The growth hormone–insulin-like growth factor axis in pregnancy

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Abstract

The growth hormone (GH)-insulin-like growth factor (IGF) axis is one of the main drivers of mammalian growth and development. Pituitary secretion of GH is pulsatile and under positive and negative hypothalamic control, as well as stimulation from gastric-secreted acyl-ghrelin. GH acts both directly via the GH-receptor (GHR) and indirectly via stimulation of IGF1 production to induce anabolic and metabolic responses at multiple target tissues. In this review, we describe the major changes to this axis during pregnancy, with increasing GH abundance in the maternal circulation across multiple species. This stimulates the secretion of IGFs, whose bioavailability is also increased by proteolytic cleavage of their circulating binding proteins during pregnancy. These changes in turn induce maternal metabolic adaptations to pregnancy and promote placental function and fetal growth, as does exogenous GH or IGF treatment in animal models of normal and compromised pregnancy. Finally, we explore alternative approaches to enhance maternal GH abundance during pregnancy to promote maternal adaptations, placental function and hence fetal growth.

Introduction

The GH–IGF axis is essential for growth and development before and after birth and has important anabolic and metabolic functions in adults. Its role during pregnancy has been less understood and is the primary focus of this review. Circulating levels of GH and IGF1 change markedly in pregnancy, and new information is emerging on how this axis contributes to maternal adaptation to pregnancy, fetal–placental growth and development, particularly in humans and rodents. Exogenous GH or IGFs can promote fetal growth in multiple non-human species, while timing, dose and context are important in avoiding adverse effects. We conclude this review by discussing alternative strategies to increase maternal GH that may assist the translation of treatments.

The non-pregnant GH–IGF axis

Regulation of circulating GH

GH is a peptide hormone secreted by somatotrophs of the anterior pituitary that promotes cell division, regeneration and growth (Fig. 1). Pulses of GH in circulation of non-pregnant adult animals reflect regulation of GH synthesis and secretion by three main factors, GHRH, SRIF, and acyl-ghrelin (reviewed by Steyn et al. 2016). This complex pattern of regulation results in episodic, high amplitude bursts of GH secretion (around 4–8/day in non-pregnant females), separated by troughs of low-level or basal secretion throughout each 24 h period. The peptide hormones GHRH and SRIF are produced in hypothalamic neurons whose axons project to the
The non-pregnant GH–IGF1 axis. Pulsatile GH secretion is the result of alternating input from GH-releasing hormone (GHRH; stimulatory, shown by black arrows) and somatostatin (SRIF; inhibitory, shown by blue blunt-ended lines). The acylated gastric peptide ghrelin can also stimulate GH secretion indirectly, by positively inducing GHRH neuronal activity and suppressing SRIF activity and directly, by stimulating the somatotrophs of the anterior pituitary. Once in the circulation, GH has direct actions in several organs and tissues, including the liver, which is also the major site of GH-mediated production of IGF1. IGF1 can exert its actions on tissues in an autocrine or paracrine manner. A number of feedback mechanisms exist to modulate GH secretion. GH suppresses its own secretion by acting on somatotrophs and at the hypothalamus to inhibit GHRH activity and promote SRIF release, which in turn inhibits ghrelin secretion. IGF1 also suppresses GH secretion by inhibiting GHRH and stimulating SRIF secretion. A full color version of this figure is available at https://doi.org/10.1530/JOE-21-0087.

Circulating patterns of GH

The pattern and abundance of GH secretion are crucial for its biological activity. In hypophysectomized rats, that lack endogenous GH secretion, greater bone growth and weight gain are observed when the same dose of GH is given in pulses compared to continuous administration (Jansson et al. 1982). Furthermore, in children, growth rates and circulating plasma IGF1 concentrations correlate positively with peak, but not basal, GH concentrations (Hindmarsh et al. 1997).

Acute changes in circulating steroids likely underlie changes in GH patterns throughout the menstrual cycle in women, where pulsatile GH release doubles during the late follicular phase compared to the early follicular phase (Faria et al. 1992). Sex steroids regulate circulating GH through multiple pathways. For example, estrogen administration in women increases circulating GH peak amplitude by suppressing GH negative feedback on hypothalamic GHRH secretion, enhancing SRIF withdrawal-induced GH release, reducing pituitary responsiveness to SRIF and increasing potency of exogenous GHRH (Bray et al. 2001, Veldhuis et al. 2004). Interestingly, in two studies of frequently sampled, conscious female rats, GH profiles did not differ between estrous cycle phases (Saunders et al. 1976, Clark et al. 1987). In addition to acute responses to steroids, the steroid hormone environment during early development induces sex-specific differences in the number of GHRH neurons and somatotrophs and their sensitivity to post-pubertal steroids (reviewed by Chown et al. 2004). These result in...
sex-specific patterns of circulating GH, including greater amplitude and more regular pulses of GH secretion in males than females in both rats and humans (reviewed by Gatford et al. 1998).

**GH signaling**

GH mediates several important physiological processes, including somatic growth and development, carbohydrate and lipid metabolism, by binding to GHR (Fig. 1). Once released into the circulation, GH binds to GH binding protein (GHBP), which is produced through the proteolytic cleavage of the extracellular domain of the GHR, and stabilizes the hormone and prolongs its bioavailability (Baumann et al. 1987, Sotiropoulos et al. 1993). GH must be released from GHBP in order to bind to membrane-bound full-length GHR, inducing downstream signaling via the JAK-STAT pathway (Smits et al. 1996, Brooks et al. 2014). The phosphorylated STAT proteins dimerize and are subsequently translocated to the nucleus where they regulate transcription of GH target proteins. Stat5b is the major mediator of GH action and upregulates transcription of IGF1 (Woelfle et al. 2003), which in turn mediates many growth-promoting actions of GH, as discussed below. IGF2 is also positively regulated by GH, such that children and adult humans with the GH-insensitivity or Laron syndrome have circulating concentrations below the 5th centile of reference values (Savage et al. 1993).

**IGFs and IGFBPs**

IGF1 and IGF2 are peptide growth factors that stimulate growth and development of vertebrates through metabolic, mitogenic, anti-apoptotic and differentiating effects on a wide range of cell types (Laron 2001, Harris & Westwood 2012). The endocrine, paracrine and autocrine actions of the IGFs are typically exerted through interactions with two different receptors, IGFIR and IGF type 2 receptor (IGF2R). IGFs can also bind to the insulin receptor (IR) and IGFIR/IR hybrid receptors (Denley et al. 2005). IGF1R has high binding affinity for IGF1, lower affinity for IGF2 and little affinity for insulin, while the IGF2R has high binding affinity for IGF2, very low affinity for IGF1 and does not bind insulin (Denley et al. 2005). Binding of IGF1 to IGF1R activates the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/AKT) and mitogen-activated protein kinase (MAPK) signaling pathways (Chitnis et al. 2008), whereas binding of IGF2 to IGF2R can either activate G-protein-coupled signaling pathways or result in IGF2 degradation (Okamoto et al. 1990).

The bioavailability and actions of the IGFs in circulation are regulated by a family of six insulin-like growth factor-binding proteins (IGFBPs). The majority of serum IGFs are bound to IGFBP-3 in a heterotrimeric complex with acid-labile subunit (ALS). A similar heterotrimeric complex can also form with IGFBP-5. These ternary complexes are mainly restricted to the vasculature and extend the half-life of the IGFs to ~15 h, compared to ~10 min for ‘free’ IGF1 not complexed to IGFBP (Guler et al. 1989). Most remaining circulating IGFs are found in binary complexes with other IGFBPs, which can exit the circulation rapidly but still extend half-life of the IGFs to ~20 to 30 min (Guler et al. 1989). Depending on the IGFBP and cell types, IGFBPs either block or promote binding of IGFs to their receptors, while IGFBP proteases regulate bioavailability by degrading IGFBPs to release IGFs (Bach 2018). Interestingly, the activity of this protease is increased in pregnancy, increasing IGF1 bioavailability (Giudice et al. 1990), as discussed later. In addition to regulation of IGF bioavailability and activity, IGFBPs also have IGF-independent activity (reviewed by Firth & Baxter 2002, Bach 2018).

Postnatally, GH is one of the main promoters of hepatic IGF1 synthesis. However, energy status and protein intake are also important regulating factors, and fasting decreases serum IGF1 concentrations (reviewed by Thissen et al. 1994). IGF1 mediates most GH effects on skeletal metabolism, including stimulation of amino acid transport and protein synthesis, free fatty acid uptake and oxidation, and inhibits protein breakdown (reviewed by Adamo & Farrar 2006). Although IGF1 can stimulate preadipocyte differentiation, at physiological concentrations its actions on mature adipose tissue appear to be limited (reviewed by Clemmons 2012). IGF1 may also be involved in the regulation of carbohydrate metabolism through inhibition of GH secretion and by enhancing insulin suppression of hepatic gluconeogenesis, reported in humans (Clemmons 2004, Yuen & Dunger 2007). In bone, IGF1 promotes chondrogenesis and increases bone formation by differentiated osteoblasts (reviewed by Giustina et al. 2008).

There is substantial evidence that both IGFs play a crucial role in placental and fetal development. IGF1 is more important than GH for fetal growth, and loss-of-function IGF1 gene mutations are associated with severe intrauterine growth restriction (Woods et al. 1996). The fact that congenital GH deficiency or resistance in humans are associated with postnatal growth failure, but not substantially reduced intrauterine growth, is consistent with IGF1 regulation being less GH-dependent during intrauterine life, although circulating IGF abundance was...
not reported (Savage et al. 1993, Wajnrajch et al. 1996). Both IGFs promote cell differentiation and growth in vitro, demonstrated in primary cultures of ovine and human placental cells (trophectoderm and fibroblasts respectively, Miller et al. 2005, Kim et al. 2008). Effects of IGFs on the placenta are likely to contribute to IGF stimulation of fetal growth; fetal and placental responses to IGFs will be covered in more detail in subsequent sections.

The pregnant GH–IGF axis

Pregnancy

Maternal changes
A female undergoes significant anatomical and physiological adaptations in order to support the developing conceptus while still being able to cope with the increased metabolic and physical demands of pregnancy (reviewed by Napso et al. 2018). Major cardiovascular adaptations in humans include a 25–30% fall in maternal systemic vascular resistance in early human pregnancy, in parallel with a ~30–50% increase in cardiac output and activation of the renin-angiotensin-aldosterone system to increase plasma and blood volumes, increasing uterine blood flow and placental perfusion (Sanghavi & Rutherford 2014). Cardiac output and stroke volume similarly increase 30–50% by mid-pregnancy in mice (Aasa et al. 2015). Pregnancy also requires adaptation of the maternal respiratory system, with increased maternal ventilation to meet increasing oxygen demand (Weinberger et al. 1980), and gastrointestinal system, where gastric contractility and gastrointestinal transit time slow down, possibly to allow a longer time for absorption of nutrients (Wald et al. 1982). Pregnancy-associated increases in maternal food intake and decreases in energy expenditure in humans and rodents also allow maintenance of a positive energy balance and supply of nutrients to the fetus (Kopp-Hoolihan et al. 1999, Ladyman & Grattan 2004, Li et al. 2021). In order to cope with the increased energy demands of pregnancy, significant alterations occur to the maternal metabolic and endocrine state. Maternal whole-body and peripheral insulin resistance develop during mid-late human pregnancy (Stanley et al. 1998) in response to placentaly produced hormones including GH (Liao et al. 2016). This state of insulin resistance ensures availability of maternal glucose for transfer across the placenta to meet fetal demands, as well as to maternal tissues (Butte 2000). Mice and rats are likewise insulin resistant during mid-late pregnancy, with reductions of 50–80% in whole-body insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp although this begins to normalize near term in mice (Ramos & Herrera 1995, Musial et al. 2016). Although whole-body insulin sensitivity decreases, the insulin sensitivity of maternal white adipose tissue increases, together with hyperplasia of insulin-secreting pancreatic β-cells in early pregnancy and increased maternal insulin secretion, which enhances insulin-stimulated lipid storage (Butte 2000, Ackermann & Gannon 2007). On average, pregnant women gain ~2.5 kg in fat mass (Kopp-Hoolihan et al. 1999), and in normal-weight women s.c. adipocyte size increases without changes in adipocyte numbers (Svensson et al. 2016). In rats, weights of different white adipose depots increase by 30–100% by late pregnancy, and gonadal (~29%) and retroperitoneal (~158%), but not mesenteric, adipocytes of late pregnant rats are larger than those of non-pregnant rats (Ladyman & Grattan 2004, Pujol et al. 2005). The majority of maternal weight gain occurs in the last half of pregnancy in humans and rodents (Fig. 2). In litter-bearing species, including mice, the total weight of fetuses and placentas accounts for approximately 30% of maternal weight at term. However, in species like humans and sheep, that typically have singleton pregnancies, only about 6–9% of maternal weight is attributable to the fully developed fetus and placenta (Fig. 2, Fowden & Moore 2012).

Fetal development
The process of conceptus growth and development within the uterus from fertilization until birth can be categorized into three phases: pre-implantation, embryonic and fetal (Fig. 2). Prior to implantation, the fertilized zygote undergoes rapid cell cleavage without increasing in size to form a blastocyst, which implants into the maternal uterine decidua (endometrium). Organ primordia form during the embryonic phase after implantation and undergo further extensive expansion and remodeling during the fetal phase of development until term birth, typically at 37–41 weeks after the last menstrual period in humans (Jukic et al. 2013). Fetal development in mice (Fig. 2) occurs during a much shorter gestation than humans (19–21 days post-conception, depending on the strain, Murray et al. 2010), with the morning after mating, when the copulation plug is detected in the female, designated as gestational day (GD)0.5.

Placental development
The placenta is the interface between the mother and fetus and performs a number of crucial functions that are essential for the maintenance of a successful pregnancy. It mediates exchange of nutrients and gases between
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For example, placenta-specific knockdown of chorionic somatomammotropin hormone in sheep embryos reduced placental expression of glucose transporter genes and fetal weight (↓22%) at early gestation (Jeckel et al. 2018), leading to reduced placental (↓52%) and fetal (↓32%) weights at late gestation (Baker et al. 2016). Both humans and mice have hemochorial placentas, where maternal blood is in direct contact with the chorionic trophoblast, and maternal and fetal circulations are separated only by fetal cell layers (Georgiades et al. 2002). Providing high-volume low-turbulence blood supply to the maternal side of the placenta requires modification of uterine spiral arteries from tight spirals with high resistance and low capacity, to wide conduits providing low resistance and high capacity (Brosens et al. 1967, Thaler et al. 1990). In humans and rodents, placental weights peak somewhat earlier than those of the fetus (Thompson et al. 2007, Mu et al. 2008), although placental remodeling and increases in transport capacity continue to term (Fig. 2).

The GH–IGF axis in normal pregnancy

The GH/IGF axis undergoes substantial changes during pregnancy in humans (Fig. 3A), and in most mammalian species studied to date, including rodents (Fig. 3B). These changes facilitate maternal adaptation to pregnancy in addition to promoting fetal and placental growth and function. Neither GH nor IGF1 cross the placenta (Laron et al. 1966, Fholenhag et al. 1994), so effects of GH and IGF1 in maternal circulation on fetal growth are via maternal and placental responses, not direct effects on the fetus.

One of the most striking changes is the increase in circulating GH, seen across multiple species, and interestingly seen both in species that express and do not express GH in the placenta. Placental GH gene expression is restricted to anthropoid primates, including humans, which express a cluster of related genes including GH and chorionic somatomammotropins, or placental lactogens and to a subset of ruminants including sheep and goat, although not cow (Papper et al. 2009). In contrast, the rodent placenta expresses multiple genes of the prolactin gene family, but not GH (Haig 2008), and signaling via the prolactin receptor is important for maternal adaptations to pregnancy, as well as pregnancy recognition, in these species (Napso et al. 2018). Prolactin and GH share some homology and are likely derived from a common ancestral gene, and at least within a species, these peptides mostly have low affinity for each other’s receptors (Soares et al. 2007). Intriguingly, the anthropoid placenta expressed GH variant, GH2, which has multiple amino acid sequence

Figure 2
Changes in fetal (green line), placental (purple dashes) and maternal weight (black dots) during pregnancy in humans (top figure) and mice (bottom graph) and periods of conceptus development. Term in humans is typically 37–41 weeks from the last menstrual period (LMP), implantation occurs around 6 days post-ovulation, and the embryonic period including gastrulation and organogenesis is completed by 10 weeks of gestation/since LMP, at the end of the first trimester. Fetal growth and maturation then continue until birth. In mice, gestation takes approximately 20 days, and implantation occurs around gestational day (GD)4.5, at the beginning of the embryonic period. Gastrulation occurs on ~GD6.5, followed by organogenesis on ~GD8.5, and the fetal growth phase begins by GD14.5 and continues until birth. Maternal, fetal and placental weight data extracted from Thompson et al. (2007), Mu et al. (2008), Kiserud et al. (2017), Ladyman et al. (2018); mouse fetal and placental weight data are in CD-1 mice (Mu et al. 2008); mouse maternal weight data is in C57BL/6j mice (Ladyman et al. 2018). A full color version of this figure is available at https://doi.org/10.1530/JOE-21-0087.
differences to the pituitary gene (GH1), binds to both human GH and prolactin receptors (Papper et al. 2009).

During early human pregnancy, pulses of pituitary-derived GH are superimposed on elevated basal GH as a result of continuous placental release of the variant form of GH (GH-V), which is the product of the GH2 gene and can be detected by late in the first trimester (Fig. 4A, Eriksson et al. 1989). At ~11 weeks of gestation, circulating GH remains pulsatile and is largely pituitary-derived, but at ~15 to 17 weeks of gestation, pituitary GH secretion becomes suppressed by negative feedback due to placental GH-V production (Fig. 4A, Eriksson et al. 1989). Unlike the pulsatile pattern of pituitary-derived GH secretion, placental GH secretion is continuous, resulting in elevated and non-pulsatile maternal circulating GH in the second half of human pregnancy (Fig. 4A, Eriksson et al. 1989). Circulating GH patterns during pregnancy have also been characterized in rodents (Fig. 4B and C). In contrast to humans, circulating GH profiles remain pulsatile throughout pregnancy in rats (Fig. 3B, Saunders et al. 1976, El-Kasti et al. 2008) and mice (Fig. 4C, Gatford et al. 2017). In these species, the increase in average circulating GH concentrations reflects elevated basal GH secretion (El-Kasti et al. 2008, Gatford et al. 2017). Although the number and amplitude of pulsatile GH secretion remain unchanged throughout murine pregnancy, they decrease in regularity (Gatford et al. 2017, Kaur et al. 2020). Despite the importance of GH during pregnancy in many mammalian species, the mechanisms driving increased maternal GH in non-human species are not yet completely understood, although the timing of changes in circulating patterns is similar to that of maternal blood perfusing the placenta, suggestive of placental signal/s inducing elevated maternal pituitary GH secretion (Gatford et al. 2017).

Changes in circulating maternal IGF concentrations during pregnancy are highly variable across species (Sferruzzi-Perri et al. 2011). In humans, reported changes in maternal IGF1 concentrations during the first two trimesters are inconsistent between studies, with some reporting a modest or gradual rise in concentrations but others finding no change or even decreased concentrations compared to pre-pregnancy levels (e.g. Gargosky et al. 1990, Mirlesse et al. 1993, Monaghan et al. 2004, Gatford et al. 2014). During the third trimester, however, the majority
of studies have reported substantially (45–200%) higher maternal circulating IGF1 concentrations compared with levels in non-pregnant women. In rodents, IGF1 concentrations gradually rise during the first half of pregnancy, followed by a rapid decline after falling below non-pregnant levels by late pregnancy (Davenport et al. 1990). In rabbits and guinea pigs, on the other hand, maternal IGF1 gradually rises during the first half of pregnancy, followed by a rapid decline after...
mid-pregnancy (Nason et al. 1996, Sohlstrom et al. 1998), while circulating IGF1 concentrations do not change during pregnancy in sheep and cattle (Wallace et al. 1997, Perry et al. 2002). Although placental IGF1 expression is minimal (Han & Carter 2000), other hormones produced by the placenta (like GH-V in humans or placental lactogen in many species) may still influence maternal IGF1 concentrations and actions. This includes increased IGF1 clearance and bioavailability to receptors as a consequence of proteolysis of IGFBPs, which in humans increases around ~6–8 weeks of gestation (Giudice et al. 1990, Boldt & Conover 2007). Similarly, in rats, circulating concentrations of most IGFBPs are significantly reduced in the latter half of pregnancy, and this likely contributes to more rapid clearance of maternal IGF1 (Davenport et al. 1990).

In contrast to IGF1, maternal IGF2 concentrations increase throughout pregnancy in most species and are higher than circulating concentrations of IGF1 (Nason et al. 1996, Sohlstrom et al. 1998, Perry et al. 2002). The placenta is a major source of maternal IGF2 in animals with a hemochorial placenta. In women, IGF2 is produced by the syncytiotrophoblast layer of the placenta, which is in direct contact with maternal blood (Han & Carter 2000, Nayak & Giudice 2003, Pringle & Roberts 2007). In humans, where circulating IGF2 remains high into adulthood, maternal IGF2 concentrations are slightly lower at 15 weeks gestation than in men and non-pregnant women, probably due to hemodilution (Gatford et al. 2014).

Mechanisms for increased GH during pregnancy

As discussed above, placental production of GH-V is responsible for the change in maternal circulating patterns of GH during human pregnancy (Figs 3 and 4A). The human GH gene cluster consists of five structurally similar genes: the pituitary GH variant GH-N (also known as GH-I), the placental variant (GH-V), chorionic somatomammotropin hormone 1 (CSH1/hCS-A), chorionic somatomammotropin hormone 2 (CSH2/hCS-B), and a pseudogene known as chorionic somatomammotropin-like hormone (Miller & Eberhardt 1983). Most other mammalian species, however, do not express a placental GH gene (Papper et al. 2009). The increases in circulating GH concentration and basal GH secretion in pregnant mice (Gatford et al. 2017) coincide with the formation of the choioallantoic placenta and initiation of placental blood flow (Pringle & Roberts 2007). This suggests, therefore, that the pregnancy-associated increase in GH secretion observed in species like rats (Fig. 4B) and mice (Fig. 4C) is driven by another placental-derived factor (Gatford et al. 2017). A number of candidate placental products might contribute to the increase in GH secretion, including two factors important for endogenous GH secretion, GHRH and ghrelin (Barnard et al. 1993, Gualillo et al. 2001). We have recently reported that circulating acyl-ghrelin does not increase during murine pregnancy and is therefore unlikely to drive increased pituitary GH secretion (Kaur et al. 2020). It is possible that placentally produced ghrelin may have local actions, since the mouse placenta expresses MBOAT4 mRNA at low levels (Kaur et al. 2020), while gene and protein expression of the acyl-ghrelin receptor, GHSR-1a, has been identified in the placenta of rats (Gualillo et al. 2001); however, this is yet to be directly demonstrated.

Responses to exogenous GH and IGF during pregnancy

Fetal growth

Maternal treatment with GH during pregnancy increases fetal growth in a number of species, provided maternal nutrient intake is not limited (Supplementary Table 1, see section on supplementary materials given at the end of this article). In studies where animals are treated with GH proteins derived from other species, results are variable, likely reflecting different receptor affinities to species-specific GH variants. For example, in the majority of published studies, bovine GH does not promote fetal growth in rats, whereas recombinant human GH given intermittently through the majority of pregnancy does promote fetal growth in rat pregnancies (Supplementary Table 1). The effects of GH treatment on fetal growth is also dependent on the pattern of administration, although this is also species-dependent. Continuous GH administration does not alter maternal IGF1 concentrations, fetal or placental weights in rats (Gargosky et al. 1991), whereas intermittent (once or twice daily) recombinant human GH administration throughout the majority of pregnancy does increase fetal growth (Jorgensen et al. 1991, Spencer et al. 1994). The mechanisms underlying differential effects of intermittent and continuous GH administration on pregnancy outcomes in rats are unclear but are consistent with pattern-dependent responses to GH in non-pregnant animals and humans (Jansson et al. 1982, Bick et al. 1992, Hindmarsh et al. 1997). In mice, continuous infusion of recombinant human placental GH (GH-V) from GD12.5 to GD18.5 did not promote fetal growth (Liao et al. 2016), and responses to exogenous pituitary GH have not been reported. It is therefore unclear whether the lack of fetal
growth promotion by GH-V reflects differences in receptor affinities between pituitary and placental GH (Ray et al. 1990), inter-species differences, or is a consequence of continuous administration. Interestingly, this dose of GH-V did induce insulin resistance in mouse dams (Liao et al. 2016), consistent with its putative effects on the mother in human pregnancy, and the whole-body and skeletal muscle insulin resistance seen in transgenic mice expressing GH-V (Barbour et al. 2002, 2004).

In pigs, where the majority of production animal studies have been performed (Supplementary Table 1), maternal GH administration via daily injections from early to mid-pregnancy increased fetal growth and birth weight when treatment was continued for the majority of gestation (Rehfeldt et al. 2001, Gatford et al. 2004). Birth weight was not increased when pigs were treated with GH only in early-mid pregnancy (e.g. Rehfeldt et al. 1993, Gatford et al. 2004), although fetuses were heavier and/or larger immediately following treatment in several studies (e.g. Steer et al. 1995, Gatford et al. 2000), suggesting that fetal growth is not maintained after maternal GH falls. In sheep, administration of a sustained release bovine GH preparation near breeding, active for ~30 days of the ~150-day pregnancy, improved birth weight by approximately 10% (Costine et al. 2005, Koch et al. 2010). Although some studies have reported a similar increase in fetal weight with intermittent bovine GH administration to ewes during late gestation, these responses are not consistent across studies (Supplementary Table 1).

Adverse effects of GH administration have also been reported in a subset of studies. Suppressed maternal appetite has been reported in sheep and pigs, particularly when high doses of GH are started in late pregnancy. In a model of IUGR induced by overfeeding young ewes, treatment with bovine GH (0.14 mg/kg twice daily) suppressed maternal appetite by 14–30% during periods of GH-treatment only (Wallace et al. 2006). Of concern, 3 of 15 ewes receiving GH between GD95 and GD125, in late pregnancy, developed severe inappetence and polyhydramnios, requiring euthanasia, with milder symptoms in another ewe resolving after GH treatment ceased (Wallace et al. 2006). Development of inappetence also required removal from study of 2 of 10 ewes treated with bovine GH (0.2 mg/kg daily) in late pregnancy (GD100–GD128) in a model of IUGR induced by placental embolization (de Boo et al. 2008). In addition to 9–20% decreases in food intake in sows that were treated with 6 mg/day rPGH starting at GD108, Cromwell and coauthors reported multiple sow deaths during and around labor, and noted that these were related to heat stress (Cromwell et al. 1992). In contrast, maternal weights were unchanged or increased, with unchanged food intake, in studies of GH-treated rats and mice and in most studies in pigs and sheep (Supplementary Table 1), consistent with the anabolic effects of GH in other settings. Finally, development of hydranencephalic brain lesions in three of five IUGR fetuses whose mothers received bovine GH (0.2 mg/kg daily) in late pregnancy (GD100–GD128) is an issue of major clinical concern (de Boo et al. 2008), while a 25% reduction in fetal brain weight was reported in IUGR fetuses from adolescent sheep pregnancies also treated with GH in late pregnancy (Wallace et al. 2006). These results contrast with increased fetal brain weight and cortical development in rats treated with GH through the second and final third of pregnancy, with evidence of earlier maturation and enhanced function for markers of cortically mediated behaviours (Clendinnen & Eayrs 1961, Zamenhof et al. 1966). Fetal brain lesions have not been reported in other studies, and progeny of GH-treated pigs have normal survival and no obvious behavioral defects. Overall, these results suggest caution in commencing maternal GH treatment in late pregnancy, particularly in the setting of IUGR, although fetal brain lesions observed by de Boo and colleagues may in part be induced by the combination of stimulated growth in the setting of restricted fetal brain blood supply due to implantation of carotid artery and jugular vein catheters (de Boo et al. 2008). It is also important to ensure fetal and progeny brain and/or neurodevelopmental outcomes, as well as maternal health, are assessed in studies of maternal GH administration, so that efficacious and safe treatment strategies can be developed.

Similar to effects of exogenous GH, exogenous maternal IGF1 treatment can promote fetal growth but results vary according to species and gestational age. In rats, continuous or intermittent administration of rIGF1 throughout or in the second half of pregnancy does not alter fetal weight (Supplementary Table 1). Interestingly, continuous IGF1 infusion in pregnant mice did remove the constraint of fetal growth due to large litter size (Gluckman et al. 1992). In guinea pigs, early-mid pregnancy treatment with IGF1 increases fetal weight near the end of treatment at GD40 (term is ~GD70), provided dams are not undernourished, and increases in fetal weight are maintained to near term at GD62 (Supplementary Table 1). Responses to maternal IGF2 treatment have been less consistent, with increases in fetal weight seen after treatment and near term in ad libitum-fed dams in some but not all studies, despite the fact that these have all been in guinea pigs and administered rhIGF2 with a similar dose, timing and administration route (Supplementary Table 1).
One feature of many studies of maternal GH or IGF1 administration which suggests potential clinical relevance is the ability of these hormones to promote fetal growth in models in which fetal growth is restricted. For example, fetal size and birth weight in pigs are constrained by adolescent first pregnancy, limited uterine capacity, large litter size and limited maternal nutrition (Town et al. 2005, Gatford et al. 2009). Consequently, birth weight decreases with increasing litter size in pigs (Gatford et al. 2004), as it does in mice (Gluckman et al. 1992). This constraint of fetal growth with increasing litter size is less pronounced in pigs treated with GH (Gatford et al. 2004). Similarly, in pregnant sheep with IUGR induced via placental embolization, twice-daily maternal GH administration increased fetal growth in terms of fetal weight and length (de Boo et al. 2008). These fetal growth-promoting effects could be mediated by GH acting directly on tissues via its own receptors or indirectly, via increased secretion of IGF1. In mice, increased maternal plasma IGF1 concentrations, either endogenously or exogenously, reduce the maternal constraint induced by litter size on fetal growth (Gluckman et al. 1992).

Placental growth and function

Growth-promoting effects of GH may be mediated by the placenta, since maternal GH treatment also increases placental growth and promotes placental function in a number of species, although not as yet reported in rats or mice (Supplementary Table 2). In pigs, maternal GH administration during early-mid pregnancy increases measures of placental size or exchange area (Sterle et al. 1995) as well as increased protein expression of glucose and amino acid transporters GLUT1 (SLC2A1) and SNAT2 (SLC38A2) in the fetal-facing placental membrane (Tung et al. 2012), which would be expected to enhance fetal nutrient supply. In sheep, short-term (10 days) maternal GH administration during mid-late pregnancy increases placental capacity for simple and facilitated diffusion (responsible for glucose transport), compared to saline-treated controls (Harding et al. 1997). The increase in simple diffusion in this study was greater than the effect on facilitated diffusion, suggesting that the improved placental transport capacity was likely the result of increased placental surface area or reduced placental barrier thickness, rather than increased activity of placental nutrient transporters (Harding et al. 1997).

Maternal treatment with IGF1 or IGF2 during pregnancy also promotes fetal growth by enhancing placental growth, fetal nutrient availability and uptake, with the strongest effects reported in guinea pigs and some ovine models of fetal growth restriction (Supplementary Table 2, Roberts et al. 2008, Sferruzzi-Perri et al. 2011). In guinea pigs, maternal IGF1 infusion during early pregnancy increases placental uptake and transfer of glucose at mid-pregnancy and near-term (Sferruzzi-Perri et al. 2007a,b). Increased placental uptake of amino acids was detected shortly after treatment, likely mediated in part by increased placental gene expression of Slc38a2 (Sferruzzi-Perri et al. 2007b). In another guinea pig study, maternal IGF2 treatment increased the volume and surface area of the labyrinth zone in the near-term placenta, which is likely to enhance placental capacity for materno-fetal nutrient and gas exchange during this crucial growth stage (Sferruzzi-Perri et al. 2006). The importance of IGF2 in promoting placental function is further highlighted in mouse studies where deletion of the placental-specific P0 Igf2 promoter, which blocks placental labyrinth-specific Igf2 production, reduces placental growth and nutrient transfer capacity and restricts fetal growth (Constância et al. 2002, Sibley et al. 2004). IGF2 is also important for placental hormone production. In a recently developed knockout mouse, tissue-specific deletion of Igf2 in the murine placental junctional zone, the placental region that produces hormones in rodents, reduces expression of the hormone placental lactogen 2 and increases expression of the steroid metabolising enzyme CYP17a1 (Aykroyd et al. 2020).

The role of IGFs in enhancing placental function is further supported by in vitro studies in trophoblast cell lines from humans, pigs and mice, where both IGF1 and 2 promote proliferation, invasion/migration and exhibit anti-apoptotic properties and are therefore likely to promote implantation and overall placental growth (Kanai-Azuma et al. 1993, Miller et al. 2005, Kim et al. 2008). The IGFs also enhance the endocrine capacity of trophoblasts in culture, such that the secretion of progesterone, hCG, and placental lactogen by human trophoblasts increases after IGF1 treatment (Maruo et al. 1995). In mice, IGF2 stimulates the differentiation of ectoplacental cone cells into endocrine trophoblast giant cells, whilst IGF2 regulates signaling pathways in ovine trophoderm cells that suggest a role in conceptus growth and differentiation (Kanai-Azuma et al. 1993, Kim et al. 2008). Therefore, the results of both in vitro and experimental animal in vivo studies support the suggestion that stimulation of the GH/IGF axis can promote fetal and placental growth, as well as enhance hormonal secretion and nutrient transport capacity of the placenta (reviewed by Sferruzzi-Perri et al. 2017).
Strategies to increase maternal GH

Despite the clear evidence that GH and IGF1 promote fetal growth and placental function, their lack of bioactivity when fed limits potential application in clinical and animal production systems. Given the adverse consequences of high circulating GH before and around mating on fertility, demonstrated in mice transgenic for an inducible ovine GH gene (Thomas et al. 2001), it is likely that strategies to increase maternal GH after establishment of pregnancy will be most successful in improving fetal growth and reproductive performance. We know in humans that interventions to manage pregnancy complications are more acceptable to patients if delivered orally than by injection (Rowan et al. 2008). Considerations around animal welfare as well as workload likewise make repeated injections unattractive in animal production systems. Alternative approaches to increase endogenous GH have therefore been explored, including some studies in pregnancy, with evidence for promotion of fetal or progeny growth without adverse maternal effects (Supplementary Table 3).

One logical target is to increase circulating abundance of hormones that promote GH synthesis and secretion, specifically GHRH or acyl-ghrelin. Administration of GHRH to pregnant sows from GD102 to GD112 induced pulses of GH in maternal circulation up to 4 h afterwards (Supplementary Table 3, Etienne et al. 1992). This preparation did not increase piglet birth weight, possibly reflecting the relatively short period of treatment, but progeny of GHRH-treated sows were heavier than control progeny from 13 days of age and a greater proportion survived to weaning (Supplementary Table 3, Etienne et al. 1992). Like GH or IGF1, the peptide hormone GHRH is not orally active, and therefore to avoid the need for repeated injections, electroporation of pregnant females with a GHRH plasmid has been evaluated in experimental settings. In rats, inducing incorporation of cDNA for a protease-resistant variant of human GHRH in dams, did not affect pregnancy outcomes at birth, but offspring were heavier than controls by 2 weeks of age (Supplementary Table 3, Khan et al. 2002). In pigs, progeny from gilts transduced with a GHRH plasmid at GD85 in their first pregnancy were heavier than control progeny from birth until reaching slaughter weight at ~4 months of age (Supplementary Table 3, Khan et al. 2003). Furthermore, increases in progeny growth rate from GHRH-transduced dams maintained over three generations, and numbers of piglets born alive were increased in litters from the second and third pregnancies, consistent with ongoing expression of the plasmid in dams (Supplementary Table 3, Person et al. 2008). Also importantly, no adverse maternal effects were reported in these studies of GHRH-transduced dams studied throughout the three pregnancies following GHRH plasmid incorporation (Person et al. 2008). The variant form of human GHRH, used to electroporate sows in the studies described above, crosses the rat placenta (Fiorotto et al. 2006), while circulating GHRH is elevated at birth in progeny of GHRH-plasmid-treated pigs (Khan et al. 2003), and circulating IGF1 is elevated in progeny of GHRH-plasmid-treated rats (Khan et al. 2002). Effects of elevated maternal GHRH are therefore likely to reflect direct effects on the developing fetus in addition to effects on the maternal GH axis. In particular, altered pituitary development in progeny might explain faster postnatal growth. In progeny of both rat and pig dams expressing high levels of GHRH, pituitary somatotroph and lactotroph numbers as well as GH and prolactin mRNA expression are higher than in controls, and circulating IGF1 is also elevated, implying higher circulating GH (Khan et al. 2002, 2003).

Treatment with acyl-ghrelin promotes GH secretion in multiple species (Kojima et al. 1999, Arvat et al. 2001, Melendez et al. 2006, Nass et al. 2008), and administration of acyl-ghrelin during pregnancy promotes birth weight and/or postnatal growth of progeny in rodents (Supplementary Table 3, Hayashida et al. 2002, Fernandez-Fernandez et al. 2005, Nakahara et al. 2006). For example, administering 9–12 nmol/day of acyl-ghrelin either by repeated injections (3/day) or via continuous infusion GD14 or GD15 until delivery increased fetal growth by ~10%, while immunising dams against ghrelin reduced fetal growth by a similar magnitude (Supplementary Table 3, Nakahara et al. 2006). In sheep, i.m. administration of acyl-ghrelin for the last 10 days of gestation resulted in higher serum GH concentrations compared to non-treated controls (Supplementary Table 3, Melendez et al. 2006). Like maternal GH administration, use of acyl-ghrelin may need to continue into later pregnancy to improve pregnancy outcomes, since acyl-ghrelin administration during only the first half of rat pregnancy, with a dose that doubled circulating GH concentrations in males, reduced litter size by 19% without altering birthweight (Supplementary Table 3, Fernandez-Fernandez et al. 2005). Interestingly, pregnant mice lacking the Mboat4 gene, which consequently cannot convert ghrelin to acyl-ghrelin (Trivedi et al. 2015), still undergo a similar increase in GH during late pregnancy as WT mice, although with somewhat lower GH concentrations at late pregnancy, suggesting that acyl-ghrelin is not required for increases in GH during pregnancy (Trivedi et al. 2015).
Maternal weight gain and litter size were also normal in Mboat4 knockout dams (Trivedi et al. 2015), although fetal weight and circulating patterns of GH have not been reported. Nevertheless, elevated acyl-ghrelin in later pregnancy can clearly promote fetal growth.

In addition to potential effects via stimulation of maternal GH, acyl-ghrelin may in part stimulate fetal growth through central stimulation of feed intake (Nakazato et al. 2001). However, although maternal feed intake is increased in acyl-ghrelin-treated pregnant mice, fetal growth is increased by maternal acyl-ghrelin treatment even when dams are pair-fed to untreated controls (Supplementary Table 3, Nakahara et al. 2006). Endogenous acyl-ghrelin also plays an important role in metabolic adaptations to maintain circulating glucose in response to calorie restriction, particularly in pregnancy, and Mboat4 knockout dams were not able to maintain pregnancies when food intake was restricted to 50% of ad libitum intake (Trivedi et al. 2017). Treatment with acyl-ghrelin might therefore potentially increase fetal growth through direct action on the placenta, increasing maternal GH, stimulating maternal food intake and/or effects on maternal metabolism.

To our knowledge, we have conducted the only study attempting to increase maternal acyl-ghrelin through an orally-active intervention during pregnancy (Kaur et al. 2020). Our approach of dietary octanoic acid (C8-MCFA) supplementation during pregnancy was based on previous studies where feeding male mice a diet enriched with the C8 medium chain fatty acid, octanoic acid (C8-MCFA), or the triglyceride containing this fatty acid, glyceryl trioctanoate, increases octanoylation of ghrelin in the stomach and the abundance of circulating acyl-ghrelin (Nishi et al. 2005, Gutierrez et al. 2008, Kirchner et al. 2009). Similarly, enteral feeding of a single meal, containing 2.8 g of octanoic acid, over a period of 3 h in humans increased plasma acyl-ghrelin concentrations by more than 30% (Ashitani et al. 2009). Although we provided C8-MCFA at levels similar to those used in previous murine studies, we did not observe increases in circulating acyl-ghrelin, changes in maternal GH profiles, or increases in fetal or placental growth in our mice (Supplementary Table 3, Kaur et al. 2020). The only dietary approach shown to elevate both circulating acyl-ghrelin and GH concentrations has been reported in non-pregnant pigs. Feeding a diet enriched with mixed medium chain fatty acids increased circulating acyl-ghrelin concentrations in young weaned pigs, growing males (~35 kg) and older growing females (~60 kg), although plasma GH concentrations in blood pooled from samples collected at 15-min intervals across a 4-h period, were only increased in the first two cohorts (Miller et al. 2016). Whether this diet can increase circulating acyl-ghrelin, GH or fetal growth if fed during pregnancy has not been evaluated and remains an important area for future investigation.

Conclusions

The maternal GH–IGF axis undergoes profound upregulation in pregnancy, with elevated circulating GH reflecting placental production in humans and pituitary production in rodents. The signal/s that induce increased maternal GH in rodents are not yet identified, although the timing of changes in circulating patterns suggests that they are induced by placently derived factor/s (Gatford et al. 2017). Lower maternal circulating GH-V and IGF1 concentrations in late pregnancy in pregnancies with a growth-restricted infant (Mirlesse et al. 1993, McIntyre et al. 2000) are probably a consequence of the fact that poor placental growth and function are major causes of fetal growth restriction (Kramer 2003), although the lack of placental GH might further reduce fetal growth and maternal adaptations to pregnancy. Exogenous GH, IGF1 and IGF2 promote fetal growth in non-human studies, at least in part by promoting placental growth and function. Some adverse maternal and fetal effects have been reported when maternal GH treatment was commenced in late gestation, with the greatest benefits seen when GH treatment is sustained from early-mid pregnancy onwards. The need to inject or infuse these peptide hormones has limited application of these therapies to promote fetal growth in clinical and animal production settings. New approaches to increase maternal GH during pregnancy are being developed, such as incorporation of GHRH cDNA into maternal somatic tissues. Additional approaches such as dietary interventions, that may be more acceptable to patients, are needed to translate the benefits of higher maternal GH during pregnancy into clinical and additional animal production settings where it would be desirable to increase fetal growth.

Supplementary materials
This is linked to the online version of the paper at https://doi.org/10.1530/JOE-21-0087.

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

Funding
During the writing of this review, H K was supported by an Australian Government Research Training Program PhD scholarship, and B S M...
was supported by a Career Development Award (APP1038009) from the Australian National Health and Medical Research Council (NHMRC). C T R was supported by a Lloyd Cox Professorial Research Fellowship from the University of Adelaide and is currently supported by an NHMRC Investigator Grant (GNT174971) and a Matthew Flinders Fellowship from Flinders University. We thank the Channel 7 Children’s Research Foundation, CRC for an Internationally Competitive Pork Industry and Pig Research & Development Corporation of Australia for support of some of our original work described in this review.

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Accepted Manuscript published online 3 September 2021

Received in final form 12 August 2021

Accepted 27 August 2021