Published in final edited form as:

Placenta. 2014 December ; 35(12): 1007–1012. doi:10.1016/j.placenta.2014.10.006.

Labor Inhibits Placental Mechanistic Target of Rapamycin Complex 1 Signaling

Susanne LAGER^{a,1,*}, Irving L.M.H. AYE^{a,*}, Francesca GACCIOLI^{a,1}, Vanessa I. RAMIREZ^a, Thomas JANSSON^a, and Theresa L. POWELL^a

^aCenter for Pregnancy and Newborn Research, Department of Obstetrics and Gynecology, University of Texas Health Science Center, San Antonio, Texas 78229, USA

Abstract

Introduction—Labor induces a myriad of changes in placental gene expression. These changes may represent a physiological adaptation inhibiting placental cellular processes associated with a high demand for oxygen and energy (*e.g.*, protein synthesis and active transport) thereby promoting oxygen and glucose transfer to the fetus. We hypothesized that mechanistic target of rapamycin complex 1 (mTORC1) signaling, a positive regulator of trophoblast protein synthesis and amino acid transport, is inhibited by labor.

Methods—Placental tissue was collected from healthy, term pregnancies (n=15 no-labor; n=12 labor). Activation of Caspase-1, IRS1/Akt, STAT, mTOR, and inflammatory signaling pathways was determined by Western blot. NFκB p65 and PPARγ DNA binding activity was measured in isolated nuclei.

Results—Labor increased Caspase-1 activation and mTOR complex 2 signaling, as measured by phosphorylation of Akt (S473). However, mTORC1 signaling was inhibited in response to labor as evidenced by decreased phosphorylation of mTOR (S2448) and 4EBP1 (T37/46 and T70). Labor also decreased NFκB and PPARγ DNA binding activity, while having no effect on IRS1 or STAT signaling pathway.

Discussion and conclusion—Several placental signaling pathways are affected by labor, which has implications for experimental design in studies of placental signaling. Inhibition of placental mTORC1 signaling in response to labor may serve to down-regulate protein synthesis and amino acid transport, processes that account for a large share of placental oxygen and glucose consumption. We speculate that this response preserves glucose and oxygen for transfer to the fetus during the stressful events of labor.

© 2014 Elsevier Ltd. All rights reserved.

Address for correspondence and reprint requests: Susanne Lager, PhD, Department of Obstetrics and Gynaecology, University of Cambridge, Box 223, The Rosie Hospital, Robinson Way, Cambridge CB2 0SW, Tel. +44(0)1223 336874, sml60@medschl.cam.ac.uk.

¹Present address: Department of Obstetrics and Gynaecology, University of Cambridge, Rosie Hospital, Robinson Way, Cambridge CB2 0SW, UK

*Susanne Lager and Irving L.M.H. Aye contributed equally to this work.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

PPAR γ ; NF κ B; MAPK; mTOR; STAT; Insulin

Introduction

The physiological process of labor has a profound impact upon placental gene expression [1, 2], as well as production of hormones and cytokines such as interleukin (IL)-1 β [2], which may alter placental function. Furthermore, the placenta is subject to hypoxia and re-oxygenation events during labor, with evidence of increased placental oxidative stress [2]. These factors can modulate placental signaling pathways and labor has previously been shown to alter activation or expression of nuclear factor (NF) κ B, the peroxisome proliferator-activated receptor (PPAR)s, and heat shock proteins in the placenta [2–4]. However given the nature of labor, other cellular signaling pathways in the placenta may also be affected.

Spontaneous labor at term is recognized as a localized inflammatory process, associated with the infiltration of leukocytes and increased pro-inflammatory cytokines in the extra-placental membranes [5]. Recent studies have also implicated labor with increased levels of pro-inflammatory cytokines in the placenta [2], which in turn regulate several downstream pathways. The higher levels of IL-1 β may be a result of increased Caspase-1 activation because it is involved in IL-1 β maturation [6]. The stress events (hypoxia/re-oxygenation) and/or the elevated levels of pro-inflammatory cytokines in the placenta may also affect other signaling pathways such as the mitogen activated protein kinases (MAPK): extracellular-signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 [7], and Signal Transducer and Activator of Transcription (STAT) signaling [8]. Leptin is increased in the placenta in response to labor [2] and may activate ERK, p38, and STAT signaling [8, 9]. Further, leptin can also modulate insulin receptor substrate 1 (IRS1) phosphorylation [10]. Yet, whether labor affects activation of these signaling pathways is currently not known.

The events of labor also have the potential to affect activation of the mechanistic target of rapamycin (mTOR) signaling pathway. mTOR has been reported to be regulated by multiple factors, including cytokines, hypoxia, and leptin [11], which are altered in the placenta during labor. mTOR is a serine/threonine kinase that is present in the cell in two complexes: mTORC1 and mTORC2. These two complexes have both distinct and overlapping functions [11]. Activation of mTORC1 results in the phosphorylation of eukaryotic initiation factor 4E binding protein 1 (4EBP1) and ribosomal protein S6 (rpS6), while mTORC2 phosphorylates Akt at S473.

The aim of this study was to determine activation/expression of Caspase-1, IRS1/Akt, STAT, MAPKs, mTOR, and PPAR γ in term human placenta from healthy pregnancies of normal weight women. We demonstrate that multiple placental signaling pathways are affected by labor, thus highlighting labor as an important confounding factor when analyzing activation of placental signaling pathways.

Patients and Methods

Ethics and study subjects

Human placental tissue samples were collected after informed and written consent. Placental samples and medical information were added to a tissue/data repository approved by the Institutional Review Board at University of Texas Health Science Center, San Antonio (HSC20100262H). De-identified tissue samples and medical information were then made available for the current study. Twenty-seven women with normal ($< 25 \text{ kg/m}^2$) pre-pregnancy or first trimester body mass index (BMI) and uncomplicated, term pregnancies (> 37 weeks of gestation) were included in this study. Of the placentas collected from laboring women, two women spontaneously went into labor, five women had labor induced by oxytocin, and five women had augmented labor.

Placenta collection

Placentas were obtained immediately after delivery. After removing decidua basalis and chorionic plate, villous tissue was homogenized in an ice-cold buffer (250 mM sucrose, 10 mM hepes, pH 7.4) containing protease and phosphatase inhibitors (Sigma-Aldrich, St. Louis, MO). The homogenates were stored at -80°C until further processing.

Western blotting

Placental homogenates were spun (16,000 g, 15 min, 4°C), supernatants collected and used for protein expression analysis; protein concentrations were determined with Pierce BCA Protein Assay kit (Thermo Scientific, Rockford, IL). Ten or 20 μg of total protein were separated on pre-cast gels from BioRad (Hercules, CA) or Invitrogen (Carlsbad, CA). After the electrophoresis, proteins were transferred to PVDF membranes (overnight, 4°C). The membranes were stained for total protein with Amido Black Stain (Sigma-Aldrich) as described [12] and blocked for 1 hour in 5% milk/TBS-tween. Primary antibodies were purchased from Abcam (Cambridge, MA), Cell Signaling Technology (Danvers, MA), or Sigma-Aldrich (Supplemental Table 1); incubations with primary antibodies were carried out overnight (4°C). Secondary antibody incubations were for 1 hour at room temperature. Immunolabeling was made visible with SuperSignal Dura West detection solution (Thermo Scientific) in a G:Box ChemiXL1.4 (SynGene, Cambridge, UK). Densitometry analysis of Amido Black Stain and target protein bands was performed with GeneTools (SynGene) or ImageJ software (1.48v; National Institutes of Health, USA). In order to adjust for potential differences in loaded amounts of protein, target protein expression was adjusted for intensity of Amido Black Stain. Validation of Amido Black Stain as loading control is presented in Supplemental figure 1. On each gel, an equalizer sample was included in order to adjust for differences in signal intensities between gels and samples from both the labor and the no-labor group were mixed. After dividing target protein expression by the intensity of the Amido Black Stain as well as adjusting for signal intensity between gels, the no-labor group was arbitrarily assigned a value of 1.0 for comparisons between groups.

NFκB p65 and PPARγ activation analysis

Nuclei from placental villous tissue were isolated using a nuclear isolation kit (Active Motif, Carlsbad, CA). Protein concentrations of the nuclear extracts were determined with Pierce BCA Protein Assay kit (Thermo Scientific). DNA binding of the transcription factor was measured in 10 μg (PPARγ) or 20 μg (NFκB p65) of nuclear extracts with TransAM Transcription Factor ELISA (Active Motif).

Statistical analysis

Differences between the two groups were evaluated statistically by t-test using GraphPad Prism 5 (version 5.04; GraphPad Software, La Jolla, CA). P-values less than 0.05 were considered significant.

Results

Clinical characteristics

Maternal pre-pregnancy/early pregnancy BMI or age did not differ between the no-labor and labor groups (Table 1). Similarly, fetal growth (as measured by birth weight and ponderal index), gestational length, or placental weights did not differ between the two groups (Table 1).

Activation of Caspase-1

Placental levels of pro-Caspase-1 (p50) did not differ significantly between the no-labor and labor groups, but active Caspase-1 (p20) was significantly higher in the placentas exposed to labor ($P<0.05$; Figure 1A–B).

Decreased PPARγ activation by labor

The DNA binding activity of PPARγ was significantly lower in nuclei isolated from placentas in the labor group compared to placenta in the no-labor group ($P<0.05$; Figure 1C).

Activation of MAPKs and NFκB signaling pathways

Phosphorylation and/or expression of ERK1/2, IκBα, JNK, and p38 were not affected by labor (Figure 2A). In contrast, the DNA binding activity of NFκB p65 significantly reduced by labor ($P<0.05$; Figure 2B).

STAT signaling is not affected by labor

Activation of the STAT signaling pathway was not affected by labor as measured by phosphorylation of STAT3 and STAT5, as well as Suppressor of cytokine signaling 3 (SOCS3) expression (Figure 3).

IRS1 signaling

The phosphorylation of IRS1 (Y612 and S307) and total IRS1 expression were not different in the two groups (Figure 4). Labor had no effect upon the IRS1 downstream target Akt, as measured by phosphorylation at T308 (Figure 4).

mTORC1 signaling is reduced by labor

Phosphorylation of mTOR (S2448) and 4EBP1 (at both T37/46 and T70) was significantly reduced by labor ($P<0.05$; Figure 5A), with total expression of mTOR also being reduced by labor ($P<0.05$; Figure 5A). There was no effect on expression or phosphorylation of rpS6 (Figure 5A). In contrast to mTORC1 signaling, mTORC2 signaling as measured by phosphorylation of Akt (S473) was activated by labor ($P<0.05$; Figure 5B). Upstream regulators of mTORC1 signaling, AMPK, glycogen synthase kinase-3 (GSK3), and tuberous sclerosis 2 (TSC2) expression or phosphorylation were not significantly different between the labor and no-labor groups (Figure 5A–B).

Discussion

In this study we show that activation of several cellular signaling pathways in the placenta are affected by labor. We show for the first time an alteration in activity of the mTOR pathway, as well as activation of Caspase-1 in response to labor. Furthermore, we confirm previously published data concerning the effects of labor on NF κ B and PPAR γ activation [3, 4].

Conflicting results concerning placental levels of IL-1 β in response to labor have been reported [2, 13], potentially due to the choice of methods for determining IL-1 β expression levels. Cindrova-Davies and co-workers measured IL-1 β by Western blot [2] while Ämmälä *et al.* used an ELISA which mostly (but not exclusively) detects the active form of IL-1 β [13]. However, it is known that active IL-1 β is formed by cleavage from its precursor by Caspase-1, which in turn also exists in active (p20) and inactive forms (p50) [6]. Our result of increased activation of Caspase-1 indirectly supports the finding of increased placental IL-1 β in response to labor [2]. In accordance with our findings, it has previously been reported that Caspase-1 in the amniotic fluid increases with labor [14].

Labor has previously been reported to affect PPAR γ , but also PPAR α and PPAR δ , with respect to mRNA expression, protein expression and/or DNA binding activity [4]. Holdsworth-Carson and coworkers show that placentas from laboring women have increased PPAR γ mRNA expression and increased abundance of PPAR γ in the nuclei, whereas PPAR γ DNA binding activity was found to be decreased compared to placentas not exposed to labor [4]. Our data also indicates that labor reduces placental PPAR γ DNA binding activity.

Labor is an inflammatory event resulting in the rupture of the fetal membranes [15]. However, it is currently less clear to what extent placental villous tissues are also affected. We observed a reduction in NF κ B p65 activation in placentas exposed to labor, which is consistent with a previous report by Lappas *et al.* [3]. On the other hand, I κ B α expression was trending lower in the laboring group, although this difference did not reach significance. These findings are in general agreement with the studies of Cindrova-Davies and co-workers demonstrating that I κ B expression is decreased with long, but not short, labor [2]. Further investigations are required to fully explore the effect of labor on the I κ B/NF κ B signaling pathway. These studies should include determination of I κ B phosphorylation, the signal to degrade this protein. I κ B expression is a known target for NF κ B induced gene transcription

[16], and hence the slight reduction in total I κ B expression may be due to decreased transcription associated with the lower NF κ B activity rather than to increased I κ B degradation.

Surprisingly, labor did not affect STAT3 or STAT5 phosphorylation despite several factors known to regulate this pathway, such as cytokines and leptin [8], are altered by labor [2]. Consistent with unaltered STAT3/STAT5 activity we observed no alteration in SOCS3 expression. Our finding concerning SOCS3 is however at odds with a previous report which could not detect SOCS3 in placenta after labor [17]. The discrepancy between our results and those of Blumenstein *et al.* are not entirely apparent, but it may be related to biological variation, and their smaller number of placentas investigated.

Placental mTORC1 signaling was significantly reduced by labor. Activation of mTORC1 has been shown to promote cell cycle progression, growth, and protein synthesis [11], functions likely not to be prioritized as this terminal organ prepares for parturition. It is interesting to note that downstream changes of mTORC1 signaling related to labor were restricted to 4EBP1. Phosphorylation of 4EBP1 occur sequentially (phosphorylation at T37/46 before T70) with both sites generally required for full protein activation [18]. Reduced phosphorylation of both sites in response to labor strongly suggests that 4EBP1 has an increased capacity to inhibit translation. Recent studies have conclusively demonstrated that mTORC1 regulation of mRNA translation is mediated by the 4EBP1 protein [19]. This suggests a global inhibition of protein synthesis in response to labor, possibly in preparation for placental expulsion.

The mTORC1 pathway is regulated by a number of signals including growth factors, inflammatory signals, and cellular energy status [11]. Phosphorylation of AMPK in response to a low cellular energy status is known to inhibit mTOR signaling [11]. However, AMPK expression and phosphorylation was not altered in response to labor and therefore an unlikely explanation to the reduced mTORC1 signaling. Phosphorylation status of other upstream regulators of mTORC1 signaling, GSK3 and TSC2, were not significantly affected by labor. However, phosphorylation of TSC2 at S1387 is only one of multiple activity regulating phosphorylation sites [20]. The precise mechanism inhibiting mTORC1 in placentas exposed to labor remains to be established. Although not examined in our study, hypoxia, a well-established inhibitor of mTOR signaling [11], may explain the decrease in mTORC1 signaling in response to labor.

The mTOR signaling pathway regulates multiple placental functions including trophoblast amino acid transport [21–24]. It is currently unknown if labor indeed affects placental amino acid transport. Labor has been demonstrated to affect other placental functions and placental metabolism. For instance, labor alters cytokine release from placental explants in response to various stimuli [25] and reduces placental fatty acid β -oxidation [26]. Labor has been demonstrated to result in a major change in placental gene expression [1, 2] and changes in cellular signaling pathways, shown by us and others [2–4]. These observations suggest that other placental functions, such as amino acid transport, may be affected as well.

In contrast to mTORC1 signaling, labor increased mTORC2 signaling as measured by phosphorylation of Akt (S473). The mTORC1 and 2 signaling pathways are influenced by largely distinct upstream regulators [11] and it is therefore plausible that the two pathways are affected in opposite directions by labor. In addition, it has previously been shown that inhibiting mTORC1 may result in increased phosphorylation of Akt (S473). For instance, in cancer cells inhibition of mTORC1 has been reported to increase Akt (S473) phosphorylation [27]. If the increased Akt (S473) phosphorylation in placentas exposed to labor is a consequence of reduced mTORC1 signaling or some other mechanism remains to be established. Such alternative mechanisms could include endoplasmic reticulum (ER) stress. It has previously been shown that phosphorylation of Akt (S473) occurs in response to ER stress [28]. Although ER stress has not directly evaluated in this study, it is plausible that the events of labor lead to increased placental ER stress.

Yung and coworkers recently reported that the speed of sample collection and processing influences placental energetics and signaling pathways [29]. Specifically, if sample collection was delayed 45 minutes with the placenta maintained in room temperature, the phosphorylation of Akt, GSK3 α and β , 4EBP1, and p70 S6K was reduced by ~30–65%, whereas phosphorylation of eIF2 α increased by almost 3-fold [29]. We believe it is unlikely that the differences in cellular signaling between the no-labor and labor groups in our study can be explained by differences in sample handling because the tissues were collected promptly and in similar fashion for both groups. This conclusion is strongly supported by the fundamentally different effects on phosphorylation of Akt (S473), a functional readout of mTORC2 signaling, in the two studies. We found phosphorylation of Akt (S473) increased markedly, but it decreased by ~60% after delayed sample collection for 30 minutes in the report from Yung and collaborators [29].

In conclusion, labor alters activation of several cellular signaling pathways in the human placenta. Whether these alterations contribute to the progression of labor and how they affect placental function remains to be determined. However, we speculate that the inhibition of mTORC1 signaling may serve the purpose to down-regulate protein synthesis and amino acid transport, processes which account for a large share of placental oxygen and glucose consumption. As a result, more glucose and oxygen may be available for transfer to the fetus during the labor process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to patients and staff at Labor and Delivery, University Hospital in San Antonio for making collection of placenta tissue possible. In addition, we are indebted to E. Miller who was responsible for tissue collection, to E. Dudley for excellent technical assistance, and to W.G.D. Frederick II for valuable comments on the manuscript. Grants supporting this study: NIDDK R01DK089989 (TLP), the Swedish Research Council (SL), and the Swedish Society of Endocrinology (SL).

References

1. Lee KJ, Shim SH, Kang KM, Kang JH, Park DY, Kim SH, Farina A, Shim SS, Cha DH. Global gene expression changes induced in the human placenta during labor. *Placenta*. 2010; 31(8):698–704. [PubMed: 20554320]
2. Cindrova-Davies T, Yung HW, Johns J, Spasic-Boskovic O, Korolchuk S, Jauniaux E, Burton GJ, Charnock-Jones DS. Oxidative stress, gene expression, and protein changes induced in the human placenta during labor. *Am J Pathol*. 2007; 171(4):1168–79. [PubMed: 17823277]
3. Lappas M, Rice GE. Transcriptional regulation of the processes of human labour and delivery. *Placenta*. 2009; 30 (Suppl A):S90–5. [PubMed: 19010537]
4. Holdsworth-Carson SJ, Permezel M, Riley C, Rice GE, Lappas M. Peroxisome proliferator-activated receptors and retinoid X receptor-alpha in term human gestational tissues: tissue specific and labour-associated changes. *Placenta*. 2009; 30(2):176–86. [PubMed: 19070893]
5. Osman I, Young A, Ledingham MA, Thomson AJ, Jordan F, Greer IA, Norman JE. Leukocyte density and pro-inflammatory cytokine expression in human fetal membranes, decidua, cervix and myometrium before and during labour at term. *Mol Hum Reprod*. 2003; 9(1):41–5. [PubMed: 12529419]
6. Stutz A, Golenbock DT, Latz E. Inflammasomes: too big to miss. *J Clin Invest*. 2009; 119(12):3502–11. [PubMed: 19955661]
7. Kyriakis JM, Avruch J. Mammalian MAPK signal transduction pathways activated by stress and inflammation: a 10-year update. *Physiol Rev*. 2012; 92(2):689–737. [PubMed: 22535895]
8. Heim MH. The Jak-STAT pathway: cytokine signalling from the receptor to the nucleus. *J Recept Signal Transduct Res*. 1999; 19(1–4):75–120. [PubMed: 10071751]
9. La Cava A, Matarese G. The weight of leptin in immunity. *Nature reviews Immunology*. 2004; 4(5):371–9.
10. Tsou RC, Bence KK. Central regulation of metabolism by protein tyrosine phosphatases. *Front Neurosci*. 2012; 6:192. [PubMed: 23308070]
11. Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012; 149(2):274–93. [PubMed: 22500797]
12. Lanoix D, St-Pierre J, Lacasse AA, Viau M, Lafond J, Vaillancourt C. Stability of reference proteins in human placenta: general protein stains are the benchmark. *Placenta*. 2012; 33(3):151–6. [PubMed: 22244735]
13. Ammala M, Nyman T, Salmi A, Rutanen EM. The interleukin-1 system in gestational tissues at term: effect of labour. *Placenta*. 1997; 18(8):717–23. [PubMed: 9364608]
14. Gotsch F, Romero R, Chaiworapongsa T, Erez O, Vaisbuch E, Espinoza J, Kusanovic JP, Mittal P, Mazaki-Tovi S, Kim CJ, Kim JS, Edwin S, Nhan-Chang CL, Hamill N, Friel L, Than NG, Mazor M, Yoon BH, Hassan SS. Evidence of the involvement of caspase-1 under physiologic and pathologic cellular stress during human pregnancy: a link between the inflammasome and parturition. *J Matern Fetal Neonatal Med*. 2008; 21(9):605–16. [PubMed: 18828051]
15. Keelan JA, Blumenstein M, Helliwell RJA, Sato TA, Marvin KW, Mitchell MD. Cytokines, prostaglandins and parturition - A review. *Placenta*. 2003; 24:S33–S46. [PubMed: 12842412]
16. Ruland J. Return to homeostasis: downregulation of NF-kappa B responses. *Nat Immunol*. 2011; 12(8):709–14. [PubMed: 21772279]
17. Blumenstein M, Bowen-Shauver JM, Keelan JA, Mitchell MD. Identification of suppressors of cytokine signaling (SOCS) proteins in human gestational tissues: differential regulation is associated with the onset of labor. *The Journal of clinical endocrinology and metabolism*. 2002; 87(3):1094–7. [PubMed: 11889171]
18. Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszyńska A, Aebersold R, Sonenberg N. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. *Genes & development*. 2001; 15(21):2852–64. [PubMed: 11691836]
19. Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM. A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature*. 2012; 485(7396):109–13. [PubMed: 22552098]

20. Huang J, Manning BD. The TSC1-TSC2 complex: a molecular switchboard controlling cell growth. *Biochem J*. 2008; 412(2):179–90. [PubMed: 18466115]
21. 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(Pt 1):449–59. [PubMed: 17463046]
22. 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 Physiol*. 2009; 296(1):C142–50. [PubMed: 18987252]
23. Roos S, Lagerlof 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 Physiol*. 2009; 297(3):C723–31. [PubMed: 19587219]
24. 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(Pt 3):609–25. [PubMed: 23165769]
25. Turner MA, Shaikh SA, Greenwood SL. Secretion of interleukin-1beta and interleukin-6 by fragments of term human placental villi: signalling pathways and effects of tumour necrosis factor alpha and mode of delivery. *Placenta*. 2002; 23(6):467–74. [PubMed: 12137744]
26. Mendez-Figueroa H, Chien EK, Ji H, Nesbitt NL, Bharathi SS, Goetzman E. Effects of labor on placental fatty acid beta oxidation. *J Matern Fetal Neonatal Med*. 2013; 26(2):150–4. [PubMed: 22928498]
27. Breuleux M, Klopfenstein M, Stephan C, Doughty CA, Barys L, Maira SM, Kwiatkowski D, Lane HA. Increased AKT S473 phosphorylation after mTORC1 inhibition is rictor dependent and does not predict tumor cell response to PI3K/mTOR inhibition. *Molecular cancer therapeutics*. 2009; 8(4):742–53. [PubMed: 19372546]
28. Yung HW, Charnock-Jones DS, Burton GJ. Regulation of AKT phosphorylation at Ser473 and Thr308 by endoplasmic reticulum stress modulates substrate specificity in a severity dependent manner. *PloS one*. 2011; 6(3):e17894. [PubMed: 21445305]
29. Yung HW, Colleoni F, Atkinson D, Cook E, Murray AJ, Burton GJ, Charnock-Jones DS. Influence of speed of sample processing on placental energetics and signalling pathways: Implications for tissue collection. *Placenta*. 2014; 35(2):103–8. [PubMed: 24406266]
30. Aye ILMH, Lager S, Ramirez VI, Gaccioli F, Dudley DJ, Jansson T, Powell TL. Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways. *Biol Reprod*. 2014; 90(6):129. [PubMed: 24759787]

- We investigate the effects of labor on placental cellular signaling pathways.
- Labor significantly reduces placental mTOR complex 1 signaling.
- Caspase-1 and mTOR complex 2 signaling was activated by labor.
- Several inflammatory related signaling pathways were not affected by labor.

Figure 1. Labor increases activation of Caspase-1 and decreases PPAR γ activation

Labor increased expression of active Caspase-1 but not pro-Caspase-1 (A). Representative Western blots shown in (B). Protein expression was adjusted for amount of total protein and the mean of no-labor group was assigned a value of 1.0; (arbitrary units, A.U.). PPAR γ DNA binding activity (C). Data are presented as mean + SEM. *P<0.05, t-test.

Figure 2. Inflammatory signaling pathways and labor

Labor did not affect expression and/or phosphorylation of ERK, IκBα ($P=0.11$), JNK ($P=0.1$), or p38 (A), but reduced NFκB p65 DNA binding activity (B). The no-labor data on DNA binding activity of NFκB p65 has been reported previously in a study on the effect of maternal BMI on placental inflammatory signaling [30]. Representative Western blots shown in (C). Protein expression was adjusted for amount of total protein and the mean of no-labor group was assigned a value of 1.0; (arbitrary units, A.U.). Data are presented as mean + SEM. * $P<0.05$, t-test.

Figure 3. Labor and STAT signaling

Labor did not affect expression and/or phosphorylation of SOCS3, STAT3, or STAT5 (**A**). Representative Western blots shown in (**B**). Protein expression was adjusted for amount of total protein and the mean of no-labor group was assigned a value of 1.0; (arbitrary units, A.U.). Data are presented as mean + SEM.

Figure 4. IRS1 signaling and labor

Labor did not affect IRS1 activation or Akt signaling (A), representative Western blots (B). Protein expression was adjusted for amount of total protein and the mean of no-labor group was assigned a value of 1.0; (arbitrary units, A.U.). Data are presented as mean + SEM.

Figure 5. Placental mTOR signaling and labor

Phosphorylation of mTOR and 4EBP1 as well as total expression of mTOR was reduced in placentas exposed to labor, while expression/phosphorylation of AMPK, GSK3, rpS6, and TSC2 were unaffected (**A**, **B**). Phosphorylation of Akt at S473 was increased by labor (**B**). Representative Western blots shown in (**C**). Protein expression was adjusted for amount of total protein and the mean of no-labor group was assigned a value of 1.0; (arbitrary units, A.U.). Data are presented as mean + SEM. * $P < 0.05$, t-test.

Table 1

Clinical Characteristics of Study Subjects

	No-labor	Labor	P-value
<i>Mother</i>			
N	15	12	-
BMI (kg/m ²)	22.3 ± 0.5	21.9 ± 0.6	0.55
Age (years)	30.2 ± 1.9	25.6 ± 1.7	0.09
Ethnicity (% Hispanic)	80%	75%	-
Delivery mode (C-section/vaginal)	15/0	2/10	-
<i>Newborn</i>			
Gestational age at delivery (weeks)	39.2 ± 0.2	39.6 ± 0.4	0.37
Fetal sex (female/male)	6/9	6/6	-
Birth weight (grams)	3387 ± 90	3523 ± 75	0.27
Ponderal index (100×g/cm ³)	2.5 ± 0.1	2.5 ± 0.1	0.80
Placental weight (grams)	703 ± 29	695 ± 34	0.86

Data are presented as mean ± SEM. Maternal BMI based on pre-pregnancy or first trimester weight.