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Metabolic regulation of kisspeptin — the link between energy balance and reproduction

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Abstract

Hypothalamic kisspeptin neurons serve as the nodal regulatory centre of reproductive function. These neurons are subjected to a plethora of regulatory factors that ultimately affect the release of kisspeptin, which modulates gonadotropin- releasing hormone (GnRH) release from GnRH neurons to control the reproductive axis. The presence of sufficient energy reserves is critical to achieve successful reproduction. Consequently, metabolic factors impose a very tight control over kisspeptin synthesis and release. This Review offers a synoptic overview of the different steps in which kisspeptin neurons are subjected to metabolic regulation, from early developmental stages to adulthood. We cover an ample array of known mechanisms that underlie the metabolic regulation of *KISS1* expression and kisspeptin release. Furthermore, the novel role of kisspeptin neurons as active players within the neuronal circuits that govern energy balance is discussed, offering evidence of a bidirectional role of these neurons as a nexus between metabolism and reproduction.

Metabolic state determines a number of behavioural and physiological adaptations to ensure survival. Situations of negative energy balance increase hunger signals, induce food- seeking mechanisms and halt physiological functions that can be spared until energy reserves are restored. Sexual maturation and reproductive success require a minimum threshold of energy reserves[1,2] owing to the high energetic cost of reproduction and are therefore subjected to very tight regulation by metabolic factors.

Reproductive function is controlled by the hypothalamic–pituitary–gonadal (HPG) axis, in which gonadotropin- releasing hormone (GnRH) neurons located in the hypothalamus control the production and release of gonadotropins that regulate gonadal function. GnRH

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secretion requires the stimulatory action of kisspeptin, produced by kisspeptin neurons located primarily in the arcuate nucleus (ARC) and the anteroventral periventricular nucleus/periventricular nucleus continuum (AVPV/PeN) of the hypothalamus. Importantly, kisspeptin neurons in the ARC (kisspeptinARC) co- express the neuropeptides neurokinin B and dynorphin A[3,4]. In these neurons, the coordinated action of neurokinin B (stimulatory) and dynorphin (inhibitory) facilitates the release of kisspeptin in a pulsatile manner, which is mirrored by GnRH and luteinizing hormone (LH) pulses[5] (FIG. 1). By contrast, kisspeptinAVPV/PeN neurons, which are almost exclusive to the female brain, are involved in the positive feedback of sex steroids leading to the pre- ovulatory LH surge[6].

The development of kisspeptin pulsatility (and therefore GnRH pulses) is essential for sexual maturation. Patients with inactivating mutations in the genes encoding kisspeptin and kisspeptin receptor (*KISS1* (REF.[7]) or KISS1R[8,9]) do not undergo puberty onset, whereas activating mutations of KISS1R[10] cause precocious puberty. External factors can accelerate or delay the timing of puberty onset by modifying kisspeptin output onto GnRH neurons. For example, metabolic factors play an essential part by relaying the energetic status of the organism to central networks that ultimately regulate reproductive function[11]. This regulation is critical for organism survival, given the high energetic burden of reproduction, that is mostly of relevance in female mammals[12]. In humans, energy excess can also alter reproductive function, leading to the advancement of puberty onset[13] and in individuals with severe obesity, to the inhibition of the reproductive axis and hypogonadism[14,15] In this context, a strong association is observed between childhood obesity and early activation of the HPG axis due to the early onset of kisspeptin–GnRH pulses. Indeed, obesity during early developmental stages has become a leading cause of precocious puberty[16–18] and polycystic ovary syndrome[19,20] in adolescent girls, with subsequent fertility impairment.

Attention has mostly been focused on the metabolic regulation of fertility; however, a bidirectional regulation where energy balance is affected by reproductive factors also exists. Changes in levels of sex hormones (oestradiol or testosterone) lead to metabolic disorders. For instance, during pregnancy, the energy demands of the mother rapidly increase, leading to the induction of hunger signals over those inducing satiety[21]. Moreover, the anorectic effect of oestradiol has been documented in several species[22,23]. In humans, the decline in levels of sex steroids in women after menopause and low levels of testosterone in men are associated with increased body weight[24–26]. However, despite this known reproductive axis- dependent regulation of energy balance, the underlying connecting mechanisms remain largely unknown.

This Review focuses on the different mechanisms by which metabolic cues can affect the maturation and function of kisspeptin neurons throughout development to modulate reproductive function. In addition, the latest advances demonstrating these neurons as active players in the regulation of metabolic function are discussed, describing the role of kisspeptin neurons as bidirectional regulatory elements between metabolism and reproduction.

The energetic state and kisspeptin

Effects on kisspeptin neuron development

Metabolic factors can influence the development and function of vital systems in an organism. First, the genome can carry adaptations to metabolic conditions that determine the pattern of gene expression, through the inheritance of epigenetic modifications that occurred in previous generations[27–29]. Second, epigenetic changes occurring in the germline in response to changes in the metabolic state of the progenitor before fertilization can be passed down to the embryo and can have effects into adulthood[27,28,30,31], affecting sexual maturation and fertility. Third, once fertilization has occurred, metabolic factors that cross the placenta can fundamentally alter the development of neurons — including those necessary for sexual maturation — during the critical window of differentiation of the brain[32–34]. This period occurs before birth in precocial animals (for example, during the first trimester in humans) and perinatally in altricial animals (for example, up until postnatal day 10 in mice and rats). During this critical period, axonal and dendritic elongation and pruning take place, to establish the synaptic connections that will form specific neuronal circuits to govern metabolism and reproduction (FIG. 2). Importantly, the location of the hypothalamus in the brain, sitting in the ventral side and exposed to a porous section of the blood–brain barrier[35–37], makes this area, and therefore the developing kisspeptin neurons, susceptible to the action of peripheral signals. Such signals can include metabolic cues from the fetus and/or the mother. Indeed, numerous studies have described impairments in axonal elongation in perinatally undernourished rodents that can affect the development of agouti-related peptide (AgRP) and proopiomelanocortin (POMC) neurons[38–40], which are essential in the control of energy balance.

Precocial

Born mature, without the need for parental care for feeding.

Altricial

Born undeveloped, requiring parental care for feeding.

By embryonic day 13.5 (E13.5) in mice, direct connections are established between kisspeptin^{ARC} neurons and GnRH neurons[41,42]. However, these connections increase progressively during postnatal development to reach a maximum plexus that connects GnRH neuronal somas and terminals at the time of puberty onset (approximately postnatal day 25) in mice[43]. Of note, in adulthood, kisspeptin^{ARC} neurons project largely to GnRH terminals, whereas kisspeptin$^{AVPV/PeN}$ neurons project to the GnRH somas[44]. Unlike kisspeptin^{ARC} neurons, which are detectable prenatally, kisspeptin expression in the AVPV/PeN is not present until postnatal day 10 in rodents[45], highlighting the existence of different developmental processes in the formation of each hypothalamic kisspeptin neuron population.

Although no direct studies have addressed the effect of metabolic cues on the differentiation and axonal elongation of kisspeptin neurons, compelling evidence in rodents indicates a long- lasting effect of perinatal metabolic insults on *Kiss1* expression throughout an animal's lifespan. For example, neonatal manipulations in energy resources in mice can be achieved by adjusting the litter size, leading to a model of neonatal energy deprivation (increased litter size) or energy excess (reduced litter size). Energy deprivation or excess leads to a statistically significant delay or advancement, respectively, in the time of puberty onset, driven by permanent changes in the hypothalamic expression of *Kiss1* (REF.[46]). Animals from small litters had increased *Kiss1* expression, whereas the animals from large litters showed decreased expression; these changes were maintained despite equal ad libitum access to food after weaning[46]. This finding strongly suggests that, similar to POMC and AgRP neurons[38–40], the kisspeptin system is also sensitive to metabolic alterations from early developmental stages and that these changes can be carried on into adulthood, leading to reproductive disorders.

These data in rodents agree with findings from human studies. Such studies in humans support a strong association between maternal obesity and hyperglycaemia during gestation with higher incidence of precocious puberty in the daughters of mothers with obesity[41,42]; however, the involvement of kisspeptin signalling in this process in humans remains to be demonstrated. Nonetheless, puberty onset depends on the re- awakening of pulsatile GnRH release, which in turn depends on the activation of kisspeptin neurons. Therefore, we can infer that, as in rodents, perinatal alterations in humans induced by the metabolic state permanently impinge on the expression of *KISS1* later in life (directly or through the action on upstream regulators), thus affecting the timing of puberty onset (discussed later).

Effects on kisspeptin neuron activity

Puberty is defined as a complex biological process involving sexual development, accelerated growth and adrenal maturation, heralded by the secretion of GnRH[47,48], which initiates the pulsatile release of gonadotropins, gonadal secretion of sex steroids and gametogenesis. A number of central and peripheral factors have been proposed to mediate puberty; however, exactly what triggers puberty onset remains elusive. Accumulating evidence suggests that hypothalamic kisspeptin is a likely candidate to serve as gatekeeper of puberty onset. For example, humans and mice with deficient kisspeptin signalling present with hypogonadotropic hypogonadism, absence of puberty onset and infertility[8,9,49]. Moreover, compelling evidence shows that *Kiss1* expression in the hypothalamus increases at the time of puberty onset in rodents[50–53]. However, most of these studies refer to the expression of *Kiss1* in whole hypothalami, whereas the specific contribution of each kisspeptin population of neurons (that is kisspeptin^{ARC} versus kisspeptin$^{AVPV/PeN}$) to puberty onset, at least in female animals given the vestigial nature of the PeN population in male animals, remains unknown. Of note, the synthesis and release of kisspeptin are critical targets for metabolic regulation induced by energy imbalances, which leads to changes in the timing of puberty onset in rodents. For example, undernutrition delays puberty onset, which is a phenotype that can be rescued by exogenous kisspeptin[51], further supporting the role of this neuropeptide as a pubertal gatekeeper sensitive to the metabolic status.

Neurokinin B (encoded by *Tac2*) is the kisspeptin co-transmitter in the ARC that participates in the auto-synaptic stimulation of kisspeptin release[54]. Importantly, this factor also acts as a metabolic target to regulate the HPG axis. In mice, the expression of *Tac2* is inhibited in conditions of negative energy balance[55,56], which further prevents the release of kisspeptin (FIG. 1). In addition to energy deficiency, a prepubertal caloric excess can advance puberty onset in a process that involves the early activation of the kisspeptin system[46]. Thus, we can consider kisspeptin neurons as the main conveyor of metabolic cues to facilitate sexual maturation.

In adulthood, once sexual maturity has been achieved, successful reproduction depends upon the presence of sufficient energy reserves. Kisspeptin neurons continue to convey metabolic signals to determine the appropriate pattern of GnRH release in adulthood. Indeed, conditions of negative energy balance such as anorexia nervosa or excessive exercise often lead to functional hypothalamic amenorrhoea[57]. Although the neuronal pathways underlying functional hypothalamic amenorrhoea in humans under energy deficit have not been addressed, studies in rodent models have shown that adult females subjected to energy-depleting conditions (for example, chronic undernutrition or fasting) display disrupted oestrous cycles and low circulating levels of gonadotropin that resemble human hypothalamic amenorrhoea, as a consequence of reduced *Kiss1* and *Tac2* expression[46,51,55]. These findings highlight the importance of kisspeptin^{ARC} neurons as conveyors of the energy state to the HPG axis.

Functional hypothalamic amenorrhoea

A condition derived from the insufficient secretion of GnRH from the hypothalamus, leading to anovulation and hypogonadotropic hypogonadism.

The effect of the metabolic state on kisspeptin neurons (described in a previous section) can critically affect sexual maturation and fertility; however, the mechanisms and factors underlying this regulation of kisspeptin neurons, from early developmental stages to adulthood, are not fully understood. This regulation could occur at a multilevel scale, including subcellular mechanisms, central networks and peripheral cues that ultimately directly affect kisspeptin neurons or act through intermediate neuronal effectors, which are described in the following sections.

Kisspeptin synthesis and release

This section summarizes what is known about the mechanisms of metabolic regulation of kisspeptin synthesis and release.

Subcellular mechanisms

Nutrient sensing.—The first mechanism of neuronal adaptation to the nutritional status of the organism is through the direct response of individual cells to metabolites. As mentioned previously, kisspeptin neurons send axonal projections to the hypophysial medial eminence[58–61], which is situated in a fenestrated area of the basal hypothalamus[35–37], and therefore this neuronal population is directly exposed to circulating nutrients. Circulating

levels of glucose and amino acids can induce a direct response on neurons through the 5'-AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) pathways, which integrate cellular metabolism and the metabolic status of the entire organism.

AMPK is a highly conserved 5'-activated protein kinase that comprises a catalytic a-subunit and two regulatory subunits (β and γ)[62]. The AMPK complex is a highly selective sensor for the AMP to ATP ratio. When glucose levels decrease during energy deficit, the increase in the AMP to ATP ratio induces the activation of AMPK by phosphorylation, which in turn increases the translocation of glucose transporters to the cell membrane, activates catalytic processes and slows down anabolism in an effort to replenish ATP levels[62,63]. AMPK is present in the hypothalamus at high levels and is involved in numerous pathways that determine the regulation of food intake and energy expenditure[64]. More importantly, the AMPK pathway is present in both kisspeptin^{ARC} and kisspeptin$^{AVPV/PeN}$ neurons[65,66], thus serving as the first-order sensor of the nutritional status of the organism to regulate reproductive function.

Studies in rodent models have demonstrated that AMPK phosphorylation is increased in kisspeptin^{ARC} neurons under negative energy balance (for example, chronic undernutrition), which leads to the inhibition of *Kiss1* expression and the resultant delay in puberty onset[65]. Additionally, the deletion of AMPKα from kisspeptin neurons in mice protects the HPG axis from the disruption induced by acute fasting[66]. Of note, although kisspeptin neurons are a nodal conveyor of metabolic and reproductive cues, glucose sensing through AMPK has also been described at different levels of the HPG axis, for example, GnRH neurons[67] and gonadotropes[68], suggesting that glucose sensing would have a role in the control of reproduction at multiple levels of the HPG axis.

Besides glucose, every cell requires amino acids for protein synthesis. The role of amino acid sensor is played by the mTOR complex, another protein kinase. In the presence of amino acids, mTOR is activated, leading to the phosphorylation of a number of protein complexes, of which S6 kinase 1 (SK61) and 4E-BP1 have a key conserved role[69,70]. Given their involvement in nutrient sensing, it is not surprising that mTOR and AMPK interact to further adapt cellular metabolism and energy status[71–74]. As with AMPK, the mTOR pathway not only actively controls food intake and energy expenditure at the hypothalamic level, but it also directly regulates reproductive function. For example, studies in rodents have shown that activation of mTOR with the amino acid leucine activates the HPG axis and advances puberty onset, whereas blockade of mTOR signalling using rapamycin causes the opposite effect through a decrease in *Kiss1* expression in the ARC while the expression of *Gnrh1* is unaffected[75]. Nonetheless, a role of mTOR signalling at the level of gonadotropes in the control of reproductive function cannot be excluded[76].

Although metabolite sensing enables the neuron to respond in real time to metabolic changes in its environment (such as circulating nutrients), the effect of these changes can reverse or cease as soon as the environment, and hence the neuron, reach a new set point of energy balance. Of note, nutrient sensing pathways also participate in the induction of epigenetic changes. Phosphorylated AMPK can induce changes in DNA methylation and

histone acetylation in response to glucose levels[77–79] (described later). This process is considered the main mechanism by which disrupted energy balance (that is, deficit or excess of nutrients) during critical developmental periods can lead to the development of metabolic and reproductive conditions during adulthood depending on the neurons and genes affected[27,28,30,31], including *Kiss1*.

Epigenetic regulation.—Numerous studies have provided information on the indirect, transgenerational epigenetic influence of sustained metabolic imbalances (for example, severe obesity or chronic undernutrition) from the parents to the offspring[80–85], as well as on the direct metabolic imprinting in an individual from early developmental periods (prenatally and perinatally)[86–90], as mentioned earlier (FIG. 2). These epigenetic modifications often contribute to the onset of obesity and reproductive impairments during postnatal development. In the past few decades, the most frequent scenario for epigenetic-induced changes in metabolism and reproduction in Western societies does not come from transgenerational or parental exposure but through childhood obesity in children of parents without obesity. This phenomenon is strikingly associated with permanent developmental alterations in energy balance and reproduction for the lifespan of the individual[18,19,28,64,83,91]. This effect strongly suggests that obesity in early developmental stages induces epigenetic changes in genes involved in reproduction and energy homeostasis, which are mostly located in the hypothalamus. Indeed, studies have shown that the decrease in the average age of menarche between the mid-nineteenth and the mid- twentieth centuries has been attributed in part to improvements in nutrition[92]. Epigenetics has been suggested as a molecular mechanism that mediates the interplay between behavioural and/or environmental factors and genetics by adapting the genome to environmental exposures[93]. In this context, the main epigenetic mechanisms that might ultimately regulate *KISS1* expression can be organized in three categories: DNA methylation, histone modification and non-coding RNAs.

DNA methylation occurs predominantly in cytosine–guanine (CpG) sites, referred to as CpG islands, through the action of methyltransferases, which leads to gene silencing or activation depending on the methylated region[93]. An extensive body of literature documents the association between obesity and diabetes mellitus with changes in DNA methylation[91,94]. Not surprisingly, the promoter region of the *Kiss1* gene is highly methylated[95–97] and developmental changes in the methylation pattern occur at the initiation of puberty in kisspeptinAVPV/PeN neurons[96,98] (FIG. 2). Importantly, methylation is not a permanent phenomenon. Studies in rodents have documented the role of ten–eleven translocation (TET) enzymes in the oxidative demethylation of DNA sites[99–101]. Moreover, the TET isoform TET2 is required for the maintenance of GnRH release[102] and preliminary studies in vivo (J. Kurian, personal communication) have indicated that they are also necessary for *Kiss1* expression. Because TET2 is phosphorylated and activated by AMPK[77–79], we can infer a direct relationship between the metabolic state of the organism and the methylation pattern of reproductive genes, that is, *Kiss1* and *Gnrh1*.

Histone modifications consist of chromatin changes that alter the accessibility of the DNA to polymerases to enable transcription, replication or DNA repair[103]. The most common modification is acetylation, which is a reversible process in which arginine or lysine residues

are acetylated on histone tails. This process is highly sensitive to the energetic state of the organism[104,105] and is also able to regulate the activity of the *Kiss1* promoter, at least in rodents[106] (FIG. 2). Indeed, the activation of the H3 histone by H3K9/14 acetylation is associated with an increase in *Kiss1* expression in all populations of kisspeptin neurons[106] and oestrogen is able to increase acetylation of the *Kiss1* promoter in kisspeptin$^{AVPV/PeN}$ neurons and facilitate deacetylation of the promoter in kisspeptin^{ARC} neurons[106]. This effect of oestrogen coincides with the differential regulation of *Kiss1* in each hypothalamic area as part of the positive feedback of sex steroids on kisspeptin$^{AVPV/PeN}$ neurons versus negative feedback on kisspeptin^{ARC} neurons. Moreover, sirtuin 1 (SIRT1), a fuel- sensing deacetylase[107], halts *Kiss1* expression in the ARC during infantile periods in rodents, therefore contributing to the proper timing of puberty onset. The activity of SIRT1 is increased by undernutrition, thus repressing *Kiss1* expression in conditions of negative energy balance[108,109].

Histone modification can also take place through methylation and the regulation of the methylated status of specific sites can substantially affect the expression of a gene. In this context, lysine-specific demethylase 1 (LSD1) has emerged as a critical link in the epigenetic regulation of obesity and ageing[110–112]. Preliminary studies uncovered an additional role for LSD1 as regulator of the timing of puberty onset. *Lsd1*-null mice display precocious puberty and elevated levels of gonadotropin (J. Gill and U.B. Kaiser, personal communication). As LSD1 is expressed in kisspeptin neurons[113], we can infer that LSD1 contributes to the metabolic regulation of *Kiss1* expression and, therefore, the energetic regulation of puberty onset and fertility (FIG. 2).

In addition to DNA methylation and histone modifications, non-coding RNAs have emerged as novel epigenetic mechanisms with critical importance in the regulation of protein synthesis through the regulation of RNA levels. Particularly, microRNAs (miRNAs) consist of RNA sequences that bind complementary mRNAs, silencing the transcript and targeting it for degradation, thereby leading to lower or absent translation of the transcript. A number of these miRNAs serve as mediators of the metabolic state of the organism to further regulate the activity of the HPG axis. Among these, the main miRNAs identified to date with documented activity in the regulation of both metabolism and reproduction are miR-7a2, Let-7a and miR-30b.

The global absence of miR-7a in a mouse model leads to hypogonadotropic hypogonadism[114]. Although the presence of miR-7a2 in kisspeptin neurons remains to be determined, its co- expression with AgRP in AgRP neurons[115] already suggests a reproductive and metabolic role for this miRNA, by modulating the output of the hunger signal AgRP (see later text). The mechanism for this effect involves, at least in part, the action of miR-7a on mTOR within the AgRP neuron[116].

Let-7a is a highly evolutionarily conserved miRNA that is upregulated in the hypothalamus of rodents in cone ditions of aberrant energy balance, that is, caloric restriction or diet-induced obesity[117]. Importantly, Let-7a and its associated protein Lin-28b (which regulates the processing of Let-7a into mature miRNA) are critical components of the neuroendocrine machinery controlling the time of puberty onset[118–120]. These findings suggest that Let-7a

must act through the transmission of the metabolic state onto kisspeptin and/or GnRH neurons.

A 2019 study provided compelling evidence for a role of miR-30b in the control of puberty onset. This miRNA is expressed in kisspeptin^{ARC} neurons and regulates the expression of kisspeptin by binding to the 3' untranslated region of the imprinted makorin 3 (*Mkrn3*) gene to repress its activity[121]. MKRN3 inhibits pubertal progression, and loss of function mutations in this gene lead to central precocious puberty in humans[122]. Because miR-30b is significantly upregulated in the hypothalamus by diet- induced obesity in mice fed a high- fat diet[117], we can infer that metabolic changes — especially those related to excess energetic state of the organism — might modulate puberty onset through the miR-30b–MKRN3–Kiss1 pathway. Whether this pathway is involved in the advancement of the timing of puberty onset observed in children with obesity remains to be deciphered.

Overall, compelling evidence from a wealth of studies suggests that kisspeptin neurons are able to directly sense the nutritional state of the organism and adapt to it to induce functional changes, for example, transcriptional and translational modifications, in response to the circulating levels of metabolites. However, despite this direct metabolic regulation, an important layer of complexity arises from the effect that regulatory networks of neuroendocrine pathways upstream of kisspeptin neurons, as well as peripheral factors, exert on kisspeptin neuron activity to precisely adapt to the nutritional environment.

Extracellular mechanisms

Neuronal factors.—The neuroendocrine regulation of food intake can occur acutely through fast- acting factors that rapidly promote hunger or satiety. Furthermore, on a long-term scale, factors can promote the increase in body weight or leanness, usually as a direct measure of the amount of energy stored in the adipose tissue[123,124]. In addition to food intake, the control of energy expenditure has a critical role in the maintenance of the overall homeostasis of the organism. Strong evidence exists that two sets of hypothalamic neurons (mostly located in the ARC) with antagonistic functions play a key part in the control of energy balance: first, the hunger-inducing AgRP neurons, which co-express neuropeptide Y and GABA; and second, POMC neurons, which co-express cocaine- and amphetamine-regulated transcript (CART), are predominantly glutamatergic and induce satiety[125,126]. In the hypothalamus, α-melanocyte-stimulating hormone (α-MSH) is the main product of POMC neurons, which binds the melanocortin 4 receptor (MC4R) to promote satiety[127] and increase energy expenditure[128], whereas AgRP antagonizes the same receptor to induce the opposite effect[129] (FIG. 3).

Dioestrus

Phase of the oestrous cycle in female mice, which precedes the ovulation phase, that is proestrus.

Situations of negative energy balance rapidly suppress the reproductive axis as a fail-safe mechanism due to the high energetic cost of reproduction. This effect occurs in part through the direct action of hunger neurons (that is, AgRP neurons through AgRP and GABA

release) on both kisspeptin^{ARC} and kisspeptin$^{AVPV/PeN}$ neurons[130]. Conditions of severe obesity, such as leptin deficiency, lead to a perceived permanent state of starvation, which also induces the activation of AgRP neurons and subsequently leads to infertility[131]. This role of AgRP neurons in reproduction is supported by studies showing that the ablation of AgRP neurons from leptin-deficient mice (*ob/ob*) is sufficient to restore fertility[131]. Moreover, in mice, the activation of AgRP neurons directly inhibits kisspeptin neurons, disrupting oestrous cycles and gonadotropin release (pulsatile and surge-like LH release) in a process that is largely mediated by GABA[130]. Altogether, these studies suggest that the main regulatory pathway by which hunger signals regulate reproductive function is through the direct inhibition of kisspeptin neurons.

Similarly, melanocortin signalling deficiency leads to severe obesity and reduced fertility in rodents[132]. The findings that the POMC^{ARC} neurons express oestrogen receptor-α[133–135] and are responsive to kisspeptin[136] led to the hypothesis that the melanocortin system might be a critical component in the central regulation of reproduction in a bidirectional POMC–kisspeptin manner. Indeed, α-MSH immunoreactive fibres are found in close apposition to kisspeptin neuron cell bodies of the ARC[137], which express MC4R[137]; however, whether these projections represent synaptic contacts that could modulate the activity of kisspeptin neurons remains unknown. Central stimulation of α-MSH signalling strongly activates the reproductive axis in rats, whereas chronic inhibition of melanocortin receptors delays puberty[137]. Interestingly, melanocortin receptor agonists are unable to modulate the firing of kisspeptin^{ARC} neurons from prepubertal and adult female mice in dioestrus in a series of cell-attached recordings[137]; however, further investigation is necessary under different sex steroid conditions to rule out a direct action of melanocortin on kisspeptin neurons. Of note, 50% of GnRH neurons of the medial preoptic area express MC4R[138], yet GnRH neurons are not the main target of α-MSH in controlling reproduction, as the stimulatory effect of α-MSH on LH secretion depends on the presence of kisspeptin signalling[137].

POMC neurons express CART, which contributes to the satiety signals from these neurons to control appetite and energy balance[139]. Importantly, CART is able to exert a strong direct depolarization of kisspeptin^{ARC} neurons in rodents[140], demonstrating that multiple stimulatory (and inhibitory, from AgRP neurons) pathways act on kisspeptin neurons to enable fine metabolic regulation of the activity of kisspeptin^{ARC} neurons.

In addition to the control of pulsatile LH release, AgRP and POMC neurons also participate in the control of the preovulatory LH surge. First, AgRP neurons contact and inhibit kisspeptin$^{AVPV/PeN}$ neurons[130] and the hypothalamic expression of *Agrp* decreases at the time of proestrus in rodents[141]. Second, although the existence of direct projections from POMC neurons to AVPV/PeN neurons has not been proven yet, the expression of *Pomc*[142] and *Mc4r*[141] increases at the time of the LH surge, which is blunted by MC4R antagonists, further suggesting the existence of metabolic mechanisms that directly affect ovulation, probably through the regulation of the activity of kisspeptin$^{AVPV/PeN}$ neurons.

Overall, although additional metabolic centres exist in the brain, both within and outside the hypothalamus (FIG. 3), to date, the two major regulators of food intake and energy balance (that is, AgRP and POMC neurons) are also the major neuronal populations that directly

transmit metabolic information to kisspeptin neurons. How these neurons sense the overall energetic state of the organism appears to be multifactorial. Although metabolite sensing contributes to the adaptation of the HPG axis to energy balance, the main regulatory process depends on, and is reinforced by, peripheral metabolic cues such as leptin, insulin or ghrelin acting directly on, or upstream of, kisspeptin neurons.

Peripheral factors.—The main peripheral signal informing the hypothalamus of the energy reserves is the adipokine leptin. Leptin is synthesized in adipocytes and secreted at levels proportional to existing adipose deposits[124]. Sufficient leptin levels are required for puberty onset, reproductive function and fertility (FIG. 1). Delayed or absent puberty, hypogonadotropic hypogonadism and infertility can result from disorders associated with reduced leptin levels[143–146]. However, despite this critical role of leptin in the maturation and maintenance of reproductive function, its mechanisms and sites of action are not fully understood. Although leptin receptors (LEPR) are expressed at multiple levels of the HPG axis, leptin action at the level of the hypothalamus is sufficient to maintain its metabolic and reproductive effects[147,148]. Regarding the control of reproduction, GnRH neurons do not express LEPR[149,150] and kisspeptin neurons express few LEPR; however, these receptors in kisspeptin neurons are not the direct target involved in the reproductive action of leptin[151].

Importantly, GABAergic neurons have been identified as the main interplay in the reproductive action of leptin[152,153], resembling the findings for the metabolic role of leptin[154]. These findings exclude a large number of glutamatergic neurons that could be direct targets of leptin to fine-tune leptin's action; however, glutamatergic neurons do not have a fundamental role in the effects of leptin on reproduction and body weight. Indeed, the deletion of LEPR specifically from GABAergic neurons largely replicated the reproductive phenotypes of female mice with deficient leptin signalling (*ob/ob* or *db/db* mice)[155,156], which show delayed or absent puberty onset, hypogonadotropic hypogonadism, disrupted oestrous cycles and infertility[152,153]. These mice also have substantially lower expression of *Kiss1* in the two main populations of hypothalamic kisspeptin neurons (AVPV/PeN and ARC)[153]. Of note, these studies showed the role of leptin action on all GABAergic neurons. Given the wide distribution of GABAergic neurons in the brain, further studies are required to decipher which specific subpopulation mediates the metabolic role and which the reproductive role of leptin.

Taken together, these findings suggest that the GABAergic action that mediates leptin's role in reproduction impinges on the kisspeptin system in female mice[153]; however, the nature of the precise GABAergic neurons that mediate this role remains unknown. Although the GABAergic-mediated action of leptin in reproduction appears to be critical for successful reproduction in female mice, male mice devoid of leptin signalling in these neurons display delayed puberty but normal reproductive function in adulthood[152]. Similar findings in *db/db* mice and in humans have also been reported[157,158]. This sexual dimorphism in the reproductive role of leptin is reasonable, given the larger energetic investment of females in reproduction.

Interestingly, studies have documented a critical contribution of the ventral pre-mammillary nucleus (PMV) to the reproductive action of leptin. First, lesions in this area prevent the

activation of the HPG axis by leptin[151]. Second, re-insertion of LEPR into the PMV of *Lepr*-deficient mice restores puberty onset and substantially improves reproductive function[151]. Although these studies offer compelling evidence supporting a role of the PMV in the reproductive role of leptin, it is important to highlight that the PMV is predominantly glutamatergic[151,154,159], which generates a puzzling discrepancy with the genetic studies described earlier that show that the reproductive role of leptin is mediated by GABAergic neurons.

One important caveat to the re-insertion studies of LEPR into *Lepr*-null mice is that the restricted expression of *Lepr* to a specific area throughout development might force the action of leptin on this area, and might not be a faithful representation of the existing neurocircuitry in wild-type animals. Nonetheless, the PMV receives projections from several brain areas, including the ARC and dorsomedial hypothalamus nuclei, which hold considerable numbers of GABAergic neurons[160]. These inputs suggest that the PMV is profusely innervated by neurons from GABAergic areas. It is therefore tempting to speculate that GABAergic LEPR-positive neurons that project to the PMV could contribute to the regulation of the reproductive role of leptin (for example, AgRP neurons). Indeed, as ARC neurons project to the PMV[160], and the selective re-insertion of LEPR into AgRP neurons of *Lepr*-null mice faithfully replicates the re-insertion of LEPR into the PMV[161], we can further speculate that in the PMV studies, AgRP and/or other GABAergic LEPR-positive neurons that project to the PMV might have contributed to the observed phenotype.

These hypotheses remain to be experimentally tested; however, if confirmed they would reconcile the ABAergic versus PMV-mediated (glutamatergic) pathways of leptin action in reproduction. Of note, this contention would not preclude a role for glutamatergic PMV LEPR-expressing neurons in the control of reproduction, although this effect would be subtle and involved in the fine-tuning of the responses of the reproductive axis to leptin. In this context, in 2018, we reported that pituitary adenylate cyclase-activating peptide neurons from the PMV, which express LEPR, directly contact both populations of kisspeptin neurons and are essential for the proper timing of puberty onset and the acquisition of full reproductive capabilities in females, probably acting as mediators of leptin's action on kisspeptin neurons[162].

In addition to leptin, several other peripheral factors transmit essential information about the energy status of the organism to the hypothalamic centres regulating metabolism and/or reproduction, among which insulin has a critical role. Insulin resistance is often associated with hypogonadotropic hypogonadism[163,164], however, the exact mechanism underlying this causative association is unknown. Insulin action in the brain is essential for gonadotropin release, as documented in brain-specific insulin receptor-deficient mice[165]. Kisspeptin^{ARC} neurons express insulin receptor[113], which suggests a likely direct effect of insulin on these neurons to transmit postprandial information on glucose levels to the HPG axis, an effect that could contribute to the onset of hypogonadotropic hypogonadism in patients with type 2 diabetes mellitus[163]. However, the specific deletion of insulin receptor from kisspeptin neurons does not affect fertility, nor does the deletion of both insulin receptor and LEPR from kisspeptin neurons[166] — as important cross-activation of downstream pathways has been documented[167]. Nonetheless, kisspeptin neuron-specific

insulin receptor-null mice exhibit a slight delay in puberty onset[166]. This finding suggests that while insulin might be involved in the maturation of the reproductive axis, it is not necessary for maintaining reproductive function in adulthood. Moreover, in studies using a rat model of streptozotocin-induced type 1 diabetes mellitus, in which the rats developed hypogonadotropic hypogonadism due to inhibited hypothalamic *Kiss1* expression, exogenous insulin was unable to restore the activity of the HPG axis[168]. This finding indicates that insulin action on kisspeptin neurons is neither necessary nor sufficient for the activation of the reproductive axis.

The orexigenic gut hormone ghrelin has also been found to be a direct regulator of kisspeptin neurons. Ghrelin levels increase with fasting, acting as an additional hunger signal at the central level. Importantly, kisspeptin^{ARC} neurons express ghrelin receptor (GHSR) in direct proportion to circulating levels of oestradiol[169] and exogenous ghrelin decreases levels of *Kiss1* mRNA in rats[170] — as expected for a hunger factor signalling insufficient energy reserves. Paradoxically, in the absence of sex steroids, which is typical of starvation states due to the subsequent hypogonadism, the expression of GHSR in kisspeptin neurons is reduced and limited to ~25% of normal levels. This observation suggests that either this subpopulation of kisspeptin neurons expressing GHSR is sufficient for ghrelin to directly inhibit the HPG axis, or that the inhibitory action of *Kiss1* expression is indirect through the documented activation of GHSR in AgRP neurons[171–173]. Nonetheless, the global deletion of ghrelin in mice does not affect fertility[174], suggesting that the role of ghrelin in reproduction is subtle and subject to compensation.

Regulation of energy balance

In this field, most of the attention has been paid to the metabolic regulation of fertility; however, the regulation of the metabolic function by reproductive cues has remained under-studied.

Kisspeptin neurons have emerged as an active player in the control of energy balance, based on several lines of evidence. First, central injections of kisspeptin reduces food intake in mice[175], jerboa[176] and rats[177,178], suggesting that kisspeptin could act as an anorexigenic factor. Second, kisspeptin neurons co-express LEPR and insulin receptor, indicating that they are a target for metabolic signals[166,179,180]. Third, *Kiss1r*- null (*Kiss1r* KO) mice develop substantial obesity compared with controls[181]. Fourth, kisspeptin neurons receive direct inputs from AgRP and POMC neurons, suggesting that they could serve as intermediate neurons in the hunger and/or satiety pathways[130,137]. Fifth, kisspeptin neurons modulate the activity of POMC and AgRP neurons through kisspeptin[136] and glutamate release in a process that is exacerbated in the presence of oestradiol[182,183] (FIG. 3). Finally, silencing of kisspeptin^{ARC} neurons in female mice increases body weight[184].

Interestingly, despite the anorexigenic action of kisspeptin found in these initial studies, further studies of kisspeptin administration in humans[185], and those in *Kiss1r* KO[181] and kisspeptin^{ARC}-silenced mice[184], do not support a significant role of kisspeptin in food intake. Moreover, the metabolic impairments observed in *Kiss1r* KO mice are restored in a mouse model of selective reinsertion of *Kiss1r* only in GnRH neurons[186]. This manipulation prevented any changes in body weight during adulthood but did not prevent the body weight

changes observed in prepubertal and young adult *Kiss1r* KO mice. This finding indicates that a large component of the metabolic phenotype observed in adult *Kiss1r* KO mice is sex steroid-dependent[186]. Furthermore, the optogenetic activation of kisspeptin^{ARC} neurons in genetic mouse models is unable to reduce food intake over a short period of time, despite the demonstrated ability to project to and stimulate neurons in the paraventricular hypothalamic (PVH) nucleus[187], a key nucleus in the control of food intake.

Overall, these studies suggest that the contribution of kisspeptin neurons to energy balance must be mediated by the regulation of energy expenditure rather than food intake, and that this role must be carried out by kisspeptin^{ARC} neurons (but not kisspeptin$^{AVPV/PeN}$ neurons) because, first, the population of kisspeptin$^{AVPV/PeN}$ neurons in the male is vestigial and, second, silencing kisspeptin^{ARC} neurons in female mice increases body weight without changing overall food intake[184]. These studies of kisspeptin^{ARC} neuron silencing uncovered a previously unknown role of kisspeptin^{ARC} neurons, as mediators of the circadian control of feeding behaviour. Thus, the loss of kisspeptin^{ARC} neurons in female mice leads to the loss of the predominantly nocturnal pattern of feeding behaviour, leading to the consumption of similar amounts of food during the dark and light phases[184].

A wealth of studies have shown that the circadian rhythm of feeding behaviour is essential to maintaining proper body weight and that the elimination of this cycle induces obesity in the face of equal calories consumed[188]. Whether the origin of the circadian pattern of feeding behaviour stems from the oscillations in the central circadian clock, that is, the suprachiasmatic nucleus (SCN), from intermediate neurons or from kisspeptin neurons themselves remains to be deciphered. Of note, the circadian genes Clock, *Per1* and *Per2* have been found to be expressed in kisspeptin^{ARC} neurons[113], suggesting that an intrinsic oscillator within these neurons is possible, which could be connected to the regulation of a master oscillator upstream, that is SCN. In this context, kisspeptin^{ARC} neurons do not appear to exert any regulatory effect on the SCN, although interactions at the level of SCN projections are possible[184].

A critical and unresolved question in the understanding of the role of kisspeptin^{ARC} neurons in the control of energy balance relates to the nature of the player carrying the main burden of this metabolic action. In this regard, although *Kiss1r* KO mice develop obesity[181], the increase in body weight observed after acute silencing of kisspeptin^{ARC} neurons[184] is substantially faster and larger than in *Kiss1r* KO mice. This finding suggests that additional factors, besides kisspeptin, must be at play in this metabolic role. The main candidate to have this role is glutamate, as glutamate from kisspeptin^{ARC} neurons directly modifies the activity of POMC and AgRP neurons[182,183] (FIG. 3). However, because the overall food intake is largely unaffected after the elimination of kisspeptin^{ARC} neurons, we can infer that the role of kisspeptin neurons through glutamate and kisspeptin release is not related to the promotion of satiety signals but to the modulation of the neuroendocrine circuits that control energy expenditure. In this context, projections from kisspeptin^{ARC} neurons to hypothalamic centres involved in energy expenditure have been described, including the PVH nucleus, the bed nucleus of the stria terminalis and the lateral hypothalamus[61,189]. Whether kisspeptin^{ARC} neurons also project to extra-hypothalamic areas involved in energy

expenditure, such as the lateral parabrachial nucleus (LPBN) and the nucleus of the solitary tract, remains to be addressed (FIG. 3).

An additional factor in the participation of kisspeptin^{ARC} neurons in this metabolic process is neurokinin B, through the binding to its putative receptor NK3R in the medial preoptic area[190]. NK3R-expressing neurons in this area form a so-called thermoregulatory centre. Kisspeptin^{ARC} neurons directly contact these neurons[190] and through the release of neurokinin B they can statistically significantly alter the temperature of the organism, causing vasomotor symptoms characterized by an increase in body temperature followed by adaptive vasodilatation of peripheral capillary veins to dissipate heat[190–192]. It is tempting to speculate that individuals with affected thermoregulatory capabilities, that is, the inability to activate brown adipose tissue or muscle shivering to induce heat, will burn fewer calories than their unaffected counterparts, further contributing to the increase in body weight observed overtime after kisspeptin^{ARC} neuron silencing.

An additional question to improve our understanding of the role of kisspeptin neurons as metabolic players relates to whether they are first order responders to key metabolic cues (such as leptin and insulin) or whether they serve as intermediate effectors within larger neuronal networks that control energy balance. In this vein, studies of selective deletion of LEPR or insulin receptor from kisspeptin neurons have clearly shown the absence of any metabolic phenotype, such as increased body weight or development of insulin resistance[166]. These findings therefore suggest that kisspeptin neurons are intermediate metabolic players rather than first- order responders. Nonetheless, it is possible that the role of kisspeptin neurons is to serve as a bypass or reinforcing mechanism for the reproductive axis to regulate metabolic function in specific conditions, such as during pregnancy. In this context, prolactin might be an additional regulatory layer on kisspeptin neurons. The majority of kisspeptin neurons express prolactin receptor[193]. Importantly, prolactin is a well-known orexigenic factor[194] during pregnancy, through a mechanism that does not involve AgRP neurons[195], suggesting that kisspeptin neurons might contribute to the metabolic actions of prolactin during pregnancy.

Finally, kisspeptin neurons are essential in the photoperiod-dependent activation of the HPG axis in mammalian seasonal breeders[196]. Most seasonal breeders also display concurrent changes in food intake, which frequently develop in opposite directions, such as a decrease in food intake during the breeding season. For instance, in sheep, short days during autumn and winter stimulate reproductive function[196], which coincides with lower voluntary food intake[197,198]. Whether this correlation is causative of the activation of kisspeptin neurons, leading to the synchronous control of reproductive function and metabolic changes, remains to be deciphered.

Conclusions

Kisspeptin expression, synthesis and release are tightly regulated by metabolic cues at multiple levels. As described in this Review, kisspeptin neurons are the main conveyor of the current metabolic status of the organism, by transmitting real-time information on circulating nutrients and stored energetic reserves to adapt reproductive capabilities. This process ensures that reproduction only happens in situations of energy surplus, mostly

in female mammals, as the population that bears the major energetic burden. However, epigenetic modifications induced by metabolic alterations, such as long periods of low food intake or obesity, can have long-lasting effects in the expression of the *Kiss1* gene. These changes can be passed on from previous generations, induced during early developmental stages or be derived from late- onset metabolic alterations.

No specific studies demonstrating the ability of metabolic factors to alter the *Kiss1* gene have been performed yet; however, the presence of methylation sites in the *Kiss1* gene and of enzymes involved in the methylation and acetylation states of DNA and chromatin in kisspeptin neurons, as well as the well-documented epigenetic effect that metabolic alterations have in a large array of studied genetic targets, indicates the existence of such interactions in kisspeptin neurons. Indeed, the expected changes derived from epigenetic alterations of the *Kiss1* gene, such as changes in the timing of puberty onset, in mouse models where the metabolic insults are no longer present, serves as a proof of persistent metabolic alterations that affect the *Kiss1* gene and/or upstream regulators.

An important system in the metabolic regulation of reproduction is the one formed by the intricate network of neuroendocrine circuits, which ultimately regulates the activity of kisspeptin neurons. Although evidence shows that AgRP and POMC neurons are critical components of this network by acting directly upstream of kisspeptin neurons, we cannot exclude additional hypothalamic centres (for example, the ventromedial nucleus) and extra-hypothalamic areas such as the LPBN or the amygdala. These areas target hypothalamic metabolic centres; however, the direct interaction with kisspeptin neurons has not been demonstrated. However, they might indirectly affect kisspeptin output through the action on other neurons, that is, AgRP and/or POMC neurons.

It is important to note that although peripheral signals transmit fundamental metabolic information to achieve successful reproduction, kisspeptin neurons do not seem to be first-order responders for the main metabolic cues, that is, leptin, insulin and ghrelin. Rather, kisspeptin neurons rely on as-yet-incompletely understood upstream neuroendocrine regulatory networks to respond to peripheral metabolic factors. Deciphering this neurocircuitry is a matter of intense research in the field.

Finally, a subject of increasing interest is the active metabolic role of kisspeptin neurons in energy balance. Some discrepancy exists between the different research models, with some presenting evidence of an anorexigenic role of kisspeptin and others indicating a predominant role in energy expenditure; however, what is clear is that kisspeptin neurons can ultimately affect energy homeostasis. Whether these discrepancies are due to the animal models or depend on specific physiological conditions of the individual remain to be deciphered. For the first time, however, a pivotal player in reproductive function has been described that influences the energetic status. This knowledge is important, given that a number of situations occur where not all the metabolic changes can be attributed to changes in the circulating levels of sex steroids, for example seasonal breeders, pregnant females or postmenopausal women.

Overall, the metabolic regulation of reproductive function is an extremely complex phenomenon composed of multiple regulatory levels, from subcellular to extracellular, as described in this Review. Unfortunately, this complexity prevents the complete understanding of the mechanism (or mechanisms) of action of metabolic cues that ensure successful reproduction. Moreover, it poses a limitation in determining which regulatory level among the ones described here has the most critical role in the control of kisspeptin neurons. Nonetheless, with time, as the neuroscience field evolves and new, more potent and precise techniques are developed, some of these questions will find an answer.

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Key points

- Metabolic factors can modulate the development and function of kisspeptin neurons at multiple developmental stages.
- These metabolic changes induced on kisspeptin neurons can be transient (for example, depending on the existing energetic reserves) or permanent (for example, epigenetic modifications).
- Kisspeptin neuron activity can be regulated by metabolic factors at subcellular, neuroendocrine and endocrine levels, which enables kisspeptin neurons to directly adapt to circulating metabolites and to the overall energetic state.
- Kisspeptin neurons serve as the main conveyor of metabolic cues to control the reproductive axis, thus determining the timing of puberty onset and reproductive success.
- Controversy exists regarding the role of kisspeptin neurons on food intake; however, mounting data suggest a predominant role of kisspeptin neurons in energy expenditure, potentially mediated by kisspeptin and/or its co-transmitters, such as glutamate.

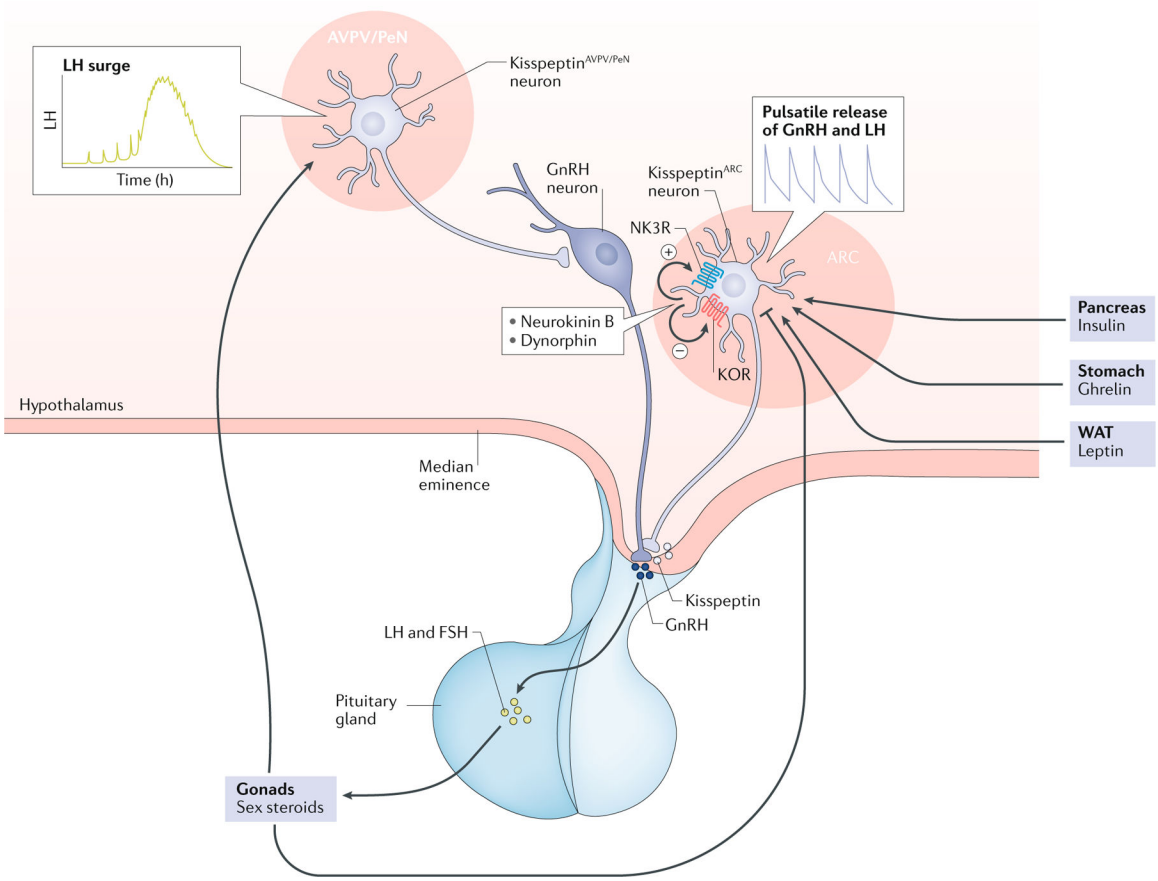

Fig. 1 |. The HPG axis with the two main populations of kisspeptin neurons.

Arcuate kisspeptin (kisspeptin^{ARC}) neuron together with neurokinin B and dynorphin are involved in the tonic (pulsatile) release of kisspeptin (positive and negative symbols indicate the effect on kisspeptin release) and, therefore, gonadotropin-releasing hormone (GnRH). By contrast, anteroventral periventricular/periventricular nucleus kisspeptin (kisspeptin$^{AVPV/PeN}$) neurons are involved in the control of the luteinizing hormone (LH) surge and are almost absent in the male brain. Major peripheral metabolic factors are depicted (leptin, insulin and ghrelin) acting at the level of the brain to regulate kisspeptin output. ARC, arcuate nucleus; FSH, follicle-stimulating hormone; HPG axis, hypothalamic–pituitary–gonadal axis; KOR, κ-opioid receptor; NK3R, neurokinin B receptor; WAT, white adipose tissue.

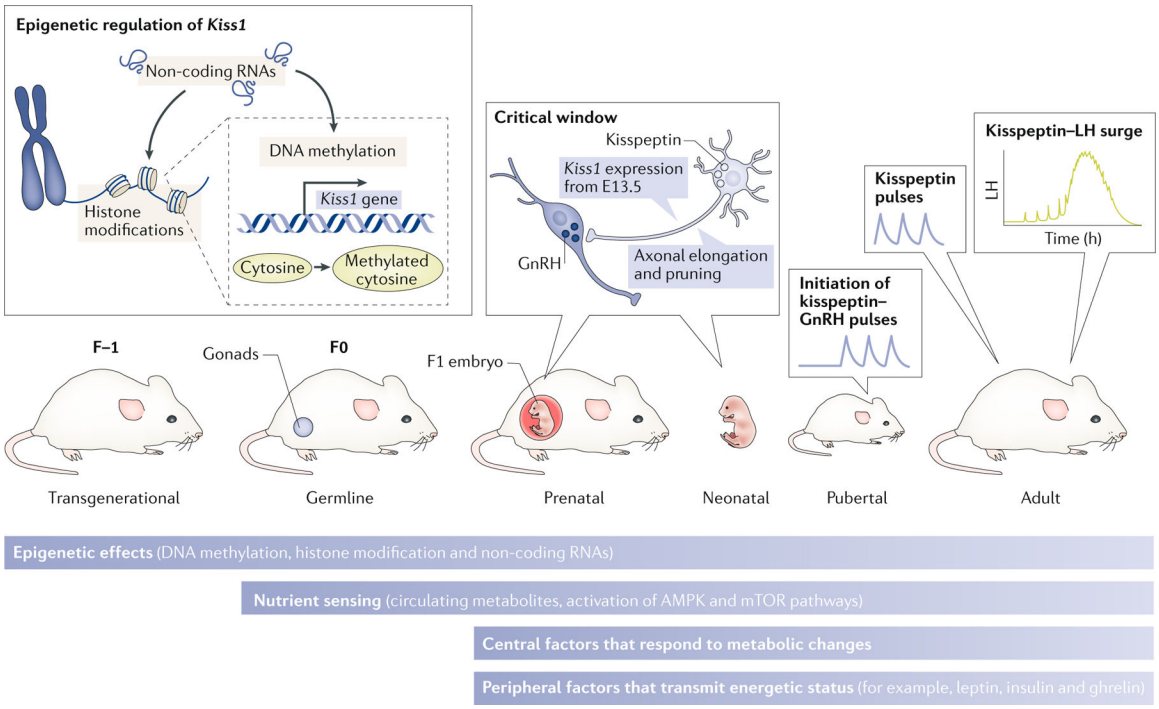

Fig. 2 |. Levels of metabolic regulation of kisspeptin neurons throughout development.

Schematic representation of the different developmental stages in which metabolic factors might affect the expression of the *Kiss1* gene. First, epigenetic effects (DNA methylation, histone modification and non-coding RNAs) might permanently affect the expression of *Kiss1*. This effect can occur transgenerationally or at any stage of development. Second, during the perinatal period there is a critical window in which conformational changes in kisspeptin neurons might happen as a consequence of the exposure to metabolic factors. These pre-existing modifications (epigenetic and conformational during development) might determine the timing of the activation of kisspeptin neurons (puberty onset) and their function in adulthood (luteinizing hormone (LH) pulses and surge). Nonetheless, during and after development, kisspeptin neurons can still be regulated by metabolic factors at different levels: epigenetic, nutrient sensing and central and peripheral factors. AMPK, 5'-AMP-activated protein kinase; E13.5, embryonic day 13.5; GnRH, gonadotropin-releasing hormone; mTOR, mammalian target of rapamycin.

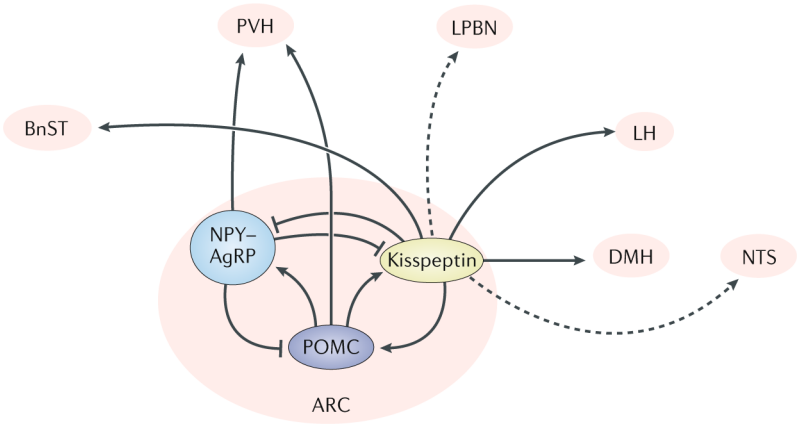

Fig. 3 |. Neuroendocrine circuits involved in the metabolic role of kisspeptin neurons.
Representation of a sagittal section of the mouse brain depicting documented (solid lines) and predicted (dotted lines) connections from kisspeptin^{ARC} neurons to and from known metabolic nuclei. AgRP, agouti-related peptide; ARC, arcuate nucleus; BnST, bed nucleus of the stria terminalis; DMH, dorsomedial hypothalamus; LH, lateral hypothalamus; LPBN, lateral parabrachial nucleus; NPY, neuropeptide Y; NTS, nucleus of the solitary tract; POMC, proopiomelanocortin; PVH, paraventricular hypothalamic nucleus.