The relationship between gut and adipose hormones, and reproduction

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BACKGROUND: Reproductive function is tightly regulated by nutritional status. Indeed, it has been well described that undernutrition or obesity can lead to subfertility or infertility in humans. The common regulatory pathways which control energy homeostasis and reproductive function have, to date, been poorly understood due to limited studies or inconclusive data. However, gut hormones and adipose tissue hormones have recently emerged as potential regulators of both energy homeostasis and reproductive function.

METHODS: A PubMed search was performed using keywords related to gut and adipose hormones and associated with keywords related to reproduction.

RESULTS: Currently available evidence that gut (ghrelin, obestatin, insulin, peptide YY, glucagon-like peptide-1, glucose-dependent insulinotropic peptide, oxyntomodulin, cholecystokinin) and adipose hormones (leptin, adiponectin, resistin, omentin, chemerin) interact with the
Introduction
The link between energy balance and fertility has long been recognized. The association of weight loss with infertility was first noted over 300 years ago. In 1694, Morton observed that amenorrhoea (period loss) was a cardinal feature of the condition which was later termed anorexia nervosa (AN) (Alexander-Mott and Lumsden, 1994). Furthermore in the 1960s, Kennedy and Mitra (1963) postulated that a critical body weight is required for reproductive capacity. Consistent with this hypothesis, the body weight of adolescent girls at menarche is relatively constant, despite variability in the age of menarche (Frisch and Revelle, 1970). Thus, energy availability exerts a permissive action on fertility; one may speculate that this represents an adaptive response which inhibits reproductive capacity during prevailing conditions of poor nutrition. The gut and adipose tissue are ideally placed to detect nutritional status; it is therefore not surprising that a number of gut- and adipose-derived hormones have been recognized recently to regulate human reproduction.

This comprehensive review serves to detail each of the gut and adipose hormones in turn and describe their relationship with reproductive capacity. To affect reproduction, these hormones have direct or indirect effects on the reproductive axis.

Reproductive axis: overview
The release of sex hormones in humans is tightly regulated by the reproductive axis (Fig. 1). This axis involves interplay between the hypothalamus, where the signals originate, the pituitary gland and the gonads. Specialized neurones within the medial preoptic area (in rodents and sheep) or the mediobasal hypothalamus (in humans and primates) release GnRH, a 10-amino acid peptide, in a pulsatile manner into the hypophyseal-portal circulation (Carmel et al., 1976; Barry, 1979; Rance et al., 1994). Through this circulation, the GnRH arrives at the anterior pituitary gonadotroph cells, which consequently release the gonadotrophin hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), into the systemic circulation (Wildt et al., 1981; Clarke and Cummins, 1982). These gonadotrophins act on the gonads to stimulate gametogenesis and release the gonadal sex steroids (androgens in males and estrogens in females). FSH predominantly acts to stimulate folliculogenesis in females and spermatogenesis in males, while LH predominantly promotes the synthesis of the sex steroids (Reichlin, 1998). In males, LH activates receptors on Leydig cells triggering the synthesis and secretion of androgens, primarily testosterone. Subsequently, testosterone stimulates spermatogenesis and the development of male secondary characteristics. In females, LH activates receptors on theca cells of the ovary triggering the release of testosterone. Testosterone is subsequently converted into estrogens by FSH-induced aromatization in adjacent granulosa cells. Meanwhile, FSH stimulates ovarian follicle production. Tight regulation of the reproductive axis is achieved by a negative feedback system whereby the gonadotrophins and sex steroids inhibit further gonadotrophin and GnRH release. Failure at any point in this reproductive axis can lead to a range of pathologies including delayed puberty and infertility.

Reproductive axis: GnRH and the GnRH pulse generator
The amino acid sequence of GnRH is structurally conserved across all mammals except the guinea pig. GnRH is synthesized and secreted by specialized GnRH neurones in the mediobasal hypothalamus in humans. These neurones have projections to the median eminence where GnRH is then released into the hypophyseal-portal circulation in a pulsatile manner (Gore, 2002). The pulsatile nature of this GnRH release is essential for the normal functioning of the reproductive axis as evidenced by the chronic (non-pulsatile) administration of GnRH to rhesus monkeys with hypothalamic lesions failing to stimulate LH and FSH release due to down-regulation of the GnRH receptor (Belchetz et al., 1978). Thereafter when hourly intermittent GnRH pulses were administered, LH and FSH release was restored (Belchetz et al., 1978). The frequency and amplitude of GnRH pulses vary throughout human development and the menstrual cycle:

Human development: the pulsatile secretion of GnRH has been noted in early gestation and continues until 6 months of age in males and 2 years in females. Thereafter GnRH pulsatility is quiescent during childhood and returns in order to trigger puberty, initially with nocturnal pulses and subsequently with daytime pulses (Gore, 2002).

Menstrual cycle: there is marked variation in GnRH pulsatility during the different phases (follicular, ovulatory and luteal) and this is necessary for normal cycling. Measurement of GnRH is difficult as it is only released into the hypophyseal-portal circulation. Therefore, downstream serum LH pulsatility is used as a surrogate marker of GnRH pulsatility. Pulsatile LH release, stimulated by GnRH, increases in frequency during the follicular phase and peaks at the time of ovulation (Gore, 2002).

The pulsatile nature of GnRH release results from the interaction of a number of complex pathways including pathways involved in energy homeostasis. For example, in women with hypothalamic amenorrhoea due to chronic energy deficiency, there is reduced frequency of GnRH pulses (Reame et al., 1985). Although GnRH neurones release GnRH inherently in a pulsatile manner (Funabashi et al., 2000), there are a number of excitatory and inhibitory neurotransmitters that modulate this secretion in vivo. These include excitatory neurotransmitters such as galanin and glutamate, inhibitory neurotransmitters such as opioids.
and gamma-aminobutyric acid, and neurotransmitters with both excitatory and inhibitory functions including acetylcholine, dopamine and serotonin. Gut and adipose hormones can act through these neurotransmitters or in some cases directly to modulate GnRH secretion and subsequent pituitary LH and FSH production. Furthermore, gut and adipose hormones can also act directly at other sites of the reproductive axis including the pituitary (not via GnRH) and the gonads. Before reviewing these effects on the reproductive axis, we will briefly cover the endometrial effects of these hormones below.

**Gut and adipose hormones: endometrial development and function**

Although not a direct component of the hypothalamo-pituitary-gonadal reproductive axis, it is important to note that gut and adipose hormones can also affect both endometrial development and function. For example, there is cyclical expression of ghrelin and its receptor in the human endometrium and suggestions of a role for ghrelin in decidualization (Tawadros et al., 2007). In addition, low endometrial expression of ghrelin and its receptor are associated with unexplained fertility (Aghajanova et al., 2010). Furthermore, in utero exposure to decreased ghrelin levels in mice leads to defects in uterine development with subsequent subfertility due to alterations in gene expression critical for implantation as well as defects in endometrial proliferation (Martin et al., 2011).

The adipose hormones leptin and adiponectin also have roles to play in the endometrial function. Leptin is secreted by endometrial cells (Gonzalez et al., 2000) and its secretion may increase the possibility of successful implantation through increases in various factors such as β3-integrin, interleukin-1 and leukemia inhibitory factor and their respective receptors (Gonzalez and Leavis, 2001; Gonzalez et al., 2003;...
Ramos et al., 2005; Dos Santos et al., 2012). Furthermore, lower circulating leptin levels are associated with recurrent spontaneous abortion (Lage et al., 1999; Laird et al., 2001). Adiponectin also appears to have a role with studies showing that adiponectin promotes syncytialization and invasion capacity of human trophoblasts (Benaitreau et al., 2010a, b). Adiponectin receptor expression in the endometrium is increased in the mid-luteal phase, the period of implantation, with reduced levels observed in the endometria of women with impaired fertility (Dos Santos et al., 2012). Further data suggests that adiponectin has important anti-inflammatory and energy-related effects on the endometrium (Takemura et al., 2006).

The expression of the peptide YY and insulin receptors (as well as the associated glucose transporter) expression has also been noted in endometrial cells with variation during the menstrual cycle, although the roles of these hormones in the endometrium requires further investigation (Xiao et al., 1998; Mioni et al., 2012).

**Gut and adipose hormones: the reproductive axis**

Each gut and adipose hormone will now be detailed in turn, in an attempt to piece together the complex relationship between gut and adipose hormones with the reproductive axis (Fig. 1). Our understanding of energy metabolism has matured greatly over the last decade partly due to the interest of scientists and clinicians in the worsening obesity epidemic. This has given us an outstanding opportunity to examine the relationship of gut and adipose hormones with reproduction.

**Methods**

A series of PubMed database searches were performed using relevant keywords either alone or in combination: gut hormones, ghrelin, obestatin, insulin, peptide YY, glucagon-like peptide 1, glucose-dependent insulinotropic peptide, oxyntomodulin, cholecystokinin, adipose hormones, leptin, adiponectin, resistin, omentin, chemerin in combination with reproduction, puberty, fertility, luteinizing hormone, follicle-stimulating hormone, estrogen, testosterone, progesterone. Relevant data were subsequently extracted from the identified papers and secondary data sources were identified by these papers. No language restrictions were applied. To ensure the inclusion of the most current data available, searches were performed up until 31 March 2013.

**Gut hormones**

**Ghrelin**

Ghrelin is a 28-amino acid peptide derived from pre-pro-ghrelin. Ghrelin was first identified in 1999 as the endogenous ligand of the growth hormone secretagogue receptor 1a (GHS-R) (Kojima et al., 1999), which was subsequently renamed the ghrelin receptor (Davenport et al., 2005). The oxyntic cells of the gastric mucosa are the predominant site of synthesis of ghrelin, although ghrelin is also produced in the intestine, pancreas, hypothalamus and pituitary (Date et al., 2000). Once acylated, ghrelin can bind to ghrelin receptors present in the periphery and pituitary gland and can also cross the blood–brain barrier to bind to central receptors in the brain (Gnanapavan et al., 2002). A full review of the pleiotropic biological roles of ghrelin is beyond the scope of this review but is covered in detail elsewhere (Stengel and Tache, 2012).

Here we summarize the main roles before detailing the reproductive actions of ghrelin (Table I and Fig. 1).

Although ghrelin can stimulate the release of growth hormone from the pituitary, its predominant actions are on feeding and metabolism. Intracerebroventricular (ICV) injections of ghrelin strongly stimulate feeding in rats and increase body weight. These actions, independent of growth hormone, occur through activation of neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons since antibodies and antagonists to these abolish ghrelin-induced feeding (Nakazato et al., 2001). This mechanism is further supported by the observation that NPY/AgRP knockout mice do not exhibit ghrelin-induced feeding (Chen et al., 2004). In humans, ghrelin levels are increased almost 2-fold immediately before a meal and subsequently fall after eating (Cummings et al., 2001). In addition, intravenous ghrelin infusion enhances appetite and increases food intake by 28% in human volunteers (Wren et al., 2001). Circulating ghrelin levels are related to current food requirements with increased levels in states of inadequate nutrition and an inverse correlation between ghrelin levels and body mass index (Otto et al., 2001; Gibson et al., 2010). Hence, ghrelin can inform higher brain centres of acute and chronic changes in peripheral energy balance. Considering the high energy demands of reproduction, it is perhaps unsurprising that evidence is emerging for the role of ghrelin as a signal of energy status in reproductive physiology. Indeed, there is now evidence for its role at all levels of the reproductive axis as detailed below.

**Effects on the gonads**

Ghrelin mRNA has been identified in the human testis (Gnanapavan et al., 2002) in both interstitial Leydig and in Sertoli cells within the seminiferous tubules (Gaytan et al., 2004). Similarly, the ghrelin receptor has also been localized to similar cell types as well as germ cells (Gaytan et al., 2004).

In females, ghrelin expression is present in ovarian hilus interstitial cells, as well as young and mature corpus lutea (Gaytan et al., 2003). Ghrelin receptor expression is more widespread in the female reproductive tract with expression identified in oocytes, follicular cells, all-stage corpus lutea and hilus interstitial cells (Gaytan et al., 2003). Hence, ghrelin and its receptor are present in the male and female gonads leading to a hypothesis that ghrelin and its receptor may indeed have a local regulatory role. This hypothesis is supported by animal studies showing that ghrelin inhibits the proliferative activity of immature Leydig cells in vivo potentially by regulating stem cell factor mRNA or other direct mechanisms (Barreiro et al., 2004). In addition in vitro studies have demonstrated a dose-dependent inhibition by ghrelin on stimulated testosterone secretion (Tena-Sempere et al., 2002). A recent study in male rats also suggests that ghrelin may serve as a modulator of spermatogenesis preventing excess build-up of germ cells through up-regulation of apoptotic and down-regulation of proliferative pathways (Kheradmand et al., 2012). In female gonads, the regulatory role of ghrelin appears to be a direct inhibitory effect on human luteal function with a shift towards luteolytic factors (e.g. prostaglandin F2α) from luteotrophic factors (e.g. prostaglandin E2 and vascular endothelial growth factor) (Tropea et al., 2007). Furthermore, ghrelin inhibits estradiol and progesterone biosynthesis by up to 25% in cultured granulosa–lutein cells via action on the GHS-R (Viani et al., 2008). Plasma ghrelin levels remain unchanged during the normal menstrual cycle suggesting that the ovarian sex steroids themselves have no feedback
effect on circulating ghrelin levels in women (Dafopoulos et al., 2009). This is further supported by a study that showed no effect of short-term estrogen administration (3–15 days) on ghrelin levels in healthy pre- and post-menopausal women (Dafopoulos et al., 2010). In summary, ghrelin can inhibit gonadal cell function/proliferation and steroidogenesis in both males and females. It is possible that this serves as an evolutionary tool to ensure there is a brake on gonadal function until ghrelin levels fall reflecting adequate nutritional intake.

**Effects on the hypothalamus and pituitary (reproductive axis)**

The actions of ghrelin higher in the reproductive axis have been studied in detail. Short-duration (2–3 h) human studies have failed to identify any

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**Table I Gut hormones: metabolic and reproductive summary.**

<table>
<thead>
<tr>
<th>Gut hormone</th>
<th>Predominant site of secretion</th>
<th>Stimulus for secretion</th>
<th>Metabolic effects</th>
<th>Reproductive Hypothalamic effects</th>
<th>Pituitary effects</th>
<th>Gonadal effects</th>
<th>Overall effect on reproductive axis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin</td>
<td>Oxyntic cells of gastric mucosa</td>
<td>Fasting</td>
<td>↑ Feeding/appetite (orexigenic) ↑ Growth hormone</td>
<td>↓ GnRH</td>
<td>↑ LH from animal explants ↑ Prolactin</td>
<td>↓ Leydig cell proliferation, testosterone secretion, luteal function and progesterone release</td>
<td>↓</td>
</tr>
<tr>
<td>Obestatin</td>
<td>Oxyntic cells of gastric mucosa</td>
<td>Fasting</td>
<td>May have similar effects to ghrelin</td>
<td>—</td>
<td>—</td>
<td>↓ Human luteal cell function</td>
<td>Unclear</td>
</tr>
<tr>
<td>Insulin</td>
<td>Pancreatic β-cells</td>
<td>Glucose intake</td>
<td>Multiple effects on glucose homeostasis</td>
<td>↑ LH in mice via GnRH ↑ LH pulse frequency in women</td>
<td>—</td>
<td>↑ Spermatozooal DNA synthesis and differentiation in newt Exogenous insulin can regenerate testis and restore fertility in hypogonadal rats</td>
<td>Permissive/↑</td>
</tr>
<tr>
<td>PYY</td>
<td>Entero-endocrine L-cells of distal ileum and colon</td>
<td>Food intake</td>
<td>Glucose homeostasis, anorectic</td>
<td>↑ GnRH release in fasted but ↓ GnRH in fed adult rat</td>
<td>Controversial. ↓ ↑ Gonadotrophins in adult male rats ↓ Gonadotrophins in prepubertal male rats (no change in females)</td>
<td>—</td>
<td>↓</td>
</tr>
<tr>
<td>GLP-1</td>
<td>Ileum and colon</td>
<td>Carbohydrate and fat</td>
<td>Insulin release, delayed gastric emptying, anorectic</td>
<td>↑ GnRH</td>
<td>↑ LH in male rats in vivo ↔ LH in healthy men</td>
<td>↓ LH-independent testosterone pulse frequency in healthy men</td>
<td>Unclear</td>
</tr>
<tr>
<td>GIP</td>
<td>K-cells of small intestine</td>
<td>Carbohydrate and fat</td>
<td>Insulin release</td>
<td>—</td>
<td>↑ LH and FSH secretion in vitro ↓ FSH (but not LH) in vivo (rat)</td>
<td>—</td>
<td>Unclear</td>
</tr>
<tr>
<td>OXM</td>
<td>Entero-endocrine L-cells of distal ileum and colon</td>
<td>Carbohydrate and fat</td>
<td>Decrease gastric acid secretion and ghrelin, anorectic</td>
<td>May act through modulation of other gut and adipose hormones</td>
<td>—</td>
<td>—</td>
<td>Unclear</td>
</tr>
<tr>
<td>CCK</td>
<td>Entero-endocrine L-cells of upper small intestine</td>
<td>Fat/protein</td>
<td>pancreatic enzyme, bile and insulin release, anorectic</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

Hypothalamic effects include studies where proven GnRH-dependent gonadotrophin changes were observed (otherwise listed as pituitary effect). The overall effect on the reproductive axis determined by review of currently available data. The “—” symbol signifies no data available. (CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin; PYY, peptide YY3-36.)
The effects of ghrelin on gonadotrophin secretion in men (Takaya et al., 2000; Nagaya et al., 2001) or women (Messini et al., 2009a, b). However, additional studies with longer durations, higher ghrelin doses and multiple ghrelin bolus (to mimic physiological pulsatile ghrelin secretion) have indeed shown suppression of both LH and FSH in men (Klüge et al., 2007, 2009; Lanfranco et al., 2008) and more recently in women (Klüge et al., 2012). The effects on FSH in these studies are less marked which may be explained by the fact that FSH release is less GnRH dependent than LH (McCann et al., 2001). Investigating the components of this LH suppression reveals that although peak levels are similar, there is a dramatic reduction in the frequency of the LH pulses after ghrelin administration suggesting upstream effects through the GnRH pulse generator (Klüge et al., 2007, 2012; Lanfranco et al., 2008). A study in young men by Lanfranco et al. (2008) provided evidence that the inhibitory effect of ghrelin on gonadotrophins is directly from the inhibition of the hypothalamic pulse GnRH pulse generator rather than the pituitary. In this study the stimulatory effect of exogenous GnRH administration (acting on the pituitary) on LH was unaffected by ghrelin; however, ghrelin inhibited LH pulsatility in response to naloxone which serves as a central stimulus for gonadotrophin secretion through the hypothalamus. Furthermore, in women ghrelin also has no effect on GnRH stimulation of LH and FSH (Messini et al., 2009a, b). The concept that ghrelin acts at the hypothalamic level of the reproductive axis is further supported by animal data showing decreased GnRH secretion from female rat hypothalamic fragments exposed to ghrelin (Fernandez-Fernandez et al., 2005a, b).

Whilst it was previously believed that the acylated form of ghrelin was the biologically active isoform, new data has shown that the unacylated isoform of ghrelin (UAG) can also mimic the actions of acylated ghrelin on LH release in male rats (Martini et al., 2006). Furthermore, given that UAG has no effect on GH secretion and hence the GHS-R, this suggests that UAGs inhibition of gonadotrophin secretion is at least partially conducted through a GHS-R-independent mechanism.

Studying the direct effect of ghrelin on the pituitary relies on animal data. Interestingly, female pituitary explants exposed to ghrelin secrete LH, an effect which is augmented by the blockade of estrogen action (Fernandez-Fernandez et al., 2007). A similar stimulatory effect has also been seen in male gonadotrophs in vitro (Fernandez-Fernandez et al., 2004). It remains unclear if these stimulatory effects are from direct action on gonadotrophs or paracrine actions from other pituitary cells, as it is yet to be established if gonadotrophs express the ghrelin receptor.

Effects on pituitary prolactin production

Outside the classic reproductive axis, ghrelin also has effects on prolactin produced by the pituitary lactotrophs. The main role of prolactin is in lactation, while high levels of prolactin can suppress the reproductive axis which appears to be through inhibition of pulsatile GnRH secretion (Page-Wilson et al., 2006). Intravenous bolus injection of ghrelin results in increases in circulating prolactin levels in men and women (Broglio et al., 2003). In women, ghrelin-stimulated prolactin production remains unchanged across the menstrual cycle (Messini et al., 2009a, b). However, D2-dopamine agonism (with bromocriptine) blocks the stimulating effect of ghrelin on prolactin in healthy women (Messini et al., 2010). This suggests that the mechanism of action of ghrelin on prolactin secretion may be mediated by inhibition of the dopaminergic system. However, against this is data in mice suggesting that ghrelin may actually activate dopaminergic neurones in the hypothalamus which one would expect to inhibit prolactin secretion (Jiang et al., 2006). Hence, additional investigation is required to clarify the precise mechanism by which ghrelin stimulates prolactin secretion in humans.

**Ghrelin receptor and leptin interactions**

The orexigenic ghrelin and the anorexigenic leptin act in opposition to each other. Therefore, perhaps unsurprisingly, animal studies have shown that both ghrelin and leptin are involved in the regulation of the arcuate ghrelin receptor. ICV ghrelin increases ghrelin receptor expression and ICV leptin decreases ghrelin receptor expression (Nogueiras et al., 2004).

**Ghrelin and kisspeptin interactions**

Kisspeptin, encoded by the KISS1 gene, is an arginine–phenylalanine amine hypothalamic peptide which acts on the kisspeptin receptor (encoded by the KISS1R gene, also known as GPR54). Kisspeptin is an essential component of the reproductive axis as demonstrated by the fact that in humans KISS1 or KISS1R inactivating mutations result in a failure to go through puberty (de Roux et al., 2003; Seminara et al., 2003; Topaloglu et al., 2012) while activating mutations of KISS1 or KISS1R result in central precocious puberty (Teles et al., 2008; Silveira et al., 2010). Furthermore, kisspeptin administration to animals and humans results in stimulation of LH release (Thompson et al., 2004; Dhiillo et al., 2005, 2007; Chan et al., 2011; George et al., 2011; Jayasena et al., 2011), an effect which is abolished by pretreatment with a GnRH antagonist, hence indicating the critical role of kisspeptin in stimulating GnRH neuronal activity (Gottsch et al., 2004).

Evidence for a link between ghrelin and kisspeptin is provided by animal studies. The predominant kisspeptin neuronal populations are located in the anteroventral periventricular (AVPV) and acuate nuclei (ARC) in rodents. AVPV kisspeptin neurones in rodents are important in generating the LH surge required for ovulation (Adachi et al., 2007). Intravenous administration of ghrelin to ovariectomized estrogen-replaced rats down-regulates KISS1 expression in the AVPV but not the ARC kisspeptin neurones (Forbes et al., 2009). This therefore suggests that high ghrelin may act to down-regulate AVPV kisspeptin expression, thus inhibiting ovulation and resulting in amenorrhea. Furthermore, the inhibition of GnRH secretion by ghrelin may indeed be partly mediated by this down-regulation of AVPV KISS1 expression. Further evidence for the close relationship between ghrelin and kisspeptin comes from a study in male rats demonstrating that injection of ghrelin reduced the duration of LH secretory responses to exogenous kisspeptin (Martini et al., 2006).

**A physiological role for ghrelin: linking energy and reproductive homeostasis peri-pubertally**

Daily ghrelin injection to prepubertal male rats delays balano-preputial separation, an external sign of pubertal development, and decreases circulating LH and testosterone concentrations (Martini et al., 2006). Therefore, ghrelin may operate as a negative modifier of male puberty during insufficient energy states. However, this inhibitory effect was not observed in prepubertal female rats under a similar protocol using timing of vaginal opening and first estrus as indices of puberty onset (Fernandez-Fernandez et al., 2005a, b). In addition, in female rats there was no effect on gonadotrophins or gonadal steroid levels at the same ghrelin doses as used for the male rats above (Fernandez-Fernandez et al., 2005b, c).
et al., 2005a, b). This suggests that female rats have a lower sensitivity to ICV ghrelin compared with male rats during the prepubertal period as determined by changes in gonadotrophins (Fernandez-Fernandez et al., 2004). In keeping with this data, doubling the doses of ghrelin (to 1 nmol/12 h) was sufficient to delay vaginal opening and ovulation in pubertal female rats (Roa et al., 2009) suggesting that both male and female puberty are delayed by ghrelin but that females are less sensitive to the negative effects of ghrelin on the reproductive axis (Roa et al., 2010). In humans, ghrelin levels increase significantly after birth, peaking at around 2 years of life. Subsequently, ghrelin levels decrease and this decrease may perhaps act as a permissive signal of adequate energy status to the reproductive axis and hence permit the completion of puberty (Soriano-Guillén et al., 2004). Further interesting data come from a study of short peri-pubertal children where ghrelin levels were measured before and after sex hormone priming for GH stimulation testing. Here pharmacological increases in sex hormone levels were associated with a marked decline in ghrelin levels in boys. This effect was not seen in girls, further suggesting that ghrelin levels are more important in male rather than female puberty (Lebenthal et al., 2006).

A physiological role for ghrelin: linking energy and reproductive homeostasis post-pubertally

While we now have a clearer picture of the effects of exogenous ghrelin on the reproductive axis, clues to the significance of endogenous ghrelin levels come from several additional studies. Endogenous ghrelin levels do not vary significantly during the normal menstrual cycle (Dafopoulos et al., 2009). However, ghrelin is increased in AN (Tolle et al., 2003). Furthermore, decreases in body weight during a diet and exercise programme result in an almost doubling of circulating ghrelin compared with those who did not perform exercise or lose weight (Leidy et al., 2004). Another study in women showed 85% higher ghrelin levels in women with amenorrhoea due to excess exercise compared with sedentary or exercising women with normal ovulation despite similar BMIs, body fat and leptin levels (De Souza et al., 2004). A further study, again controlling for BMI differences, showed higher ghrelin levels (with associated lower downstream gonadal steroid levels) in amenorrheic athletes compared with athletes or non-athletes with normal menstrual cycles (Christo et al., 2008). To examine this further, a recent study showed that young amenorrheic athletes have increased ghrelin and decreased LH pulse parameters when compared with eumenorrheic athletes or non-athletes. This suggests that higher ghrelin causes a decrease in GnRH pulsatility (as LH pulsatility is a surrogate for GnRH pulsatility) (Ackerman et al., 2012). Disordered eating behaviours also increase ghrelin levels (despite similar leptin levels) in normal weight women with hypothalmic amenorrhoea suggesting a direct link to abnormal dietary behaviours and not just weight or excess exercise (Schneider et al., 2008). Putting these studies together suggests that fasting and abnormal eating as well as over-exercise behaviours may all result in higher ghrelin levels. These higher ghrelin levels may then play a physiological role through GnRH/LH inhibition to suppress the reproductive axis resulting in the associated menstrual disturbances observed in women with these behaviours.

Ghrelin and polycystic ovarian syndrome

Polycystic ovarian syndrome (PCOS) is characterized by hyperandrogenism, anovulation and polycystic ovaries and is frequently associated with insulin resistance. Circulating fasting ghrelin levels in women with PCOS are lower than in weight-matched controls and negatively correlated with insulin sensitivity (Schoff et al., 2002; Moran et al., 2004; Glintborg et al., 2006; Barber et al., 2008; Pandis et al., 2010). Furthermore, it has been observed that women with PCOS have a less marked decrease in post-prandial ghrelin than women without PCOS (Moran et al., 2004; Zwisler-Korczala et al., 2008). These findings suggest an altered ghrelin homeostasis in PCOS which may contribute to the abnormal appetite regulation often observed in these women (Moran et al., 2004). In terms of reproductive hormones, the LH/FSH ratio, which is frequently elevated in PCOS, has been shown to be negatively correlated with ghrelin levels (Bideci et al., 2008). In addition, a negative correlation has also been observed between ghrelin and hirsutism score as well as between ghrelin and androgen levels, such as androstenedione, suggesting a link between ghrelin and steroidal synthesis/action (Pagotto et al., 2002; Pandis et al., 2005, 2010). To explore this link further, studies have observed that anti-androgen treatment or oral contraceptives (containing ethinyl estradiol and norethisterone) increase circulating ghrelin levels in women with PCOS suggesting that androgen excess may be involved in the regulation of ghrelin levels (Gambineri et al., 2003; Sagoz et al., 2009). However, another study did not observe any effect of exogenous short-term estrogen administration. In addition, given that ghrelin inhibits gonadotrophin secretion, predominantly LH rather than FSH, and inhibits ovarian steroidogenesis (Viani et al., 2008; Kluge et al., 2012) then it is possible that the lower ghrelin levels in PCOS may therefore favour androgen production (Repaci et al., 2011). It is important to note however that there also exist reports of no differences in circulating ghrelin levels between women with PCOS and weight-matched controls (Orio et al., 2003; Kale-Gurbuz et al., 2013) and a report of elevated ghrelin levels in PCOS (Wasako et al., 2004). The explanation for these results remains unclear; however, the body of recent evidence favours a lower circulating ghrelin in PCOS with possible links to the observed abnormal reproductive hormone levels.

Ghrelin: summary

In conclusion, there is now extensive data supporting the role of ghrelin not only as an established orexigenic hormone in metabolism but also in reproduction. Studies suggest both stimulatory (pituitary in vitro) but predominantly inhibitory (hypothalamus via kisspeptin, and gonads) actions of ghrelin on the reproductive axis (Table 1). The exact action of ghrelin may therefore vary between reproductive tissues and hormonal milieus. In terms of ghrelin's overall role, multiple lines of evidence suggest that ghrelin serves as an important link between metabolism and reproduction by informing higher brain centres of changes in peripheral energy balance. This role of ghrelin appears to be key in the regulation of puberty as well as menstrual cyclicity depending on energy balance. Future studies will almost certainly continue to demonstrate the importance of ghrelin in reproduction and enhance our understanding of this key link to metabolism.

Obestatin

Obestatin (23 amino acid hormone) originates from the same precursor peptide as ghrelin, namely pre-pro-ghrelin (Zhang et al., 2005). Furthermore, obestatin immunoreactivity has been localized to the same secretory granules in human gastric endocrine cells as ghrelin (Gronberg et al., 2008; Tsolakis et al., 2009). The literature remains controversial as to whether the physiological functions of obestatin are similar or opposite
to those of ghrelin (Zhang et al., 2005). In terms of reproductive roles, obestatin may have a role in regulating luteal cell function as recently evidenced by reduced progesterone and vascular endothelial growth factor release from primary cells isolated from human corpora lutea exposed to obestatin in vitro (Romani et al., 2012) (Table I). However, earlier work suggests that obestatin can stimulate proliferation, apoptosis and progesterone release from porcine ovarian granulosa cells (Meszarosova et al., 2008). These different observed ovarian effects of obestatin may indeed relate to species variation. Animal studies have also demonstrated that obestatin administration can increase the expression of the ghrelin gene in the hypothalamus of chickens, and can also modify the response of the hypothalamic and ovarian ghrelin system to food restriction (Sirotkin et al., 2012). Further studies especially in humans, given the species differences identified above, are therefore required to clarify the suggested inhibitory roles of obestatin on ovarian cell function and on hypothalamic reproductive function through stimulation of hypothalamic ghrelin expression.

### Insulin

Insulin is a hormone produced by pancreatic β-cells and has multiple roles in peripheral and central energy homeostasis beyond the scope of this review. Its best known role is in stimulating glucose uptake from the blood into peripheral tissues. Insulin receptors are distributed widely throughout the body including in the central nervous system. Here there is distinct expression in the hypothalamus and pituitary (Havrannová et al., 1978; Werther et al., 1987; Marks et al., 1990). Evidence suggesting a role for insulin in the central control of body weight and reproduction emerged over a decade ago. Mice with neurone-specific disruption of the insulin receptor gene (NIRKO mice) developed diet-sensitive obesity as well as impaired spermatogenesis or ovarian follicle maturation resulting in impaired fertility (Bruning et al., 2000). This effect on reproduction was as a result of a 60% reduction of circulating LH in males and a 90% reduction in females despite maintained pituitary sensitivity to GnRH, essentially hypergonadotrophic hypogonadism (Bruning et al., 2000). Interestingly, mouse models with insulin receptor knockout exclusively on GnRH neurones show no impairment of fertility suggesting that the GnRH neurones do not respond directly to insulin (Divall et al., 2010). Hence, the site of insulin action on the reproductive axis may not be at the level of the GnRH neurone or alternatively the associated metabolic derangements of the NIRKO mice may explain their impaired fertility. Contrary to the former explanation, however, is evidence of direct modulation of GnRH-expressing neurones by insulin; the insulin receptor has been identified at protein and mRNA levels in GnRH neuronal cell lines, furthermore strong c-fos expression (a marker of neuronal activation) in GnRH neuronal cell lines is observed after insulin stimulation, via activation of the mitogen-activated protein (MAP) kinase extracellular signal-regulated kinase (ERK)1/2 pathway (Salvi et al., 2006).

One emerging alternative pathway by which insulin may interact with the hypothalamic reproductive apparatus is the direct action of insulin together with leptin on hypothalamic pro-opiomelanocortin (POMC) neurones. POMC neurones are critical regulators of energy homeostasis responding to circulating adiposity signals such as insulin and leptin (Cheung et al., 1997; Baskin et al., 1999; Benoit et al., 2002). POMC neurones are also involved in the hypothalamic pathways linking energy balance and reproduction (Hill et al., 2008). Female mice lacking both insulin and leptin receptors exclusively in POMC neurones demonstrate elevated serum testosterone levels and ovarian abnormalities (more degenerating follicles and cysts), which result in reduced fertility (Hill et al., 2010). Interestingly, there were no significant differences in levels of LH, prolactin, estrogen or GnRH expression in these POMC insulin and leptin knockout mice indicating normal gonadotroph and GnRH neuronal function; the reduced fertility may be merely a result of peripheral insulin resistance resulting in hyperandrogenaemia (Popovetsky and Piper, 1994) or subtle modulation of the GnRH pulse generator without detectable LH or GnRH expression changes. Furthermore, reduced fertility was not seen in mice with single deletions of either the insulin or leptin receptor, indicating the importance of the co-ordinated action of the insulin and leptin receptor (Hill et al., 2010). Another possible pathway involves insulin-inhibiting hypothalamic NPY neurones. As NPY neurones normally inhibit GnRH production, insulin can therefore relieve this GnRH inhibition via NPY inhibition (Sato et al., 2005). Further work is required to establish the precise cell type(s) targeted by insulin in the reproductive axis; but currently available studies above support a permissive action by insulin on hypothalamic GnRH neurones via leptin, POMC and NPY neurones.

Increased circulating insulin levels in mice (achieved by hyperinsulinaemic clamp studies) are associated with increased LH secretion through increased expression and secretion of GnRH independent of glycaemia (Burcelin et al., 2003; Kim et al., 2005). Interestingly, an investigation in humans has revealed a sexual dimorphism in response to insulin. The mean LH levels and LH pulse amplitudes were not affected over a 12 h hyperinsulinaemic euglycaemic clamp study in healthy women (although there was a trend towards LH increases) (Moret et al., 2009). However, this study revealed significant increases in LH pulse frequency suggesting an effect at the level of the hypothalamic GnRH neurone as such a modulation cannot be exerted purely at the level of the pituitary gonadotroph (Moret et al., 2009). This is consistent with previous animal data but is in contrast to a study observing no effect on LH pulsatility in healthy women under similar hyperinsulinaemic clamp conditions (Patel et al., 2003). The explanation for this difference in results is unclear but may relate to the higher body mass index (with associated increased basal insulin levels) and higher achieved clamp insulin levels in the study by Patel et al.

In contrast to the female data, a recent similar hyperinsulinaemic euglycaemic clamp study in healthy men failed to demonstrate any similar effect on the pattern of LH secretion (Pesant et al., 2012), although similar studies in male rats resulted in a dose-dependent rise in LH secretion (Burcelin et al., 2003). This suggests a human sexual dimorphism in the sensitivity of the reproductive axis to insulin signals. Given that men do not undergo the metabolic stresses of pregnancy, it is possible that the male reproductive axis is less sensitive than that of the female to nutritional insulin status.

Variations in circulating insulin also have an effect on other gut and adipose hormones that have a role in reproduction, suggesting a complex interplay: Hyperinsulinaemia increases serum leptin concentrations in a dose-dependent manner during euglycaemic clamp studies in healthy men (Boden et al., 1997). Furthermore, leptin potentiates the effect of insulin on GnRH secretion (Burcelin et al., 2003) and improves peripheral glucose homeostasis in obese mice by increasing hypothalamic insulin sensitivity (Koch et al., 2010). Conversely, insulin decreases plasma ghrelin levels (Saad et al., 2002), indicating that insulin is a dynamic modulator of both circulating leptin and ghrelin.
Studies in women with PCOS (who characteristically have more frequent LH pulses, higher LH levels and higher LH/FSH ratios than normal women with regular menstrual cycles) have revealed no effect of a 12 h hyperinsulinaemic euglycaemic clamp on LH secretion (Patel et al., 2003; Mehta et al., 2005; Moret et al., 2009). Furthermore, LH responses to GnRH were not influenced by insulin administration (Patel et al., 2003), suggesting that insulin resistance and compensatory hyperinsulinaemia are not the direct causes of the abnormal hypothalamic GnRH pulse generator activities in PCOS patients (although the presence of marginally raised basal insulin concentrations in the PCOS group compared with healthy controls does not allow full confirmation of this).

**Insulin and the gonads**

Studies of impaired spermatogenesis in diabetic rats have revealed insulin expression in the testis (Gomez et al., 2009). In addition, human ejaculated spermatozoa express and secrete insulin (Aquila et al., 2005). These discoveries led to further work in male β-cell deficient diabetic rats attempting to rescue their impaired fertility resulting from decreased LH and testosterone and testicular morphological abnormalities (Schoeller et al., 2012). Exogenous insulin was able to regenerate testes and restore fertility in diabetic rats despite the inability of plasma insulin to traverse the blood–testis barrier; this suggests that insulin acts on the hypothalamus and pituitary to normalize sex hormone levels which in turn stimulate testicular regeneration (Schoeller et al., 2012).

There is evidence that insulin can also directly stimulate ex vivo spermatogenesis in addition to its actions on the reproductive axis. Insulin treatment of seminiferous tubule segments stimulates spermatozoal DNA synthesis (Soder et al., 1992) and differentiation in the newt testis (Nakayama et al., 1999). Furthermore, the insulin/forkhead box class O (FOXO) signalling pathway has been identified as crucial for the synthesis of locally acting prostaglandins to promote various related reproductive processes in rodents including oogenesis, oocyte maturation, ovulation and sperm guidance (Edmonds et al., 2010).

**Insulin: summary**

In summary, current evidence suggests stimulatory roles for insulin at both a hypothalamic GnRH level (with a greater effect in women than men) and a gonadal level (gametogenesis) (Table I). Whether the former effect is predominantly permissive remains to be seen, and further investigation of the insulin-leptin-grelin-POMC-NPY interplay with regard to reproduction is likely to provide some answers in future.

**Peptide YY3-36 (PYY)**

PYY is secreted by the entero-endocrine L-cells of the distal ileum and colon. PYY secretion is stimulated by food intake and conversely suppressed by fasting (Wren and Bloom, 2007; Woods and D’Alessio, 2008). PYY can cross the blood–brain barrier (Nonaka et al., 1999). Furthermore, the Y2 receptors of hypothalamic NPY neurones resulting in their inhibition and a subsequent decrease in food intake in rodents (Batterham and Bloom, 2003). Consistent with this, a 90-min PYY infusion to humans inhibits their subsequent food intake by 36% compared with saline (Batterham et al., 2002).

Studies regarding the effects of PYY on the reproductive axis have so far produced contrasting results and a possible sexual dimorphism. PYY administration inhibited GnRH secretion by hypothalamic fragments from ad libitum fed male rats but stimulated GnRH secretion from fasted male rats (Pinilla et al., 2006). Furthermore, PYY increased gonadotrophin secretion in male rat pituitaries and the effect was potentiated by fasting. Similar effects were observed in females but to a lesser degree. ICV PYY administration to adult male rats stimulated gonadotrophin secretion in vivo, an effect that was augmented by fasting and blocked by GnRH antagonist (Pinilla et al., 2006). However, a later study by the same group observed decreased LH secretion after ICV PYY in adult intact and orchidectomized male rats (Pinilla et al., 2007). Studies in prepubertal rats (Day 25) have also found inhibition of LH secretion after ICV PYY administration in male but no change in LH in females (Fernandez-Fernandez et al., 2005a, b; Pinilla et al., 2007). This suggests a sexual dimorphism in response to PYY in prepubertal and adult rats. Another study in ovariectomized ewes demonstrated that PYY infusion delayed the estradiol-induced LH surge (Clarke et al., 2005). Putting these animal studies together suggests the importance of gender as well as nutritional, pubertal and gonadal status for the action of PYY on the reproductive axis where evidence suggests it acts at the hypothalamic level.

Human data on PYY and the reproductive axis are scarce. In AN, the reproductive axis usually reverts to a hypothalamic hypogonadal state (see recent comprehensive review by Miller, 2011). PYY levels in girls with AN are over 3-fold higher than healthy controls, suggesting that elevated PYY may contribute to their reduced food intake and could potentially affect the reproductive axis (Misra et al., 2006). Another cross-sectional study looking at 87 children in various stages of puberty showed that fasting PYY levels were lowest in mid-puberty (Lloyd et al., 2010). Indeed, this may fit with the model that exogenous growth hormone suppresses PYY secretion (Tovar et al., 2004) as growth hormone levels increase during puberty.

The aforementioned prepubertal animal data suggest that PYY may have an overall inhibitory action on the reproductive axis (Fernandez-Fernandez et al., 2005a, b; Pinilla et al., 2007). Hence, it is conceivable that a decrease in circulating PYY (due to increasing growth hormone) may have some role (together with a plethora of other factors) in relieving inhibition on the reproductive axis to allow human pubertal development. Further work is required to clarify this hypothesis as well as the exact stimulatory and inhibitory actions of PYY on the reproductive axis in relation to sex, pubertal stage and fasting status (Fig. 1).

**Glucagon-like peptide 1**

Glucagon-like peptide 1 (GLP-1) is a 29-amino acid amidated peptide hormone secreted by the entero-endocrine L-cells in the distal ileum and colon in response to food intake during which levels can increase up to 20-fold (Turton et al., 1996). GLP-1 serves an important role in glucose homeostasis by stimulating glucose-induced insulin synthesis and secretion as well as preserving pancreatic β-cell mass and delaying gastric emptying (Drucker, 1998). Furthermore, GLP-1 receptors have been identified centrally in the human and rat brain, including the cerebral cortex, hypothalamus (e.g. the medial and lateral preoptic areas; regions rich in GnRH neurones), pituitary, caudate putamen and cerebellum (Shinizu et al., 1987; Small et al., 2002; Alvarez et al., 2005). GLP-1 can traverse the blood–brain barrier and peripheral GLP-1 infusions can therefore act in the brain to enhance satiety and reduce subsequent spontaneous food intake by 12% in healthy humans (Flint et al., 1998).

The first evidence for a role of GLP-1 in reproduction came from knock-out mice. Female GLP-1 receptor knockout mice exhibit pubertal delay, while male GLP-1 receptor knockout mice have reduced gonadal

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**References**

Patel et al., 2003; Mehta et al., 2005; Moret et al., 2009; Nakayama et al., 1999; Schoeller et al., 2012; Nakayama et al., 1999; Batterham and Bloom, 2003; Wren and Bloom, 2007; Woods and D’Alessio, 2008; Nonaka et al., 1999; Batterham and Bloom, 2003; Shima et al., 1987; Small et al., 2002; Alvarez et al., 2005; Tovar et al., 2004; Lloyd et al., 2010; Miller, 2011; Misra et al., 2006; Tovar et al., 2004; Clarke et al., 2005.
weights (MacLusky et al., 2000). However, gonadal sex steroid levels are normal and both sexes are fertile (MacLusky et al., 2000).

GLP-1 administration to GnRH cell lines results in a concentration-dependent increase in GnRH release (Beak et al., 1998). Furthermore, ICV injection of GLP-1 to male rats produces a prompt increase in plasma LH (Beak et al., 1998). However, 6-h GLP-1 infusions to healthy men result in no change in LH levels or LH pulse frequency and amplitude (Jeibmann et al., 2005). This difference between the response to GLP-1 in rodent and human studies may be explained by the fact that the human studies only achieved post-prandial physiological GLP-1 levels rather than the supraphysiological levels achieved by ICV injection in rats. However, GLP-1 infusion in men reduced the frequency of testosterone pulses by over 50% in the 6 h time course (Jeibmann et al., 2005), suggesting an inhibitory effect on testosterone secretion independent of changes in LH. Furthermore, animal studies provide evidence of a central LH-independent pathway affecting testosterone secretion via changes in steroidalogenic acute regulatory protein (Ogilvie et al., 1999) or catecholamine-mediated mechanisms (Selvage and Rivier, 2003).

In summary, there is evidence that GLP-1 has stimulatory actions at a hypothalamic-pituitary level of the reproductive axis in animals (Table I). These effects have not been observed in humans and so further work is needed to confirm/investigate this as well as the possibility that GLP-1 may have LH-independent inhibitory effects on testosterone secretion. Furthermore, clarification of the relationship between GLP-1 and other gut/adipose hormones (which also interact with the reproductive axis) may provide a clearer picture of the relationship between GLP-1 and reproduction.

**Glucose-dependent insulinotropic polypeptide**

Glucose-dependent insulinotropic polypeptide (GIP) is secreted in response to oral glucose intake by K-cells located throughout the small intestine but predominantly in the duodenum. GIP stimulates insulin secretion and promotes β-cell proliferation, lipoprotein lipase activity and fatty acid synthesis in adipocytes (Yip and Wolfe, 2000). The role, if any of GIP on the reproductive axis, remains unclear with little data available in rodents or humans. An early study in 1985 showed that GIP administration on dispersed anterior pituitary cells from ovariectomized rats induced both LH and FSH release, although GIP was not as potent as GnRH (Ottlez et al., 1985). However, in vivo work on male rats showed that ICV GIP administration produced a significant decrease in plasma FSH but no effect on LH (Ottlez et al., 1985). These contrasting findings of GIP stimulation of rodent gonadotrophins in vitro but inhibition in vivo clearly require future study together with the investigation of the role of GIP (if any) in human reproduction. However, once again it is important to note that the gut/adipose hormones closely interact with each other and so there are conceivably indirect effects through each other on reproduction (e.g. via insulin).

**Oxyntomodulin and cholecystokinin**

Oxyntomodulin (OXM) and cholecystokinin (CCK) are further gut hormones that may play roles in the reproductive axis, although these are currently undefined due to little or no published data. Given the lack of data it is indeed also possible that they play no direct role. However, given their involvement in pathways involving food intake or other gut/adipose hormones, it is feasible that they may affect the reproductive axis indirectly.

OXM is a 37-amino acid peptide co-secreted post-prandially with GLP-1 and PYY from the entero-endocrine L-cells. Similarly to GLP-1 and PYY, OXM has anorectic effects. Peripheral oxyntomodulin reduces food intake in rats (Dakin et al., 2004) as well as in normal weight and obese humans (Cohen et al., 2003; Wynne et al., 2006). In addition, pre-prandial levels of the orexigenic hormone ghrelin are significantly suppressed by OXM in healthy humans (Cohen et al., 2003).

CCK was the first gut hormone that was shown to reduce food intake in rats (Gibbs et al., 1997) and humans (Kissileff et al., 1981). It has other roles including delaying gastric emptying, stimulating pancreatic enzyme release and stimulating gall bladder contraction. CCK is predominantly secreted by the entero-endocrine I-cells in the mucosal epithelium of the small intestine in response to food intake. CCK is composed of a varying number of amino acids depending on post-translational modification of the original CCK gene product (e.g. CCK 58, CCK 33, CCK 8). The only finding in the reproductive field is that fasting CCK levels are significantly higher in the luteal phase compared with the follicular phase of the menstrual cycle, although the exact physiological importance of this remains unclear (Frick et al., 1990).

**Adipose hormones**

**Leptin**

The discovery of leptin in the 1990s was hailed as a major breakthrough in our understanding of human metabolism, but leptin has since been recognized to also play an influential role in human reproduction. Leptin is a glycoprotein synthesized and secreted by adipocytes acting on the leptin receptor (Zhang et al., 1994). Circulating leptin levels positively correlate with fat mass. Levels of leptin secretion are significantly reduced following weight loss (Maffei et al., 1995) and acute starvation in humans (Chan et al., 2003, 2006). Leptin-deficient ob/ob mice are severely obese and infertile (Zhang et al., 1994); a similar phenotype of childhood onset morbid obesity and infertility is observed in humans with mutations of leptin or its receptor (Farooqi and O’Rahilly, 2006).

Leptin has a number of metabolic effects on the brain, the best characterized of which is in the arcuate nucleus of hypothalamus (ARC). The ARC contains two discrete subpopulations of first-order leptin-responsive neurons. One neuronal population expresses the orexigenic NPY, whilst another neuronal population expresses the anorectic peptide alpha-melanocyte-stimulating hormone (α-MSH) which is derived from POMC (Cheung et al., 1997; Broberger et al., 1998). Leptin activates POMC neurons (thus increasing α-MSH secretion), and inhibits the activity of NPY/agouti-related peptide (AgRP) neurons. Therefore, during starvation, low levels of leptin allow compensatory increases in NPY/AgRP and a reduction in α-MSH, thus increasing food intake. Leptin is therefore a circulating marker of fat reserves, which acts directly on the human hypothalamus to inhibit food intake; low circulating leptin therefore signals low levels of adipose tissue. Therefore, experimental evidence suggests that leptin regulates food intake by altering the balance between orexigenic and anorectic hypothalamic factors.

**Reproductive effects of leptin**

The most striking feature of genetic leptin deficiency is severe obesity. However, mice and humans with congenital leptin deficiency also have...
As discussed above, GnRH neurones do not express the leptin receptor. Kisspeptin as a mediator of leptin effects on reproduction

GnRH; two candidate factors have been investigated to date: kisspeptin and glutamate. Kisspeptin signalling may therefore be essential for fertility.

Kisspeptin as a mediator of leptin effects on reproduction

The expression of KISS1 is influenced by nutritional status. In pre-pubertal rats which have been food deprived for 72 h, the hypothalamic expression of KISS1/mRNA is markedly reduced (Castellano et al., 2005). Furthermore, in a model of chronic undernutrition in pre-pubertal rats, daily ICV administration of kisspeptin from post-natal days 30 to 37 restores the delayed vaginal opening of these animals and increases the suppressed levels of plasma LH, FSH and estradiol (Castellano et al., 2005). We have also demonstrated that administration of kisspeptin to women with hypothalamic amenorrhoea due to weight loss or low body weight, acutely and potently stimulates reproductive hormone secretion (Jayasena et al., 2009, 2010).

A number of observations suggest that kisspeptin is well placed to participate in energy homeostasis within the hypothalamus. Using double-label fluorescent immunohistochemistry, kisspeptin fibres in ewes have been shown to be in close apposition with the appetite-stimulating population of NPY and POMC neurons within the Arc of hypothalamus (Backholer et al., 2010). ICV administration of an α-MSH-like agonist (which inhibits food intake and is a hypothalamic signal of energy sufficiency) stimulates food intake and is a hypothalamic signal of energy insufficiency) inhibits the stimulatory effects of kisspeptin on GnRH neurons (Wu et al., 2009).

Kisspeptin signalling may be regulated by the intracellular regulator of metabolism, mammalian target of rapamycin protein (mTOR), a ubiquitously expressed serine–threonine protein kinase which plays a vital role in the regulation of cell growth and differentiation (Schmelze and Hall, 2000). When nutrient availability is low, mTOR activity falls thus inhibiting the high-energy demand cell cycle. Interestingly, ICV administration of leucine (which stimulates mTOR signalling) leads to an increase in plasma LH in female peri-pubertal rats (Cota et al., 2006; Roa et al., 2009).

Conversely, when mTOR activation is blocked (by rapamycin), the expression of KISS1/mRNA in the arcuate becomes almost undetectable and plasma levels of LH fall (Roa et al., 2009). Kisspeptin may also be regulated by the nuclear factor, CAMP responsive element-binding protein–1 regulated transcription coactivator–1 (Crtc1). Mice with genetic inactivation of Crtc1 are obese and infertile (Altarejos et al., 2008). Conversely, leptin induces dephosphorylation resulting in activation of Crtc1, which increases KISS1 promoter activity. Crtc1 may therefore be required for leptin to activate kisspeptin signalling within the hypothalamus, which is required for fertility.

Glutamate signalling within the ventral pre-mammillary nucleus as a mediator of leptin effects on reproduction

The dependence of leptin-mediated reproductive effects on kisspeptin has been challenged recently (Donato et al., 2011). Cre-lox technology was used to specifically reduce the expression of the leptin receptor in kiss1-expressing neurones of mice; surprisingly however, these animals had an entirely normal reproductive phenotype. During the same study, re-expression of leptin receptor selectively in glutamatergic PMV neurons of mice with genetic inactivation of the leptin receptor induced pubertal development in females (but not in males). In addition, lesioning of the PMV reduced the efficacy of exogenous leptin to restore fertility in leptin-deficient female mice. This experiment suggests that kisspeptin signalling may not be essential for leptin-dependent activation of GnRH. This is further evidenced by a recent study in mice observing that leptin signalling in kisspeptin neurones does not actually arise until sexual maturation is complete (Cravo et al., 2013). Further work is required to elucidate the role of PMV glutamatergic signalling in human reproduction, and whether other intermediaty signals are required for leptin to regulate reproduction.

Leptin signalling within the ovaries

A growing body of evidence suggests that leptin may also play a role in regulating ovarian function. Leptin expression has been observed in human granulosa and cumulus cells (Antczak and Van Blerkom, 1997; Cioffi et al., 1997). In vitro studies suggest that leptin attenuates the induction of steroidogenesis within bovine granulosa cells by insulin (Spicer and Francisca, 1997). The same authors also observed that leptin inhibited insulin-induced synthesis of progesterone and androstenedione in theca cells, but potentiated insulin-induced theca cell proliferation (Spicer and Francisca, 1998). Furthermore, Karamouti et al. (2008) observed that leptin modulates the actions of growth hormone and insulin-like growth factor–1 (IGF–1) on human ovarian steroidogenesis in vitro. Collectively, these data suggest that leptin may play a role in regulating human ovarian steroidogenesis.
Some interesting data have also emerged regarding the influence of circulating sex steroids on circulating levels of leptin in humans. Serum levels of leptin are significantly elevated during the luteal phase of human menstrual cycle, when compared with the follicular phase (Hardie et al., 1997). Serum leptin is elevated during combined estradiol and progesterone supplementation during the follicular phase of menstrual cycle, whereas pure estradiol supplementation does not stimulate leptin secretion (Messinis et al., 2001). Furthermore, serum leptin levels are reduced following bilateral ovariectomy in women (Messinis et al., 1999). These data suggest a number of possibilities: ovarian sex steroids may potentiate leptin secretion from adipocytes; alternatively, ovarian leptin production may contribute to the the circulating levels of leptin observed in women.

It is important to recognize that the ovarian actions of leptin may be paracrine in nature, and unrelated to the actions of adipose-derived leptin. Nevertheless, leptin clearly appears to be implicated in the regulation of ovarian steroidogenesis. Further work is needed to elucidate the physiological significance of leptin signalling within the ovaries.

**Leptin: summary**

In summary the adipose-derived hormone, leptin, is necessary for human reproduction, since genetic models of leptin deficiency and starvation-induced leptin suppression are associated with hypogonadism due to hypothalamic GnRH deficiency. Leptin may act in the hypothalamus to modulate GnRH function through glutamate signalling or through a recently identified hormone called kisspeptin. Leptin is also expressed in the human ovaries, where it modulates steroidogenesis. The studies collectively suggest that leptin acts both centrally and peripherally to modulate the human reproductive system.

**Adiponectin**

Adiponectin is a 244-amino acid protein secreted exclusively by adipocytes in white adipose tissue (Maeda et al., 1996). Adiponectin is the most abundant protein secreted by adipocytes with levels over 1000-fold greater than leptin (Stefan and Stumvoll, 2002; Gavras et al., 2003a, b). However, circulating levels of adiponectin are lower in obesity and increase with weight loss, unlike other adipose hormones (Goodman et al., 2004). Adiponectin levels are regulated in conditions with disturbed GnRH pulsatility such as hypogonadotrophic hypogonadism (Lanfranco et al., 2004) and AN (Modan-Moses et al., 2007). A potential inhibitory role of adiponectin on GnRH neuropeptide activity is further evidenced by adiponectin significantly reducing GnRH secretion from immortalized GT1-7 hypothalamic GnRH neurons via activation of adenosine monophosphate-activated protein kinase (AMPK) and a subsequent reduction of ERK (Wen et al., 2011). In addition, recent evidence suggests that adiponectin may also inhibit GnRH neurons via down-regulation of KISS1 gene transcription through AMPK and subsequent decreased specificity protein 1 (SP1) translocation (Wen et al., 2012).

**Effects on the hypothalamus**

Adiponectin can cross the blood–brain barrier (Qi et al., 2004). Furthermore, AdipoR1 is expressed in the lateral hypothalamus of humans (Psilopanagioti et al., 2009). Circulating adiponectin levels are elevated in conditions with disturbed GnRH pulsatility such as hypogonadotrophic hypogonadism (Lanfranco et al., 2004) and AN (Modan-Moses et al., 2007). A potential inhibitory role of adiponectin on GnRH neuropeptide activity is further evidenced by adiponectin significantly reducing GnRH secretion from immortalized GT1-7 hypothalamic GnRH neurons via activation of adenosine monophosphate-activated protein kinase (AMPK) and a subsequent reduction of ERK (Wen et al., 2008; Cheng et al., 2011). In addition, recent evidence suggests that adiponectin may also inhibit GnRH neurons via down-regulation of KISS1 gene transcription through AMPK and subsequent decreased specificity protein 1 (SP1) translocation (Wen et al., 2012).

**Effects on the pituitary**

There are high levels of adiponectin and adiponectin receptor expression in the pituitary gland especially in the gonadotrophs (Psilopanagioti et al., 2009). ICV adiponectin administration suppresses the LH pulse (mean and peak LH levels but not frequency) in male rats but this most likely occurs through GnRH inhibition as described above (Cheng et al., 2011). A direct pituitary action has been demonstrated by adiponectin inhibiting both basal and GnRH-stimulated LH secretion in rodent pituitary explants with the latter effect explained by decreased GnRH receptor expression (Rodriguez-Pacheco et al., 2007; Lu et al., 2008).
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<td>↓ Ovarian IGF-induced progesterone and estrogen production in rat ↓ Choriogonadotrophin-stimulated testosterone secretion in rat testis</td>
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<tr>
<td>Resistin</td>
<td>Adipocytes and macrophages</td>
<td>Inflammatory stimuli</td>
<td>↑ Insulin resistance, role in inflammatory pathways</td>
<td>Resistin mRNA expressed</td>
<td>Resistin mRNA expressed with peak expression during puberty (mice)</td>
<td>↑ hCG-stimulated testosterone secretion in vitro Gonadotrophins ↑ resistin expression in Leydig and Sertoli cells</td>
</tr>
<tr>
<td>Omentin</td>
<td>Adipocytes, placenta, ovary</td>
<td>Inflammatory stimuli</td>
<td>↑ Insulin sensitivity, inflammatory pathways</td>
<td>—</td>
<td>—</td>
<td>Unclear (lower levels in PCOS)</td>
</tr>
<tr>
<td>Chemerin</td>
<td>Adipocytes and liver</td>
<td>Inflammatory stimuli</td>
<td>Adipocyte differentiation, glucose homeostasis, inflammatory pathways</td>
<td>—</td>
<td>—</td>
<td>↓ Progesterone and estradiol</td>
</tr>
</tbody>
</table>

Hypothalamic effects include studies where proven GnRH-dependent gonadotrophin changes were observed (otherwise listed as pituitary effect). The overall effect on the reproductive axis determined by review of currently available data. The "—" symbol signifies no data available. (PCOS, polycystic ovary.)
Effects on the gonads
Adiponectin and its receptors are also expressed in the human ovary and these mediate the actions of adiponectin to stimulate production of IGF-induced progesterone and estrogen (Chabrolle et al., 2009). The expression of both adiponectin receptor genes (AdipoR1 and AdipoR2) is increased in the mid-luteal phase when implantation typically occurs (Takemura et al., 2006), while the human placenta also expresses the adiponectin and AdipoR2 genes (Caminos et al., 2005).

The effects of adiponectin on female steroidogenesis are predominantly via action on AdipoR2 receptors on ovarian theca cells (Lagaly et al., 2008) and on both AdipoR1 and AdipoR2 receptors on granulosa cells (Chabrolle et al., 2009). Recombinant LH administration stimulates ovarian theca cell AdipoR2 expression in vitro (Lagaly et al., 2008) and ovarian adiponectin production in vivo (Gutman et al., 2009). Adiponectin may also have a role in IVF conception with adiponectin receptor expression in human ovarian cumulus cells being positively related to fertility outcome, while adiponectin administration to the mouse cumulus—oocyte complex improves oocyte quality (Richards et al., 2012). Furthermore, another study observed a positive association between circulating serum adiponectin levels on the day of IVF oocyte collection and successful embryo implantation (Bersinger et al., 2006).

A meta-analysis of studies looking at women with PCOS demonstrated lower circulating adiponectin levels in PCOS women and this was related to their increased insulin resistance but not to their testosterone levels (Toulis et al., 2009). This is reasonable to expect given the insulin-sensitizing actions of adiponectin and its negative correlation with obesity.

Adiponectin expression is observed in the male rat gonads, predominantly in the interstitial Leydig cells with regulation by metabolic signals such as glucocorticoids and thyroid as well as gonadotrophins (Caminos et al., 2008). Adiponectin receptor expression has also been identified in the rat testis, with AdipoR1 expression being prominent in the seminiferous tubules, while AdipoR2 expression is regulated by gonadotrophins (Caminos et al., 2008). Furthermore, in this study recombinant adiponectin administration to rat testicular tissue in vitro was found to significantly inhibit human choriogonadotrophin-stimulated testosterone secretion.

Adiponectin: summary
Overall, currently available data seem to suggest a largely inhibitory role of adiponectin on the hypothalamus, pituitary and male gonads but a stimulatory or at least permissive role in the female gonads (Table II). Further study is required to investigate these effects as well as to investigate the more subtle interplay between different sites of adiponectin expression, circulating adiponectin levels and the sex steroid ratios mentioned earlier.

Resistin
Resistin is secreted as a 94-amino acid polypeptide by adipocytes and macrophages and has a regulatory role in insulin sensitivity which explains the derivation of its name, resistin (Steppan et al., 2001). Insulin signal transduction pathways can be blocked by resistin, while other roles of resistin include inhibition of glucose transporters and hepatic glucose production via reduced AMPK activity (Stojkova, 2010). Interestingly, circulating resistin levels are not associated with obesity, weight loss or insulin resistance in humans (Lee et al., 2003; Monzillo et al., 2003). In addition, no link has been established to date between serum/follicular-fluid resistin levels or gene expression and PCOS where there is impaired insulin sensitivity (Panidis et al., 2004; Seow et al., 2005; Arikan et al., 2010; Zhang et al., 2011). Work over the last 10 years suggests that the predominant role of resistin in humans is in inflammatory pathways rather than glucose homeostasis. Inflammation in humans results in high circulating resistin levels with cytokines being able to induce resistin expression (Lehrke et al., 2004). In fact several recent studies link resistin levels with various inflammatory diseases, including type 2 diabetes (Yin et al., 2012), multiple sclerosis (Kraszula et al., 2012) and childhood chronic kidney disease (Nehus et al., 2012).

Circulating resistin levels are unchanged throughout the normal menstrual cycle (Dafopoulos et al., 2009). Although Chu et al. observed elevated resistin levels in obese post-menopausal women compared with obese and non-obese premenopausal women, a further study by Hong et al. found no difference between pre- and post-menopausal resistin levels independent of BMI (Chu et al., 2006; Hong et al., 2007). Interestingly, a recent study revealed elevated circulating resistin levels in post-menopausal breast cancer compared with age-matched controls (Dalamaga et al., 2013). Exogenous estrogen administration has been observed to have no effect on serum resistin levels in premenopausal women (Rechberger et al., 2004; Chalvatzas et al., 2009) but may either increase (Chu et al., 2006a, b) or not affect levels in post-menopausal women (Chalvatzas et al., 2009). The latter study also demonstrated unchanged serum resistin levels post-ovariectomy (Chalvatzas et al., 2009). Animal studies offer some clues as to the exact relationship between resistin and sex steroids with resistin messenger RNA (mRNA) levels in perigonadal adipose tissue being increased in diestrous without differences in plasma levels (Gui et al., 2004). However, perigonadal resistin mRNA levels were unaffected by ovariectomy (Nogueiras et al., 2003) but then lowered by estrogen treatment in ovariectomized mice (Gui et al., 2004; D’Eon et al., 2005; Huang et al., 2005) suggesting a role for estrogen as a negative modulator of resistin gene expression. To examine this further, we can examine data from studies involving the main sites of the reproductive axis.

Resistin expression has been detected in interstitial Leydig cells and Sertoli cells within adult rodent seminiferous tubules (Nogueiras et al., 2004). This expression of resistin is increased by gonadotrophins while, in terms of functionality, in vitro studies have demonstrated dose-dependent increases in basal and hCG-stimulated testosterone secretion after resistin administration (Nogueiras et al., 2004). Resistin expression in white adipose tissue and serum resistin levels increase in pregnant rats (Caja et al., 2005) with evidence of stimulation by progesterone in early pregnancy and inhibition in late pregnancy by estradiol (Caja and Puerta, 2007). Furthermore, chorionic gonadotrophin injection to non-pregnant rats increases resistin expression in white adipose tissue, further confirming a role for chorionic gonadotrophin and LH (as hCG acts on the LH receptor) in resistin expression (Caja et al., 2005). Centrally, resistin mRNA expression has been observed in the mouse hypothalamus and pituitary, with pituitary levels increasing to a peak during puberty in contrast to the constant levels observed in the hypothalamic during development (Morash et al., 2002).

The significance of these studies remains unclear, although it implies a role for resistin in testosterone production and pubertal hypothalamic–pituitary signalling. In addition, feedback may exist with LH and progesterone stimulating and estradiol inhibiting white adipose tissue resistin
expression. Further work is needed to clarify if resistin has some role as an endocrine mediator linking energy status with the reproductive axis.

Omentin

Omentin is a recently identified adipose hormone, expressed not only in human adipose tissue, but also strongly expressed in the placenta and ovary (Schafler et al., 2005). Current data suggest that omentin acts by activating 5′ adenosine AMPK and akt pathways as well as by binding to lactoferrin resulting in a range of effects in various cell types (Suzuki et al., 2001; Yang et al., 2006; Maruyama et al., 2012). Insulin-stimulated glucose uptake by adipocytes is enhanced by omentin in vitro (Yang et al., 2006). Furthermore, omentin has recently identified anti-inflammatory roles in vascular smooth muscle cells (Kazama et al., 2012). Plasma levels and gene expression of omentin are lower in obesity, in impaired glucose tolerance and in subjects with type 2 diabetes mellitus (de Souza Batista et al., 2007; Pan et al., 2010). There is currently limited data on the reproductive effects of omentin. A recent study observed lower circulating omentin levels in overweight insulin-resistant women with PCOS; furthermore 6 months of metformin treatment not only decreased insulin resistance (as expected) but also increased circulating omentin levels (Tan et al., 2010). Future work may clarify the role (if any) of omentin in PCOS and may explain the high omentin expression observed in the placenta and ovary.

Chemerin

Chemerin is another recently identified adipose hormone with roles in adipogenesis, energy metabolism and inflammation. Increased levels in obesity have been linked to the development of type 2 diabetes mellitus (Ernst et al., 2010; Ernst and Sinal, 2010). Chemerin expression is highest in human white adipose tissue and liver (Bozaoglu et al., 2007); however, significant expression has also been identified in human placenta (Goralski et al., 2007) as well as more recently in human ovarian follicles (granulosa, theca cells and follicle fluid) with follicular-fluid levels of chemerin being over 2-fold higher than plasma levels (Reverchon et al., 2012). Furthermore, this study also demonstrated that recombinant chemerin administration in vitro significantly decreased insulin-like growth factor-1 induced progesterone and estradiol secretion from human granulosa cells (Reverchon et al., 2012). Another recent study demonstrated that chemerin also suppresses FSH-induced progesterone and estradiol secretion from rodent cultured pre-antral follicles and granulosa cells in a PCOS-rat model, suggesting that chemerin may be involved in the negative regulation of FSH-induced follicular steroidogenesis in PCOS (Wang et al., 2012).

Overall these studies suggest a direct negative effect of chemerin on ovarian steroidogenesis (Table II). There is currently no available data on the role (if any) of chemerin in the tests and on higher apparatus of the reproductive axis. Furthermore, the explanation for the high chemerin expression observed in the placenta remains elusive.

Future research and final remarks

Gut and adipose hormones are currently proving an area of great scientific interest predominantly as a result of the obesity epidemic. This has led to increasing numbers of studies relating these metabolic hormones with the reproductive axis and so our understanding has improved greatly since Morten first observed the association between AN and amenorrhoea in 1694 (Alexander-Mott and Lumsden, 1994).

The gut and adipose hormones have direct effects on the reproductive axis (Fig. 1), while it is also important to bear in mind the effects they have on each other which play indirect roles on the reproductive axis. The key inhibitory hormone is ghrelin, although emerging inhibitory roles have been identified for obestatin, adiponectin and chemerin. Leptin and insulin are the key stimulatory players. The roles of other hormones such as PYY, GLP-1 and GIP remain controversial with roles seemingly dependent on pubertal age, sex, species and reproductive axis location (Tables I and II). These controversies will undoubtedly be investigated in depth in the coming years and furthermore the reproductive roles of less-studied hormones such as omentin, chemerin and cholecystokinin will emerge.

This review has served to detail the relationship between established, as well as emerging, gut and adipose hormones and the reproductive axis. In addition, many of the studies mentioned have associated these hormones with various reproductive pathologies. Combining this knowledge of pathology with our better understanding of healthy metabo-lo-reproductive physiology will hopefully aid the future management of patients with reproductive pathologies. These reproductive pathologies often have a great psychological and social impact on those involved and so improving our understanding and management is of great importance.

Authors’ roles

A.N.C. performed the literature search, extracted data, wrote the manuscript and gave final approval of the manuscript. C.N.J. performed the literature search, extracted data, wrote the leptin section of manuscript, critically reviewed the complete manuscript and gave final approval of the manuscript. W.S.D. designed the review, supervised the writing, critically reviewed the complete manuscript and gave final approval of the manuscript.

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