REVIEW

The role of kisspeptin neurons in reproduction and metabolism

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Abstract

Kisspeptin is a neuropeptide with a critical role in the function of the hypothalamic–pituitary–gonadal (HPG) axis. Kisspeptin is produced by two major populations of neurons located in the hypothalamus, the rostral periventricular region of the third ventricle (RP3V) and arcuate nucleus (ARC). These neurons project to and activate gonadotrophin-releasing hormone (GnRH) neurons (acting via the kisspeptin receptor, Kiss1r) in the hypothalamus and stimulate the secretion of GnRH. Gonadal sex steroids stimulate kisspeptin neurons in the RP3V, but inhibit kisspeptin neurons in the ARC, which is the underlying mechanism for positive- and negative feedback respectively, and it is now commonly accepted that the ARC kisspeptin neurons act as the GnRH pulse generator. Due to kisspeptin's profound effect on the HPG axis, a focus of recent research has been on afferent inputs to kisspeptin neurons and one specific area of interest has been energy balance, which is thought to facilitate effects such as suppressing fertility in those with under- or severe over-nutrition. Alternatively, evidence is building for a direct role for kisspeptin in regulating energy balance and metabolism. Kiss1r-knockout (KO) mice exhibit increased adiposity and reduced energy expenditure. Although the mechanisms underlying these observations are currently unknown, Kiss1r is expressed in adipose tissue and potentially brown adipose tissue (BAT) and Kiss1rKO mice exhibit reduced energy expenditure. Recent studies are now looking at the effects of kisspeptin signalling on behaviour, with clinical evidence emerging of kisspeptin affecting sexual behaviour, further investigation of potential neuronal pathways are warranted.

Key Words

- Kiss1
- hypothalamus
- fertility
- energy homeostasis
- glucose metabolism

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Discovery and distribution of kisspeptin neurons

Kisspeptin and kisspeptin neurons

In humans, kisspeptin refers to a family of neuropeptides resulting from the cleavage of a 145 amino acid precursor peptide encoded by the *KISS1* gene (Lee *et al.* 1996, Ohtaki *et al.* 2001). Kisspeptin is thought to be active predominantly as a 54 amino acid peptide, while in mice, it exists as 52 amino acids (Ohtaki *et al.* 2001, Terao *et al.* 2004). The first observation of kisspeptin's function occurred in melanoma cell lines, where it acted as a metastasis inhibitor and thus it was initially known as 'metastin' (Lee *et al.* 1996). In humans, kisspeptin was found to be expressed in cell populations within the placenta, and later in the testes, ovaries, pancreas and small intestine (Kotani *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001). Significantly, kisspeptin

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was discovered and mapped in the brain, expressed in the rodent hypothalamus, specifically within neurons predominantly located in the arcuate nucleus (ARC) and the anteroventral periventricular nucleus (AVPV) extending into the periventricular nucleus (PEN) (Gottsch *et al.* 2004) – now referred collectively as the rostral periventricular region of the third ventricle (RP3V) (de Croft *et al.* 2012). In humans, kisspeptin neurons are found in the infundibular nucleus and preoptic area (POA), which are analogous to the rodent ARC and RP3V respectively (Rometo *et al.* 2007).

The kisspeptin receptor

Several years after the initial discovery of kisspeptin, a G-protein-coupled receptor that binds to the peptide with strong affinity was discovered, initially known as GPR54 (Lee *et al.* 1999, Kotani *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001). Following the discovery of kisspeptin's importance in the reproductive axis, the kisspeptin receptor GPR54 has since come to be known as Kiss1r (Gottsch *et al.* 2009*a*). The Kiss1r receptor is expressed within the rodent hypothalamus predominantly in the POA (Lee *et al.* 1999, Herbison *et al.* 2010) – notably on the majority of gonadotrophin-releasing hormone (GnRH) neurons (Irwig *et al.* 2004, Han *et al.* 2005) – and also the ARC (Lee *et al.* 1999). Similar, data are apparent in monkeys (Shahab *et al.* 2005, Kim *et al.* 2009) and sheep (Smith *et al.* 2009, 2011). Outside of the hypothalamus, Kiss1r can be found consistently in the hippocampus (Lee *et al.* 1999, Kotani *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001, Herbison *et al.* 2010) and outside the brain in the anterior pituitary, pancreas, liver and adipose tissue (Kotani *et al.* 2001, Muir *et al.* 2001, Ohtaki *et al.* 2001).

Kisspeptin is critical for reproduction

The neuroendocrine control of fertility

The reproductive 'axis' depends on the dynamic interplay between neural and hormonal signals originating from three primary sources: the anterior hypothalamus, where GnRH is synthesised and secreted in pulses; the anterior pituitary, where GnRH pulses stimulate pituitary gonadotrophin (luteinising hormone (LH) and follicle-stimulating hormone (FSH)) secretion; and the gonads, which respond to the trophic actions of gonadotrophins by secreting sex steroids and producing gametes (Clarke *et al.* 2011, Navarro & Tena-Sempere 2011). In turn, these sex steroids 'feedback' to the GnRH neurons in the hypothalamus to regulate their activity (Smith 2013) collectively forming the hypothalamic–pituitary–gonadal (HPG) axis. Since the discovery and characterisation of GnRH neurons, the search for the required inter-neuronal pathway linking steroid hormone feedback mechanism to these neurons was a priority – because GnRH neurons lack the prerequisite steroid hormone receptors (Herbison & Theodosis 1992) – in particular oestrogen receptor (ER)-α, which is known to be necessary for oestrogen to exert negative and positive feedback. Moreover, the existence and characterisation of an extrinsic GnRH pulse generator was one of the neuroendocrine field's most polarising challenges.

Two studies, published almost simultaneously, showed that kisspeptin/Kiss1r signalling was a major stimulus for the secretion of GnRH and gonadotrophins (de Roux *et al.* 2003, Seminara *et al.* 2003). Kisspeptin also seems to have an important role in the onset of puberty. Puberty is a phenomenon that is triggered by increasing pulsatility of GnRH secretion from the hypothalamus. Moreover, hypogonadotrophic hypogonadism is a condition characterised by impaired pubertal development and infertility that occurs when the pulsatility of GnRH is insufficient or absent. The initial studies that discovered kisspeptin's reproductive importance by Seminara *et al.* (2003) and de Roux *et al.* (2003) observed consanguineous families that had members with idiopathic hypogonadotrophic hypogonadism. Seminara and colleagues analysed the genome of these families to determine a genetic contributor to this condition and discovered affected individuals were homozygous for a 'L148S' mutation in the *GPR54* gene – Kiss1r. Seminara *et al.* (2003) also created a mouse model deficient in Kiss1r, and it was found that these mice expressed an identical phenotype of hypogonadotrophic hypogonadism, characterised by small testes in male mice, small ovaries in females as well as an absence of follicular maturation and a delay in vaginal opening. However, exogenous administration of GnRH to these mice reverted them back to a relatively normal phenotype, which suggested that kisspeptin acts by stimulating GnRH release (Seminara *et al.* 2003). From these findings, it was concluded that Kiss1r may be integral for the normal function of GnRH secretion and for puberty. Since it was known that the peptide from the *Kiss1* gene (kisspeptin) had a strong affinity for this receptor, it was also suggested at this time that this peptide could be the stimulus for GnRH secretion. In addition to causing hypogonadotrophic hypogonadism, mutations in the *Kiss1r* gene also seem

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to be involved in precocious puberty: a condition in which the onset of puberty occurs much earlier than usual. Initial studies identified an autosomal dominant activating mutation in the *Kiss1r* gene in individuals with this condition (Teles *et al.* 2008).

After the initial evidence of kisspeptin's reproductive functions, many studies – primarily in rodents, but some in sheep, monkey and goat – allowed for the further expansion of knowledge of kisspeptin biology. Gottsch *et al.* (2004) examined the effects of direct kisspeptin administration to the lateral cerebral ventricle of the mouse brain. They found that both LH and FSH were stimulated by the presence of kisspeptin – even at doses as low as 1 fmol. Furthermore, administration of a GnRH antagonist (acyline) prevented kisspeptin's stimulatory effect, providing the first evidence that kisspeptin acts solely via GnRH neurons to stimulate gonadotrophin release (Gottsch *et al.* 2004). Further studies of GnRH neurons themselves revealed their affinity to kisspeptin peptide. Irwig *et al.* (2004) analysed GnRH neurons in male rats with immunocytochemical techniques and found that in response to exogenous kisspeptin treatment, 86% of the GnRH neurons coexpressed Fos (a marker of neuronal transcription and hence activation), as opposed to less than 1% of GnRH neurons activated in untreated controls. Furthermore, the authors used double-label *in situ* hybridization to determine the proportion of GnRH neurons that expressed *Kiss1r* mRNA. It was found that 77% of neurons did, suggesting the majority of GnRH neurons express the kisspeptin receptor, thus allowing direct activation of kisspeptin (Irwig *et al.* 2004).

The electrophysiological properties of GnRH neurons in response to kisspeptin were analysed in a study by Han *et al.* (2005). When looking in adult male and female mice, they observed a significant depolarisation of more than 90% of GnRH neurons following administration of kisspeptin, consistent with similar percentage of GnRH neurons that expressed *Kiss1r* mRNA (Han *et al.* 2005). In addition to this, juvenile (postnatal day 8–19) and pre-pubertal (postnatal day 26–33) mice treated with kisspeptin only experienced a depolarisation in 27% and 40% of GnRH neurons respectively, suggesting that the number of kisspeptin-responsive GnRH neurons increases over pubertal development.

Characterisation of kisspeptin neurons

Clarkson & Herbison (2006) investigated the neuroanatomical arrangement and development of kisspeptin neurons using male and female mice. They found that kisspeptin neuronal cell bodies existed primarily in the ARC and RP3V. They also noticed a sexual dimorphism in the number of kisspeptin neurons present in the RP3V, with females having drastically more than males (Clarkson & Herbison 2006). Dual immunofluorescence revealed close appositions between kisspeptin fibres and GnRH cell bodies, which first appeared in pre-pubertal mice (postnatal day 25) and grew in number throughout further pubertal development (Clarkson & Herbison 2006). Moreover, projections from kisspeptin neurons extend throughout midline/periventricular hypothalamic areas and extending to the median preoptic nucleus (Clarkson *et al.* 2009). Recent CLARITY processing of *Kiss1*-CRE mouse brains confirmed this distribution and was able to conclude projections of kisspeptin terminals from both ARC and RP3V kisspeptin populations to preoptic areas. In addition, RP3V kisspeptin neurons project to the ARC and ARC kisspeptin neurons project to the RP3V, in addition to lateral hypothalamic regions (Yeo *et al.* 2016).

We further investigated the neuroanatomical arrangement of kisspeptin neurons and the effects they have on GnRH secretion. Looking in ewes, we found that kisspeptin treatment significantly stimulated GnRH secretion into the hypophyseal portal system (Smith *et al.* 2011), which travels directly to the anterior pituitary, allowing exquisite control over the release of gonadotrophins. Moreover, the GnRH neuron terminals in the median eminence are apposed to projections from kisspeptin neurons (Smith *et al.* 2011), establishing a novel 'axo-axonal' method of kisspeptin-GnRH control, where kisspeptin stimulates GnRH release (d'Anglemont de Tassigny *et al.* 2008, Smith *et al.* 2011, Uenoyama *et al.* 2011). Using immunohistochemical and neuro-tracing techniques, we followed these fibres to their origin, which was found to be the ARC. This further strengthened the theory of kisspeptin's integral role in the HPG axis.

Effects of sex steroids on kisspeptin neurons

It has long been known that oestrogens have both negative and positive feedback effects on GnRH secretion; however, the mechanisms through which a single hormone could change modes of feedback were not fully understood. Moreover, the neuroanatomical pathway in which this feedback was achieved was also unknown because the GnRH neurons in mammals do not express any of the requisite receptors (Herbison & Theodosis 1992). Following the discovery of kisspeptin's critical reproductive role, the hypothesis arose that feedback

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effects could be mediated by kisspeptin neurons. Our studies looked at the expression of *Kiss1* mRNA in both the ARC and RP3V in response to gonadal steroids (Smith *et al.* 2005*a*,*b*) and noted differential regulation in these regions, particularly oestradiol stimulation of RP3V kisspeptin neurons and inhibition of ARC kisspeptin neurons. Moreover, we found that at the time of the preovulatory LH surge Kiss1 mRNA expression increased in the RP3V but decreased in the ARC (Smith *et al.* 2006). This was preliminary evidence that kisspeptin neurons in the ARC mediate negative feedback, while those in the RP3V mediate positive feedback. The precise mechanism by which oestradiol, acting through ERα, achieves both positive and negative feedback in kisspeptin neuron populations is perplexing but may relate to classical (for positive) and non-classical (for negative) receptor signalling (Gottsch *et al.* 2009*b*). Additionally, divergent epigenetic regulation of the *Kiss1* promoter region between the ARC and AVPV appears to play a significant role (Tomikawa *et al.* 2012). Here, oestrogen acting through ERα induces histone acetylation of the *Kiss1* promoter and enhanced gene expression in the AVPV – but deacetylation in the ARC (Tomikawa *et al.* 2012). On top of this, it is now clear that progesterone receptor is vital for the positive feedback-induced LH surge (Mittelman-Smith *et al.* 2017) particularly its expression on kisspeptin neurons (Stephens *et al.* 2015, Gal *et al.* 2016) and androgen receptor may play a modulating role (Walters *et al.* 2018). Also important is that although ERα signalling in RP3V kisspeptin neurons is vital for positive feedback, ERα signalling in ARC kisspeptin neurons is not a complete requirement for negative feedback, as indicated in mice with kisspeptin cell-specific deletion of ERα (Dubois *et al.* 2015). In these mice, oestradiol treatment resulted in the expected lack of change in *Kiss1* mRNA in the ARC, but surprisingly the negative feedback regulation of LH concentration remained (Dubois *et al.* 2015). This suggests the existence of redundant negative feedback pathway independent of kisspeptin signalling. In addition, positive feedback is more important in females due to it being the mechanism responsible for the preovulatory LH surge – which does not occur in males – a fact that is reflected by females having a much greater number of kisspeptin neurons in the RP3V (Clarkson & Herbison 2006, Kauffman *et al.* 2007). The role of kisspeptin in the male RP3V is still, to our knowledge, unresolved.

In the ARC kisspeptin neurons, it is now accepted that the negative feedback regulation by gonadal sex steroids enables these neurons to act as the long sought-after GnRH pulse generator (Fig. 1). Previous immunohistochemical data detailed the neural machinery, involving the co-expression of the neuropeptides neurokinin B (NKB) and dynorphin (Dyn) within ARC kisspeptin neurons and autosynaptic control of these 'KNDy' neurons (Goodman *et al.* 2007, Navarro *et al.* 2009, Lehman *et al.* 2010). The model stipulates that NKB acts on KNDy neurons to drive pulsatility and Dyn acts as the 'brake' halting pulses and kisspeptin is the final output to GnRH neurons. In mice and rats, communication within and between KNDy neurons exists because they express NKB and dynorphin receptors (Navarro *et al.* 2009) in addition to an ARC network of reciprocal projections (Burke *et al.* 2006, Clarkson *et al.* 2009) and possible cell-to-cell gap junction synchronisation (Ikegami *et al.* 2017). Pharmacological data in sheep support the KNDy hypothesis because central administration of NKB receptor antagonists reduces LH pulse frequency (Goodman *et al.* 2013, Li *et al.* 2015) and NKB or dynorphin receptor antagonists increase LH pulses (Goodman *et al.* 2013). Similar data are also apparent in goats, with electrophysiological multiple-unit activity recordings directed at KNDy neurons showing 'volleys' of activity consistent with GnRH pulse generator activity (Wakabayashi *et al.* 2010, 2013) and appropriate responses to kisspeptin and NKB receptor agonists (Yamamura *et al.* 2014, 2015). Despite this, recent data in sheep show persistent LH pulses in the presence of NKB receptor antagonists paired with a constant infusion of kisspeptin (Clarke *et al.* 2018), indicating KNDy neurons may not be the sole pulse generator in this species. Moreover, there is still debate and uncertainty as to how three neuropeptides are released from a single neuron with temporal specificity to interact and modulate secretion of a kisspeptin/GnRH pulse. Notwithstanding, new data show optogenetic control of ARC kisspeptin neurons and the generation of LH/GnRH pulses (Han *et al.* 2015), inhibition of pulses (Clarkson *et al.* 2017) and – more importantly – the resetting of ongoing pulsatility (Clarkson *et al.* 2017) providing the strongest evidence for ARC kisspeptin neurons as the hypothalamic GnRH pulse generator.

Kisspeptin regulation of energy balance and metabolism

Effect of metabolism on fertility

Reproduction is a resource-costly activity and as such its success is dependent on sufficient energy and nutrient reserves (Pasquali *et al.* 2007, Evans & Anderson 2012). As a result, there is a well-established relationship between

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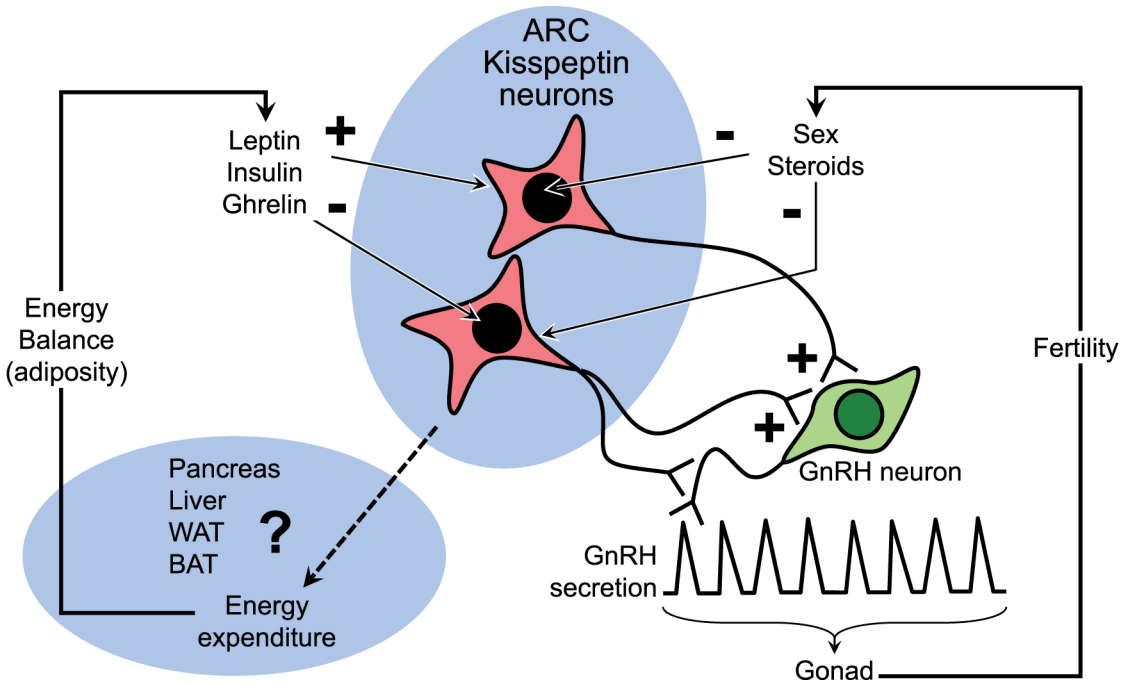

Figure 1
The potential function of kisspeptin neurons in mediating the relationship between energy balance and reproduction. Kisspeptin neurons in the arcuate nucleus (ARC) of the hypothalamus control reproduction through stimulation (+) of gonadotropin-releasing hormone (GnRH) neurons to regulate gonadal sex steroid production, which then has an inhibitory effect (–) on kisspeptin neurons. Kisspeptin neurons are also regulated by changes in energy balance. Levels of circulating metabolic hormones such as leptin, insulin and ghrelin are relayed onto kisspeptin neurons. Kisspeptin neurons also regulate energy expenditure and adipose tissue levels although the mechanisms underlying these observations are unknown.

metabolism and reproduction with states of altered energy balance associated with suppressed reproductive function. Luo *et al.* (2016) observed 72-h fasted female rats had decreased GnRH and *Gnrh* mRNA levels. Similar results were observed in food-restricted male rats (Compagnucci *et al.* 2002) and in mice (Castellano *et al.* 2005). Impaired fertility as a consequence of reduced food intake has also been reported in larger animal models; caloric-restricted ewes exhibited reduced LH concentration and reduced FSH secretion (Thomas *et al.* 1990). Humans with anorexia nervosa, a psychological condition resulting in persistent energy intake restriction, often have suppressed HPG axis activity – females with amenorrhoea, and males with reduced testosterone concentrations (Katz & Vollenhoven 2000). These results indicate reproduction is sensitive to states of both short- and long-term negative energy balance.

Reproduction also appears to be sensitive to positive energy balance. Diet-induced obesity in rodents was coupled with reduced fertility and hypogonadism in males (Sanchez-Garrido *et al.* 2014). Similarly, diet-induced obesity in female mice impairs fertility by impacting systemic inflammation (Skaznik-Wikiel *et al.* 2016) but also by reducing hypothalamic *Gnrh* gene expression (Tortoriello *et al.* 2004). With the increasing prevalence of overweight and obesity, it is not surprising that evidence is now emerging that positive energy balance also has a suppressive effect on reproduction in humans. Obesity in women is associated with alterations to negative feedback control of gonadotrophin secretion, increased risk of infertility and miscarriage and lower *in vitro* fertilisation success rates (Metwally *et al.* 2007). In males, obesity is known to reduce total testosterone levels and cause sexual dysfunction (Loret de Mola 2009).

Effect of metabolism on hypothalamic kisspeptin

Given the critical role that kisspeptin plays in governing the HPG axis, and its location within the ARC, it is not surprising that *Kiss1* gene expression is also perturbed during periods of altered energy balance. Castellano *et al.* (2005) reported male and female pre-pubertal rats undergoing short-term under-nutrition had significantly reduced whole hypothalamic *Kiss1* mRNA and increased hypothalamic *Kiss1r* mRNA compared to non-fasted littermates. LH response following central administration of kisspeptin was enhanced, indicating increased sensitivity, but lower output of kisspeptin during periods

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of negative energy balance. Conversely, the effect of overnutrition or increased adiposity on kisspeptin expression is not as well investigated. Quennell *et al.* (2011) reported female *ob/ob* mice have reduced *Kiss1* mRNA in the ARC, but not the RP3V. Moreover, DBA/2J mice with diet-induced obesity had a marked decrease in *Kiss1* mRNA in the RP3V and ARC, however, Dudek *et al.* (2016) did not observe any changes in whole hypothalamic *Kiss1* mRNA levels in obese mice but did show an increase in hypothalamic *Kiss1r* mRNA in mice with streptozotocin-induced diabetes.

It is clear that kisspeptin neurons are sensitive to changes in metabolic status, but how this is mediated is contentious. Both NPY/AgRP and POMC/CART neurons in the ARC (our 'first order' orexigenic and anorexigenic neuronal populations respectively; Barsh & Schwartz (2002) project to kisspeptin neurons in the ARC of sheep (Backholer *et al.* 2010, Padilla *et al.* 2017) and mice (Backholer *et al.* 2010, Padilla *et al.* 2017) allowing changes in metabolic status to be integrated into kisspeptin-mediated control of reproduction. Additionally, several studies report kisspeptin neurons themselves are also sensitive to metabolic hormones, particularly insulin, leptin (Cravo *et al.* 2013, Qiu *et al.* 2011, 2015) and ghrelin (Frazao *et al.* 2014). However, the physiological relevance of these inputs to kisspeptin neurons, particularly in relation to the effect of leptin cues on the onset of puberty (Donato *et al.* 2011), and also insulin (Evans *et al.* 2014) have been debated. To this point, we would like to stress caution with interpretation of mouse models employing Cre-lox technology as negative phenotypic results could be explained by imperfect recombination efficacy. In addition, the data presented above are derived from sheep and mouse models using an array of techniques (immunohistochemistry, Cre-lox transgenics and electrophysiology) each with limitations potentially contributing to inconsistencies. In any case, we feel it is clear that *Kiss1* mRNA expression and subsequent control of GnRH release is modulated by metabolic status; however, the precise mechanisms and exact neuronal pathway are still to be unequivocally confirmed (Fig. 1).

Kisspeptin signalling regulates energy balance

While most of the research so far has focused on the effect of energy balance on kisspeptin, kisspeptin signalling in turn has recently emerged as a regulator of energy balance. Indeed, it is not uncommon for neuroendocrine circuits to have reciprocal control over both reproduction and metabolism (Rosenbaum & Leibel 1998).

Disrupted energy balance in Kiss1r KO mice

In our publication (Tolson *et al.* 2014), we were the first to report an altered metabolic phenotype in Kiss1r KO mice. Specifically, adult Kiss1r KO female mice had significantly increased body weights and adiposity (assessed using dual energy X-ray absorptiometry) (Fig. 2), impaired glucose regulation and reduced energy expenditure (measured using metabolic cages) from 10 weeks of age. The results from this study contradict that of an earlier one by Lapatto *et al.* (2007) who reported no significant changes in body weight in either Kiss1KO or Kiss1rKO mice at 9–12 weeks of age, compared to WT littermates. In our data, bodyweight changes in Kiss1rKO mice do not manifest until 8–9 weeks of age and were only apparent in females (Tolson *et al.* 2014) despite changes in adiposity in both sexes. Moreover, a follow-up study (Tolson *et al.* 2016) showed that although no body mass changes were evident, Kiss1rKO mice had significantly increased adiposity and decreased energy expenditure as early as

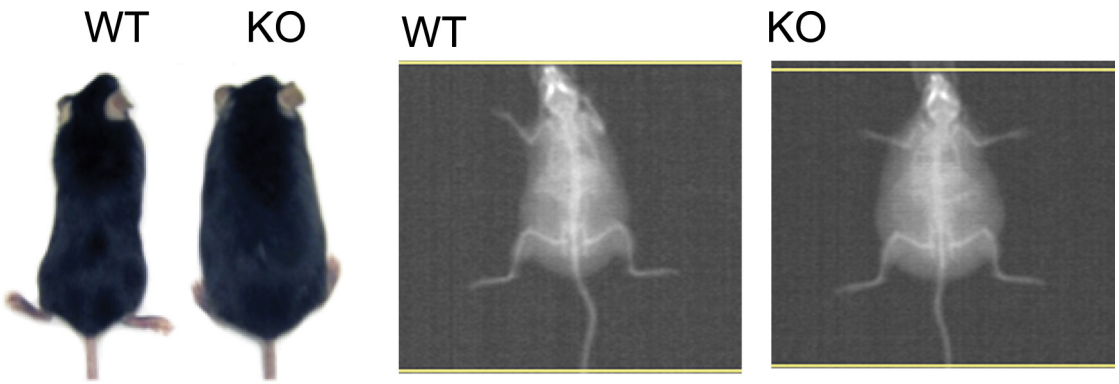

Figure 2
Obese phenotype in Kiss1rKO mice. Body weight phenotype comparison between female and WT and Kiss1rKO (KO) mice at 20 weeks of age. The obese phenotype of Kiss1rKO mice compared to WT is highlighted by dual energy X-ray absorptiometry imaging.

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6 weeks of age. These data suggest that the emergence of an obese phenotype in Kiss1rKO mice at maturity is likely to be a result of changes in metabolic function during early life, potentially even from birth. Our recent data confirmed the findings of Tolson and reported only female Kiss1rKO mice at maturity showed abnormal body weights and increased white adipose tissue mass suggesting that the altered metabolic phenotype in Kiss1r KO mice is sexually dimorphic, with females showing a greater phenotype than males (De Bond *et al.* 2016). Importantly, the increased adiposity was also evident in gonadectomised mice (males and females), suggesting the regulation of metabolism by kisspeptin neurons is not simply sex steroid dependent (Tolson *et al.* 2014) and due to some inherent characteristic of kisspeptin signalling.

Action via NPY/AgRP and POMC/CART

Kisspeptin appears able to directly innervate NPY/AgRP and POMC/CART neurons in a reciprocal manner, but the nature of these effects is subject to debate. In sheep, exogenous kisspeptin increased *Npy* mRNA expression and decreased *Pomc* mRNA (Backholer *et al.* 2010). Alternatively, in mouse brain sections, electrophysiological recordings indicate an increase in activity of POMC neurons, and a decrease in NPY neurons, in response to kisspeptin treatment (Fu & van den Pol 2010). This difference in response may indicate species differences (sheep versus mouse) or differences in experimental technique (*in situ* hybridisation versus electrophysiology), but they point to uncertainty surrounding the direct effect of kisspeptin on food intake neural circuitry. Thompson (Thompson *et al.* 2004) reported that ICV administration of kisspeptin in rats had no effect on food intake and similar data are apparent in sheep (Clarke *et al.* 2012). Unexpectedly, Kiss1rKO mice actually had reduced food intake, despite increased adiposity (Tolson *et al.* 2014, De Bond *et al.* 2016). We also reported gonadectomised Kiss1r KO mice showed no significant change in mRNA expression of *Npy*/*Agrp* or *Pomc*/*Cart* in the ARC compared to WT littermates (De Bond *et al.* 2016). These data potentially indicate the effects of kisspeptin signalling on energy balance are possibly the result of peripheral – rather than central – pathways.

Action via peripheral tissue

As hypothalamic regulators of appetite were found to not be the sole contributor to the altered metabolic phenotype in Kiss1rKO mice, the focus naturally shifts to peripheral kisspeptin signalling. Kiss1r is expressed in areas of the brain extending that of GnRH neuron control and also in the periphery, specifically the pancreas, liver and adipose tissue (Kotani *et al.* 2001). Kisspeptin has a proposed role in regulating the magnitude of the insulin response to glucose through a direct stimulatory effect on islet beta cells (Hauge-Evans *et al.* 2006). In the liver, glucagon-induced kisspeptin production inhibits insulin secretion in the mouse, with similar data determined *in vitro* with human pancreas – dependent on Kiss1r expression (Song *et al.* 2014), indicating kisspeptin may be pro-diabetic. What is confusing, however, is that kisspeptin appears to be capable of inhibiting or stimulating insulin secretion from islets (Hauge-Evans *et al.* 2006, Vikman & Ahren 2009, Song *et al.* 2014). Nevertheless, it is doubtful these observations are sufficient to fully explain the altered metabolic phenotype in Kiss1rKO mice because adiposity, hyperleptinaemia and reduced energy expenditure all occur at 6 weeks of age (Tolson *et al.* 2016), while impairments in glucose tolerance present at older ages (18–20 weeks) and could potentially be a consequence of the obese state. Thus, despite initial investigations in the liver and pancreas, the importance of kisspeptin signalling in other peripheral tissues such as WAT and BAT, the latter known to regulate energy expenditure through thermogenesis (Napolitano & Fawcett 1958, Saito *et al.* 2009) and possesses *Kiss1r* mRNA (Smith JT and Kauffman AS, unpublished observation), has not previously been reported and must be investigated (Fig. 1).

Conclusions and future directions

Despite a wealth of work highlighting the essential function of kisspeptin signalling in reproduction – specifically the control of GnRH neurons – little evidence exists regarding the potential regulatory role that kisspeptin signalling plays in controlling energy balance and metabolism. In particular, the mechanisms underlying the altered energy balance in the absence of kisspeptin signalling remains to be elucidated. There are clear links between circadian rhythms and kisspeptin signalling (Yap *et al.* 2016), and these may prove important in impairments in energy expenditure, which are more pronounced during the dark phase in Kiss1rKO mice (Tolson *et al.* 2014). Importantly, to our knowledge, there are no current reports of human obesity associated with *KISS1* or *KISS1R* mutations. Nevertheless, there is recent evidence currently emerging of kisspeptin affecting behaviour, particularly human male sexual and emotional brain processing (Comninos *et al.* 2017). Female sexual behaviour in mice also appears to involve processing via

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kisspeptin neurons, potentially independent of Kiss1r (Hellier *et al*. 2018). Loss of kisspeptin signalling also appears to reduce anxiety-related behaviours in mice (Delmas *et al*. 2018). Another emerging effect of kisspeptin is the enhancement of memory capabilities in mice (Jiang *et al*. 2015). Despite these findings, the nature in which kisspeptin may bring about these limbic effects is not fully understood. It remains to be seen whether kisspeptin's role in these behavioural traits is directly linked to kisspeptin neural circuitry involved in governing energy balance, metabolism and predisposition to obesity.

Declaration of interest

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

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