REPRODUCTION

REVIEW

Kisspeptin and energy balance in reproduction

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Abstract

Kisspeptin is vital for the neuroendocrine regulation of GNRH secretion. Kisspeptin neurons are now recognized as a central pathway responsible for conveying key homeostatic information to GNRH neurons. This pathway is likely to mediate the well-established link between energy balance and reproductive function. Thus, in states of severely altered energy balance (either negative or positive), fertility is compromised, as is *Kiss1* expression in the arcuate nucleus. A number of metabolic modulators have been proposed as regulators of kisspeptin neurons including leptin, ghrelin, pro-opiomelanocortin (POMC), and neuropeptide Y (NPY). Whether these regulate kisspeptin neurons directly or indirectly will be discussed. Moreover, whether the stimulatory role of leptin on reproduction is mediated by kisspeptin directly will be questioned. Furthermore, in addition to being expressed in GNRH neurons, the kisspeptin receptor (*Kiss1r*) is also expressed in other areas of the brain, as well as in the periphery, suggesting alternative roles for kisspeptin signaling outside of reproduction. Interestingly, kisspeptin neurons are anatomically linked to, and can directly excite, anorexigenic POMC neurons and indirectly inhibit orexigenic NPY neurons. Thus, kisspeptin may have a direct role in regulating energy balance. Although data from *Kiss1r* knockout and WT mice found no differences in body weight, recent data indicate that kisspeptin may still play a role in food intake and glucose homeostasis. Thus, in addition to regulating reproduction, and mediating the effect of energy balance on reproductive function, kisspeptin signaling may also be a direct regulator of metabolism.

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Kisspeptin governs puberty onset and reproduction

Kisspeptin is a hypothalamic neuropeptide that drives fertility by stimulating gonadotropin-releasing hormone (GNRH) neurons (Gottsch *et al*. 2004, Han *et al*. 2005). A product of the *KISS1* gene, kisspeptin, is cleaved from an initial 145 amino acid precursor to a 54 amino acid peptide in humans (Kotani *et al*. 2001, Ohtaki *et al*. 2001) and a 52 amino acid peptide in mice (Terao *et al*. 2004). In humans, smaller isoforms of 14 and 13 amino acids have also been isolated, each sharing the common C-terminal sequence (Kotani *et al*. 2001, Ohtaki *et al*. 2001). Kisspeptin binds to the once orphaned G-protein-coupled receptor-54 (Kotani *et al*. 2001), now commonly referred to as *Kiss1r* (Gottsch *et al*. 2009).

Two independent research groups discovered the essential role of kisspeptin in reproduction almost simultaneously in 2003, when *Kiss1r* mutations were isolated in cases of idiopathic hypogonadotropic hypergonadism (de Roux *et al*. 2003, Seminara *et al*. 2003). Seminara *et al*. (2003) were also the first to examine *Kiss1r* null mice, which shared the infertility and had no other discernible phenotype. It is now universally accepted that kisspeptin is fundamental to GNRH-driven fertility and the key pieces of evidence for this include the following: i) the stimulatory effect of kisspeptin is blocked by GNRH antagonists (Gottsch *et al*. 2004, Irwig *et al*. 2004, Matsui *et al*. 2004, Shahab *et al*. 2005); ii) injections of kisspeptin directly in to the vicinity of GNRH neuron stimulate luteinizing hormone (LH) secretion (Patterson *et al*. 2006); iii) kisspeptin activates GNRH neurons *in vivo* (Irwig *et al*. 2004, Matsui *et al*. 2004) and *in vitro* (Han *et al*. 2005, Pielecka-Fortuna *et al*. 2008); iv) kisspeptin immunoreactive fibers appose GNRH neuron cell bodies (Kinoshita *et al*. 2005, Clarkson & Herbison 2006, Smith *et al*. 2008) and their terminals within the median eminence (Smith *et al*. 2011); v) kisspeptin stimulates GNRH release into the portal circulation of sheep (Smith *et al*. 2011) and the isolated mediobasal hypothalamus (d'Anglemont de Tassigny *et al*. 2008); and finally vi) almost all GNRH neurons express *Kiss1r* (Irwig *et al*. 2004, Han *et al*. 2005, Smith *et al*. 2009). Importantly, the effects of kisspeptin are absent in *Kiss1r* knockout (KO) mice, showing specificity to this receptor (Messager *et al*. 2005, Dungan *et al*. 2007, Kauffman *et al*. 2007).

It is worthy to note that using a genetic ablation approach, the importance of the kisspeptin system in mice has been challenged. Mice with ablated kisspeptin neurons presented with normal fertility

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(Mayer & Boehm 2011). However, it is questionable whether a complete loss of kisspeptin cells was achieved. It is likely that this result reflects redundancy in kisspeptin neurons and signaling as genetically targeted mice with 50 and 95% reductions in *Kiss1* transcript still maintain, albeit impaired in females, fertility (Popa *et al*. 2013). In addition, the DBA/2J mouse strain possess less than one-tenth the level of *Kiss1* mRNA in the brain than the C57BL/6 mice (Quennell *et al*. 2011), yet are fertile. Thus, these data may highlight the importance of kisspeptin in reproduction, in that it is synthesized in excess to ensure reproductive success.

One of the primary functions of kisspeptin appears to be as an interneuronal bridge between systemic levels of sex steroids and GNRH neuron regulation (Smith 2013; Fig. 1). In rodents, kisspeptin-producing cells are found in the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC) (Smith 2013). In sheep, kisspeptin neurons are located in the dorsolateral region of the preoptic area (POA) (perhaps a homologous population to the rodent AVPV) and the ARC (Estrada *et al*. 2006, Smith *et al*. 2007), and estrogen regulation of kisspeptin has been extensively studied in these regions in both rodents and sheep (Smith 2013). Both neuronal populations are important in the generation of estrogen-positive feedback and sex steroid-negative feedback signals to GNRH neurons. The former, critical for the GNRH/LH surge and ovulation in females and the latter, involved in the tonic/pulsatile regulation of GNRH secretion in both sexes (Simerly 2002, Herbison 2008). Specifically, sex steroids robustly regulate kisspeptin neurons and those in the ARC forward signals applicable to negative feedback regulation of GNRH in mice (Smith *et al*. 2005*a*, 2005*b*). In the female rodent, AVPV kisspeptin cells are critical for positive feedback regulation of GNRH (Smith *et al*. 2005*a*, 2006*b*). In sheep, key differences are apparent in feedback regulation of GNRH compared with rodents. Thus, estradiol (E_2)-induced positive feedback appears to be mediated by kisspeptin neurons in both the ARC (Estrada *et al*. 2006, Smith *et al*. 2009) and the POA (Smith *et al*. 2009, Hoffman *et al*. 2011).

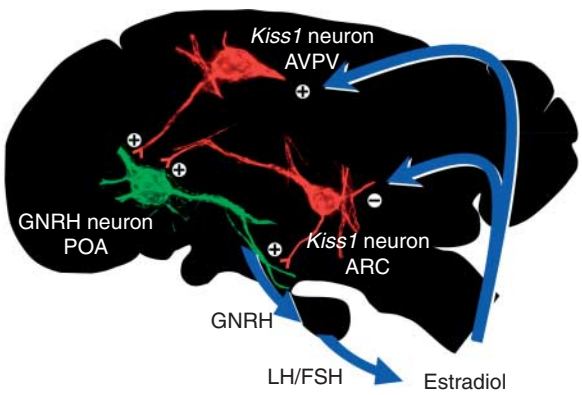

Figure 1 The proposed negative and positive feedback mechanism exerted by ovarian steroids on the regulation of kisspeptin neurons. In rodents, *Kiss1* neurons are located in both AVPV and ARC and stimulate (+) GNRH neurons. In the ARC, *Kiss1* neurons project directly to GNRH cell bodies and their terminals in the median eminence. Moreover, estradiol (E_2) inhibits (−) ARC *Kiss1* neurons consistent with negative feedback control of GNRH neurons. In the AVPV, E_2 stimulates (+) *Kiss1* neurons, facilitating positive feedback regulatory control and the preovulatory GNRH/LH surge. AVPV *Kiss1* neurons only project directly to GNRH cell bodies.

Metabolic control of fertility

The appropriate regulation of energy balance is important for fertility. Successful reproduction requires adequate resources within the individual organism. Thus, the ability to control reproduction and metabolism simultaneously ensures that offspring are born into an environment with sufficient energy supplies to maintain survival of both the mother and the offspring (Evans & Anderson 2012). As a result, there is a clear association of the effects of energy balance on reproduction, whereby perturbations in energy balance, including obesity and frequently result in fertility impairment (Pasquali *et al*. 2007). In most cases, it is evident that negative energy balance, when less energy (food) is consumed than is expended in metabolism, inhibits the reproductive axis. Ewes subjected to restricted feeding exhibited a significant decrease in mean LH concentration, LH pulse frequency, and follicle-stimulating hormone (FSH) concentrations compared with normal fed ewes (Thomas *et al*. 1990). In male rats, food restriction decreased LH, FSH, and testosterone levels compared with *ad libitum* fed controls (Compagnucci *et al*. 2002). Similar studies are also evident in mice, showing reduced fertility as a consequence of undernutrition (Castellano *et al*. 2005, Luque *et al*. 2007). Overall, these studies demonstrate that lean animals with a reduced food intake are often hypogonadotropic.

At the other end of the metabolic spectrum, diet-induced obesity (DIO) also has an effect on reproductive status. This is especially relevant now because obesity is reaching epidemic proportions and is one of the most serious public health issues facing the developed world. In male mice with DIO, sperm motility and fertility are compromised compared with normal-weight controls (Ghanayem *et al*. 2010). In adult female DBA/2J mice, which are susceptible to DIO, there were decreases in pregnancy rates and GNRH expression compared with controls (Tortoriello *et al*. 2004). These studies highlight that obesity can also lead to reduced fertility.

The effect of energy status on fertility is mediated by kisspeptin

As both metabolic state and energy balance are important for reproduction, it would be reasonable to assume that kisspeptin neurons can provide the link

between energy status and fertility. The role of kisspeptin expression in altered energy states has been investigated in states of undernutrition in mice and streptozotocin-induced diabetes in rats. Fasting in prepubertal rats led to a significant reduction in whole hypothalamic *Kiss1* but increased *Kiss1r* expression compared with normal fed rats (Castellano *et al*. 2005). Using a shorter period of fasting (48 h) in adult male mice, both *Kiss1* and *Kiss1r* mRNA expression was reduced compared with fed controls (Luque *et al*. 2007). In a different altered energy state, an induced diabetic rat model, there was a significant decrease in *Kiss1* mRNA compared with controls (Castellano *et al*. 2006). In follow-up studies isolating the distinct populations of *Kiss1*, fasting reduced expression in the AVPV, but not the ARC, in adult female ovariectomized (OVX) rats (Kalamatianos *et al*. 2008). Alternatively, chronic calorie restriction in female rats at the age of puberty reduced *Kiss1* expression in the ARC, but not the AVPV (Roa *et al*. 2009). In OVX ewes, the effect of body weight status was seen on *Kiss1* mRNA expression in both the ARC and the rostal, POA region, and was reduced in ewes made lean (Backholer *et al*. 2010). Thus, both populations of kisspeptin neurons are potential targets for negative energy balance.

To further support the role of kisspeptin in mediating the effects of energy status on fertility, there are functional data where exogenous kisspeptin administration can rescue the hypothalamic–pituitary axis in these conditions. Fasted prepubertal male and female rats showed a significant increase in LH levels when they were centrally administrated with kisspeptin (Castellano *et al*. 2005). Furthermore, in a male diabetic rat model, kisspeptin administration led to a significant increase in LH levels compared with a vehicle control (Castellano *et al*. 2006). Thus, in conditions of negative energy balance, treatment with kisspeptin may overcome the reduced endogenous expression of *Kiss1* and rescue any deficit in reproductive function. Alternatively, such a relationship does not automatically preclude a role for kisspeptin in mediating the effects of energy status. Kisspeptin may simply bypass the neuronal pathways that exert inhibitory metabolic actions on GNRH secretion, thus the data above should be considered with caution.

With regard to positive energy balance, data are relatively scarce. However, in one recent study, *Kiss1* expression was investigated in a DIO mouse model. Here, female DBA/2J mice made obese by maintenance on a high-fat diet from weaning to adulthood had reduced *Kiss1* mRNA in the ARC and the AVPV compared with chow-fed controls. Consistent with this, kisspeptin neuron number (as detected by immunohistochemistry) was also reduced in the latter (Quennell *et al*. 2011).

From the aforementioned data, it is clear that energy balance has profound effects on the reproductive axis and these appear to be mediated, at least in part, by kisspeptin expression and signaling in the hypothalamus. This immediately raises the question as to what metabolic signals govern this effect. Metabolic control involves multiple factors acting on and within the hypothalamus (Barsh & Schwartz 2002), and it is possible that any one or the combined effect of many signals is important for the regulation of kisspeptin and in turn fertility.

Metabolic regulators of kisspeptin

Leptin

The adipose hormone leptin is a critical component for energy balance. Leptin is secreted in proportion to fat stores and acts within the brain to signal adequate energy stores and satiety (Halaas *et al*. 1995). Adequate leptin concentrations are also known to be essential for the reproductive axis, gating the onset of puberty (Cheung *et al*. 1997, Chehab 2000) again through action in the brain (de Luca *et al*. 2005). Despite the acceptance of leptin as a requisite for puberty and fertility, the neuroanatomical pathway linking leptin signaling to GNRH neurons is not yet fully understood. GNRH neurons do not possess the signaling isoform of the leptin receptor (LepRb; Quennell *et al*. 2009). Therefore, interneuronal pathways that are sensitive to leptin and converge on GNRH neurons are required and kisspeptin neurons are recognized as a primary candidate.

We were the first to demonstrate leptin's regulatory control of the kisspeptin system using leptin-deficient *ob/ob* mice (Smith *et al*. 2006*a*). Owing to the lack of circulating leptin, these mice are obese but experience a condition of perceived negative energy balance and are infertile. Male *ob/ob* mice had significantly reduced expression of *Kiss1* mRNA in the ARC compared with WT littermates and this was partially corrected when exogenous leptin was administered to the periphery. Importantly, all mice in this study were castrated to remove the confounding regulatory effects of endogenous gonadal steroids, which are reduced in *ob/ob* mice. The study also confirmed the presence of *Lepr* (*LepRb*) mRNA on 40% of kisspeptin neurons in the ARC, indicating that leptin regulation of gene expression is likely to occur directly on kisspeptin neurons (Fig. 2).

In support of this study, female *ob/ob* mice were also shown to have reduced expression of *Kiss1* mRNA in the ARC compared with WT controls (Quennell *et al*. 2011). Moreover, this reduction was again shown in OVX mice and also in OVX mice with baseline estrogen replacement (Quennell *et al*. 2011). Similar confirmation of leptin regulation of kisspeptin has been reported in rats using a streptozotocin-induced diabetes model (resulting in hypoinsulinemia and hypoleptinemia; Castellano *et al*. 2006). Here, central leptin administration was able to restore the otherwise reduced expression of *Kiss1* mRNA in the whole hypothalamus. In guinea pigs, leptin can induce depolarization of kisspeptin neurons in the

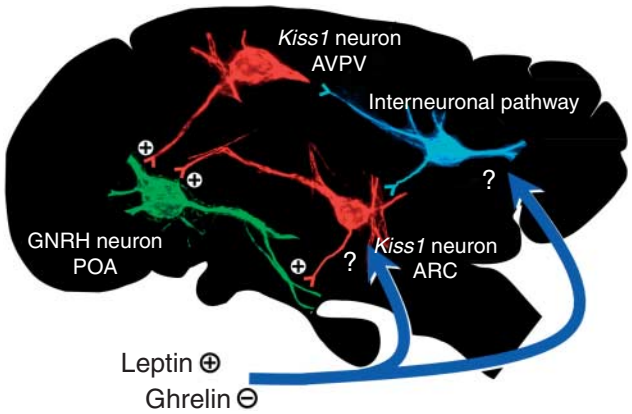

Figure 2 The potential role of leptin and ghrelin on *Kiss1* neuron regulation. Leptin stimulates (+) *Kiss1* expression in both AVPV and ARC, but direct or indirect action on *Kiss1* neurons is currently debated (?). Data suggest a direct role on ARC *Kiss1* neurons, but not those in the AVPV. Equally, data show an interneuronal pathway linking leptin signaling to *Kiss1* neurons. Alternatively, ghrelin inhibits (−) *Kiss1* neurons and does so in the AVPV via a yet to be determined indirect mechanism (?). There is currently no evidence to claim a direct action of ghrelin on ARC *Kiss1* neurons (?), but this remains a possibility.

ARC, of which 36% express *Lepr* (Qiu *et al*. 2011). Finally, similar data are also apparent in sheep. In OVX ewes made lean through dietary restriction, reduced *Kiss1* expression in the ARC was again partially restored with central leptin administration (Backholer *et al*. 2010). Interestingly, in this study, a similar effect was also observed in the POA kisspeptin neurons and virtually all these neurons (both POA and ARC) expressed *Lepr* mRNA (Backholer *et al*. 2010).

Despite the evidence for a direct effect of leptin on kisspeptin neurons, more recent discoveries have cast doubt as to whether this is indeed the case. Subsequent neuroanatomical data in female sheep show a complete absence of leptin-induced pSTAT3 responses (indicating the presence of functional LepR) in either POA or ARC kisspeptin neurons (Louis *et al*. 2011). This same publication also showed an absence of LepRb (detected using *Lepr*–EGFP transgenic mice) in immunoreactive kisspeptin neurons in the AVPV. Moreover, a very limited proportion (0–6%) of ARC kisspeptin neurons (visualized using *Tac2*–EGFP transgenic mice) coexpressed pSTAT3 immunoreactivity (Louis *et al*. 2011). In this model, however, the number of 'kisspeptin neurons' visualized via *Tac2*–EGFP appeared limited and so it is conceivable that the authors may have underestimated this population and reduced their ability to detect LepRb colocalization. Indeed, other studies using similar techniques have shown leptin activation (pSTAT3) in 15% of ARC kisspeptin neurons (Cravo *et al*. 2011). Despite this, the notion of kisspeptin neurons receiving direct input from leptin signaling has certainly become a contentious one. This was originally brought to the fore in a paper demonstrating that the onset of puberty is unaltered in a female mouse model with a targeted disruption of LepR selectively in *Kiss1* neurons (Donato *et al*. 2011). Of interest here is that functional LepR signaling (pSTAT3 immunoreactivity) was confirmed in 13–20% of ARC kisspeptin neurons in control ($LepR^{flox/flox}$) mice. As predicted, receptor expression was markedly reduced, but not completely abolished, in *Kiss1-Cre* $LepR^{flox/flox}$ mice. So it is possible that the remaining (but severely limited) LepR expression on kisspeptin neurons following cre-lox recombination may still be adequate for the relay of leptin signaling to kisspeptin neurons and in-turn fertility. Of note here is the apparent redundancy in *Kiss1* expression required for fertility in mice (Popa *et al*. 2013). Equally, it is noted in *Kiss1-Cre* $LepR^{flox/flox}$ mice that LepR deletion was also apparent in the ovary and testes (Donato *et al*. 2011). *Kiss1* expression has been documented in the gonads (Gaytan *et al*. 2009, Tariq *et al*. 2013) and also other brain regions during development (Gottsch *et al*. 2011), so the specificity of LepR deletion may be compromised. This, paired with the possibility of developmental compensatory mechanisms in the transgenic model, should not be ignored. In order to overcome many of these issues, a subsequent study examined LepR-null mice where LepR was re-expressed selectively in kisspeptin cells. These mice showed no improvement to the infertile phenotype of LepR-null mice (Cravo *et al*. 2013), indicating that leptin signaling in kisspeptin neurons is not sufficient for fertility in mice.

Although it remains to be disproven as to whether leptin can act directly on kisspeptin neurons, indirect actions remain a likely possibility (Fig. 2). Indeed, in both studies refuting the direct role of leptin on kisspeptin neurons, a neuronal population expressing LepRb located in the ventral premammillary nucleus (PMV) was implicated (Donato *et al*. 2011, Louis *et al*. 2011). Notably, lesions of the PMV prevent the restoration of fertility following leptin treatment in *ob/ob* mice and 're-expression' of LepRb in the PMV of female *LepR*-null mice is sufficient to induce sexual maturation (Donato *et al*. 2011). Moreover, PMV neurons appear to make close contacts with kisspeptin and GNRH neurons (Donato *et al*. 2011, Louis *et al*. 2011) and a yet to be characterized population of LepRb neurons is also present in close vicinity of *Kiss1* neurons in both ARC and AVPV (Louis *et al*. 2011). So coordinated interplay between the PMV–kisspeptin–GNRH systems is likely. Overall, it can be concluded that PMV is a key site for leptin's permissive action at the onset of puberty and supports the hypothesis that leptin's role in controlling metabolism (via the ARC) and reproduction is anatomically dissociated (Coppari *et al*. 2005).

In spite of wealth of evidence demonstrating the effect of leptin on promoting fertility, and the involvement of kisspeptin neurons in mediating this effect (direct or indirect), the contribution of additional modulators of metabolism should not be ignored (see below).

Data also suggest that leptin may not be the sole critical metabolic factor predicating the restoration of fertility in models of negative energy balance. In sheep, the restoration of *ad libitum* feeding in food-restricted ewes rescues pulsatile LH secretion but does so prior to any change in circulating leptin concentrations (Szymanski *et al*. 2007). Similarly, restoration of leptin to normal basal levels in caloric restricted female rats does not restore *Kiss1* mRNA or plasma LH levels (True *et al*. 2011). Although it could be argued in the latter that a required threshold of leptin was not reached because higher 'pharmacological' levels of leptin replacement in this study did maintain LH at control values (True *et al*. 2011). Nevertheless, alternative regulators of metabolism are very likely involved in kisspeptin regulation and should be explored.

Insulin

Insulin, the product of the pancreatic β cells, is vital for the control of carbohydrate and fat metabolism and also plays a role in the hypothalamus to regulate energy balance (Schwartz *et al*. 1992). Moreover, central insulin signaling promotes fertility (Bruning *et al*. 2000) and, like leptin, appears to regulate GNRH neurons through an interneuronal mechanism (Divall *et al*. 2010). In food-restricted ewes, the rescue of pulsatile LH secretion via restoration of *ad libitum* feeding (which occurs prior to any change in circulating leptin, see above) is preceded by an increase in circulating insulin concentrations (Szymanski *et al*. 2007), leading to the hypothesis that LH pulses are reinitiated by changes in availability of metabolic fuels and insulin.

Further, mice that are lacking insulin receptors selectively in kisspeptin neurons (IR^{Kiss} mice) experience a delay in puberty (Qiu *et al*. 2013). Specifically, female IR^{Kiss} mice had delayed vaginal opening and first estrous, while males had reduced testis mass at postnatal day 31. These data indicate that kisspeptin neurons are likely mediators for the effects of insulin on reproduction. However, this phenotype appears to be limited to puberty onset because measures of adult fertility in these mice (levels of LH, FSH, sex steroids, as well as fertility) appeared unperturbed (Qiu *et al*. 2013). Consistent with the latter, insulin treatment does not appear to restore *Kiss1* mRNA expression in the whole hypothalamus of diabetics rats (Castellano *et al*. 2006). Thus, the role of kisspeptin neurons in mediating the effect of insulin on the reproductive axis still requires further clarification.

Ghrelin

Ghrelin is a stomach hormone commonly associated with the neural control of appetite and metabolism (Andrews 2011, Briggs & Andrews 2011). However, unlike leptin or insulin, ghrelin operates as an orexigenic factor. Ghrelin also affects the reproductive system. For example, central ghrelin injection to OVX rats, or OVX rats treated with E_2, suppressed LH concentration and pulse frequency (Furuta *et al*. 2001, Ogata *et al*. 2009). Similar inhibitory effects on LH secretion were observed throughout the estrus cycle (Fernandez-Fernandez *et al*. 2005). Here, ghrelin significantly inhibits GNRH release from hypothalamic explants and ghrelin suppressed GNRH-induced LH release *in vitro* (Fernandez-Fernandez *et al*. 2005). The central inhibitory effects of ghrelin on LH secretion also occurs in sheep (Iqbal *et al*. 2006). Thus, it appears that conditions of negative energy balance increase plasma ghrelin concentrations and in turn suppress the reproductive axis.

The mechanisms through which central ghrelin inhibits the reproductive system remain unresolved, although kisspeptin neurons in the hypothalamus may be a primary target. Previous studies highlight that ghrelin could inhibit LH secretion by directly suppressing the effects of kisspeptin on the reproductive axis. Ghrelin significantly reduced the duration of the LH secretory response to kisspeptin-10 (Martini *et al*. 2006). Moreover, during fasting, exogenous ghrelin treatment, or the combination of both, expression of *Kiss1* mRNA in the AVPV is reduced (Forbes *et al*. 2009), without affecting *Kiss1* mRNA in the ARC, indicating that ghrelin may target these kisspeptin neurons to suppress LH secretion.

Ghrelin acts on the growth hormone secretagogue receptor (GHSR) in the brain to elicit changes in physiological functions. Although ghrelin suppresses LH secretion and regulates *Kiss1* mRNA, there is no clear neuroanatomical evidence linking GHSR neural circuits to reproductive neural circuits. We first examined direct coexpression of the GHSR and GNRH or kisspeptin neurons using a *GHSR*–eGFP reporter mouse line, which is currently the best model available to visualize GHSR expressing neurons. We showed for the first time that neither GNRH nor kisspeptin neurons in the AVPV express *GHSR*–eGFP, so any effect of ghrelin on these kisspeptin neurons must be indirect (Smith *et al*. 2013; Fig. 2). Importantly, we realize that these findings are reliant on the validity of the *GHSR*–eGFP model. In our study, we observed that only half of the *GHSR*–eGFP cells in the AVPV coexpressed *Ghsr* mRNA (as determined by *in situ* hybridization) and we remain cautious with the GHSR–eGFP mouse model and the interpretation of our data. Moreover, far fewer eGFP cells were localized to the ARC than expected. Given this, we can make no claim to the degree of GHSR–eGFP coexpression in kisspeptin neurons of the ARC. It remains likely that ARC kisspeptin neurons could coexpress GHSR and receive direct ghrelin input (Fig. 2) because expression of mRNA for both genes is prominent in this area (Gottsch *et al*. 2004, Zigman *et al*. 2006).

Hypothalamic regulators of kisspeptin

If leptin, insulin, or ghrelin do not directly act on kisspeptin neurons, what are the possible indirect mechanisms? A realistic possibility may be the neuronal systems within the ARC responsible for integrating these peripheral metabolic signals and relaying effects on food intake and energy expenditure to higher brain centers. These neurons, termed 'first-order neurons' consist of neuropeptide Y (NPY)/agouti-related peptide (AgRP) neurons and pro-opiomelanocortin (POMC)/cocaine and amphetamine-regulated transcript (CART) neurons (Barsh & Schwartz 2002). The majority of NPY/AgRP and POMC/CART neurons in the ARC contain LepRb, insulin receptor, and GHSR (Willesen *et al*. 1999, Barsh & Schwartz 2002, Perello *et al*. 2012). Whether they are actively involved in the metabolic control of kisspeptin neurons is yet to shown.

NPY/AgRP neurons

A recent study suggests that NPY/AgRP neurons are involved in an important link between reproduction and metabolism (Wu *et al*. 2012). These neurons are orexigenic and critical to initiate food intake (Aponte *et al*. 2011, Atasoy *et al*. 2012) and genetic ablation of AgRP neurons in adulthood results in starvation (Luquet *et al*. 2005). In order to examine the mechanisms underpinning hyperphagia in genetically obese and infertile *ob/ob* mice, Wu *et al*. (2012) discovered that ablating NPY/AgRP neurons in these mice caused a prolonged period of reduced food intake and remarkably restored fertility in both males and females. Consistent with this is the inhibitory effect of NPY on GNRH/LH secretion (Barker-Gibb *et al*. 1995, Xu *et al*. 2009). Interestingly, NPY may inhibit or stimulate LH secretion according to the steroid milieu in rats, inhibiting in OVX models, but stimulating in intact (Kalra & Crowley 1984). In sheep, NPY only appears to have an inhibitory role on gonadotropin release (Barker-Gibb *et al*. 1995).

Despite the association, the effect of NPY on kisspeptin neurons is far from clear. In sheep, kisspeptin neurons receive neuroanatomically defined inputs from NPY/AgRP neurons (Backholer *et al*. 2010). However, in NPY KO mice, the expression of *Kiss1* mRNA appears to be reduced to levels similar to that during a fasted state (Luque *et al*. 2007). This is somewhat counterintuitive given that fasting, which reduces kisspeptin expression, stimulates the hypothalamic expression of NPY (Hahn *et al*. 1998). Leptin also appears to suppress NPY expression (Ahima 2000) but increases the expression of *Kiss1* (Smith *et al*. 2006*a*). Nevertheless, the stimulatory role of NPY on *Kiss1* has also been shown in a hypothalamic cell line *in vitro* (Luque *et al*. 2007). Thus, the relationship between NPY and kisspeptin appears highly complex and one wonders what specific role AgRP may be playing (Fig. 3), particularly in regard to data obtained from NPY KO mice. Specific actions of AgRP on kisspeptin neurons are yet to be shown.

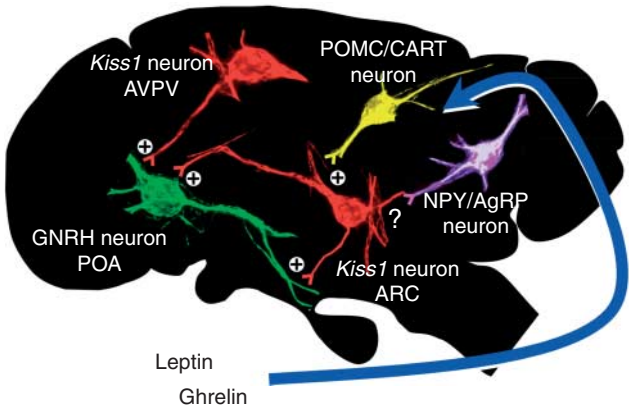

Figure 3 The potential involvement of NPY/AgRP and POMC/CART neurons in *Kiss1* neuron regulation. Regulators of metabolic function (leptin and ghrelin) regulate NPY/AgRP and POMC/CART neurons, which are neuroanatomicaly and functionally connected to *Kiss1* neurons. At this stage, NPY/AgRP regulation of *Kiss1* neurons is unclear (?). On the other hand, products of POMC/CART neurons appear to stimulate (+) *Kiss1* neurons.

POMC/CART neurons

Juxtaposed to the NPY/AgRP neurons in the ARC are the POMC/CART neurons. These represent the major anorexigenic pathway in the control of food intake and energy expenditure (Barsh & Schwartz 2002). Like their counterparts, POMC/CART neurons also send projections to kisspeptin neurons in sheep (Backholer *et al*. 2010) and mice (Cravo *et al*. 2011, True *et al*. 2013). In mice, subsets of kisspeptin neurons also express melanocortin receptor type 4 (Cravo *et al*. 2011) and melanocortin (one of the neuropeptides produced from these neurons) agonist (MTII) stimulates LH release in luteal phase ewes and increases *Kiss1* mRNA expression in the POA (Backholer *et al*. 2009). Of note, *Kiss1* mRNA in the ARC was reduced in response to MTII (Backholer *et al*. 2009). The latter, while counterintuitive, could be explained due to a downstream effect of the possible reinstatement of E_2 levels following treatment as the experiment was performed in ovary intact seasonally anestrous ewes.

Very recent data have demonstrated that CART may also play a specific role in the regulation of kisspeptin. Using electrophysiological recordings from *Kiss1*–GFP mice, CART has been shown to postsynaptically depolarize kisspeptin neurons in the ARC (True *et al*. 2013). Thus, the stimulatory role of positive energy balance mediators on kisspeptin and fertility may also involve this neuropeptide (Fig. 3). Importantly, the authors here noted that CART could also directly activate GNRH neurons, using *GNRH*–GFP rats (True *et al*. 2013). This relationship was previously noted in mice for

both products of POMC/CART neurons but also with NPY, showing Y1 receptor-mediated suppression of GNRH neuron activity (Roa & Herbison 2012). Thus, although evidence shows that first-order neuron regulation of kisspeptin is possible, these neurons can also provide a kisspeptin-independent route through which neuropeptide metabolic cues can directly regulate GNRH and fertility.

Kisspeptin-mediated control of energy balance?

While most of the focus of current research has been on the role of kisspeptin in relaying metabolic signals to the reproductive axis, little attention has been paid to the potential role for kisspeptin as a regulator of energy balance. It is not uncommon for neuroendocrine systems to possess reciprocal control of feeding behavior and reproduction (Small *et al*. 2002, Tena-Sempere 2007). Moreover, *Kiss1r* is expressed in a number of brain areas that do not contain GNRH (Herbison *et al*. 2010), as well as in several peripheral tissues (Kotani *et al*. 2001), including metabolic tissues such as pancreas and adipose tissue (Brown *et al*. 2008). Thus, it is possible that kisspeptin signaling may have additional roles beyond the control of reproduction. However, this has not yet been shown.

Kiss1 and *Kiss1r* KO mice do not appear to have any difference in body weights compared with WT littermates (Lapatto *et al*. 2007), and initial studies on rats found no effects of central kisspeptin administration on food intake, bodyweight, or the hypothalamic expression of NPY, AgRP, POMC, or CART (Castellano *et al*. 2005). Similarly, kisspeptin treatment had no effect on food intake in sheep (Clarke *et al*. 2012), so any contribution of kisspeptin in the control of energy balance seemed unlikely. However, it its worth noting here that DBA/2J mice, which are much more susceptible to high-fat DIO and infertility, have substantially less *Kiss1* mRNA in the AVPV and ARC (Quennell *et al*. 2011). Moreover, body weights in *Kiss1* and *Kiss1r* KO mice have only been reported before full maturity, and the examination of the entire metabolic characteristics of these mice has not yet been performed.

In opposition to this precedent, kisspeptin neurons send afferents to first-order NPY/AgRP and POMC/CART neurons (Backholer *et al*. 2010). These neurons may possess *Kiss1r* because it is expressed in cells within the ARC (Lee *et al*. 1999), which are not kisspeptin neurons (Smith *et al*. 2011). Moreover, electrophysiological recordings in mice show that kisspeptin can directly excite POMC/CART neurons and indirectly inhibit NPY/AgRP neurons, via a mechanism based on enhancing GABA-mediated inhibitory synaptic tone (Fu & van den Pol 2010). The net effect of such kisspeptin regulation would be to decrease food intake and increase metabolism. Consistent with this, central administration of kisspeptin was recently shown to increase meal intervals, reducing nocturnal food intake in mice (Stengel *et al*. 2011). Alternatively, central administration of kisspeptin in sheep was reported to inhibit POMC and increase NPY mRNA expression in the ARC (Backholer *et al*. 2010). Although counterintuitive, this effect may relate to possible antagonistic properties of the continuous kisspeptin infusion (which lasted for 20 h). Such treatment has been previously shown to desensitize *Kiss1r*-induced GNRH release (Seminara *et al*. 2006) and reduced *Kiss1r* mRNA expression on GNRH neurons (Li *et al*. 2012). Nevertheless, similar data (kisspeptin treatment stimulating NPY secretion) are also apparent using a cell line (Kim *et al*. 2010). So the precise role of kisspeptin in regulating the POMC/CART and/or NPY/AgRP systems is clouded and awaits closer inspection.

In another layer of complexity, recent data have shown that saporin ablation of kisspeptin neurons in the ARC prevents the known effects of OVX and E_2 replacement on bodyweight in rats (Mittelman-Smith *et al*. 2012*a*). Apart from their role in reproduction, E_2 is known to act in the brain via ERα to alter body composition by decreasing food intake and increasing energy expenditure (Xu *et al*. 2011). Thus, it appears that ARC kisspeptin neurons are required for the orexigenic effect of OVX. In addition, a follow-up study with kisspeptin neurons ablation demonstrated that E_2 and kisspeptin appear to have apposing actions on skin temperature (Mittelman-Smith *et al*. 2012*b*), perhaps predicating a role for kisspeptin in thermogenesis and energy expenditure. While encouraging, these data are at odds with the proposed role for kisspeptin, which (like E_2) is to decrease food intake and increase energy expenditure. Of significance here is that the ablation of kisspeptin neurons would also eliminate NKB and dynorphin signaling (as these are coexpressed in these neurons (Goodman *et al*. 2007)). The roles that these other neuropeptides have on energy balance and their potential role in the above phenomenon need to be confirmed.

Finally, a role for kisspeptin on energy balance may occur outside the hypothalamus. As stated above, *Kiss1r* is expressed in the pancreas (Kotani *et al*. 2001) and adipose tissue (Brown *et al*. 2008). In the former, kisspeptin has been shown to play a physiological role. Kisspeptin appears to be capable of stimulating insulin release *in vitro* in mice and *in vivo* in rats (Bowe *et al*. 2009). These data, however, have been challenged by similar studies displaying an inhibitory effect of kisspeptin on insulin secretion in isolated mouse islets (Vikman & Ahren 2009). Clearly, more work is necessary to decipher the role that kisspeptin signaling is playing here and in other potential peripheral tissues.

Conclusion

Kisspeptin is a vital component for the neuroendocrine regulation of GNRH secretion. As such, it has been a

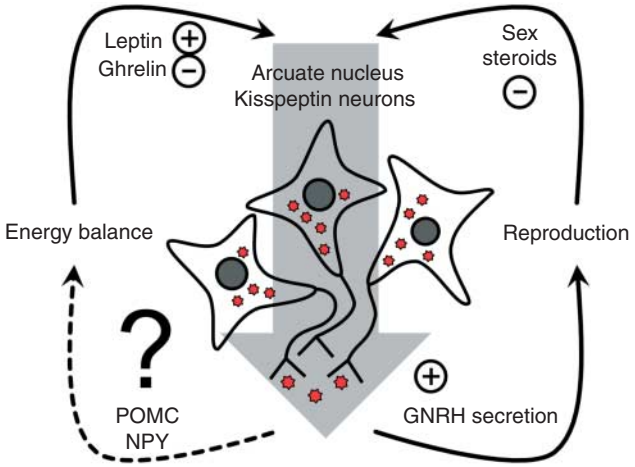

Figure 4 The relationship between energy balance and reproduction. We propose that kisspeptin neurons in the arcuate nucleus are central to this integrated regulatory loop, forming a link between energy balance and reproduction. Concerning reproduction, kisspeptin neurons stimulate (+) GNRH secretion and ultimately gonadal steroid production. These hormones then feed back (−) and regulate kisspeptin output. Concerning energy balance, metabolic signals such as leptin and ghrelin modulate fertility via kisspeptin regulation. Kisspeptin may potentially regulate energy balance circuits (stimulating POMC and inhibiting NPY) in a feedback mechanism similar to that of reproduction. But whether kisspeptin has clear effects on energy balance is yet to be shown (?).

focus for the central pathway responsible for conveying key homeostatic information to GNRH neurons (Fig. 4). Multiple studies have implicated kisspeptin signaling as a conduit for the well-established link between energy balance and reproductive function. While the precise metabolic pathway is yet to be fully understood, the peripheral signals leptin, insulin, and ghrelin are likely to play a role, as are POMC/CART and NPY/AgRP neurons in the ARC. Finally, a direct role for kisspeptin in mediating energy balance is now gathering momentum. Future studies are required to confirm this possibility and determine its physiological relevance.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

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