol valerate and estradiol acetate, undergo hydrolysis rapidly following absorption to release E_2 into the systemic circulation, while the microcrystalline structure of micronised estradiol (principally as estradiol hemihydrate) facilitates accelerated absorption by its larger compound surface area and thus minimizing first-pass metabolism (12). Conversely, transdermal application of E_2, which has moderate skin permeability, avoids first-pass effect, and hence generates an E_2:E_1 (estrone, a metabolite of E_2) profile similar to normal physiology, whereas E_1 concentrations are higher after oral E_2 administration (13). However, the weak potency of E_1 does not have significant impact on the overall estrogenic bioactivity (14).

In contrast, CEE and EE are non-physiological because of their different molecular structure and properties. EE—a near-universal component of combined oral contraceptives (COCs)—is a potent synthetic E_2 analog with a 17α-ethinyl substitution that binds to estrogen receptors α and β with high affinity, prevents the oxidation of the 17β-hydroxy group, as well as irreversibly inhibits CYP enzymes involved in the metabolism of steroids, resulting in a very reactive intermediate (12). CEEs are urine derivatives from pregnant horses and is a complex mix of numerous estrogenic compounds with varying receptor affinity, pharmacokinetics and biologic potency, as well as other non-estrogenic steroids with unknown effects (12). Additionally, both EE and CEE have considerably greater hepatic stimulatory effect, altering the synthesis of various proteins including angiotensinogen, SHBG and coagulation factors.

Given these fundamental pharmacological differences, biological effects are expected to be dissimilar, and hence the adverse effects observed in older trials employing non-physiological estrogen would not be generalisable to all estrogen formulations. Indeed, emerging data are demonstrating comparatively greater safety and efficacy associated with E_2 use.

In a population-based, case-control study of ∼400 postmenopausal women aged 30–79 years using oral hormone therapy, CEE use was significantly associated with increased venous thrombosis risk (odds ratio 2.08) and a trend toward increased myocardial infarction (MI) risk when compared with E_2 (15). Further investigations demonstrated a higher endogenous thrombin potential-based normalized activated protein C (APC) sensitivity ratio as one of the mechanisms for the elevated clotting propensity observed in CEE users. This is in line with a recent large UK observational study of general female population aged 40–79 which found that among oral HRT, CEE(+MPA) had the greatest risk while E_2(+dydrogesterone) had the lowest risk (16). Likewise, Danish Osteoporosis Prevention Study showed no evidence of increased thrombotic or stroke risk in women with recent menopause onset who received E_2(± norethisterone) replacement and followed for up to 16 years (17). Moreover, a significant reduction in combined end-point of mortality and hospitalisations for congestive heart failure or MI was demonstrated.

Similarly, recent HRT intervention trials in younger women with premature ovarian failure have reported encouraging data with E_2 therapy (**Table 1**). Improvement in BMD, particularly at lumbar spine, was consistently observed across studies, with E_2 demonstrating superiority to COCs (19, 21). Furthermore, E_2 has beneficial effects on several cardiovascular and uterine parameters, which could have far-reaching impact on long-term cardiovascular health and possibly fertility treatment outcomes,

TABLE 1 | Summary of hormone therapy studies in **(A)** young women with premature ovarian failure (recent HRT trials), and **(B)** transgender females (retrospective & cross-sectional studies).

References	Study design	HRT regimen	Subjects	Key findings	Remarks
(A)					
Popat et al. (18)	3-year prospective, randomized, double-blind, single-center, placebo-controlled clinical trial.	Estradiol patch 100 μg/d & cyclical oral MPA 10 mg/d for 12 d/mo, ± Testosterone (T) patch 150 μg/d.	145 women with spontaneous 46, XX primary ovarian insufficiency vs. 70 healthy female controls.	Normalization of bone mineral density (BMD): ↑2.45% at neck of femur (NoF), ↑7.7% at lumbar spine (LS). Increase in bone formation markers.	Transdermal T did not provide additional benefit. 5 subjects (4 received T) had skin irritation, redness, hirsutism, & oily skin.
Cartwright et al. (19)	Open-label randomized trial comparing effects of HRT and combined oral contraceptive pill (COCP) on bone density.	HRT (Estradiol 2 mg/d & levonorgestrel 75 μg for 12d/mo) vs. COCP (EE 30 μg & levonorgestrel 150 μ for 21d/mo followed by 7-day break).	50 women with spontaneous POI recruited, of whom 30 elected for estrogen therapy (HRT = 15, COCP = 15). 36 completed the trial (no treatment 52%; HRT 60%; COCP 80%).	HRT significantly ↑ BMD at LS at 2 years, compared to COCP, while NoF and total hip BMD remained stable in all treated subjects. BMD decreased at all sites in untreated women.	HRT is superior to COCP in improving bone density at LS. No adverse cardiovascular events reported.
University of Edinburgh group (20–22)	12-month open-label randomized controlled crossover trial, comparing effects of physiological HRT vs. COCP on:	Estradiol patch (100 μg/d for week 1, 150 μg/d for weeks 2–4) & vaginal progesterone (200 mg/12 h for weeks 3–4) vs. COCP (EE 30 μg/d & norethisterone 1.5 mg/d for weeks 1–3 followed by 7 "pill-free" days).	34 women with primary ovarian failure (POF) attributed to chemotherapy or radiotherapy, idiopathic or surgical treatment, or Turner syndrome.		Only 4 clearly withdrew because of intolerance to the treatment, which was adverse reaction to the patch adhesives.
O'Donnell et al. (20)	Uterine health		17 women completed study; data from 25 subjects were analyzed.	Significant beneficial effect on endometrial thickness, and trend toward greater uterine volume.	HRT could benefit women with POF seeking infertility treatment by improving uterine physical characteristics.
Crofton et al. (21)	Skeletal health		18 women completed study.	Significant improvement in LS BMD *z*-scores observed in HRT but not COCP group, & only HRT was associated with an increase in bone formation markers.	The positive correlation of HRT's beneficial effect on LS BMD to E_2 levels underscores the importance of ensuring treatment adequacy.
Langrish et al. (22)	Cardio-vascular health		18 women completed the study.	HRT was associated with lower mean 24-h systolic & diastolic BP throughout the treatment period, along with reduced plasma angiotensin II and serum creatinine.	Compared to COCP, physiological HRT could have beneficial long-term cardiovascular health benefits.
Torres-Santiago et al. (23)	12-month randomized clinical trial to assess the metabolic effects of oral vs. transdermal E_2.	Cyclical estradiol (oral or transdermal) for weeks 1–3, with doses titrated to normal E_2 range in both groups, & MPA 10 mg from days 14–21 each month.	40 women with Turner syndrome; 20 in each treatment arm.	No significant difference in body composition, lipid oxidation, and lipid concentrations.	Oral and transdermal E_2 exert similar metabolic effects when titrated to normal E_2 range. No adverse event reported.
(B)					
Asscheman et al. (24)	Observational cohort study	Various estrogen regimens	966 Transgender females; mean age at therapy initiation was 31.4 ± 11.4 years; median follow-up of 18.5 years.	Current EE use was associated with 3-fold increase in risk of cardiovascular mortality.	No increased risk was observed in former EE users who had changed to other formulations & lower doses of E_2.
Dittrich et al. (25)	Retrospective cohort study	Monthly injections of gonadotrophin-releasing hormone agonist, & oral estradiol valerate 6 mg/d for 2 years.	60 transgender females, mean age 38.3 ± 11.3 years, treated with	One venous thrombosis occurred in a 62-year-old patient with known homozygous methylenetetrahydrofolate reductase mutation (genetic predisposition to thrombosis).	Increase in LS and NoF BMD observed. Overall safe and effective treatment.

(Continued)

TABLE 1 | Continued

References	Study design	HRT regimen	Subjects	Key findings	Remarks
Ott et al. (26)	Retrospective cohort study	Transdermal E_2 (2 × 100 μg/week); + oral cyproterone acetate & finasteride if yet to undergo sex reassignment surgery (SRS).	162 transwomen, mean age 36.6 ± 10.9 years, mean follow up period of 64.2 ± 38.0 months.	None developed VTE under cross-sex hormone therapy.	Notably 8.0% of subjects had thrombophilic defect (activated protein C resistance).
Arnold et al. (27)	Retrospective chart review	Oral estradiol therapy (4–8 mg/d & spironolactone pre-SRS, 2–4 mg/d only post-SRS).	676 transwomen, mean age 33.2 ± 10.8 years, treated for a mean of 1.9 years.	1 case of VTE; incidence of 7.8 events per 10,000 person-years.	Subject was in her 20s with severe obesity (BMI of 37 kg/m^2).
Getahun et al. (28)	Electronic medical record-based cohort study	All types of estrogens, with subgroup analyses of "only estradiol or estradiol first" (E_2 group), & "only non-estradiol or non-estradiol first" (non-E_2 group) within the estrogen initiation cohort.	2,842 transwomen, including 853 in estrogen initiation cohort. Mean follow-up of 4.0 years.	Adjusted hazard ratio (HR) for ischaemic stroke were 25.4, 2.8, and 1.8 in non-E_2 group, E_2 group, and overall cohort, respectively. Adjusted HR for VTE were 2.8 and 2.1 in E_2 group and overall cohort, respectively. Not calculated for non-E_2 group due to small numbers.	Although detailed comparison of risks between various estrogen formulations is not possible from limited data, it is notable that non-E_2 group had a 9-fold higher risk than E_2-group for ischemic stroke.
Seal et al. (29)	Controlled, retrospective case audit	Various types of estrogen formulations.	165 transgender women, mean age 45.7 ± 10.0 year, with a mean follow-up of 8.95 ± 4.87 years.	VTE occurred in 1.2%, more frequently in those treated with CEE vs. estradiol valerate.	Nearly 8-fold increased VTE risk with CEE use compared to estradiol valerate.
van Kesteren et al. (30)	Retrospective, descriptive study	EE 100 μg/d & cyproterone acetate 100 mg/d; in subjects age >40 years, transdermal E_2 was preferred (since 1989).	816 transwomen, mean age 41, treated for 7,734 patient-years.	36 cases of VTE were attributed to hormone therapy; all but one were oral EE users.	The switch to transdermal E_2 in age >40 years nearly ameliorated VTE risk.
Asscheman et al. (31)	Retrospective medical chart review	EE 100 μg/d and cyproterone acetate 100 mg/d.	303 transwomen, treated for 1,333 patient-years.	19 cases (6.3%) of VTE	EE-based therapy was associated with 45-fold increased risk of VTE. Higher risk was also found in age >40 years.
Wierckx et al. (32)	Multicentre 1-year prospective study	Transwomen <45 years received estradiol valerate 4 mg/d whereas those >45 years received transdermal E2 100 μ/d. All had cyproterone 50 mg/d.	Ghent: 47 subjects, mean age 31.7 ± 14.8; Oslo: 6 subjects, mean age 19.3 ± 2.4.	No cardiovascular or VTE events.	Low risk for adverse events at 1-year follow-up, which is significant considering earlier reports of high incidence of VTE during the first year of cross-sex hormone therapy.
Wierckx et al. (33)	Cross-sectional study	Various estrogen formulations	214 transwomen on average treatment period of 7.4 years.	5% had VTE; half occurred in the first year of therapy; Only 2 subjects were on EE or CEE, whilst at least 3 were using transdermal E_2 at the time of incident.	Findings deviate from other studies. Possibly confounded by high prevalence of risk factors (smoking, immobilization, clotting disorder).
Nota et al. (34)	Retrospective medical records review	EE (pre-2001) and more natural estrogens (post-2001).	2,517 transwomen, median age 30 years, with a mean follow-up duration of 9.07 years.	Standardized incidence ratios of VTE, comparing to reference women, were 5.52 and 3.92 in transwomen initiated on estrogen therapy pre-2001 and post-2001, respectively.	The change in estrogen prescribing practice away from EE therapy led to a substantial decline in incidence of VTE.

respectively (20, 22). These studies also provided evidence for dose titration to achieve physiological serum E_2 levels (21, 23). More importantly, both oral and transdermal E_2 therapy were safe and well-tolerated in these trials.

Despite that, the current evidence base remains disproportionately influenced by older randomized controlled trials which employed non-physiological estrogens, with little regards for their differential effects. In a recent Cochrane review

examining the risk of cardiovascular events in HRT trials, WHI and HERS—both of which employed CEE in intervention arms—accounted for 79%(425/540) of stroke, 88%(312/353) of VTE, and 90%(149/166) of pulmonary embolism events (35). That would inevitably serve to confuse clinicians with a skewed picture of HRT-associated risks being presented. More clarity is certainly needed.

Furthermore, most guidelines on the management of female hypogonadism (e.g., NICE 2017) continue to list COCs as reasonable replacement therapy—with the exception of those for Congenital Hypogonadotropic Hypogonadism, which only recommend E_2-based HRT (36). In contrast, WHO guidance for the treatment of male hypogonadism has long emphasized that only native testosterone should be prescribed, rather than synthetic androgens. Similarly, HRT prescribing practices in transgender medicine have also evolved over the past 2–3 decades following accumulating data of the significantly lower risk with E_2 therapy compared to EE/CEE. Another commonality between guidance for androgen replacement in males and E_2 replacement in trans-females is that it emphasizes the importance of monitoring serum sex hormone with the aim of achieving physiological levels. By contrast, guidance for both young hypogonadal women and older post-menopausal women do not recommend biochemical monitoring (37).

DATA FROM TRANSGENDER CLINICAL STUDIES ON COMPARATIVE SAFETY OF ESTROGEN PRODUCTS

For individuals receiving cross-sex hormone treatment, the major goal is to suppress endogenous sex hormone levels and thus reduce biological secondary sexual characteristics, and to replace sex hormone levels consistent with those of the affirmed sex. Importantly, there are no fundamental sex differences in response to sex steroids, and the principles of treatment are very similar to that of HRT in hypogonadal patients. Hence such data are wholly applicable to cis-gender patients.

In 1989, a key publication from a major center in Netherlands on the estrogen treatment outcomes in transwomen reported an alarming 45-fold increase in risk of VTE with EE compared with cisgender controls (31). This finding triggered a change in the treatment protocol to switch patients of age >40 years to transdermal E_2 in order to lower VTE risk. Although that led to an overall reduction in adverse events, the VTE risk remained high at 20-fold, largely because EE was still being used by a significant proportion of entire cohort, albeit at lower doses (30). It was also concerning that a 3-fold increased risk of cardiovascular mortality was found to be independently associated with long-term users of EE, consistent with the deleterious effects on haemostatic cascade induced by EE (24). Similarly, CEE proved to be greatly unsafe, with an 8-fold increased risk of VTE compared with E_2 in transwomen seen in a large transgender service in UK (29).

On the other hand, E_2 demonstrates an excellent safety profile in several transgender studies (**Table 1**). In a large US cohort of ∼700 subjects on E_2 4–8 mg/day for a mean duration of 1.9 years, only a single case of VTE occurred (27). Similarly, in a German cohort of 60 subjects on a relatively high oral E_2 dose of 6 mg/day, including three with underlying thrombotic tendency, only one VTE event was observed (25). Furthermore, in an Austrian study of 162 subjects on transdermal E_2, no VTE was observed over a median follow-up of 64.2 ± 38.0 months despite a high prevalence of smokers (∼60%) and the presence of confirmed thrombophilic disorder (APC resistance) in nearly 10% (26).

EE has been shown to induce APC resistance similar to that of factor V Leiden mutation, as well as increase in plasma protein C and a decrease in plasma protein S, in a dose-dependent manner. Additionally, non-physiological estrogens lead to elevated inflammatory markers such as C-reactive protein and interleukin-6, which could contribute to the prothrombotic milleu (38). Besides first-pass liver effect driving haemostatic dysregulation, higher prothrombotic tendencies are present with other modes of administration (transdermal and transvaginal) as well, providing evidence for a direct pathway induced by the molecular structure (39, 40).

17β-ESTRADIOL REPLACEMENT FACILITATES TREATMENT INDIVIDUALIZATION

Another major advantage of E_2 over EE/CEE is the feasibility for dose adjustment according to serum E_2 concentration. This is important as bioequivalence between different administrative forms (oral tablet, gel, and patch) is not well-established and subject to wide interindividual variation (13). Moreover, titrating to robust physiological E_2 levels has been correlated with positive outcomes on metabolic parameters and carotid intima media (23, 41). Normative E_2 values derived from healthy normally menstruating females would serve as a good guide to dosing (14).

CONCLUSION

The choice of estrogen formulation is vital to ensure optimisation of safety and treatment efficacy. Compelling data from recent literature supports the use of E_2 over EE/CEE to avoid the excessive vascular risk that the latter formulations are associated with. Further studies should seek to build on available evidence and provide greater clarity on estrogen replacement to empower clinicians and patients to make better therapeutic decisions.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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OPINION
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Androgens and Anemia: Current Trends and Future Prospects

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INTRODUCTION

The regulatory effects of androgens on haematopoiesis have been recognized since the early twentieth century. Castration of male rats causes anemia (1) which is reversible after treatment with androgens (2). A classical clinical study by Vahlquist provided indirect evidence that men had intrinsically higher haematocrit (Hct) levels when compared with women; it was observed that pre-menopausal women did not have higher Hct levels when compared with post-menopausal women, and did have an increased Hct in response to iron supplementation (3). Anecdotal data from competitive athletes suggest that androgen misuse can improve performance, partly through increasing the VO_2max (Hb-mediated blood oxygen carrying capacity), albeit at the expense of an increased risk of arterial and venous thrombosis (4). Equally, women suffering from hyperandrogenic endocrinopathies such as congenital adrenal hyperplasia and Cushing's syndrome, may exhibit relative erythrocytosis (5, 6). The above historic findings underpin the androgens on stimulating bone morrow erythropoiesis.

TESTOSTERONE (T) AND OTHER ANDROGENS

T, the principal circulating androgen in men, is a steroid hormone biosynthesized from cholesterol with its biological action mediated through binding and activation of androgen receptors in androgenic tissues after conversion, by 5α-reductase enzyme, to its more potent form dihydrotestosterone (DHT) (7). Since 1935, when the first T was isolated (8) different T preparations and routes were developed including several forms of the longer-acting T esters such T enanthate, T cypionate, and subsequently T undecanoate (9–11).

By 1970, several synthetic steroidal androgens were also available comprising groups of 17α-alkylated androgens, 1-methyl androgens, and nandrolone (12). As most of the oral 17α-alkylated androgens have hepatotoxic effect, they were not suitable as long term options for androgen replacement therapy (12). However, some of these androgens (such as danazol and stanozolol) along with other 1-methyl androgen (like methenolone) were used to treat aplastic anemia and anemia due to myelofibrosis (13–15).

Unlike T, nandrolone (19-nor testosterone) is metabolized by 5α-reductase to its much weaker metabolite 5α-dihydronandrolone (DHN), giving it a very high ratio of anabolic to androgenic activity (16). Due to this anabolic effect, it can be used in certain clinical indications such as severe burns, HIV-associated cachexia, and chronic obstructive lung diseases (17). Nevertheless, this anabolic potency unfortunately makes nandrolone and its derivatives (such as Trenbolone) an attractive substance to misuse for physique- and performance-enhancing purposes.

ANDROGENS AND ERYTHROPOIESIS

Animal and human studies suggested a direct and indirect stimulatory effect of androgens on erythropoiesis, though the exact mechanism of such relation remains vaguely understood. Androgens administration results in an increase in the erythroid cell mass, the colony forming units for erythrocytes (CFU-E), and the production and secretion of Erythropoietin (EPO) (6) while androgen deprivation causes a reduction in red blood cell indices due to reduced proliferation of marrow erythroid precursors (18).

Androgens are converted to 17-keto-steroids capable of increasing the synthesis of mRNA in the nucleus causing differentiation of bone marrow cells from EPO-non-responsive to EPO responsive (6). Moreover, androgens enhances the glucose uptake resulting in glycolysis and gene transcription and mRNA synthesis in erythroid (19–21).

T may increase Hct by inhibiting secretion of hepcidin, the principal iron regulatory peptide, thereby leading to an increase in bioavailable iron (22) but may also enhance the incorporation of iron into the red blood cells (23) and improve red blood cells survival (24). Finally, the finding of raised insulin like growth factor 1 (IGF-1) levels in those receiving androgens suggested a potential link between androgens and an IGF-1 driven erythroid progenitor cells proliferation and differentiation (25, 26).

The effect of T on erythropoiesis is most pronounced during puberty, with prepubertal Hb being similar in boys and girls, but increases in boys after age 13 years in tandem with increasing T concentrations (6, 27). Boys with delayed puberty have Hb levels similar to those of prepubertal boys and girls, and treatment with T normalizes hemoglobin levels to those observed in the late male puberty (28, 29).

HYPOGONADISM AND ANEMIA OF CHRONIC KIDNEY DISEASE (CKD)

Prior to the development of EPO therapy in the late 1980s, androgens were the only option for treating CKD-related anemia in men. Patients with CKD may have impairment of bone mineral density, muscle bulk, energy levels, quality of life, and sexual function, with adverse cardiovascular outcomes, especially in those with concurrent diabetes (30); while these features are likely multi-factorial in origin, they also occur during hypogonadism. Low T is prevalent among CKD patients and may contribute to renal anemia (31, 32). Up to two thirds of men on hemodialysis (HD) have serum T levels in the hypogonadal range, resulting from abnormalities at all levels in the hypothalamic-pituitary-testicular axis (33–36). Unpublished data from a cohort of 113 HD & 85 pre-dialysis (preD) stage 4 and 5 CKD patients reported a subnormal T levels were in 76% of pre-dialysis and 80% in HD males with a significant inverse correlation of Dα dose with total T (R −0.253; $p < 0.01$) and free T (−0.29; <0.01) (37).

T concentrations have been found to inversely correlate with all-cause and cardiovascular—related mortality, as well as with markers of inflammation in patients with dialysis dependent end stage renal disease (32, 38). Even in those with non-dialysis dependent kidney disease, low total T was associated with higher mortality (39). However, it remains unclear if T treatment in those with CKD-anemia and hypogonadism, could positively influence mortality in this high risk cohort.

The fact that hypogonadism is a well-established cause for anemia and reduced responsiveness to EPO in men with CKD may suggest a possible role for T therapy as an adjunct or an alternative to EPO in some men with CKD-related anemia (40). This is particularly relevant in some healthcare systems where CKD patients are unable to afford EPO therapy which might result in anemia requiring blood transfusion, especially while there is a lacking evidence that EPO improves morbidity or mortality in CKD (41).

The Cochrane systemic review for the use of androgen in treating anemia of CKD did not find sufficient evidence to confirm the benefit of androgen therapy in treating anemia of CKD (42). The Cochrane systemic review excluded 20 studies for either being non-randomized controlled trials, of <6 months duration, if treatment and control groups data were indistinct or if it was unknown if participants received any androgen therapy for the preceding 6 months. The review only included data from 8 studies with sample sizes ranging between 9 and 37 participants. Limitations of the included studies include lack of matching for hypogonadism at baseline, and the absence of titrating T doses to reach therapeutic levels. In a meta-analysis of 4 randomized controlled trial including men over the age of 50 years, nandrolone was non-inferior to EPO for the treatment of anemia of CKD, especially in developing countries where EPO might be unavailable (43).

It has been argued that androgen therapy may potentiate the effectiveness of EPO treatment and reduces the minimal EPO dose necessary to maintain satisfactory Hb level in patients undergoing HD (44). Additional benefits of T in patients with CKD may include improvement of sexual function (45), muscle mass, functional performance, quality of life and subsequently, could ameliorate frailty (46). However, these potential benefits require formal investigation to assess the clinical benefits vs. risk of therapy.

HYPOGONADISM AND UNEXPLAINED ANEMIA IN THE ELDERLY

Anemia in the elderly men may increase morbidity and mortality risk. In a retrospective study of men aged over 65 years admitted with acute myocardial infarction, lower Hct levels were associated with an increase in the 30-day mortality while treating the anemia improved the mortality rates (47). Similar observation of high mortality was reported in a cohort of anemia patients presenting with new onset heart failure (48).

Increasing age *per se* was previously thought to be a risk factor for developing anemia, since anemia prevalence dramatically rises after the age of 50 years and affects up to 20% of 85 year olds (49). Several causes for anemia in the elderly have been reported in the non-institutionalized United States population in the third National Health and Nutrition Examination Survey; nutritional factors, anemia of CKD, blood loss, bone marrow myelodysplasia were causative of anemia in around

2/3 of the population while in the remaining 1/3, anemia was unexplained (49, 50). Currently, the patho-physiological basis for "aging" anemia remains incompletely understood and the term "unexplained" anemia is still a common diagnosis in older men (51).

Hypogonadism and aging may both cause anemia, sarcopenia, and osteoporosis (52) with longitudinal and cross-sectional studies consistently showing a declining serum levels of T in aging men.

An analysis of the European male aging study (EMAS) data found that primary hypogonadism (PH) was strongly related to aging but not obesity, while secondary hypogonadism (SH) was linked to obesity, irrespective of age (53). In most aging men, "functional hypogonadism" is a reflection of the physiological burden of obesity, inflammation and accumulating comorbidities on the hypothalamus -pituitary-gonadal axis, however; it is unclear if this effect is adaptive (diverts resources from reproduction to survival), maladaptive (exacerbated disease-related frailty), or neutral. Indeed, the EMAS found that 1–2% of the general population of elderly men can show a clinical picture of PH or late onset hypogonadism (LoH), characterized by low T and raised gonadotrophins, attributed to age-related primary testicular failure (52, 54). Men with elevated serum follicle stimulating hormone (FSH) and normal levels of luteinizing hormone (LH) and T may have a reduced spermatogenesis with entirely normal testicular androgen production. In some men, a compensated dysregulation of gonadal function "compensated hypogonadism" is characterized by raised LH with normal T, reflecting a state of age-related health deterioration with potential progression to primary hypogonadism (55).

Whereas, organic secondary hypogonadism (low-normal LH+FSH) is biochemically indistinguishable from non-gonadal illness effect, PH can be easily diagnosable even in the acute (or chronic) disease settings, subject to the potential for the LH+FSH rise being blunted with intercurrent illness and/or medication (especially opiates).

In a retrospective study of 67 hospitalized elderly men (56), PH was found to be a surprisingly common cause of anemia among an unselected population of older male medical inpatients, 20.6% (7/34) of total cases with anemia and 43% (7/16) of those with otherwise unexplained anemia. Older men with PH and anemia therefore constitute a homogeneous group that can be reliably diagnosed and would thus represent an excellent potential cohort for examining the effects of T replacement therapy on haematopoiesis and other markers of frailty.

EFFECT OF T THERAPY ON ANEMIA

Studies have examined Hb changes in patients receiving T replacement therapy; some specifically trialing T therapy with the primary aim of improve anemia, with an accumulating body of evidence. In a randomized double blinded placebo-controlled study by Dhinsda et al., T therapy suppressed hepcidin with a marked increase in the Hb, EPO and expression of ferroportin and transferrin receptors in hypogonadal patients with type 2 diabetes (57). The National Institute of Health (NIH)-funded T Trials examined the effects of T therapy in hypogonadal men aged $>$ 65 years (58). Using a double-blinded, placebo-controlled design, 12 months of daily T gel treatment increased Hb levels by at least 1.0 g/dL in $\sim$52% of more men with hypogonadism and a known cause of anemia when compared with placebo. Furthermore, in the 64 older men with unexplained anemia; Hb improved by at least 1.0 g/dL in 54% of men vs. 15% in the placebo group. Nevertheless, androgen deficiency (AD) is typically overlooked in guidelines on the investigation of anemia (59).

It is important to note that most clinical guidelines recommend that Hct and prostate specific antigen (PSA) are assessed prior to initiating T therapy (60).

FUTURE CONSIDERATIONS

As life-expectancy increases, aging and frailty have become increasing health priorities worldwide. For instance, the number of people over 65 years of age is projected to reach 15–20% of the entire population by 2030 (61, 62). Unexplained anemia is common in older men, hypogonadism accounts for a proportion of these cases. Hypogonadism may negatively impact men's health and aggravate frailty, morbidity and mortality. Androgen therapy also has potential to treat anemia of CKD in hypogonadal men as an adjunct to EPO. Given the long-standing risks associated with T therapy, further studies are required to determine which men would most benefit functionally from T therapy without exposing them to unnecessary adverse effects or long-term complications.

AUTHOR CONTRIBUTIONS

AA-S drafted the structure of the article while AM and AA collected the relevant data as per AA-S guidance. AM and AA wrote the initial draft which was revised, edited, and expanded by AA-S. CJ supervised the manuscript's writing process and approved the final version.

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OPINION
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The Lived Experience of Klinefelter Syndrome: A Narrative Review of the Literature

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INTRODUCTION

Klinefelter syndrome (KS) or 47, XXY is a chromosomal disorder in males. Persons with KS have an additional X chromosome creating karyotype 47, XXY and 46, XY/47, XXY mosaics. According to existing epidemiological studies KS is one of the most common genetic disorders, affecting ∼1 in 500 men [see (1, 2)]. Whilst there can be phenotypic variation between individuals, physical traits associated with the syndrome can include small testes, a less muscular body, less facial and body hair, broader hips, and increased breast tissue (1). This physiological background and associated traits can generate questions relating to gender identity and a proportion of KS individuals will not identify as male, instead identifying as female, non-binary or intersex[1] [see (3)].

Learning difficulties, low self-confidence and issues relating to social interaction are also reported in relation to those with KS (4–6). Whilst a number of physical and developmental issues are therefore associated with KS, infertility is a common feature of the disorder (7). Estimates suggest that over 95% of those with KS are infertile (8), although some men with KS can seek to have biological children using advanced assisted reproductive technologies such as surgical sperm retrieval followed by intracytoplasmic sperm injection (ICSI) (9). Such approaches are however high risk and uncertain, and those with KS may also be faced with decisions about the use of donor sperm, adoption, or remaining childless (10). This review examines the existing psychosocial evidence around the impact of KS, exploring what we know about KS and its relevance for health care for this group.

METHOD

In order to identify literature for this review we searched the following key databases: Academic Search Premier, CINAHL, MEDLINE, PsychINFO. The search terms "Klinefelter's syndrome+ Psychosocial[2]" were used to reflect our interest in the psychological and social aspects pertaining to the disorder and specifically to the lived experience of those with KS. The results of these databases were limited to English articles in scholarly academic journals in the last 20 years.

[1]Whilst we refer to men within this paper, given that primarily those with KS will identify as male, we are aware that not all will identify as male, and have chosen this terminology for clarity and to reflect the medical literature on this topic.

[2]We have chosen to use Klinefelter syndrome as a descriptor of the disorder within our work, in line with NHS guidance (https://www.nhs.uk/conditions/klinefelters-syndrome/), but it is also widely called Klinefelter's syndrome. The papers we have included use of a mix of "Klinefelter" and "Klinefelter's". For our search using "Klinefelter syndrome+ psychosocial" brought up only 11 results across all time in the databases we searched so despite our preference to call it Klinefelter syndrome we have chosen to use the search terms "Klinefelter's syndrome + psychosocial" in order to maximise results as they then include "Klinefelter" and "Klinefelter's."

There were 47 results generated from this search and identified articles were screened using the inclusion of criteria of being about the patient or lived experience of KS. After screening, 15 results were included for the review, although a further 2 were initially discounted due to not being accessible, but on accessing did not fully meet the inclusion criteria after screening so were not included. Given the small number of results obtained, a Google Scholar search was also conducted, using the same search terms and the first 5 pages of these results were screened (beyond page five revealed the papers were not relevant to the search) which resulted in a further five inclusions. Four further papers were included following identification by reference chaining (11). In total 22 papers were included, as detailed in **Table 1** below. These were all papers which met the inclusion criteria specified above and were therefore extracted for the review. An inductive coding approach was adopted as part of the use of qualitative content analysis. This approach is advocated as a useful method when the body of evidence is perceived as limited at the outset of the analysis and when dealing with topics which could be described as sensitive [see (31)]. This inductive approach involves open coding, specifically writing notes and headings during the initial reading phase of the review articles, and these open codes then these headings are grouped into broader "umbrella" categories. As Elo and Kyngas (31) note, "The purpose of creating categories is to provide a means of describing the phenomenon, to increase understanding and to generate knowledge" (:111). From this analysis our overall categories, which we will refer to hear as themes, were then generated, these include; Diagnosis- Issues and timings; Outcomes for those with Klinefelter syndrome[3]; Experiences with health care professionals.

DIAGNOSIS—ISSUES AND TIMINGS

Much of the literature examined discusses the challenges of getting and managing a diagnosis for KS. Fewer than 10% of cases of KS are diagnosed before puberty (4, 25), with only 6% diagnosed before aged 10 and 21% diagnosed before aged 20 (16). The mean age of diagnosis is suggested to be 27 (32) and aspects such as poor learning at school, subsequent challenges around employment and low socio-economic status are believed to be correlated to late or under diagnosis (29). A delay in diagnosis also remains problematic for health aspects including infertility (20). Many boys with KS report growing up with an unexplained sense of "feeling different" (16) and receiving a KS diagnosis, it has been reported as being a "relief" (29). Diagnosis can be a point of acceptance and understanding for patients (10).

Whilst diagnosis can then be a relief for those with KS, literature relating to the experience of parents of boys with KS shows that diagnosis can be uncertain and complex which can be a source of frustration for parents (14, 30). Even though parents may struggle to obtain a diagnosis for their children, particularly where there is an absence of "typical" physical symptoms associated with KS, they are not always well prepared to receive a genetic diagnosis when it is ultimately obtained (13, 14).

OUTCOMES FOR THOSE WITH KLINEFELTER SYNDROME

Quality of life (QoL) outcomes are reported as being worse for men with KS than for the general population (17, 22, 23, 25, 27, 28). There are also higher rates of anxiety and depression found in people with KS (18, 25) and sleep related problems (21). The phenotypic severity influences the psychosocial outcomes for patients (27) and a higher number of physical features attributed to KS inversely relates to QoL (5).

Turriff et al. (29) found that infertility along with psychosocial challenges were viewed as a major issue for those with KS. It is suggested that 50% of adult men with KS will yield viable sperm as a result of advances in reproductive technologies (26). There is however a desire from paediatricians and parents of KS children to see fertility preservation being used for minors who have KS (19). Parents are often concerned about sexuality, masculinity and fertility after a diagnosis, with the fathers of KS boys seen as particularly concerned about their son's sexual development and functioning (14). Evidence suggests that gender identity can be an issue for those with KS, with some reporting they neither feel, or look either masculine or feminine (23).

Physical health outcomes for those with KS can include lower physical activity levels and higher BMIs (27) as well as an increased risk of osteoporosis, diabetes as well as breast and other cancers (4). This increases both morbidity but also premature mortality (27) and those with KS have a decreased life expectancy of between 2–6 years (15). Whilst there is no cure for KS, many of these health issues are viewed as being best managed through early diagnosis of KS and relevant ongoing healthcare (15, 20).

EXPERIENCES WITH HEALTH CARE PROFESSIONALS (HCPS)

There is seen to be widespread lack of knowledge about KS by HCPs (5, 14, 29), with a "haphazard" approach taken to the informing of parents around the diagnosis of KS (12). Information given to those who have KS is seen to be inconsistent and HCPs are often viewed as lacking insight into the realities of KS (29). Given that KS is not heritable, parents may lack knowledge of what KS is, demonstrating the need for good quality professional support to plan for the care of their children with KS (14, 16). However, common misconceptions around KS are reported as being conveyed from HCPs, such as parents being told their sons are more likely to be gay as a result of having KS (14) despite the contested nature of evidence about differential rates of people identifying as gay among those with KS when compared to the general population (23, 27).

Knowledge amongst healthcare professionals around treatment options is also now seen to be outdated (14) and not evidence based, due to lack of research around testosterone

[3]The concept of outcomes from the analysis within this paper, and these relate to psychosocial outcomes within this paper, as per the aims and objectives of the article, but we do for ease of discussion refer to this as simply "outcomes" within the paper.

TABLE 1 | Study characteristics of included papers.

References	Method, sample size, and country of research
Abramsky et al. (12)	Phone interviews with health care professionals ($n = 29$) and Questionnaires with parents ($n = 23$) Conducted in the UK
Bhartia and Ramachandran (13)	Patient experience ($n = 1$ auto-ethnographical reflections) and clinician testimonies ($n = 2$). Conducted in the UK
Bojesen and Gravholt (4)	Epidemiological study of KS patients from the UK and Denmark. Cohort of 4,800 patients in the UK and 900 patients in Denmark.
Bourke et al. (14)	Qualitative semi-structured interviews conducted with parents of children with KS ($n = 15$). Conducted in Australia.
Bourke et al. (15)	Practice commentary piece- drawing on practitioner experience in Australia and review of relevant literature.
Close et al. (16)	Triangulated mixed methods study, using semi- structured interviews and online questionnaires with parents of children with KS. Purposive sample of $n = 40$. Conducted in America.
Close et al. (5)	Cross sectional study of boys with KS, samples was $n = 43$. Study included physical examination, hormone analysis and psychosocial questionnaire. Conducted in America.
de Ronde et al. (17)	Questionnaires sent to attendees at Dutch outpatient clinic ($n = 40$)
Geschwind et al. (18)	Discussion of existing studies around neurobehavioral and psychosocial issues and includes pilot data from their study of $n = 15$ adults with KS. Participants completed measures of personality and motivation and measures of problem behaviors. Conducted in America.
Gies et al. (19)	Questionnaire study with clinicians ($n = 49$) and parents ($n = 18$) about fertility preservation. Conducted in Belgium.
Grace (10)	Patient testimony of their experience of diagnosis of KS. Patient based in America.
Groth et al. (20)	Evidence synthesis of studies on KS in PubMED. No details of the number of papers included were provided. Study conducted in Denmark.
Fjermestad and Stokke (21)	Self report data from men ($n = 53$) with sex chromosome aneuploidies (SCA) Data collected via Health Survey–Short Form (SF-36),the Pittsburgh Sleep Quality Index and the Personal Wellbeing Index
Herlihy et al. (22)	Discussion paper based on existing evidence around KS. Conducted in Australia.
Herlihy et al. (23)	Self-completion question with men with KS ($n = 87$) in Australia.
Herlihy et al. (24)	Discussion paper based on review of current evidence around screening for KS. Conducted in Australia.
Nahata et al. (25)	Retrospective study of those diagnosed with KS at Boston Children's hospital. Study conducted in America.
Paduch et al. (26)	Review of existing evidence around KS for urology practice. Study conducted in America.
Skakkebæk et al. (27)	$N = 132$ men with KS were assessed via surveys for demographics, socioeconomic status, health problems and behaviors, sexual function, medical follow-up, and mental and physical quality of life (MQoL and PQoL, respectively). The population group was assessed against a control group ($n = 313$). The study was conducted in Denmark.
Turriff et al. (28)	Self-report survey with people with KS aged 14–75, recruited via online networks ($n = 310$).
Turriff et al. (29)	Online questionnaire with open ended questions, part of a wider study into KS. $N = 310$ completed the study but the responses for the open-ended questions ranged from $n = 169$—$n = 210$ due to incomplete data. Participants were aged 14–75. Conducted in America.
Whitmarsh et al. (30)	Interview study with families of those with genetic disorders. For the KS group they interviewed, six mothers, three fathers, one grandmother ($n = 10$). Study was conducted in America.

replacement or other management interventions (4, 5). The existing literature suggests that those with KS would be best served by multidisciplinary and coordinated health care (20, 27, 29, 32) supported by more training and education for HCPs (14). In light of a lack of quality information forthcoming from HCPs, parents of children with KS are seen to turn to the internet for help and advice (5), and others have noted the importance of support groups for those with KS, as a mechanism to help with the uncertainty of what having KS will mean for their lives (15).

DISCUSSION

This narrative review suggests that a lack of or late diagnosis remains a critical problem in relation to KS. Whilst prenatal screening techniques may improve future diagnosis (33), current low levels of diagnosis remain problematic, particularly for the possibility of improving physical and mental health outcomes (25). This is particularly important as those with KS are reported to have poorer health outcomes than the general population across a range of measures, including quality of life (23, 25, 27) and comorbidities result in a decreased life expectancy for those with the disorder. The perception that all persons with KS will demonstrate "textbook" signs is viewed as compromising the ability of patients to obtain a diagnosis (34). Early diagnosis allows for more extensive options for children and adolescents to preserve their fertility, which is seen as one of the key concerns for patients, although this remains an area in need of further research (19). Diagnosis itself can be a relief for patients, which is similar to other long-term health conditions [see (35–37)] although the literature details that uncertainty can also spring from a KS diagnosis, perhaps connected to the perceived lack of knowledge by HCPs reported within the literature.

The experience with healthcare for persons with KS is described as poor (5, 14, 29), ranging from a lack of information to misinformation, due to a perceived lack of

expertise among HCPs around KS. There is a consensus in the literature around the importance and value of the multidisciplinary team as a means of providing care to KS patients (20). Coordinated approaches to care are currently seen to be lacking despite evidence of the effectiveness of such approaches being noted in relation to other illnesses (38, 39). Questions of gender identity are noted within the literature (23) but not extensively explored; how those with KS identify and how this then intersects with their experiences of healthcare remains an important area for future consideration.

Given the prevalence of KS within the population, greater research focus on the disorder in the future, particularly in relation to reproductive health and the psychosocial impact of KS, would have a significant impact for patients and their families. There are inevitably limitations to a short review of this nature, and not all papers which may be relevant to KS, particularly those which are more clinically focused [such as (32)] appeared within our search, thereby illustrating a well-recognized limitation of literature keyword search based review algorithms. The voices of those with KS appear to be currently lacking from the literature, which could be further marginalizing, so future research should attempt to capture the lived experience of those with KS and use participatory methods where possible to embed this lived experience centrally within research. Developing a priority setting partnership for those with KS to identify and rank key research areas for the future would be fruitful, and co-production of research agendas would help with inclusion of this otherwise hidden group. Attempts to move forward research and care for those with KS should then begin with a central focus on what matters to those with KS and seek to make positive improvements to their diagnosis, outcomes and encounters with healthcare professionals.

AUTHOR CONTRIBUTIONS

EH led the analysis and drafting and all other authors equally contributed to the writing, editing, and refining of the work. All authors contributed to the preparation of the manuscript.

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