

# Human placental growth hormone variant in pathological pregnancies.

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## Abstract

Growth hormone (GH), an endocrine hormone primarily secreted from the anterior pituitary, stimulates growth, cell reproduction and regeneration and is a major regulator of postnatal growth. Humans have two *GH* genes which encode two versions of GH proteins: a pituitary version (GH-N/*GHI*), and a placental GH variant (GH-V/*GH2*) which is expressed in the syncytiotrophoblast and extravillous trophoblast cells of the placenta. During pregnancy, placental GH replaces pituitary GH in the maternal circulation at mid-late gestation as the major circulating form of GH. This remarkable change in spatial and temporal GH secretion patterns is proposed to play a role in mediating maternal adaptations to pregnancy. Placental GH is associated with fetal growth and its circulating concentrations have been investigated across a range of pregnancy complications. However, progress in this area has been hindered by a lack of readily accessible and reliable assays for measurement of placental GH. This review will discuss the potential roles of placental GH in normal and pathological pregnancies and will touch on the assays used to quantify this hormone.

## Introduction

Growth hormone (GH) is a classical endocrine hormone secreted primarily by the somatotrophic cells in the anterior pituitary which exerts widespread effects on multiple tissues within the body, including increasing the mineralization of bone and muscle mass, promoting lipolysis and gluconeogenesis in the liver, and stimulating glucose homeostasis and immune system function. Somatic growth is regulated by GH stimulation of hepatic insulin-like growth factor-1 (IGF-1), with IGF-1 acting as an endocrine factor to promote growth. However, GH can also exert additional effects on growth that are independent of IGF-1 (1). In addition to pituitary-derived GH production, GH is also secreted via a number of extra-pituitary sites, including the brain, immune system, mammary gland, testis and placenta, where it has localised autocrine/paracrine effects (2-5), adding further complexity to GH functions.

During pregnancy there is a fundamental change in how the GH/IGF-1 axis functions. Humans have two *GH* genes which produce two versions of GH, a pituitary protein (GH-N) to which effects on postnatal growth can be ascribed, and a placental variant (GH-V). In pregnancy, GH-V is expressed from the placenta and is the predominant form of this hormone in the maternal circulation. Whether GH-V contributes to fetal growth has been the subject of some debate. However, there is now reasonably clear evidence to suggest that GH-V is associated with fetal growth and correlates with the increases in circulating IGF-1 observed during pregnancy. However, the physiology of GH-V remains far from understood. This review will cover potential roles for GH-V in normal and pathological pregnancies and will touch on the assays used to quantify this hormone.

## The growth hormone locus

The human *GH* gene family includes five tandemly arranged and highly related genes in a 47-kb cluster on the long arm of chromosome 17 (q22-q24) (6-8). These include *GHI* (GH-N), *GH2* (GH-V), and three chorionic somatomammotropin (CS) genes (also known as placental lactogens), *CSH1* (CS-A), *CSH2* (CS-B) and *CSHL1* (CS-L) (Figure 1). Each gene is composed of five exons (1 to 5) and four introns (A to D) occurring at identical positions (9,10). The five genes share 90% to 95% sequence nucleotide identity in the coding regions, and are thought to have arisen by gene duplication (11). The *CSH1* and *CSH2* genes encode identical mature chorionic somatomammotropin proteins. The *GH2* gene was originally thought to be a pseudogene until expression of *GH2* mRNA was identified in the human placenta in 1987 (12). The *CSHL1* gene undergoes complex alternative splicing leading to multiple mRNA transcripts, the majority of which are non-functional (13).

A locus control region (LCR) located upstream of the *GHI* gene controls tissue specific expression of the locus (14,15) (Figure 1). *GHI* is expressed primarily in pituitary somatotroph cells and at certain extrapituitary sites (5), while the remaining four genes are expressed in the placenta. GH-V is expressed in syncytiotrophoblastic layer and the extravillous trophoblast cells of the placenta (16,17). Distinct patterns of chromatin modification and complex chromosome looping are associated with differential activation of the human *GH* genes in the pituitary and the placenta (18,19).

## Growth hormone isoforms

Several transcript variants and isoforms of GH-N and GH-V exist (20). A 22 kDa GH-N isoform is the most abundant and major bioactive form of GH, comprising 85-90% of circulating GH-N, while a 20 kDa GH-N accounts for approximately 10% of the pituitary GH-N transcripts. Similarly, 22 kDa GH-V is the main circulating form of GH-V, while the expression of the 20 kDa GH-V seems to be variable in normal and pathological conditions. 20 kDa GH-V is generated from a 45-bp deletion produced by the use of an alternative splice

acceptor site within exon 3 of the *GH2* gene, similar to that in the *GH1* gene (21-23). However, the transcript encoding 20 kDa GH-V is not detected in all placentas, which may partly explain the previous unsuccessful attempts in detecting this transcript (24). In rats, 20 kDa GH-V has reduced lactogenic and diabetogenic activities compared with pituitary 22 kDa GH-N but retains growth-promoting and anti-lipogenic properties (25).

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Although GH-N and GH-V stem from the same gene cluster and their proteins only differ by 13 amino acids, they are quite different in certain aspects (Table 1). GH-V is more basic and contains an N-linked glycosylation site at asparagine 140, resulting in a 25 kDa isoform (26). Although GH-N lacks consensus sequences for N-linked glycosylation, an O-glycosylation has been reported for the 23-24 kDa GH-N (20,27-29). Other GH-N isoforms and fragments have been reported, including some fragments which may inhibit aspects of GH function. These may arise from alternative splicing and post translational modification such as proteolysis, deamidation, phosphorylation, acetylation, and aggregation (20,27,30,31). Whether GH-V undergoes this full range of modifications has not been investigated in detail. As described below, GH-N and GH-V also have different affinities for the prolactin receptor. Importantly, GH-N is secreted in the anterior pituitary by somatotrophic cells in a pulsatile manner. The secretion rate of GH-N changes rapidly, regulated by a series of positive and negative stimuli (GH releasing hormone (GHRH) and somatostatin). However, GH-V is specifically expressed in the syncytiotrophoblast and invasive extravillous trophoblast cells of the human placenta (16,17), and secretion is continuous, which has important implications for physiological adjustment to gestation. Similar to GH-N, GH-V secretion is inhibited by hyperglycaemia (32). However, GH-V is not regulated by GHRH, ghrelin or somatostatin. The secretion and the maternal level of GH-V is closely related to the formation of the syncytiotrophoblast (33). GH-V concentrations are also affected by fetal gender (34). Two early studies observed that maternal body mass index (BMI) is negatively correlated to circulating GH-V concentrations during different stages of pregnancy (35,36).

### **The growth hormone receptor**

GH mediates anabolic effects on the body by interacting with a specific GH receptor (GHR) on the plasma membrane of target cells. The GHR is a member of the Type I cytokine receptor family and has three domains characteristic of this family: an extracellular binding domain, a transmembrane domain and a cytoplasmic domain (37,38). The GHR exists as a constitutive dimer consisting of two identical GHR subunits (39). GH possesses two asymmetric binding sites which interact with the GHR. Initial binding occurs at a high affinity site (Site 1). This facilitates binding at the lower affinity site (Site 2), and leads to a conformational change in the receptor and subsequent activation of signal transduction (40). The GHR lacks intrinsic tyrosine kinase activity, and therefore relies on the recruitment of additional non-receptor tyrosine kinases to mediate signal transduction such as janus kinase (JAK) and cellular sarcoma kinase (c-SRC). Key signalling pathways activated include JAK-STAT (signal transducer and activator of transcription), mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3-K) pathways.

GH-N and GH-V bind the GHR with similar affinity and share similar physiological effects on somatotrophic, lactogenic and lipolytic properties, as well as the effect on immunoregulatory process (26,41,42). In humans, GH-N can also bind and activate the prolactin receptor, but GH-V binds the prolactin receptor poorly and its lactogenic effects are greatly reduced compared with GH-N (43).

### **Placental growth hormone assays**

Accurate detection and quantification of GH-V in clinical studies can be challenging due to the high level of sequence similarity between *GH* locus genes and proteins. The mature GH-N, GH-V and CS proteins share over 80-93% identity in amino acid sequence, and GH-N and GH-V proteins differ by only 13 amino acids, thus development of sensitive and specific GH-V assays can be problematic. Sensitive and specific assays have been established in many labs. However, the lack of reliable commercial assays to measure this hormone has limited the number of studies conducted.

Early studies used highly sensitive radioimmunoassays (RIAs) and modifications of these are still in use. GH-V was first detected in maternal blood using two monoclonal antibodies (K24 and 5B4) (44,45). 5B4 reacts with an N-terminal epitope and recognizes both GH-N and GH-V; K24 reacts with an internal epitope and exclusively recognizes GH-N. An indirect estimate of GH-V concentration was derived by determining the difference between measurements obtained from RIAs using these two antibodies (44,46). With use of purified recombinant GH-V, two monoclonal antibodies (E8 and 7C12) were produced by Georges Hennen's group that were highly specific for GH-V (47,48). E8 does not react with GH-N, chorionic somatomammotropin or prolactin, and GH-V concentrations measured with 5B4 and E8 have a high degree of correlation ( $r=0.93$ ) (46). Antibody 7C12 exhibits some cross reactivity with GH-N. E8 and 7C12 have been used to measure GH-V using a  $^{125}\text{I}$ -labeled sandwich immunoassay (48). In addition, we developed an enzyme-linked immunosorbent assay (ELISA) using these antibodies, following biotinylation of 7C12 (49). Wu *et al.* have reported a immunofluorometric assay with a panel of high affinity monoclonal antibodies specific for human GH-V developed in the lab (50). This has been used in several subsequent studies. Other assays which assess circulating GH-V protein or mRNA have been reported (51-54). Commercial ELISAs are available; however, we have had limited success with these, and previously utilised kits which exhibited good specificity in our hands have been discontinued over recent years (e.g. Diagnostic Systems Laboratories, Inc). We highly recommend verifying any commercial assays prior to use using an independent source of recombinant GH-N and GH-V with proven activity, to assess sensitivity and cross-reactivity.

### **Placental growth hormone in normal pregnancy**

A vast range of substances are secreted from the placenta during human pregnancy. As described above, GH-V is specifically expressed in the trophoblast cells of the human placenta. Perhaps the most remarkable characteristic of GH-V secretion is the reciprocal exchange of GH-N for GH-V in the maternal circulation as pregnancy progresses. In humans, pulsatile pituitary-derived GH is the dominant form of GH in maternal circulation prior to 15 weeks of gestation (55). GH-V is detected from as early as 5 weeks gestation and levels increase significantly to reach peak levels at approximately 36-37 weeks gestation (Figure 2) (56-58). At approximately 17 weeks gestation, GH-V replaces GH-N completely, resulting from the negative feedback by GH-V and IGF-1 (43,59). With the onset of labour, there is a rapid fall of GH-V concentrations in the maternal circulation, which occurs within 1 h after birth, contributed to by the 15 minute half-life of GH-V in blood and placental origin of the hormone (57).

GH-V is thought to play a key role in maternal adaptations to pregnancy and fetal growth (60). Firstly, it is thought that GH-V stimulates fetal growth and regulates maternal circulating IGF-1 concentrations during pregnancy. Previous studies have demonstrated that GH-V is positively associated with fetal growth (48,56,60-62). A positive correlation between GH-V and IGF-1 was also observed in a number of longitudinal and cross-sectional studies (56,60,63). Secondly, GH-V promotes the adaptation to pregnancy of blood vessels supplying the placenta (16), and relaxes the arteries supplying the uterus (64); the effect of these changes is an increase in blood flow to the fetus. In addition, it is postulated that GH-V

induces maternal insulin resistance to ensure supply of nutrients is adequate for the growing fetus. This is evidenced by studies in non-pregnant transgenic mice whereby GH-V over-expression was shown to induce severe insulin resistance and altered body composition including significant increase in bone density and reduced fat mass (65). Recently, we examined the dose response relationship for GH-V administration in a mouse model of normal pregnancy (66). Continuous GH-V treatment did not affect maternal or fetal growth, but treatment at the higher dose range significantly increased maternal fasting plasma insulin concentration with impaired insulin sensitivity, suggesting that GH-V is a likely mediator of the insulin resistance observed in pregnancy (66).

Earlier work had suggested that GH-V was only secreted into the maternal circulation as GH-V had yet to be detected in fetal blood. It was therefore thought that GH-V impacted on fetal growth by regulating the maternal substrate supply via IGF-1 (63). However, conflicting data exist on the relationship between maternal IGF-1 concentrations and fetal growth during pregnancy (56,67). As circulating maternal IGF-1 is not closely related to fetal growth, GH-V may influence fetal growth through alternative mechanisms. In support of this, the presence of the GHR in the placenta and the stimulation of trophoblast proliferation and invasion by GH-V have been observed in several studies (16,68-71). Thus, it is argued that GH-V may play a role in placental function and the process of placentation, but the mechanism remains to be fully defined.

Using a highly sensitive enzyme-linked immunosorbent chemiluminiscent assay, GH-V has been detected in umbilical cord samples in a cross-sectional study (72). This was the first evidence that GH-V exists in the fetal circulation, and is contrary to the popular belief that GH-V is secreted by the placenta only into the maternal circulation. A further study by Higgins *et al.* also observed the presence of GH-V in fetal circulation and at concentrations similar to the previous report (73). It is thought that only substances under 1 kDa can cross the placental barrier (74). Therefore, GH-V may be secreted directly from the syncytiotrophoblast into the fetal circulation rather than cross the placental from maternal circulation, or it may be actively transported across. However, fetal GH-V levels are much lower than maternal levels, and do not appear to be related to fetal growth and placental size (73). Given the above findings, the current understanding of GH-V function in the fetus needs to be re-evaluated.

### **Regulation of placental growth hormone**

The regulation of GH-V is still unclear. Several stimulators and inhibitors of pituitary GH secretion, including GHRH, ghrelin and somatostatin, have been shown to have no effect on circulating GH-V (75-77). Other studies have found GH-V secretion may be related to maternal glucose levels. Glucose inhibits GH-V secretion *in vitro* and in placental explants (32,78). *In vivo*, hypoglycaemia was induced in insulin-dependent diabetic pregnancies and a marked increase in maternal circulating GH-V concentrations was observed (79).

Leptin is expressed in the placenta and fetal tissues (80) and is thought to have physiological effects on the placenta and its function given the marked state of leptin resistance during pregnancy (81,82). Some studies have reported that maternal leptin is negatively correlated with GH-V (36), but the mechanisms are not well defined, and leptin does not stimulate GH-V release in placental explants (78).

### **Placental growth hormone in pathological pregnancies**

GH-V has been associated with a number of pathological pregnancy conditions. Clear associations are seen with abnormal fetal growth in mid-late pregnancy; however, associations with other major pregnancy pathologies such as gestational diabetes mellitus

(GDM) and pre-eclampsia (PE) have been inconsistent or absent (Table 2). More recently, associations with gestational trophoblastic disease and Down syndrome have also been observed. Limitations with some of these studies include lack of experimental power due to small sample numbers, and variations in the time of sampling. As described above, a further challenge has been the lack of commercially available and reliable detection assays.

### 1. Abnormal fetal growth

The placenta receives blood supply from both the maternal and the fetal systems, and thus has two separate circulations: the uteroplacental circulation and the fetoplacental circulation. The maternal blood flow is supplied by the uterine and ovarian arteries while the fetal blood flow is derived from the umbilical arteries. Between these two circulatory systems, an exchange of oxygen and nutrients takes place in the intervillous space in the placenta. Any impairment of maternal and/or fetal blood flow and the placenta can lead to a reduced blood supply to the fetus, resulting in fetal growth restriction (FGR). The aetiology of FGR is multifactorial and involves maternal, fetal and placental factors. Of all the factors, “placental insufficiency” is believed to be a dominant contributor (83). Placental insufficiency includes inappropriate maternal/fetal blood flow, reduced nutrient transfer and morphological abnormalities of the placenta (84).

The majority of studies conducted mid-late pregnancy have found a positive association between maternal GH-V and fetal growth. Additionally, they demonstrate lower concentrations of GH-V in pregnancies complicated by FGR (85). Mirlesse *et al.* found reduced concentrations of GH-V in maternal plasma samples taken after 33-39 weeks amenorrhea in 22 cases of FGR (86). In a study conducted by McIntyre *et al.*, blood samples were obtained from FGR pregnancies at 28-30 weeks gestation and 36-38 weeks gestation, and lower concentrations of GH-V were observed at both time points compared to normal pregnancies (48). One study found no differences in GH-N in maternal and cord serum and amniotic fluid between average for gestation age, small for gestational age (SGA) or large for gestational age (LGA) pregnancies at birth. However, only pituitary GH-N was measured and it was not clear whether the assay also detected placental GH-V (87).

Studies conducted earlier in pregnancy show an association with LGA pregnancies, but not SGA or FGR. We carried out a nested case-control study using samples from the Screening for Pregnancy Endpoints (SCOPE) biobank and found that there was a significant increase in maternal GH-V in LGA pregnancies at 20 weeks of gestation when compared to control pregnancies (49), but there was no change in maternal serum GH-V in pregnancies associated with SGA infants. In addition, maternal serum GH-V concentrations were positively correlated to birth weight (49). Similarly, Sifakis *et al.* found no difference in serum GH-V concentrations between SGA and non-SGA groups at 11-13 weeks gestation (88).

Several studies have investigated *GH-V/GH2* mRNA expression (52,89,90). Mannik *et al.* described the expression of *GH2* in the placenta from SGA and LGA pregnancies (90). Placental samples were collected from 72 pregnancies after caesarean section or vaginal delivery. Compared with babies born of normal birth weight, the expression of *GH2* was approximately 1.1-fold lower in placentas from SGA pregnancies but there was no difference in *GH2* between LGA and controls (90). This is consistent with Barrio *et al.* who found *GH2* mRNA expression was decreased in placentas from SGA pregnancies (89). In contrast, Whitehead *et al.* found that *GH2* expression was increased in maternal peripheral blood and term placenta from pregnancies complicated by SGA; the reason for this opposing trend is not clear (52).

Schiessl *et al.* observed impaired uterine blood flow is correlated with low serum concentrations of placental GH-V in FGR pregnancies and suggested that lower concentrations of GH-V might contribute to the impaired uteroplacental circulation (64). This



may be mediated through secondary regulation of IGF-1, as IGF-1 has been demonstrated to directly alter human myometrial arterial tone as assessed via wire myography (91). However, whether GH-V has similar impacts on the placental or myometrial arteries is not known.

The impact on GH-V on fetal growth can also be seen in conditions associated with GH resistance. Laron syndrome is a rare condition which results from inactivating mutations in the GHR. It is characterized by high levels of circulating GH and very low levels of IGF-1, as well as a lack of response to GH stimulation (92-94). Birth lengths of individuals with Laron Syndrome are well below average (95,96). In contrast, birthweights tend to be normal, although some studies have noted decreased birthweight with Laron Syndrome (95,97,98). In female *Ghr* knockout mice, litter size, fetal size and birth weight of pups are significantly reduced (99,100). *Igf-1* knockout animals exhibit more pronounced reproductive deficits when compared to *Ghr* knockout animals (101). In the absence of GH stimulation, IGF-1, produced locally may be one factor that compensates for GH (102), whereas quantitative reproductive deficits in *Ghr* knockout animals reflect absence of GH-dependent IGF-1 production and other consequences of eliminating GH signalling.

While GH resistance models provide evidence of an impact of GH-V on fetal growth, a clear picture is not seen with genomic alterations in the *GH* gene cluster, which lead to different phenotypes. It has been reported that genetic deletion of the chorionic somatomammotropin/placental lactogen genes and *GH-V* results in severe growth retardation in one case (103), but has little impact on fetal growth in others (104,105). This may indicate that neither hormone is required for normal fetal growth. However, it is likely that other *GH* locus hormones not disrupted by the deletion compensate for the function of these genes (7).

The mechanisms by which GH-V may contribute to fetal growth are varied. As described above, the aetiology of FGR is complex. The placenta acts an interface between the maternal and fetal circulation, with the rates of placental blood flow dependent upon placental vascularisation and angiogenesis. Although a number of factors have been implicated in angiogenesis, vascular endothelial growth factor-A (VEGF-A), fibroblast growth factor 2 (FGF-2), and placental growth factor (PlGF) are key factors involved in placental vascularisation (106,107). Failure of vascularisation and angiogenesis leads to increased vascular resistance and reduced blood flow, and is associated with high-risk pregnancy complications including FGR and PE (108,109).

GH-N is a proangiogenic factor which promotes endothelial cell proliferation, migration and tube formation *in vitro* (110,111), upregulates VEGF-A expression (110), and enhances angiogenesis and vascularisation *in vivo* (110,112). Further, *in vivo* and *in vitro* data suggest that proteolytic fragments cleaved from GH are anti-angiogenic while the intact pituitary GH-N and GH-V proteins are angiogenic (31,112). These N-terminal fragments inhibit the activation and phosphorylation of MAPK, and reduce the pro-angiogenic effects of VEGF and FGF-2 (31,112). It is likely that GH-V has similar functions but this has yet to be demonstrated experimentally.

Placental vascularisation begins with the invasion of trophoblast into the uterus; impaired trophoblast invasion of the myometrial spiral arteries is deemed a crucial factor in the pathogenesis of FGR (113). The precise mechanisms that regulate trophoblast invasion are largely unknown with several proteinases, cytokines, and growth factors involved. Lacroix *et al.* determined that both GH-V and GH-N can stimulate trophoblast invasion, but GH-V was more efficient in stimulating invasiveness (16). This result implicates an autocrine or paracrine role for GH-V in the regulation of trophoblast invasion.

Placental nutrient transport capacity plays a key role in the development of FGR. All substrates that pass between the maternal and fetal circulation must go through the placental



exchange barrier which consists of a number of layers: syncytiotrophoblast, discontinuous inner cytotrophoblast layer, basal lamina of the trophoblast, connective (mesenchymal) tissue of the villus, basal lamina of the endothelium and endothelium of the fetal placental capillary in the tertiary villus (114). The microvillous plasma membrane of the syncytiotrophoblast and basal plasma membrane are thought to be the most important membranes of the placental barrier as they represent the rate limiting steps in the transport process. Among the substrates the fetus requires, amino acids appear to be important determinants of fetal growth. There are several amino acid transporters in the microvillous plasma membrane of the placenta, but the system A amino acid transporter, which transports non-essential neutral amino acids, has drawn the most attention. In 1988, Dicke and Henderson first described a defect of system A amino acid transport in FGR pregnancies (115). Later studies demonstrated that reduced activity or expression of system A amino acid transporter was associated with FGR (116,117), and also related to the severity of FGR (118). Consistent with an effect on placental transport, maternal GH treatment increases placental capacity for simple diffusion and stimulates fetal growth (119,120), and increases fetal body weight and length in sheep FGR models after placental embolization (121). Another animal study observed increased placental nutrient transporter expression following maternal GH intervention, not accompanied by alterations of the placental structure (122). These findings suggest that GH-V may influence placental nutrient transport, in addition to altering blood flow and placental morphology.

One explanation for changes in circulating GH-V in pregnancies complicated by growth restriction, which warrants attention, is that circulating GH-V concentrations may just reflect placental mass, rather than changes in GH-V expression *per se*. This has particular relevance for FGR where placental mass is often reduced. GH-V is predominantly secreted from the placental syncytiotrophoblast layer. Placental defects would therefore lead to both compromised fetal growth and reduced GH-V secretion. However, studies investigating *GH2* placental mRNA expression also argue for compromised regulation of GH expression in certain pregnancy pathologies (89,90). For example the study by Mannik *et al.* found that placental *GH2* mRNA expression was altered in the majority of placentas from pregnancies resulting in the birth of SGA new-borns (90). Thus it is plausible that both placental mass and regulation of *GH2* gene expression contribute to changes in systemic GH-V during pregnancy.

## **2. Gestational diabetes mellitus**

GDM is a condition in which women without previously diagnosed diabetes exhibit glucose intolerance during pregnancy (123). It is associated with multiple gestational and neonatal complications, including macrosomia, dystocia, stillbirth, hypoglycaemia and respiratory distress (124). Both the fetus and the mother have increased risk for developing diabetes in later life (125). The aetiology of GDM is unclear. Normal pregnancy is accompanied by insulin resistance that begins mid-pregnancy and progresses through the third trimester (126). Pregnancy-associated insulin resistance appears to result from a combination of increased maternal adiposity and the anti-insulin effects of hormones produced by the placenta (127). Delivery of the baby and placenta leads to a rapid decline in this insulin resistant phenotype, suggesting that the major contributors to this state of resistance are placental hormones. Insulin resistance is a characteristic feature of GDM and it has been suggested that GH-V may play a role in the development of the insulin resistance characteristic of GDM pregnancies. Animal studies have demonstrated that GH-V induces insulin resistance by increasing fasting and postprandial hyperinsulinemia (65). Previously we found that GH-V reduced maternal insulin sensitivity in dose-dependent manner (66). However, limited studies suggest GH-V secretion is regulated by glucose levels. Patel *et al.* observed a dose-dependent

inhibition of GH-V secretion by glucose in human placental explants and in trophoblast cultures (32). Conversely, Bjorklund *et al.* described an increase in GH-V during a hyperinsulinemic hypoglycemic clamp in pregnant Type 1 diabetes patients (79). Despite an interest in the involvement of GH-V in GDM, no studies to date have demonstrated aberrant levels in pregnancies complicated by GDM (128). Recently, we also found that there was no difference in circulating GH-V measurements in maternal serum from GDM pregnancies at 20 weeks. However, GDM cases who delivered LGA babies had significantly higher serum GH-V concentrations compared to non-diabetic control cases, although the numbers of GDM cases with LGA babies were small (129). Verhaeghe *et al.* observed no difference in plasma GH-V in women with a normal versus an abnormal glucose tolerance test at 24-29 weeks gestation (35). Finally, Mannik *et al.* found that the placental mRNA expression profile of *GH2* was not different in GDM pregnancies, but also observed a trend towards increased *GH2* mRNA expression in GDM pregnancies associated with LGA infants (130).

Other studies have investigated associations with Type 1 (T1D) and 2 (T2D) diabetes mellitus. McIntyre *et al.* found that maternal GH-V concentrations were positively correlated with maternal glycaemia in women with established T1D or T2D, particularly in the post-prandial state (48). There was no difference in total GH-V between women with normal glucose tolerance and diabetic patients, but free GH-V (calculated as the ratio of GH binding protein (GHBP) to GH-V) was decreased in maternal serum of T2D pregnancies and increased in T1D pregnancies (48). However, Higgins *et al.* (73) did not observe any differences between GH-V concentrations in women with normal glucose tolerance and diabetic patients. A study by Ringholm *et al.* found that GH-V concentrations were similar in women with T1D delivering LGA infants when compared with T1D alone, except at 8 weeks where GH-V concentrations were slightly lower in women with LGA infants (131). Fuglsang *et al.* demonstrated that the increase in insulin requirements during pregnancy in T1D was not related to GH-V concentrations (132,133).

### 3. Pre-eclampsia

PE is one of the leading causes of maternal, fetal, and neonatal mortality and morbidity, affecting 3–5% of pregnancies worldwide. Clinically it is characterised by maternal hypertension and proteinuria. Impaired trophoblast invasion and placental angiogenesis are key pathogenic mechanisms involved in PE (113,134). As GH-V may influence the placentation process it was hypothesised that aberrant GH-V expression might be associated with PE. However, results from available studies are conflicting. Papadopoulou *et al.* analysed samples in pairs of maternal serum and amniotic fluid from 25 PE pregnancies with combined FGR at 16-22 weeks of gestation (54). They found that GH-V concentrations in both serum and amniotic fluid were significantly higher in pregnancies complicated by FGR associated with PE. In a cross-sectional study, Mittal *et al.* observed that maternal circulating concentrations of GH-V at 20-42 weeks gestation were higher in women with PE than in normal pregnant women, and women whose pregnancies were complicated with PE and SGA had lower maternal serum concentrations of GH-V compared women whose pregnancies were complicated by PE alone (72). Contrary to their findings, Sifakis *et al.* observed no difference in maternal serum GH-V concentration at first trimester in a case control study from 60 PE cases and 120 controls (135). We also observed that GH-V was not altered in maternal serum from PE pregnancies at 20 weeks in a nested case-control study (136). At the molecular level, Mannik *et al.* found that the placental mRNA expression profile of *GH2* was significantly decreased in PE pregnancies at term (130).

## Other conditions

Additional studies have demonstrated that maternal serum and amniotic concentrations of GH-V are increased in the second trimester in pregnancies affected by chromosomal anomalies, including Down syndrome, when compared with controls (137-141). Adding GH-V measurements to the triple screening test for chromosome abnormalities, which measures choriongonadotropin,  $\alpha$ -foetoprotein and oestrogen, increases the detection rate of Down Syndrome from 65.6 to 71.9% (140). However, these results are not consistent with two studies that measured GH-V in the first trimester. Sifakis *et al.* observed that maternal serum GH-V in the first trimester was significantly lower in trisomy 18 and 21 compared to euploid pregnancies (142). In addition, Frendo *et al.* observed decreased *GH2/GH-V* mRNA expression in placentas first trimester pregnancies (143).

In pregnancies affected by Down syndrome, some defects in placentation, especially the formation of syncytiotrophoblast layer, have been demonstrated *in vitro* (143,144). As GH-V is secreted in the syncytiotrophoblast layer, and expression is diminished (143), lower serum GH-V concentrations would be expected in Down syndrome pregnancies. The explanation for this discrepancy is not clear. However, other placental products have been demonstrated to change between the first and second trimester in trisomy 21 relative to euploid pregnancies (142).

GH-V has also been detected in gestational trophoblastic disease (GTD) and ectopic pregnancies (145,146). However, it is unclear whether it is associated with these disorders. GTD refers to a group of pregnancy-related tumours involving placental villous trophoblasts which include choriocarcinoma, hydatidiform mole, invasive mole, placental-site trophoblastic and epithelioid trophoblastic tumours. A recent study found that GH-V was expressed in the majority of these; however, sample numbers were small (145). An association with cancer is not surprising given the wealth of literature which links pituitary GH-N with various cancers (147,148).

## Summary

The GH/IGF-1 axis is closely associated with human reproduction and fetal growth. GH-V may play an important role in the pathology of several pregnancy complications, in respect to fetoplacental blood supply, placental nutrient transport and the process of placentation. Clear associations are seen with both FGR and LGA fetuses in mid-late pregnancy, but associations with other major pregnancy pathologies such as GDM and PE have been inconsistent or lacking. GH is a classical endocrine hormone which also has autocrine and paracrine functions, and it is possible that inconsistent associations between circulating GH-V and GDM or PE is due to local actions not being reflected by systemic levels. Consequently, autocrine and/or paracrine functions of GH-V in the placenta may play an important role in the pathology of these complications, regardless of circulating levels. For now the exact physical and pathological effect of GH-V in pregnancy remains largely unknown and further investigation will be required to delineate associative versus functional effects relating to pregnancy outcomes.

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### Figure Legends

**Figure 1.** The human *GH* gene family is a cluster of five genes in 17q22-24 which includes *GH1* (GH-N), *CSHL1* (CS-L), *CSH1* (CS-A), *GH2* (GH-V) and *CSH2* (CS-B). *GH1* is expressed in the pituitary as well as other extra-pituitary sites. The remaining four genes are expressed in the placenta.

**Figure 2.** Relative changes of placental GH-V, pituitary GH-N and IGF-1 in the maternal circulation during pregnancy.

**Table 1.** Characteristic differences between placental GH-V and pituitary GH-N

**Table 2.** Summary of studies investigating the association of GH-V with different pregnancy pathologies [SGA, small for gestational age; FGR, fetal growth restriction; LGA, large for gestational age; GDM, gestational diabetes mellitus; T1D, Type 1 diabetes; T2D, Type 2 diabetes PB, peripheral blood; PE, Pre-eclampsia; DS, Down syndrome].



961 **Table 2.** Summary of studies investigating the association of GH-V with different pregnancy  
962 pathologies

Sample	Timing	Association	Reference
<b><i>Small for gestational age/fetal growth restriction</i></b>			
Maternal serum	11-13 wks	Not associated	Sifakis, 2012 (88)
Maternal serum, SGA	20 wks	Not associated	Liao, 2016 (49)
Maternal serum, FGR	28-30 & 36-38 wks	Decreased	McIntyre, 2000 (48)
Maternal plasma, FGR	33 -39 wks amenorrhea	Decreased	Mirlesse, 1993 (86)
Placental and peripheral blood mRNA	28 & 36 wks (PB); term (placenta)	Increased	Whitehead, 2013 (52)
Maternal serum, FGR	Various	Decreased	Caufriez 1993 (60)
Placental RNA	Term	Decreased GH2, transcript variant 1	Männik, 2010 (90)
Placental RNA, SGA	Term	Decreased	Barrio 2009 (89)
Maternal serum, FGR	?	Decreased	Schiesl 2007 (64)
<b><i>Large for gestational age</i></b>			
Maternal serum	20 wks	Increased	Liao, 2016 (49)
Placental RNA	Term	Not associated	Männik, 2010 (90)
<b><i>Gestational diabetes mellitus</i></b>			
Maternal serum	20 wks	Not associated	Liao, 2017 (129)
Maternal serum (GDM +LGA)	20 wks	Increased in GDM + LGA group vs. GDM alone	Liao, 2017 (129)
Maternal plasma	24-29 wks	Not associated	Verhaeghe, 2002 (35)
Placental RNA	Term	Not associated	Männik, 2012 (130)
<b><i>Type 1 diabetes</i></b>			
Maternal serum, cord blood	36 wks (MS); term (CB)	Not associated	Higgins, 2012 (73)
Maternal serum (T1D +LGA)	8, 14, 21, 27 & 33 wks	Small decrease at 8 wks in T1D + LGA group vs. T1D alone, otherwise not associated	Ringholm, 2015 (131)
Maternal serum (free GH-V)	28-30 & 36-38 wks	Increased	McIntyre, 2000 (48)
<b><i>Type 2 diabetes</i></b>			
Maternal serum (free GH-V)	28-30 & 36-38 wks	Decreased	McIntyre, 2000 (48)
<b><i>Pre-eclampsia</i></b>			
Maternal serum	11-13 wks	Not associated	Sifakis, 2011 (135)
Maternal serum	20 wks	Not associated	Liao, 2017 (136)
Maternal serum and amniotic fluid (PE + FGR)	16- 22 wks	Increased in PE + FGR vs. normal	Papadopoulou, 2006 (54)

		pregnancies	
Maternal serum	20-42 wks	Increased in PE; decreased in PE+ SGA babies vs. PE alone	Mittal, 2007 (72)
Placental RNA	Term	Decreased GH2, transcript variant 1	Männik, 2012 (130)
Maternal serum	?	Decreased	Schiessl 2007 (64)
Gestational trophoblastic disease			
Tissue and sera samples	n/a	n/a	Hübener, 2017 (146)
<b><i>Ectopic pregnancy</i></b>			
Tissue and sera samples	n/a	n/a	Hübener, 2015 (145)
<b><i>Chromosome trisomies/ Down syndrome</i></b>			
Maternal serum, DS	8-14 weeks	Decreased	Christiansen, 2009 (141)
Maternal serum, trisomy 18 & 21	11-13 wks	Decreased	Sifakis, 2010 (142)
Maternal serum, DS	16-23 wks	Increased	Papadopoulou, 2008 (138)
Amniotic fluid, DS	16-23 wks	Increased	Sifakis, 2009 (139)
Maternal serum, trisomy 18 & 21	Second trimester	Increased	Moghadam, 1998 (137)
Maternal serum, DS	Second trimester	Increased	Baviera, 2004 (140)
Placental RNA, DS	12–35 wks	Decreased	Frendo, 2000 (143)

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