Biology of Reproduction, 2019, 100(4), 872–884
doi:10.1093/biolre/ioy249
Review
Advance Access Publication Date: 21 November 2018

Review

Novel roles of mechanistic target of rapamycin signaling in regulating fetal growth†

Madhulika B. Gupta [1,2,3] **and Thomas Jansson** [4,*]

[1]Department of Pediatrics, University of Western Ontario, London, Ontario, Canada; [2]Department of Biochemistry, University of Western Ontario, London, Ontario, Canada; [3]Children's Health Research Institute, London, Ontario, Canada and [4]Department of Obstetrics and Gynecology, Division of Reproductive Sciences, University of Colorado | Anschutz Medical Campus, Aurora, Colorado, USA

*__Correspondence:__ Department of Obstetrics & Gynecology, University of Colorado | Anschutz Medical Campus (CU Anschutz), Research Complex-2; Mail Stop 8613, 12700 East 19th Avenue, Room P15-3000E, Aurora, CO 80045, USA. Tel: +1 303 724 8622; Fax: +1 303 724 3512; E-mail: Thomas.jansson@ucdenver.edu

†__Grant support:__ Supported by grants from NIH (HD089980, HD093950 HD065007, HD068370, and HD078376).
Edited by Dr. Myriam Hemberger

Received 12 September 2018; Revised 8 November 2018; Accepted 19 November 2018

Abstract

Mechanistic target of rapamycin (mTOR) signaling functions as a central regulator of cellular metabolism, growth, and survival in response to hormones, growth factors, nutrients, energy, and stress signals. Mechanistic TOR is therefore critical for the growth of most fetal organs, and global mTOR deletion is embryonic lethal. This review discusses emerging evidence suggesting that mTOR signaling also has a role as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth. In the fetus, liver mTOR governs the secretion and phosphorylation of insulin-like growth factor binding protein 1 (IGFBP-1) thereby controlling the bioavailability of insulin-like growth factors (IGF-I and IGF-II), which function as important growth hormones during fetal life. In the placenta, mTOR responds to a large number of growth-related signals, including amino acids, glucose, oxygen, folate, and growth factors, to regulate trophoblast mitochondrial respiration, nutrient transport, and protein synthesis, thereby influencing fetal growth. In the maternal compartment, mTOR is an integral part of a decidual nutrient sensor which links oxygen and nutrient availability to the phosphorylation of IGFBP-1 with preferential effects on the bioavailability of IGF-I in the maternal–fetal interface and in the maternal circulation. These new roles of mTOR signaling in the regulation fetal growth will help us better understand the molecular underpinnings of abnormal fetal growth, such as intrauterine growth restriction and fetal overgrowth, and may represent novel avenues for diagnostics and intervention in important pregnancy complications.

Summary Sentence

Emerging evidence suggest that mTOR signaling in the fetal liver, trophoblast, and decidua serves as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth.

Key words: decidua, developmental origins of health and disease, fetal development, insulin-like growth factor, intrauterine growth restriction, kinases, metabolism, nutrition, placenta, placental transport, pregnancy, syncytiotrophoblast.

Introduction

Fetal growth is broadly determined by the genetic growth potential of the fetus and the availability of oxygen and nutrients. Abnormal fetal growth affects 10–15% of all pregnancies in the developed world [1, 2] and occurs when the fetus fails to achieve its genetically determined growth potential (intrauterine growth restriction) or exceeds its growth determined genetically (fetal overgrowth). Abnormal fetal growth is not only associated with increased perinatal morbidity and mortality but also increases the risk of developing obesity, diabetes, and cardiovascular disease in childhood and later in life [3–12]. Thus, understanding the molecular mechanisms regulating fetal growth in normal and complicated pregnancies is of fundamental importance and of significant public health interest. Whereas the role of endocrine factors and nutrients in regulating fetal growth has been the focus of multiple excellent overviews [13–22], this review discusses emerging evidence implicating mechanistic target of rapamycin (mTOR) signaling as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth.

Mechanistic TOR is a serine/threonine kinase that regulates cell survival, metabolism, growth, and proliferation [23–27]. Mechanistic TOR exists in two complexes, mTOR complex (mTORC) 1 and 2, with the protein raptor associated with mTORC1 and rictor associated with mTORC2. mTORC1 regulates protein translation mediated by phosphorylation of S6K1 and 4EBP1 [23–28]. mTORC2 phosphorylates Akt, PKCα, and serum and glucocorticoid-regulated kinase 1 (SGK1), and regulates the actin skeleton, cell-cycle progression, anabolism, and cell survival [29–31]. Deptor is an endogenous inhibitor of both mTORC1 and 2 [32].

It is well established that mice lacking either *mtor* [33, 34], *raptor* [35], or *rictor* [36] die early in development, demonstrating the critical role of mTORC1 and 2 for embryonic development and growth. In contrast, whole-body *deptor* mutant KO mice are viable, fertile, and normal in size [37]. Moreover, there is a wealth of evidence that mTOR signaling plays an important role in the growth of individual fetal tissues and organs such as the intestine [38, 39], beta cell [40, 41], and skeletal muscle [42–45]. Similarly, decreased tissue growth is associated with inhibition of mTOR signaling in the fetal brown adipose [46], brain [47], heart [48], and thymus [49]. Whereas restricted fetal liver growth is not associated with mTOR inhibition following 48 h of starvation in the rat [50], inhibition of fetal liver mTOR signaling has been reported in other animal models of IUGR, including in the naturally occurring runt in pigs [51] and following maternal nutrient restriction in the baboon [52].

In this review, we will summarize recent data suggesting that mTOR signaling in specific tissues plays an important role in regulating overall fetal growth in response to changes in the availability of oxygen, nutrients, and growth factors by influencing global homeostatic systems. The mechanisms involved include mTOR regulation of placental function and influencing the maternal and fetal insulin-like growth factor (IGF) axis by regulating IGF binding protein 1 (IGFBP-1) secretion and phosphorylation. Both IGF-I [53, 54] and IGF-II [55, 56] are key regulators of fetal growth, and both growth factors are abundantly present in the maternal circulation and at the maternal–fetal interface [13] and regulate placental function [13, 57]. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II [58], we will focus on this specific IGF.

First, we will discuss the molecular mechanisms by which mTOR and the amino acid response (AAR) signaling pathway govern the secretion and phosphorylation of IGFBP-1 in the fetal liver. Because changes in the abundance and phosphorylation of IGFBP-1 have profound effects on the bioavailability of IGFs, fetal liver mTOR and AAR signaling link oxygen and nutrient delivery to fetal growth. Second, we will briefly review how trophoblast mTOR responds to a large number of growth-related signals, including amino acids, glucose, oxygen, folate, and growth factors, to regulate trophoblast mitochondrial respiration, nutrient transport, and protein synthesis, thereby influencing fetal growth. Third, we will examine the emerging evidence suggesting that mTOR functions as a decidual nutrient sensor which links oxygen and nutrient availability to increased phosphorylation of IGFBP-1 with preferential effects on the bioavailability IGF-I in the maternal–fetal interface and in the maternal circulation. We will conclude by presenting an overall model placing mTOR signaling as a critical hub in the overall homeostatic regulation of fetal growth and discussing how this model may help us better understand the molecular underpinnings of abnormal fetal growth. Finally, we will briefly speculate how this new knowledge could lead to novel avenues for diagnostics and intervention in important pregnancy complications.

Fetal liver mTOR and AAR signaling pathways link oxygen and nutrient availability to fetal growth

The bioavailability of fetal IGF-I is tightly regulated by IGFBP-1, which is primarily secreted by the fetal liver [59]. Phosphorylation of IGFBP-1 at three serine residues (Ser101, 119, and 169) is known to markedly increase its affinity for binding IGF-I [60], thus affecting the ability of IGF-I to interact with the IGF receptor, resulting in inhibition of IGF-I function [61, 62]. While phosphorylation of human IGFBP-1 does not alter the affinity for IGF-II [58], the affinity of phosphorylated human IGFBP-1 for IGF-I is 6 to 10- fold higher than for the nonphosphorylated protein [62–64] and hypoxia causes increased phosphorylation of IGFBP-1 with up to 300-fold higher affinity for IGF-I [65]. In addition, phosphorylation makes IGFBP-1 more resistant to proteolysis [61, 66]. Functionally, phosphorylation increases the capacity of IGFBP-1 to inhibit IGF-I-stimulated cell proliferation, DNA synthesis, amino acid transport, and apoptosis [67–69]. We have shown that hepatic IGFBP-1 phosphorylation induced in response to hypoxia caused a profound increase in its affinity for IGF-I, resulting in a marked inhibition of IGF-I-dependent cellular proliferation [65, 70]. IGFBPs also influence cell function by mechanisms that are independent of their ability to alter IGF–receptor interaction [71]. For example, IGFBP-1 contains RGD sequences that mediate binding to $\alpha5\beta1$ integrin, and this interaction stimulates cell migration independent of IGF-I [72].

There are numerous observations indirectly supporting a mechanistic link between increased IGFBP-1 secretion and restricted fetal growth. For example, mouse fetuses overexpressing *igfbp1* are growth restricted [73–75], clearly demonstrating a cause-and-effect relationship between IGFBP-1 and fetal growth in this species. In addition, IUGR is associated with elevated fetal IGFBP-1 [76] and increased IGFBP-1 phosphorylation at three specific residues in human fetuses [52, 77, 78]. Importantly, using liver tissue from growth restricted and control baboon fetuses we reported that IUGR is associated with increased fetal liver IGFBP-1 abundance and phosphorylation [52].

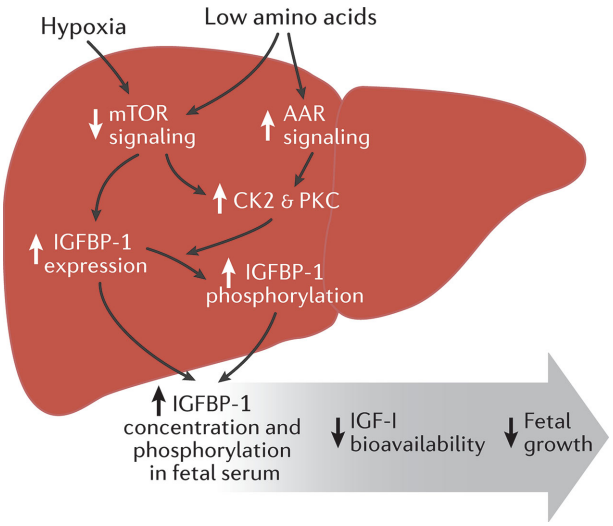

Figure 1. Liver mTOR as a link between decreased oxygen and nutrient availability and restricted fetal growth. Inhibition of fetal liver mTOR signaling and activation of AAR are mechanistically linked to increased IGFBP-1 secretion and IGFBP-1 phosphorylation in primary fetal hepatocytes, and we propose that these changes precede the development of IUGR. Both insulin-like growth factor I (IGF-I) and IGF-II are key regulators of fetal growth. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II only IGF-I is depicted in the figure (see the text). AAR, amino acid response pathway; CK2, casein kinase 2; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I; IUGR, intrauterine growth restriction; mTOR, mechanistic target of rapamycin; PKC, protein kinase C.

It is well established that IGFBP-1 secretion is regulated by nutrient and oxygen availability [79–82]; however, the underlying molecular mechanisms are largely unexplored. Moreover, how the phosphorylation of IGFBP-1 is regulated has, until recently, remained unknown. Based on studies in cultured HepG2 cells and primary fetal baboon hepatocytes, we demonstrated that inhibition of mTOR is required for increased IGFBP-1 secretion and phosphorylation in response to hypoxia [83, 84] and the enhanced IGFBP-1 secretion following decreased amino acid availability [85]. In contrast, IGFBP-1 hyperphosphorylation in response to amino acid deprivation is mediated by activation of the AAR signaling pathway [85] (Figure 1). The AAR signal transduction pathway is activated by limitation or imbalance of essential amino acids [86], resulting in increased levels of uncharged tRNA species, which bind to general control nonderepressible 2 (GCN2) kinase. As a result, the translation initiation factor eIF2α is phosphorylated, which leads to inhibition of global translation, but increased translation of activating transcription factor (ATF) 4. ATF4 increases the expression of a small group of genes involved in transport, metabolism, and oxidative stress [86].

Moreover, it was demonstrated that CK2 and PKC constitute the key kinases, regulated by mTOR and AAR, responsible for IGFBP-1 serine phosphorylation [52, 84, 87] (Figure 1). An important role of CK2 in the phosphorylation of IGFBP-1 was further supported by extensive co-localization between these two proteins in HepG2 cells (Figure 2). CK2 is a ubiquitous kinase that phosphorylates substrates characterized by multiple acidic residues surrounding the threonine or serine residue [88–90]. CK2 exists in tetrameric structures consisting of two catalytic subunits (α or α', in any combination) and two regulatory β-subunits.

Collectively, we propose that inhibition of fetal liver mTOR signaling and activation of AAR result in increased IGFBP-1 secretion and IGFBP-1 phosphorylation and constitute a key molecular link between decreased oxygen and nutrient availability and reduced fetal growth (Figure 1). Preliminary data suggest that these changes in the fetal liver occur prior to the development of IUGR in response to maternal nutrient restriction in nonhuman primates [91].

Mechanistic TOR regulates trophoblast function in response to an array of upstream signals

The placenta constitutes the main interface between mother and fetus and represents the primary site for maternal–fetal exchange. The syncytiotrophoblast, a highly specialized multinucleated epithelial cell layer covering the surface of the chorionic villi, produces a multitude of hormones, mediates nutrient transport, and forms a physical and immunological barrier between the maternal and fetal circulations. Thus, the syncytiotrophoblast is strategically positioned as a large maternal–fetal interface, which determines nutrient supply to the fetus. Moreover, a wide array of cellular signaling pathways in the syncytiotrophoblast modulate and integrate placental growth and function in response to maternal and fetal cues [92].

Upstream signals influencing trophoblast mTOR signaling

mTORC1 integrates a large number of metabolic signals, including hormones and growth factors, such as insulin, IGF-I and EGF, cellular ATP levels, hypoxia, DNA damage, amino acids, glucose, and fatty acids, to regulate cellular metabolism, growth, and proliferation [93, 94]. In contrast to mTORC1, mTORC2 predominantly responds to insulin/PI3K signaling [93]. These signals are likely to regulate mTOR signaling also in trophoblast cells as confirmed for growth factors [95], fatty acids [96], and glucose [97]. In addition, corticosterone administration to pregnant mice has been reported to inhibit placental mTORC1 and mTORC2, as evidenced by a decrease in the ratio of the degree of phosphorylation/total abundance for 4EBP1 and S6K and Ser 473 phosphorylation of Akt [98]. Moreover, adiponectin decreases trophoblast mTOR signaling activity by inhibiting insulin signaling [99–101]. We have recently reported that mTORC1 and mTORC2 are novel folate sensors in the placenta and beyond [102]. Specifically, folate deficiency in pregnant mice caused a marked inhibition of mTORC1 and mTORC2 signaling in multiple maternal and fetal tissues, downregulation of placental amino acid transporters, and fetal growth restriction [103]. In addition, folate deficiency in cultured primary human trophoblast (PHT) cells resulted in inhibition of mTORC1 and mTORC2 signaling and decreased the activity of key amino acid transporters [104]. Folate sensing by mTOR in PHT cells is independent of the accumulation of homocysteine and requires the proton-coupled folate transporter (PCFT, SLC46A1). These findings, which provide a novel link between folate availability and cell function, growth, and proliferation, may have broad biological significance given the critical role of folate in normal cell function.

In summary, trophoblast mTORC1 has an array of upstream regulators, including free fatty acids, amino acids, glucose, ATP, and oxygen (Figure 3), and it is likely that the placental levels of these nutrients are changed in conditions such as placental insufficiency, maternal undernutrition, or obesity [105, 106]. It has been proposed that the placenta integrates a multitude of maternal and fetal nutritional cues with information from intrinsic nutrient-sensing signaling pathways to match fetal demand with maternal supply by regulating maternal physiology, placental growth, and nutrient transport,

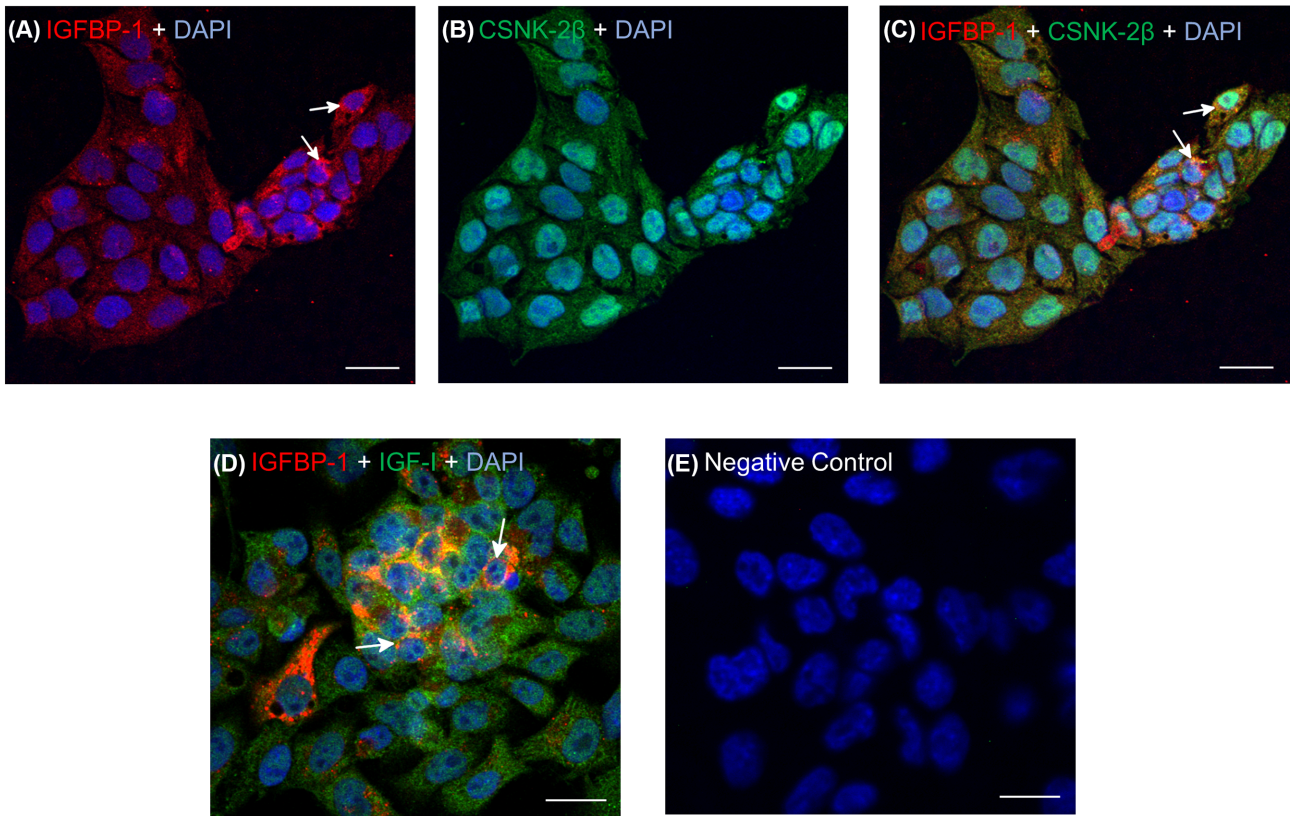

Figure 2. Dual Immunofluorescence staining for the co-localization of IGFBP-1 and CSNK-2β and the co-localization of IGFBP-1 and CSNK-2β. Human hepatocellular carcinoma (HepG2) cells were stained with anti-mouse IGFBP- 1 (monoclonal antibody 6303), anti-rabbit IGF-I, and anti-rabbit CSNK-2β antibodies. Corresponding secondary antibodies were Alexa anti-mouse 660 (shown green) and anti-rabbit 568 (red). Images were captured via confocal microscopy. (A and B) IGFBP-1 (red) is predominantly localized in the perinuclear region of the cells (A, white arrows), whereas CSNK-2β (green) is detected throughout the cell (B). (C) Merged channel image shows co-localization (yellow) predominantly in the perinuclear region (white arrows). (D) Co- localization of IGFBP-1 (red) and IGF-I (green) in HepG2 cells, indicating a positive control, with perinuclear localization of IGFBP-1 signal (white arrows). (E) Dual immunofluorescence with no primary antibodies depicting a negative control where no staining was visualized. Scale bars: 20 μm. Reproduced from [84] with permission. CK2-2β, casein kinase 2-2β; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I.

and that trophoblast mTOR plays a critical role in this homeostatic regulatory loop [92, 107, 108].

Mechanistic TOR regulates key trophoblast functions

mTORC1 is the master regulator of protein synthesis mediated by phosphorylation of p70S6 kinase (S6K1), activating the protein translation initiation, and eiF4E binding protein (4EBP), which allows 5′cap-dependent translation [109]. mTORC1 is also an important regulator of cellular lipid, nucleotide, and glucose metabolism. For example, mTORC1 stimulates de novo lipid synthesis by SREBP activation [110] and promotes a switch from oxidative phosphorylation to glycolysis, thereby shunting glucose into the pentose phosphate pathway and generating critical intermediates such as ribose-5 phosphate needed by growing and proliferating cells [110]. In addition, mTORC 1 inhibits autophagy by phosphorylation of ULK, a key activator of autophagy [111], and stimulates mitochondrial biogenesis mediated by the transcription factor PGC1α [112]. mTORC2, on the other hand, promotes cell proliferation and survival by phosphorylating a number of the AGC protein kinase family members including Akt, PKCα, and SGK1, which regulate cytoskeletal remodeling and cell migration [93].

Most of the information pertaining to mTOR regulation of cell function has been generated in various nonplacental cell lines. However, it is likely that mTOR signaling has similar functions in, for example, PHT cells. It was recently reported that mTORC1, but not mTORC2, is a positive regulator of oxidative phosphorylation mediated by effects of mitochondrial biogenesis [113]. In addition, using human placental villous explants and PHT cells, we have identified a novel role for mTOR signaling as a regulator of nutrient transport in mammalian cells [95, 97, 104, 114–116]. Specifically, we reported that inhibition of both mTORC1 and/or mTORC2 downregulates trophoblast System A and L amino acid transport activity by affecting the plasma membrane trafficking of specific System A (SNAT2) and System L isoforms (LAT1) [95]. Furthermore, it was demonstrated that Nedd4–2, an E3 ubiquitin ligase, is required for the regulation of plasma membrane trafficking of amino acid transporter isoforms by mTORC1, but not mTORC2 [116]. In contrast, regulation of amino acid transporter trafficking by mTORC2 in PHT cells is mediated by the Rho GTPases Cdc42 and Rac1, which influence the actin skeleton (Rosario et al, unpublished observations). The powerful effects of mTOR signaling on nutrient transport are not limited to amino acids. For example, both mTORC1 and 2 are positive regulators of trophoblast folate uptake by modulating the

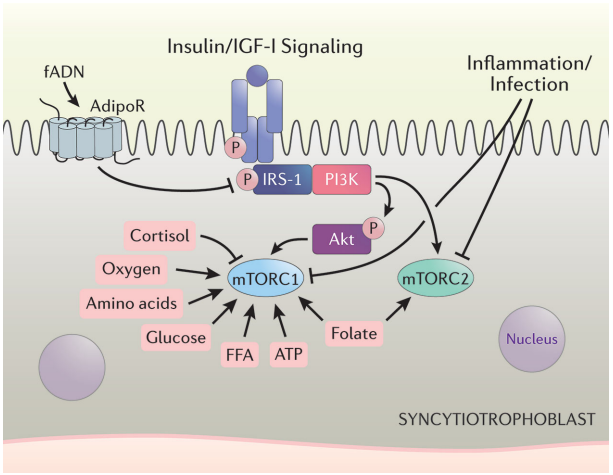

Figure 3. mTORC1 signaling is influenced by a multitude of upstream regulators. AAR, amino acid response pathway; AdipoR, adiponectin receptor; Akt, protein kinase B; ATP, adenosine triphosphate; fADN, full length adiponectin; FFA, free fatty acids, IRS-1, insulin receptor substrate 1; mTORC1, mechanistic target of rapamycin complex 1; mTORC2, mechanistic target of rapamycin complex 2; PI3K, phosphoinositide 3-kinase.

cell surface expression of folate receptor-α (FR-α) and the reduced folate carrier [104].

Taken together, a diverse set of metabolic signals impinges on trophoblast mTOR signaling, which regulates key placental functions, which in turn influence fetal growth and development. For example, mTOR regulation of trophoblast oxidative phosphorylation influences ATP availability with potential profound effects on all active transport processes. In addition, mTOR directly regulates placental transport of amino acid and folate, thereby affecting the fetal availability of these critical nutrients and fetal growth. Moreover, mTOR regulates placental protein synthesis directly and indirectly (by modulating ATP availability) with expected consequences for placental growth.

Placental mTOR signaling and abnormal fetal growth

A consistent relationship exists between changes in placental mTORC1 signaling and altered fetal growth in women and across a range of animal models of IUGR and fetal overgrowth (Table 1). Specifically, placental mTORC1 is altered in pregnancy complications associated with abnormal fetal growth and in animal models where maternal nutrient availability has been altered experimentally. Placental mTORC1 activity is inhibited in human IUGR [117, 118] and activated in placentas of large babies born to obese mothers [119]. Furthermore, placental mTORC1 activity has been reported to be decreased in hyperthermia-induced IUGR in the sheep [120], in response to a maternal low protein diet in the rat [121] and maternal calorie restriction in the baboon [122]. In general, placental nutrient transport, specifically placental amino acid transport, is regulated in the same direction as mTOR signaling (Table 1). However, Sferruzzi-Perri and co-workers reported that undernutrition in pregnant mice resulted in inhibition of placental mTOR signaling, using S6K phosphorylation as a functional readout, but increased transplacental amino acid transport [123]. The reasons for this contrasting finding remain to be established but may be related to the moderate calorie restriction used in the study of Sferruzzi-Perri et al [123].

Mechanistic TOR functions as a decidual nutrient sensor and regulates IGFBP-1 secretion and phosphorylation

In the maternal compartment, the decidua is a major site of IGFBP-1 synthesis and secretion. Locally in the placental barrier IGFBP-1 inhibits trophoblast invasion [124, 125]. Furthermore, the decidua constitutes the major source for maternal circulating IGFBP-1 in pregnancy [126, 127]. Serum IGF-I concentrations are decreased in mothers delivering IUGR babies [128] and most [128–138], but not all [139–142], studies show that IUGR or low birth weight is associated with increased maternal serum IGFBP-1 levels. Because maternal IGF-I is a powerful positive regulator of placental function and growth [143–145], alterations in the maternal IGF-I/IGFBP-1 levels in pregnancies complicated by IUGR may directly contribute to the restricted fetal growth.

Hypoxia and leucine deprivation markedly increased IGFBP-1 phosphorylation and decreased IGF-I bioactivity in cultured human endometrial stromal cells decidualized in vitro [146]. Moreover, IGFBP-1 phosphorylation is increased in decidualized stromal mesenchymal cells in human IUGR [147]. We examined decidua and maternal plasma collected at delivery from appropriate-for-gestational age (AGA) and IUGR pregnancies and maternal plasma collected in late first trimester from women who later delivered an AGA or IUGR infant. It was demonstrate that decidual mTOR is markedly inhibited, AAR is activated, and IGFBP-1 abundance and phosphorylation are increased in IUGR [147]. Moreover, IGFBP-1 hyperphosphorylation in maternal first trimester plasma is associated with the development of IUGR [147].

This data suggest that the decidua functions as a nutrient sensor linking limited oxygen and nutrient availability to increased phosphorylation of IGFBP-1, mediated by mTOR and AAR signaling. Hyperphosphorylation of maternal plasma IGFBP-1 may serve as a novel early biomarker of IUGR. These observations are consistent with the possibility that IGFBP-1 phosphorylation constitutes a link between decreased decidual oxygen and nutrient availability and reduced fetal growth, mediated by diminished IGF-I bioavailability, resulting in inhibition of trophoblast invasion [125, 148, 149] and placental function (Figure 4).

Interestingly, compelling data generated in a genetic mouse model of spontaneous preterm birth, involving a conditional deletion of the tumor suppressor *p53* in uterine tissues, implicate activation of mTORC1 signaling in spontaneous and inflammation-induced preterm birth [150–152]. Specifically, decidual *p53* deficiency resulted in premature decidual senescence mediated by mTORC1 activation, leading to preterm birth and fetal death, outcomes that were prevented with mTORC1 inhibitors [150–152].

Mechanistic TOR signaling as a critical hub in the overall homeostatic control of fetal growth: the example of IUGR

The evidence presented above suggest that mTOR signaling in the fetal liver, trophoblast, and decidua serves as a critical hub in the overall homeostatic control of fetal growth, adjusting the fetal growth trajectory according to the ability of the maternal supply line to support fetal growth. This concept can be illustrated using IUGR as an example (Figure 5). The most common cause of IUGR in Western societies is believed to be placental insufficiency due to a lack of normal gestational increase in uteroplacental blood flow caused by suboptimal trophoblast invasion. It is often assumed that the failure

Table 1. Examples of studies reporting placental mTORC1 signaling and amino acid transport capacity in association to maternal nutrition and fetal growth.

	Placental mTORC1 activity	Placental amino acid transport activity	References
Human IUGR	Decreased	Decreased	[115, 158, 159]
Low protein diet in the rat with IUGR	Decreased	Decreased	[121]
Maternal nutrient restriction in the baboon with IUGR	Decreased	Decreased	[122]
Human GDM with fetal overgrowth	Increased	Increased	[184, 185]
Human obesity with fetal overgrowth	Increased	Increased	[183]
High fat diet mouse with fetal overgrowth	Increased	Increased	[101, 186, 187]

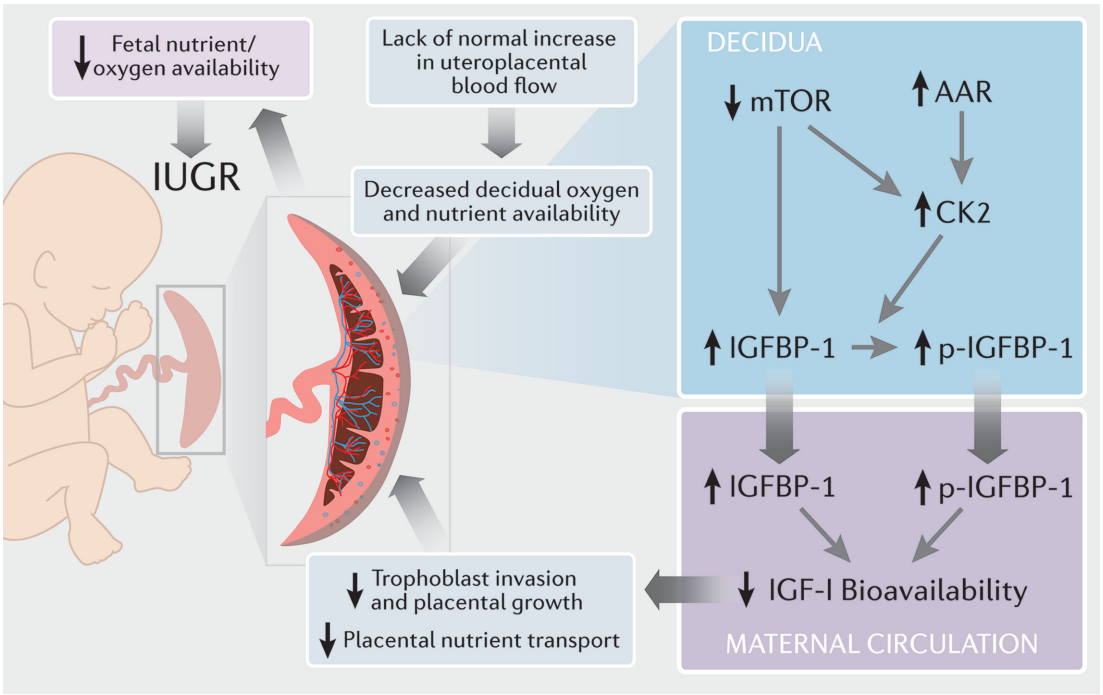

Figure 4. Decidual nutrient sensing. A model linking decreased nutrient and oxygen availability in the decidua in early pregnancy to the development of IUGR. Both insulin-like growth factor I (IGF-I) and IGF-II are key regulators of placental function, and both growth factors are abundantly present in the maternal circulation and at the maternal–fetal interface. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II only IGF-I is depicted in the figure (see the text). AAR, amino acid response pathway; CK2, casein kinase 2; IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I; IUGR, intrauterine growth restriction; mTOR, mechanistic target of rapamycin.

of uteroplacental blood flow to increase normally directly causes the restricted fetal growth. However, an array of adaptive responses in the decidua, trophoblast, and fetus as a consequence of the initial change in uteroplacental blood flow, some of which are mediated by inhibition of mTOR (Figure 5), are likely to play important roles.

One consequence of the lack of normal increase in uteroplacental blood flow is that nutrient and oxygen availability decreases in the decidua, trophoblast, and, ultimately, in the fetus, which inhibits mTOR signaling in these tissues. In the decidua, mTOR inhibition results in the increased release of hyperphosphorylated IGFBP-1, which effectively binds IGF-I, decreasing the bioavailability of this important growth factor at the maternal–fetal interface and in the maternal circulation. Because IGF-I promotes placental growth [143–145] and function, specifically amino acid and glucose transport in cultured trophoblast cells [114, 153–157] and across the placenta in vivo [143], the result is a decreased placental growth and placental nutrient transfer contributing to the development of IUGR. The predominant placental response to a lack of normal increase in uteroplacental blood flow is inhibition of mTORC1 and mTORC2 signaling [115, 158, 159], downregulation of placental nutrient transport, including decreased activity of amino acid [160–165] and folate transporters [166], decreased mitochondrial function [113], and protein synthesis, which directly contributes to decreased fetal nutrient availability and IUGR.

A regulatory loop involving mTOR inhibition and IGFBP-1 phosphorylation—similar to what is present in the decidua—exists in the fetal liver. Inhibition of fetal liver mTOR signaling and activation of AAR result in increased IGFBP-1 secretion and IGFBP-1 phosphorylation, which may occur prior to the development of IUGR [91], and constitute a key molecular link between decreased oxygen and nutrient availability and reduced fetal growth.

Trophoblast mTOR signaling may regulate placental secretion of factors that influence maternal and/or fetal physiology. This hypothesis is supported by our preliminary observations linking trophoblast mTOR signaling to fetal liver IGFBP-1 secretion and phosphorylation [167]. Specifically, incubation of HepG2 cells, an established model for fetal hepatocytes, in conditioned media from PHT cells in which raptor (mTORC1 inhibition) or rictor (mTORC2

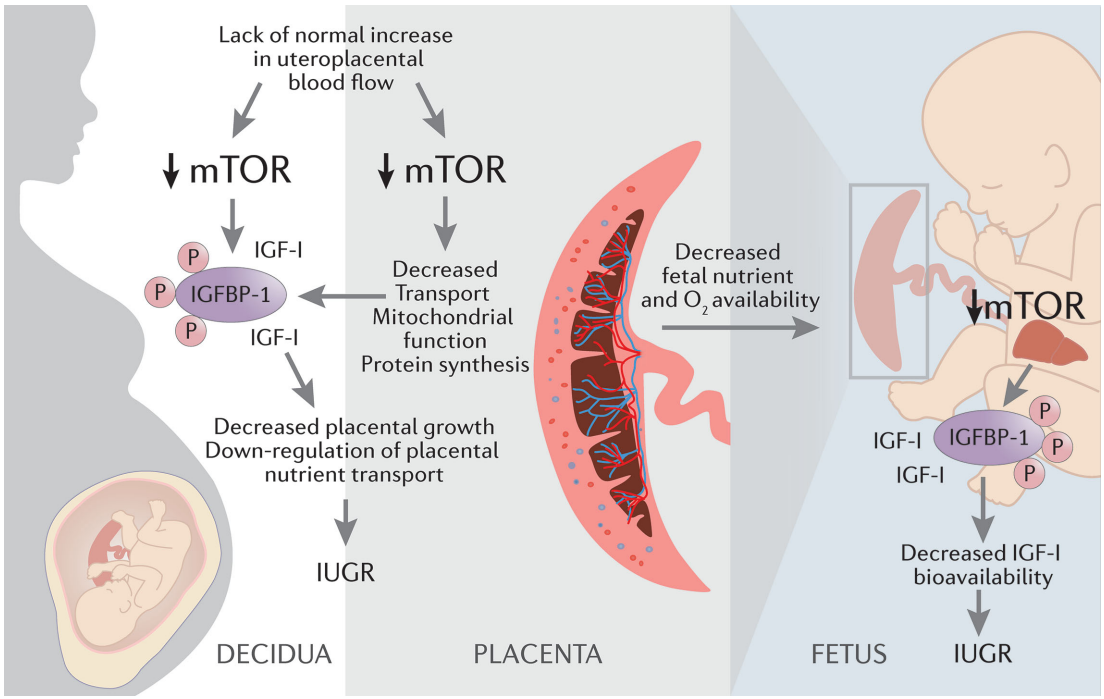

Figure 5. A model placing mTOR signaling as a critical hub in the overall homeostatic regulation of fetal growth. IGFBP-1 binds both IGF-I and IGF-II. However, because phosphorylation of IGFBP-1 increases the affinity for binding IGF-I but not IGF-II only IGF-I is depicted in the figure (see the text). IGFBP-1, insulin-like growth factor binding protein 1; IGF-I, insulin-like growth factor I; IUGR, intrauterine growth restriction; mTOR, mechanistic target of rapamycin.

inhibition) had been silenced, caused an increase in IGFBP-1 secretion and phosphorylation [167].

The proposal that decidual, trophoblast, and/or fetal liver mTOR signaling plays an important role in regulating fetal growth has yet to be systematically tested in rigorous animal experiments involving approaches for tissue-specific, inducible targeting of the genes in the mTOR pathway. However, when *s6k1* expression was rescued in the placenta of *s6k1*–/– mice using tetraploid embryo complementation, the fetal growth restriction in *s6k1*–/– mice was completely rescued [168, 169], strongly implicating a key role of trophoblast mTORC1 signaling in determining fetal growth in the mouse. Furthermore, some MTOR gene variants in humans may be associated with major functional deficits resulting in early pregnancy growth failure and miscarriage, which is supported by reported associations between a single nucleotide polymorphism in the MTOR gene and recurrent spontaneous abortion [170]. Moreover, an important role of mTOR in the regulation of fetal growth is further supported by animal experiments showing that administration of the mTORC1 inhibitor rapamycin at embryonic day 11 in mice causes spontaneous abortions and fetal lethality around embryonic day 16 [171]. The fact that MTOR gene variants yet to be associated to birth weight in large GWAS studies [172, 173] may suggest a marginal role for mTOR signaling in regulating fetal growth in women. However, a lack of association between variants in a particular gene and a phenotype in GWAS studies cannot be taken as evidence that the gene in question is unimportant in determining the phenotype. This point is best illustrated by efforts in the past 20 years to identify genes responsible for the heritability of type 2 diabetes: the total number of associated variants explains only a small proportion of the heritability of this disease. More importantly, however, no gene variants in, for example, insulin (INS), insulin receptor (INSR), PI3-kinase (PIK3CA), GLUT 2 (SLC2A2), or GLUT 4 (SLC2A4) have been associated with type 2 diabetes risk even in the most recent GWAS study involving more than 600,000 subjects [174], which cannot lead to the conclusion that insulin, the insulin receptor, PI3 kinase, Glut 2, and Glut 4 are inconsequential for the regulation of glucose homeostasis. In analogy, no firm conclusion with respect to the importance of mTOR signaling in the regulation of fetal growth can be drawn from the fact that no genetic variant at the MTOR locus has been shown to associate with birth weight in GWAS.

One important implication of this model is that intervention strategies to alleviate or prevent IUGR must take mTOR-mediated adaptive responses in the decidua, trophoblast, and fetal liver into account and are unlikely to be successful if they attempt to correct isolated fetal deficits associated with IUGR. Based on the concept that decreased fetal amino acid availability represents a key mechanism underpinning the development of IUGR, maternal amino acid supplementation has been contemplated [175, 176] as a strategy to prevent and treat IUGR. It is possible that the positive effects of maternal supplementation with branched chain amino acids on fetal growth that has been reported in animals with normal sized and IUGR fetuses [45, 177, 178] may be due to activation of mTOR in the decidua, trophoblast, and the fetus. Given the recent successful development of in vivo trophoblast-specific gene targeting approaches in mice [179–181], it may be possible to design interventions that activate placental mTOR signaling in IUGR in the future. Drug discovery aiming at identifying mTOR activators or inhibitors of DEPTOR may lead to the development of drugs useful in IUGR. Because mTOR activation promotes cancer cell proliferation, survival, metabolic transformation, and metastasis, a number of drugs have been developed for use in cancer [182]. Albeit speculative, it is possible that some of these drugs could be considered in selected cases of fetal overgrowth in maternal obesity and gestational diabetes, conditions associated with placental mTOR

activation and enhanced placental function [183]. Identifying biomarkers for IUGR in early pregnancy could improve the clinical management of these patients by allowing early intervention, preventing some of the perinatal complications associated with this condition. IUGR is associated with inhibition of mTOR signaling and increased IGFBP-1 phosphorylation in the decidua, and our data suggest that IGFBP-1 hyperphosphorylation in first trimester maternal plasma may serve as a novel predictive IUGR biomarker.

Acknowledgments

We thank KIMEN Design4Research (kimendesign4research.com) for the graphic design of figures.

References

1. Romo A, Carceller R, Tobajas J. Intrauterine growth retardation (IUGR): epidemiology and etiology. *Pediatr Endocrinol Rev* 2009; **6**(Suppl 3):332–336.
2. Chauhan SP, Rice MM, Grobman WA, Bailit J, Reddy UM, Wapner RJ, Varner MW, Thorp JM, Jr, Leveno KJ, Caritis SN, Prasad M, Tita ATN et al. Neonatal morbidity of small- and large-for-gestational-age neonates born at term in uncomplicated pregnancies. *Obstet Gynecol* 2017; **130**(3):511–519.
3. Hochner H, Friedlander Y, Calderon-Margalit R, Meiner V, Sagy Y, Avgil-Tsadok M, Burger A, Savitsky B, Siscovick DS, Manor O. Associations of maternal prepregnancy body mass index and gestational weight gain with adult offspring cardiometabolic risk factors. *Circulation* 2012; **125**(11):1381–1389.
4. Parsons TJ, Power C, Manor O. Fetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. *BMJ* 2001; **323**(7325):1331–1335.
5. Boney CM, Verma A, Tucker R, Vohr BS. Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus. *Pediatrics* 2005; **115**(3):e290–e296.
6. Whitaker RC. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. *Pediatrics* 2004; **114**(1):e29–e36.
7. Reynolds RM, Allan KM, Raja EA, Bhattacharya S, McNeill G, Hannaford PC, Sarwar N, Lee AJ, Bhattacharya S, Norman JE. Maternal obesity during pregnancy and premature mortality from cardiovascular event in adult offspring: follow-up of 1 323 275 person years. *BMJ* 2013; **347**(1):f4539–f4539.
8. Cnattingius S, Villamor E, Lagerros YT, Wikstrom AK, Granath F. High birth weight and obesity—a vicious circle across generations. *Int J Obes (Lond)* 2012; **36**(10):1320–1324.
9. Lawlor DA, Smith GD, O'Callaghan M, Alati R, Mamun AA, Williams GM, Najman JM. Epidemiologic evidence for the fetal overnutrition hypothesis: findings from the mater-university study of pregnancy and its outcomes. *Am J Epidemiol* 2007; **165**(4): 418–424.
10. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science* 2004; **305**(5691):1733–1736.
11. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of in utero and early-life conditions on adult health and disease. *N Engl J Med* 2008; **359**(1):61–73.
12. Gluckman PD, Hanson MA. The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab* 2004; **15**(4):183–187.
13. Sferruzzi-Perri AN, Owens JA, Pringle KG, Roberts CT. The neglected role of insulin-like growth factors in the maternal circulation regulating fetal growth. *J Physiol* 2011; **589**(1):7–20.
14. Sferruzzi-Perri AN, Vaughan OR, Forhead AJ, Fowden AL. Hormonal and nutritional drivers of intrauterine growth. *Curr Opin Clin Nutr Metab Care* 2013; **16**(3):298–309.
15. Gicquel C, Le Bouc Y. Hormonal regulation of fetal growth. *Horm Res* 2006; **65**(Suppl 3):28–33.
16. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev* 2006; **27**(2):141–169.
17. Newbern D, Freemark M. Placental hormones and the control of maternal metabolism and fetal growth. *Curr Opin Endocrinol Diabetes Obes* 2011; **18**(6):409–416.
18. Harding JE, Johnston BM. Nutrition and fetal growth. *Reprod Fertil Dev* 1995; **7**(3):539–547.
19. Fowden AL, Forhead AJ. Endocrine interactions in the control of fetal growth. *Nestle Nutr Inst Workshop Ser* 2013; **74**:91–102.
20. Forbes K, Westwood M. Maternal growth factor regulation of human placental development and fetal growth. *J Endocrinol* 2010; **207**(1):1–16.
21. Evain-Brion D. Hormonal regulation of fetal growth. *Horm Res* 1994; **42**(4-5):207–214.
22. Fowden AL, Forhead AJ. Endocrine regulation of feto-placental growth. *Horm Res* 2009; **72**(5):257–265.
23. Peng T, Golub TR, Sabatini DM. The immunosuppressant rapamycin mimics a starvation-like signal distinct from amino acid and glucose deprivation. *Mol Cell Biol* 2002; **22**(15):5575–5584.
24. Tee AR, Blenis J. mTor, translational control and human disease. *Sem Cell Dev Biol* 2005; **16**(1):29–37.
25. Martin DE, Hall MN. The expanding TOR signaling network. *Curr Opin Cell Biol* 2005; **17**(2):158–166.
26. Hay N, Soneneberg N. Upstream and downstream of mTOR. *Genes Dev* 2004; **18**(16):1926–1945.
27. Jacinto E, Hall MN. Correction: TOR signalling in bugs, brain and brawn. *Nat Rev Mol Cell Biol* 2003; **4**(2):117–126.
28. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**(2):274–293.
29. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005; **307**(5712):1098–1101.
30. Garcia-Martinez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). *Biochem J* 2008; **416**(3):375–385.
31. Facchinetti V, Ouyang W, Wei H, Soto N, Lazorchak A, Gould C, Lowry C, Newton AC, Mao Y, Miao RQ, Sessa WC, Qin J et al. The mammalian target of rapamycin complex 2 controls folding and stability of Akt and protein kinase C. *EMBO J* 2008; **27**(14):1932–1943.
32. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl VM, Gray NS, Sabatini DM. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. *Cell* 2009; **137**(5):873–886.
33. Murakami M, Ichisaka T, Maeda M, Oshiro N, Hara K, Edenhofer F, Kiyama H, Yonezawa K, Yamanaka S. mTOR is essential for growth and proliferation in early mouse embryos and embryonic stem cells. *Mol Cell Biol* 2004; **24**(15):6710–6718.
34. Gangloff YG, Mueller M, Dann SG, Svoboda P, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, Thomas G. Disruption of the mouse mTOR gene leads to early postimplantation lethality and prohibits embryonic stem cell development. *Mol Cell Biol* 2004; **24**(21):9508–9516.
35. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, Brown M, Fitzgerald KJ, Sabatini DM. Ablation in mice of the mTORC components raptor, rictor or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not to S6K1. *Dev Cell* 2006; **11**(6):859–871.
36. Shiota C, Woo JT, Lindner J, Shelton KD, Magnuson MA. Multiallelic disruption of the rictor gene in mice reveals that mTOR complex 2 is essential for fetal growth and viability. *Dev Cell* 2006; **11**(4):583–589.
37. Caron A, Mouchiroud M, Gautier N, Labbe SM, Villot R, Turcotte L, Secco B, Lamoureux G, Shum M, Gelinas Y, Marette A, Richard D et al.

Loss of hepatic DEPTOR alters the metabolic transition to fasting. *Mol Metab* 2017; **6**(5):447–458.
38. Wang Y, Zhang L, Zhou G, Liao Z, Ahmad H, Liu W, Wang T. Dietary L-arginine supplementation improves the intestinal development through increasing mucosal Akt and mammalian target of rapamycin signals in intra-uterine growth retarded piglets. *Br J Nutr* 2012; **108**(8):1371–1381.
39. Wang C, Zhang R, Zhou L, He J, Huang Q, Siyal FA, Zhang L, Zhong X, Wang T. Intrauterine growth retardation promotes fetal intestinal autophagy in rats via the mechanistic target of rapamycin pathway. *J Reprod Dev* 2017; **63**(6):547–554.
40. Elghazi L, Blandino-Rosano M, Alejandro E, Cras-Meneur C, Bernal-Mizrachi E. Role of nutrients and mTOR signaling in the regulation of pancreatic progenitors development. *Mol Metab* 2017; **6**(6):560–573.
41. Rachdi L, Aiello V, Duvillie B, Scharfmann R. L-Leucine alters pancreatic -cell differentiation and function via the mTor signaling pathway. *Diabetes* 2012; **61**(2):409–417.
42. Zhu MJ, Ford SP, Nathanielsz PW, Du M. Effect of maternal nutrient restriction in sheep on the development of fetal skeletal muscle. *Biol Reprod* 2004; **71**(6):1968–1973.
43. Calkins KL, Thamotharan S, Dai Y, Shin BC, Kalhan SC, Devaskar SU. Early dietary restriction in rats alters skeletal muscle tuberous sclerosis complex, ribosomal s6 and mitogen-activated protein kinase. *Nutr Res* 2018; **54**:93–104.
44. Rhoads RP, Baumgard LH, El-Kadi SW, Zhao LD. Physiology and endocrinology symposium: roles for insulin-supported skeletal muscle growth1,2. *J Anim Sci* 2016; **94**(5):1791–1802.
45. Wang CX, Chen F, Zhang WF, Zhang SH, Shi K, Song HQ, Wang YJ, Kim SW, Guan WT. Leucine promotes the growth of fetal pigs by increasing protein synthesis through the mTOR signaling pathway in longissimus dorsi muscle at late gestation. *J Agric Food Chem* 2018; **66**(15):3840–3849.
46. Ma X, Han M, Li D, Hu S, Gilbreath KR, Bazer FW, Wu G. L-Arginine promotes protein synthesis and cell growth in brown adipocyte precursor cells via the mTOR signal pathway. *Amino Acids* 2017; **49**(5):957–964.
47. Liang Q, Luo Z, Zeng J, Chen W, Foo SS, Lee SA, Ge J, Wang S, Goldman SA, Zlokovic BV, Zhao Z, Jung JU. Zika virus NS4A and NS4B proteins deregulate Akt-mTOR signaling in human fetal neural stem cells to inhibit neurogenesis and induce autophagy. *Cell Stem Cell* 2016; **19**(5):663–671.
48. Zhu Y, Pires KM, Whitehead KJ, Olsen CD, Wayment B, Zhang YC, Bugger H, Ilkun O, Litwin SE, Thomas G, Kozma SC, Abel ED. Mechanistic target of rapamycin (Mtor) is essential for murine embryonic heart development and growth. *PLoS One* 2013; **8**(1):e54221.
49. Wang HX, Shin J, Wang S, Gorentla B, Lin X, Gao J, Qiu YR, Zhong XP. mTORC1 in thymic epithelial cells is critical for thymopoiesis, T-cell generation, and temporal control of gammadeltaT17 development and TCRgamma/delta recombination. *PLoS Biol* 2016; **14**(2):e1002370.
50. Boylan JM, Sanders JA, Gruppuso PA. Regulation of fetal liver growth in a model of diet restriction in the pregnant rat. *Am J Physiol Regul Integr Comp Physiol* 2016; **311**(3):R478–R488.
51. Long B, Yin C, Fan Q, Yan G, Wang Z, Li X, Chen C, Yang X, Liu L, Zheng Z, Shi M, Yan X. Global liver proteome analysis using iTRAQ reveals AMPK-mTOR-autophagy signaling is altered by intrauterine growth restriction in newborn piglets. *J Proteome Res* 2016; **15**(4):1262–1273.
52. Abu Shehab M, Damerill I, Shen T, Rosario FJ, Nijland M, Nathanielsz PW, Kamat A, Jansson T, Gupta MB. Liver mTOR controls IGF-I bioavailability by regulation of protein kinase CK2 and IGFBP-1 phosphorylation in fetal growth restriction. *Endocrinology* 2014; **155**(4):1327–1339.
53. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 1993; **75**(1):73–82.
54. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). *Cell* 1993; **75**:59–72.
55. DeChiara TM, Efstratiadis A, Robertson EJ. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. *Nature* 1990; **345**(6270):78–80.
56. Efstratiadis A. Genetics of mouse growth. *Int J Dev Biol* 1998; **42**:955–976.
57. Sferruzzi-Perri AN, Owens JA, Pringle KG, Robinson JS, Roberts CT. Maternal insulin-like growth factors-I and -II act via different pathways to promote fetal growth. *Endocrinology* 2006; **147**(7):3344–3355.
58. Westwood M, Gibson JM, White A. Purification and characterization of the insulin-like growth factor-binding protein-1 phosphoform found in normal plasma. *Endocrinology* 1997; **138**(3):1130–1136.
59. Han VK, Matsell DG, Delhanty PJ, Hill DJ, Shimasaki S, Nygard K. IGF-binding protein mRNAs in the human fetus: tissue and cellular distribution of developmental expression. *Horm Res* 1996; **45**(3-5):160–166.
60. Abu Shehab M, Iosef C, Wildgruber R, Sardana G, Gupta MB. Phosphorylation of IGFBP-1 at discrete sites elicits variable effects on IGF-I receptor autophosphorylation. *Endocrinology* 2013; **154**(3):1130–1143.
61. Gibson JM, Aplin JD, White A, Westwood M. Regulation of IGF bioavailability in pregnancy. *Mol Hum Reprod* 2001; **7**(1):79–87.
62. Jones JI, Busby WH, Jr, Wright G, Smith CE, Kimack NM, Clemmons DR. Identification of the sites of phosphorylation in insulin-like growth factor binding protein-1. Regulation of its affinity by phosphorylation of serine 101. *J Biol Chem* 1993; **268**:1125–1131.
63. Jones JI, D'Ercole AJ, Camacho-Hubner C, Clemmons DR. Phosphorylation of insulin-like growth factor (IGF)-binding protein 1 in cell culture and in vivo: effects on affinity for IGF-I. *Proc Natl Acad Sci USA* 1991; **88**(17):7481–7485.
64. Westwood M. Role of insulin-like growth factor binding protein 1 in human pregnancy. *Rev Reprod* 1999; **4**(3):160–167.
65. Seferovic MD, Ali R, Kamei H, Liu S, Khosravi JM, Nazarian S, Han VK, Duan C, Gupta MB. Hypoxia and leucine deprivation induce human insulin-like growth factor binding protein-1 hyperphosphorylation and increase its biological activity. *Endocrinology* 2009; **150**(1):220–231.
66. Dolcini L, Sala A, Campagnoli M, Labo S, Valli M, Visai L, Minchiotti L, Monaco HL, Galliano M. Identification of the amniotic fluid insulin-like growth factor binding protein-1 phosphorylation sites and propensity to proteolysis of the isoforms. *FEBS J* 2009; **276**(20):6033–6046.
67. Frost RA, Tseng L. Insulin-like growth factor-binding protein-1 is phosphorylated by cultured human endometrial stromal cells and multiple protein kinases in vitro. *J Biol Chem* 1991; **266**:18082–18088.
68. Yu J, Iwashita M, Kudo Y, Takeda Y. Phosphorylated insulin-like growth factor (IGF)-binding protein-1 (IGFBP-1) inhibits while non-phosphorylated IGFBP-1 stimulates IGF-I-induced amino acid uptake by cultured trophoblast cells. *Growth Horm IGF Res* 1998; **8**(1):65–70.
69. Siddals KW, Westwood M, Gibson JM, White A. IGF-binding protein-1 inhibits IGF effects on adipocyte function: implications for insulin-like actions at the adipocyte. *J Endocrinol* 2002; **174**(2):289–297.
70. Gupta MB. The role and regulation of IGFBP-1 phosphorylation in fetal growth restriction. *J Cell Commun Signal* 2015; **9**(2):111–123.
71. Clemmons DR. Role of IGF binding proteins in regulating metabolism. *Trends Endocrinol Metab* 2016; **27**(6):375–391.
72. Jones JI, Gockerman A, Busby WH, Jr, Wright G, Clemmons DR. Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. *Proc Natl Acad Sci USA* 1993; **90**(22):10553–10557.
73. Ben Lagha N, Seurin D, Le Bouc Y, Binoux M, Berdal A, Menuelle P, Babajko S. Insulin-like growth factor binding protein (IGFBP-1) involvement in intrauterine growth retardation: study on IGFBP-1 overexpressing transgenic mice. *Endocrinology* 2006; **147**(10):4730–4737.
74. Watson CS, Bialek P, Anzo M, Khosravi J, Yee SP, Han VK. Elevated circulating insulin-like growth factor binding protein-1 is sufficient to cause fetal growth restriction. *Endocrinology* 2006; **147**(3):1175–1186.
75. Rajkumar K, Barron D, Lewitt MS, Murphy LJ. Growth retardation and hyperglycemia in insulin-like growth factor binding protein-1 transgenic mice. *Endocrinology* 1995; **136** (9):4029–4034.
76. Giudice LC, de Zegher F, Gargosky SE, Dsupin BA, de las Fuentes L, Crystal RA, Hintz RL, Rosenfeld RG. Insulin-like growth factors and

their binding proteins in the term and preterm human fetus and neonate with normal and extremes of intrauterine growth. *J Clin Endocrinol Metab* 1995; **80**:1548–1555.

77. Abu Shehab M, Khosravi J, Han VK, Shilton BH, Gupta MB. Site-specific IGFBP-1 hyper-phosphorylation in fetal growth restriction: clinical and functional relevance. *J Proteome Res* 2010; **9**(4):1873–1881.
78. Abu Shehab M, Inoue S, Han VK, Gupta MB. Site specific phosphorylation of insulin-like growth factor binding protein-1 (IGFBP-1) for evaluating clinical relevancy in fetal growth restriction. *J Proteome Res* 2009; **8**(11):5325–5335.
79. Popovici RM, Lu M, Bhatia S, Faessen GH, Giaccia AJ, Giudice LC. Hypoxia regulates insulin-like growth factor-binding protein 1 in human fetal hepatocytes in primary culture: suggestive molecular mechanisms for in utero fetal growth restriction caused by uteroplacental insufficiency. *J Clin Endocrinol Metab* 2001; **86**:2653–2659.
80. Tazuke SI, Mazure NM, Sugawara J, Carland G, Faessen GH, Suen LF, Irwin JC, Powell DR, Giaccia AJ, Giudice LC. Hypoxia stimulates insulin-like growth factor binding protein 1 (IGFBP-1) gene expression in HepG2 cells: a possible model for IGFBP-1 expression in fetal hypoxia. *Proc Natl Acad Sci USA* 1998; **95**(17):10188–10193.
81. Jousse C, Bruhat A, Ferrara M, Fafournoux P. Physiological concentration of amino acids regulates insulin-like-growth-factor-binding protein 1 expression. *Biochem J* 1998; **334**(1):147–153.
82. Averous J, Maurin AC, Bruhat A, Jousse C, Arliguie C, Fafournoux P. Induction of IGFBP-1 expression by amino acid deprivation of HepG2 human hepatoma cells involves both a transcriptional activation and an mRNA stabilization due to its 3'UTR. *FEBS Lett* 2005; **579**(12):2609–2614.
83. Damerill I, Biggar KK, Abu Shehab M, Li SS, Jansson T, Gupta MB. Hypoxia increases IGFBP-1 phosphorylation mediated by mTOR inhibition. *Mol Endocrinol* 2016; **30**(2):201–216.
84. Singal SS, Nygard K, Dhruv MR, Biggar K, Shehab MA, Li SS, Jansson T, Gupta MB. Co-localization of insulin-like growth factor binding protein-1, casein kinase-2β, and mechanistic target of rapamycin in human hepatocellular carcinoma cells as demonstrated by dual immunofluorescence and in situ proximity ligation assay. *Am J Pathol* 2018; **188**(1):111–124.
85. Malkani N, Jansson T, Gupta MB. IGFBP-1 hyperphosphorylation in response to leucine deprivation is mediated by the AAR pathway. *Mol Cell Endocrinol* 2015; **412**:182–195.
86. Kilberg MS, Shan J, Su N. ATF4-dependent transcription mediates signaling of amino acid limitation. *Trends Endocrinol Metab* 2009; **20**(9):436–443.
87. Malkani N, Biggar K, Shehab MA, Li SS, Jansson T, Gupta MB. Increased IGFBP-1 phosphorylation in response to leucine deprivation is mediated by CK2 and PKC. *Mol Cell Endocrinol* 2016; **425**:48–60.
88. Hanif IM, Shazib MA, Ahmad KA, Pervaiz S. Casein kinase II: an attractive target for anti-cancer drug design. *Int J Biochem Cell Biol* 2010; **42**(10):1602–1605.
89. Montenarh M. Cellular regulators of protein kinase CK2. *Cell Tissue Res* 2010; **342**(2):139–146.
90. Litchfield DW. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. *Biochem J* 2003; **369**(1):1–15.
91. Sutherland A, Li C, Nathanielsz PW, Jansson T, Gupta MB. Inhibition of mTOR, activation of amino acid response (AAR) and IGFBP-1 hyperphosphorylation in the fetal liver precede the development of IUGR in baboons following maternal nutrient restriction. *Reprod Sci* 2018; **25**:76A Abstract.
92. Jansson T, Powell TL. Role of placental nutrient sensing in developmental programming. *Clin Obstet Gynecol* 2013; **56**(3):591–601.
93. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. *Cell* 2017; **169**(2):361–371.
94. Roux PP, Topisirovic I. Signaling pathways involved in the regulation of mRNA translation. *Mol Cell Biol* 2018; **38**(12):e00070–18.
95. Rosario FJ, Kanai Y, Powell TL, Jansson T. Mammalian target of rapamycin signalling modulates amino acid uptake by regulating transporter cell surface abundance in primary human trophoblast cells. *J Physiol* 2013; **591**(3):609–625.
96. Lager S, Jansson T, Powell TL. Differential regulation of placental amino acid transport by saturated and unsaturated fatty acids. *Am J Physiol Cell Physiol* 2014; **307**(8):C738–C744.
97. Roos S, Lagerlöf O, Wennergren M, Powell TL, Jansson T. Regulation of amino acid transporters by glucose and growth factors in cultured primary human trophoblast cells is mediated by mTOR signaling. *Am J Physiol Cell* 2009; **297**(3):C723–C731.
98. Vaughan OR, Fisher HM, Dionelis KN, Jefferies EC, Higgins JS, Musial B, Sferruzzi-Perri AN, Fowden AL. Corticosterone alters materno-fetal glucose partitioning and insulin signalling in pregnant mice. *J Physiol* 2015; **593**(5):1307–1321.
99. Jones HN, Jansson T, Powell TL. Full-length adiponectin attenuates insulin signaling and inhibits insulin-stimulated amino acid transport in human primary trophoblast cells. *Diabetes* 2010; **59**(5):1161–1170.
100. Aye IL, Gao X, Weintraub ST, Jansson T, Powell TL. Adiponectin inhibits insulin function in primary trophoblasts by PPARα-mediated ceramide synthesis. *Mol Endocrinol* 2014; **28**(4):512–524.
101. Aye IL, Rosario FJ, Powell TL, Jansson T. Adiponectin supplementation in pregnant mice prevents the adverse effects of maternal obesity on placental function and fetal growth. *Proc Natl Acad Sci USA* 2015; **112**(41):12858–12863.
102. Silva E, Rosario FJ, Powell TL, Jansson T. Mechanistic target of rapamycin is a novel molecular mechanism linking folate availability and cell function. *J Nutr* 2017; **147**(7):1237–1242.
103. Rosario FJ, Nathanielsz PW, Powell TL, Jansson T. Maternal folate deficiency causes inhibition of mTOR signaling, down-regulation of placental amino acid transporters and fetal growth restriction in mice. *Sci Rep* 2017; **7**(1):3982.
104. Rosario FJ, Powell TL, Jansson T. Mechanistic target of rapamycin (mTOR) regulates trophoblast folate uptake by modulating the cell surface expression of FR-? and the RFC. *Sci Rep* 2016; **6**(1):31705.
105. Cetin I, Corbetta C, Sereni LP, Marconi AM, Bozzetti P, Pardi G, Battaglia FC. Umbilical amino acid concentrations in normal and growth-retarded fetuses sampled in utero by cordocentesis. *Am J Obstet Gynecol* 1990; **162**(1):253–261.
106. Economides DL, Nicolaides KH. Blood glucose and oxygen tension levels in small-for-gestational-age fetuses. *Am J Obstet Gynecol* 1989; **160**(2):385–389.
107. Jansson T, Powell TL. IFPA 2005 Award in Placentology Lecture. human placental transport in altered fetal growth: does the placenta function as a nutrient sensor?—a review. *Placenta* 2006; **27**(Suppl A):91–97.
108. Diaz P, Powell TL, Jansson T. The role of placental nutrient sensing in maternal-fetal resource allocation. *Biol Reprod* 2014; **91**(4):82.
109. Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev* 1999; **13** (11):1422–1437.
110. Duvel K, Yecies JL, Menon S, Raman P, Lipovsky AI, Souza AL, Triantafellow E, Ma Q, Gorski R, Cleaver S, Vander Heiden MG, MacKeigan JP et al. Activation of a metabolic gene regulatory network downstream of mTOR complex 1. *Mol Cell* 2010; **39**(2):171–183.
111. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nat Cell Biol* 2011; **13**(2):132–141.
112. Cunningham JT, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* 2007; **450**(7170):736–740.
113. Rosario FJ, Gupta MB, Myatt L, Powell TL, Glenn JP, Cox LA, Jansson T. Mechanistic target of rapamycin complex 1 is a positive regulator of genes encoding electron transport chain proteins and oxidative phosphorylation, which is inhibited in placentas of growth restricted fetuses. *Sci Rep* 2018; In press.
114. Roos S, Kanai Y, Prasad PD, Powell TL, Jansson T. Regulation of placental amino acid transporter activity by mammalian target of rapamycin. *Am J Physiol Cell* 2009; **296**(1):C142–C150.
115. Roos S, Jansson N, Palmberg I, Säljö K, Powell TL, Jansson T. Mammalian target of rapamycin in the human placenta regulates leucine

transport and is down-regulated in restricted fetal growth. *J Physiol* 2007; **582**(1):449–459.
116. Rosario FJ, Dimasuay KG, Kanai Y, Powell TL, Jansson T. Regulation of amino acid transporter trafficking by mTORC1 in primary human trophoblast cells is mediated by the ubiquitin ligase Nedd4-2. *Clin Sci* 2016; **130**(7):499–512.
117. Roos S, Jansson N, Palmberg I, Saljo K, Powell TL, Jansson T. Mammalian target of rapamycin in the human placenta regulates leucine transport and is down-regulated in restricted fetal growth. *J Physiol* 2007; **582**(1):449–459.
118. Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones DS, Burton GJ. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am J Pathol* 2008; **173**(2):451–462.
119. Gaccioli F, White V, Capobianco E, Powell TL, Jawerbaum A, Jansson T. Maternal overweight induced by a diet with high content of saturated fat activates placental mTOR and eIF2alpha signaling and increases fetal growth in rats. *Biol Reprod* 2013; **89**(4):96: 1–11.
120. Arroyo JA, Brown LD, Galan HL. Placental mammalian target of rapamycin and related signaling pathways in an ovine model of intrauterine growth restriction. *Am J Obstet Gynecol* 2009; **201**(6): 616.e1–616.e7.
121. Rosario FJ, Jansson N, Kanai Y, Prasad PD, Powell TL, Jansson T. Maternal protein restriction in the rat inhibits placental insulin, mTOR, and STAT3 signaling and down-regulates placental amino acid transporters. *Endocrinology* 2011; **152**(3):1119–1129.
122. Kavitha JV, Rosario FJ, Nijland MJ, McDonald TJ, Wu G, Kanai Y, Powell TL, Nathanielsz PW, Jansson T. Down-regulation of placental mTOR, insulin/IGF-I signaling, and nutrient transporters in response to maternal nutrient restriction in the baboon. *FASEB J* 2014; **28**(3):1294–1305.
123. Sferruzzi-Perri AN, Vaughan OR, Coan PM, Suciu MC, Darbyshire R, Constancia M, Burton GJ, Fowden AL. Placental-specific Igf2 deficiency alters developmental adaptations to undernutrition in mice. *Endocrinology* 2011; **152**(8):3202–3212.
124. Irwin JC, Giudice LC. Insulin-like growth factor binding protein-1 binds to placental cytotrophoblast alpha5beta1 integrin and inhibits cytotrophoblast invasion into decidualized endometrial stromal cultures. *Growth Horm IGF Res* 1998; **8**(1):21–31.
125. Lacey H, Haigh T, Westwood M, Aplin JD. Mesenchymally-derived insulin-like growth factor 1 provides a paracrine stimulus for trophoblast migration. *BMC Dev Biol* 2002; **2**(1):5.
126. Martina NA, Kim E, Chitkara U, Wathen NC, Chard T, Giudice LC. Gestational age-dependent expression of insulin-like growth factor-binding protein-1 (IGFBP-1) phosphoisoforms in human extraembryonic cavities, maternal serum, and decidua suggests decidua as the primary source of IGFBP-1 in these fluids during early pregnancy. *J Clin Endocrinol Metab* 1997; **82**:1894–1898.
127. Fang Q, Wang YX, Zhou Y. Insulin-like growth factor binding protein 1 and human embryonic development during 6 - 10 gestational weeks. *Chin Med J (Engl)* 2004; **117**:488–491.
128. Holmes R, Montemagno R, Jones J, Preece M, Rodeck C, Soothill P. Fetal and maternal plasma insulin-like growth factors and binding proteins in pregnancies with appropriate or retarded fetal growth. *Early Hum Dev* 1997; **49**(1):7–17.
129. Hall K, Hansson U, Lundin G, Luthman M, Persson B, Povoa G, Stangenberg M, Ofverholm U. Serum levels of somatomedins and somatomedin-binding protein in pregnant women with type I or gestational diabetes and their infants. *J Clin Endocrinol Metab* 1986; **63**(6):1300–1306.
130. Hills FA, English J, Chard T. Circulating levels of IGF-I and IGF-binding protein-1 throughout pregnancy: relation to birthweight and maternal weight. *J Endocrinol* 1996; **148**(2):303–309.
131. Wang HS, Lim J, English J, Irvine L, Chard T. The concentration of insulin-like growth factor-I and insulin-like growth factor-binding protein-1 in human umbilical cord serum at delivery: relation to fetal weight. *J Endocrinol* 1991; **129**(3):459–464.
132. Boyne MS, Thame M, Bennett FI, Osmond C, Miell JP, Forrester TE. The relationship among circulating insulin-like growth factor (IGF)-I, IGF-binding proteins-1 and -2, and birth anthropometry: a prospective study. *J Clin Endocrinol Metab* 2003; **88**(4):1687–1691.
133. Gibson JM, Westwood M, Lauszus FF, Klebe JG, Flyvbjerg A, White A. Phosphorylated insulin-like growth factor binding protein 1 is increased in pregnant diabetic subjects. *Diabetes* 1999; **48**(2):321–326.
134. Langford K, Blum W, Nicolaides K, Jones J, McGregor A, Miell J. The pathophysiology of the insulin-like growth factor axis in fetal growth failure: a basis for programming by undernutrition? *Eur J Clin Invest* 1994; **24**(12):851–856.
135. Olausson H, Lof M, Brismar K, Forsum E, Sohlstrom A. Maternal serum concentrations of insulin-like growth factor (IGF)-I and IGF binding protein-1 before and during pregnancy in relation to maternal body weight and composition and infant birth weight. *Br J Nutr* 2010; **104**(6):842–848.
136. Baldwin S, Chung T, Rogers M, Chard T, Wang HS. Insulin-like growth factor-binding protein-1, glucose tolerance and fetal growth in human pregnancy. *J Endocrinol* 1993; **136**(2):319–325.
137. Fowler D, Albaiges G, Lees C, Jones J, Nicolaides K, Miell J. The role of insulin-like growth factor binding protein-1 phosphoisoforms in pregnancies with impaired placental function identified by doppler ultrasound. *Hum Reprod* 1999; **14**(11):2881–2885.
138. Larsen T, Main K, Andersson AM, Juul A, Greisen G, Skakkebaeck NE. Growth hormone, insulin-like growth factor I and its binding proteins 1 and 3 in last trimester intrauterine growth retardation with increased pulsatility index in the umbilical artery. *Clin Endocrinol (Oxf)* 1996; **45**(3):315–319.
139. Bhatia S, Faessen G, Carland R, Balise RL, Gargosky SE, Druzin M, El-Sayed Y, Wilson DM, Giudice LC. A longitudinal analysis of maternal serum insulin-like growth factor I (IGF-I) and total and non-phosphorylated IGF-binding protein-1 in human pregnancies complicated by intrauterine growth restriction. *J Clin Endocrinol Metab* 2002; **87**(4):1864–1870.
140. Sifakis S, Akolekar R, Kappou D, Mantas N, Nicolaides KH. Maternal serum insulin-like growth factor (IGF-I) and binding proteins IGFBP-1 and IGFBP-3 at 11–13 weeks' gestation in pregnancies delivering small for gestational age neonates. *Eur J Obstet Gynecol Reprod Biol* 2012; **161**(1):30–33.
141. Qiu C, Vadachkoria S, Meryman L, Frederick IO, Williams MA. Maternal plasma concentrations of IGF-1, IGFBP-1, and C-peptide in early pregnancy and subsequent risk of gestational diabetes mellitus. *Am J Obstet Gynecol* 2005; **193**(5):1691–1697.
142. Clapp 3rd JF, Schmidt F, Paranjape A, Lopez B. Maternal insulin-like growth factor-I levels (IGF-I) reflect placental mass and neonatal fat mass. *Am J Obstet Gynecol* 2004; **190**(3):730–736.
143. Sferruzzi-Perri AN, Owens J, Standen P, Taylor RL, Robinson JS, Roberts CT. Early pregnancy maternal endocrine insulin-like growth factor i programs the placenta for increased functional capacity throughout gestation. *Endocrinology* 2007; **148**(9):4362–4370.
144. Sohlstrom A, Fernberg P, Owens JA, Owens PC. Maternal nutrition affects the ability of treatment with IGF-I and IGF-II to increase growth of the placenta and fetus, in guinea pigs. *Growth Horm IGF Res* 2001; **11**(6):392–398.
145. Thongsong B, Bonkobara M, Matsumoto M, Jang JS, Matsuki N, Inaba M, Ono K. Effects of insulin-like growth factor-I on maternal and fetal plasma amino acid levels in pregnant rats. *J Vet Med Sci* 2002; **64**(9):859–861.
146. Shehab MA, Biggar K, Singal SS, Nygard K, Shun-Cheng Li S, Jansson T, Gupta MB. Exposure of decidualized HIESC to low oxygen tension and leucine deprivation results in increased IGFBP-1 phosphorylation and reduced IGF-I bioactivity. *Mol Cell Endocrinol* 2017; **452**: 1–14.
147. Gupta MB, Abu Shehab M, Nygard K, Biggar K, Singal SS, Santoro N, Powell TL, Jansson T. IUGR is associated with marked hyperphosphorylation of decidual and maternal plasma IGFBP-1. *J Clin Endocrinol Metab* 2018 Aug 16. doi: 10.1210/jc.2018-00820. [Epub ahead of print].

148. Shibuya H, Sakai K, Kabir-Salmani M, Wachi Y, Iwashita M. Polymerization of insulin-like growth factor-binding protein-1 (IGFBP-1) potentiates IGF-I actions in placenta. *J Cell Physiol* 2011; **226**(2):434–439.
149. Mayama R, Izawa T, Sakai K, Suciu N, Iwashita M. Improvement of insulin sensitivity promotes extravillous trophoblast cell migration stimulated by insulin-like growth factor-I. *Endocr J* 2013; **60**:359–368.
150. Deng W, Cha J, Yuan J, Haraguchi H, Bartos A, Leishman E, Viollet B, Bradshaw HB, Hirota Y, Dey SK. p53 coordinates decidual sestrin 2/AMPK/mTORC1 signaling to govern parturition timing. *J Clin Invest* 2016; **126**(8):2941–2954.
151. Cha J, Bartos A, Egashira M, Haraguchi H, Saito-Fujita T, Leishman E, Bradshaw H, Dey SK, Hirota Y. Combinatory approaches prevent preterm birth profoundly exacerbated by gene-environment interactions. *J Clin Invest* 2013; **123**(9):4063–4075.
152. Hirota Y, Cha J, Yoshie M, Daikoku T, Dey SK. Heightened uterine mammalian target of rapamycin complex 1 (mTORC1) signaling provokes preterm birth in mice. *Proc Natl Acad Sci USA* 2011; **108**(44):18073–18078.
153. Kniss DA, Shubert PJ, Zimmerman PD, Landon MB, Gabbe SG. Insulin-like growth factors. Their regulation of glucose and amino acid transport in placental trophoblasts isolated from first-trimester chorionic villi. *J Reprod Med* 1994; **39**:249–256.
154. Fang J, Mao D, Smith CH, Fant ME. IGF regulation of neutral amino acid transport in the BeWo choriocarcinoma cell line (b30 clone): evidence for MAP kinase-dependent and MAP kinase-independent mechanisms. *Growth Horm IGF Res* 2006; **16**(5-6):318–325.
155. Karl PI. Insulin-like growth factor-1 stimulates amino acid uptake by the cultured human placental trophoblast. *J Cell Physiol* 1995; **165**(1):83–88.
156. Karl PI, Alpy KL, Fisher SE. Amino acid transport by the cultured human placental trophoblast: effect of insulin on AIB transport. *Am J Physiol* 1992; **262**(4):C834–C839.
157. Jones H, Crombleholme T, Habli M. Regulation of amino acid transporters by adenoviral-mediated human insulin-like growth factor-1 in a mouse model of placental insufficiency in vivo and the human trophoblast line BeWo in vitro. *Placenta* 2014; **35**(2):132–138.
158. Yung HW, Calabrese S, Hynx D, Hemmings BA, Cetin I, Charnock-Jones S, Burton GJ. Evidence of placental translation inhibition and endoplasmic reticulum stress in the etiology of human intrauterine growth restriction. *Am J Pathol* 2008; **173**(2):451–462.
159. Chen YY, Rosario FJ, Shehab MA, Powell TL, Gupta MB, Jansson T. Increased ubiquitination and reduced plasma membrane trafficking of placental amino acid transporter SNAT-2 in human IUGR. *Clin Sci* 2015; **129**(12):1131–1141.
160. Glazier JD, Cetin I, Perugino G, Ronzoni S, Grey AM, Mahendran D, Marconi AM, Pardi G, Sibley CP. Association between the activity of the system A amino acid transporter in the microvillous plasma membrane of the human placenta and severity of fetal compromise in intrauterine growth restriction. *Pediatr Res* 1997; **42**(4):514–519.
161. Mahendran D, Donnai P, Glazier JD, D'Souza SW, Boyd RDH, Sibley CP. Amino acid (System A) transporter activity in microvillous membrane vesicles from the placentas of appropriate and small for gestational age babies. *Pediatr Res* 1993; **34**(5):661–665.
162. Jansson T, Ylvén K, Wennergren M, Powell TL. Glucose transport and system A activity in syncytiotrophoblast microvillous and basal plasma membranes in intrauterine growth restriction. *Placenta* 2002; **23**(5):392–399.
163. Norberg S, Powell TL, Jansson T. Intrauterine growth restriction is associated with a reduced activity of placental taurine transporters. *Pediatr Res* 1998; **44**(2):233–238.
164. Jansson T, Scholtbach V, Powell TL. Placental transport of leucine and lysine is reduced in intrauterine growth restriction. *Pediatr Res* 1998; **44**(4):532–537.
165. Paolini CL, Marconi AM, Ronzoni S, Di Noio M, Fennessey PV, Pardi G, Battaglia FC. Placental transport of leucine, phenylalanine, glycine, and proline in intrauterine growth-restricted pregnancies. *J Clin Endocrinol Metab* 2001; **86**(11):5427–5432.
166. Chen YY, Gupta MB, Grattton R, Powell TL, Jansson T. Down-regulation of placental folate transporters in intrauterine growth restriction. *J Nutr Biochem* 2018; **59**:136–141.
167. Jansson T, Eliasson L, Rosario FJ, Powell TL, Gupta MG. Remote control of fetal metabolism by placental mTOR signaling. *Reprod Sci* 2012; **19** 151A (Abstract).
168. Um SH, Frigerio F, Watanabe M, Picard F, Joaquin M, Sticker M, Fumagalli S, Allegrini PR, Kozma SC, Auwerx J, G T. Erratum: Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 2004; **431**(7005):200–205.
169. Um SH, Sticker-Jantscheff M, Chau GC, Vintersten K, Mueller M, Gangloff YG, Adams RH, Spetz JF, Elghazi L, Pfluger PT, Pende M, Bernal-Mizrachi E et al. S6K1 controls pancreatic beta cell size independently of intrauterine growth restriction. *J Clin Invest* 2015; **125**(7):2736–2747.
170. Xiang H, Liu S, Zong C, Li Z, Liu Y, Ma X, Cao Y. A single nucleotide polymorphism in the MTOR gene is associated with recurrent spontaneous abortion in the Chinese female population. *Syst Biol Reprod Med* 2015; **61**(4):205–210.
171. Hennig M, Fiedler S, Jux C, Thierfelder L, Drenckhahn JD. Prenatal mechanistic target of rapamycin complex 1 (m TORC1) inhibition by rapamycin treatment of pregnant mice causes intrauterine growth restriction and alters postnatal cardiac growth, morphology, and function. *J Am Heart Assoc* 2017; **6**(8):005506–25.
172. Horikoshi M, Beaumont RN, Day FR, Warrington NM, Kooijman MN, Fernandez-Tajes J, Feenstra B, van Zuydam NR, Gaulton KJ, Grarup N, Bradfield JP, Strachan DP et al. Genome-wide associations for birth weight and correlations with adult disease. *Nature* 2016; **538**(7624):248–252.
173. Beaumont RN, Warrington NM, Cavadino A, Tyrrell J, Nodzenski M, Horikoshi M, Geller F, Myhre R, Richmond RC, Paternoster L, Bradfield JP, Kreiner-Moller E et al. Genome-wide association study of offspring birth weight in 86 577 women identifies five novel loci and highlights maternal genetic effects that are independent of fetal genetics. *Hum Mol Genet* 2018; **27**(4):742–756.
174. Xue A, Wu Y, Zhu Z, Zhang F, Kemper KE, Zheng Z, Yengo L, Lloyd-Jones LR, Sidorenko J, Wu Y, eQTLGen C, McRae AF et al. Genome-wide association analyses identify 143 risk variants and putative regulatory mechanisms for type 2 diabetes. *Nat Commun* 2018; **9**(1):2941.
175. Brown LD, Green AS, Limesand SW, Rozance PJ. Maternal amino acid supplementation for intrauterine growth restriction. *Front Biosci (Schol Ed)* 2011; **3**:428–444.
176. Lin G, Wang X, Wu G, Feng C, Zhou H, Li D, Wang J. Improving amino acid nutrition to prevent intrauterine growth restriction in mammals. *Amino Acids* 2014; **46**(7):1605–1623.
177. Mogami H, Yura S, Itoh H, Kawamura M, Fujii T, Suzuki A, Aoe S, Ogawa Y, Sagawa N, Konishi I, Fujii S. Isocaloric high-protein diet as well as branched-chain amino acids supplemented diet partially alleviates adverse consequences of maternal undernutrition on fetal growth. *Growth Horm IGF Res* 2009; **19**(6):478–485.
178. Teodoro GF, Vianna D, Torres-Leal FL, Pantaleao LC, Matos-Neto EM, Donato J, Jr, Tirapegui J. Leucine is essential for attenuating fetal growth restriction caused by a protein-restricted diet in rats. *J Nutr* 2012; **142**(5):924–930.
179. Harris LK. Could peptide-decorated nanoparticles provide an improved approach for treating pregnancy complications? *Nanomedicine* 2016; **11**(17):2235–2238.
180. Tobita T, Kiyozumi D, Ikawa M. Placenta-specific gene manipulation using lentiviral vector and its application. *Placenta* 2017; **59**(Suppl 1):S37–S43.
181. Renaud SJ, Karim Rumi MA, Soares MJ. Review: Genetic manipulation of the rodent placenta. *Placenta* 2011; **32**(Suppl 2):S130–S135.
182. Martelli AM, Buontempo F, McCubrey JA. Drug discovery targeting the mTOR pathway. *Clin Sci* 2018; **132**:543–568.
183. Jansson N, Rosario FJ, Gaccioli F, Lager S, Jones HN, Roos S, Jansson T, Powell TL. Activation of placental mTOR signaling and amino acid transporters in obese women giving birth to large babies. *J Clin Endocrinol Metab* 2013; **98**:105–113.

184. Jansson T, Ekstrand Y, Björn C, Wennergren M, Powell TL. Alterations in the activity of placental amino acid transporters in pregnancies complicated by diabetes. *Diabetes* 2002; **51**:2214–2219.
185. Sati L, Soygur B, Celik-Ozenci C. Expression of mammalian target of rapamycin and downstream targets in normal and gestational diabetic human term placenta. *Reprod Sci* 2016; **23**:324–332.
186. Rosario FJ, Powell TL, Jansson T. Activation of placental insulin and mTOR signaling in a mouse model of maternal obesity associated with fetal overgrowth. *Am J Physiol Regul Integr Comp Physiol* 2016; **310**:R87–R93.
187. Jones HN, Woollett LP, Barbour N, Prasad PP, Powell TL, Jansson T. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J* 2009; **23**:271–278.