Biochemical Regulation of Placental Transport Proteins and Implications for Fetal Growth

Crystal Huang

CHEM 4496 - Research Techniques in Biochemistry

Department of Chemistry, Temple University

Abstract

The placenta is critical for successful fetal growth during pregnancy, regulating the transport of nutrients and other substrates between the mother and the developing fetus. Its implications for fetal growth can be determined by analyzing the mechanisms and regulation of placental transport protein systems. Abnormal activity of these transporters can lead to abnormal fetal growth and development, but their specific mechanisms are not completely understood. This review aims to examine the factors that increase and decrease the activity of Glucose Transporter Proteins (GLUTs), amino acid transporters, Fatty Acid Transporter Proteins (FATPs), and Organic Anion Transporters (OATs)/Organic Cation Transporters (OCTs) and their contributions to adverse health outcomes. Decreased activity of these transporters generally results in decreased fetal growth, and vice versa. It is important to understand these mechanisms as their individual and combined actions can clarify how maternal health influences fetal health.

Section 1.1 - Introduction

Fetal growth and development during pregnancy rely heavily on the placenta. By acting as a precise selective barrier between the mother and the fetus, specialized placental transport proteins regulate the exchange of nutrients, hormones, waste products, and drugs. The outer layer of the placenta consists of trophoblasts, cells that mediate nutrient and waste transport. Syncytiotrophoblasts, a specialized type of trophoblast, form the entire outer layer of placental villous trees. Since they are in direct contact with the maternal blood supply, as seen in Figure 1, these cells are the main sites of oxygen and nutrient exchange.[1] The transport proteins can be localized to either the microvillous membrane (MVM) of the syncytiotrophoblasts, the basolateral membrane (BM) of the syncytiotrophoblast, or both. The microvillous membrane is the maternal-facing side, while the basolateral membrane is the fetal-facing side. These transport proteins help control the molecular environment within fetal circulation, which is essential for proper growth before and after birth.

However, disruptions to the normal functioning of these proteins can lead to irregular fetal growth. For example, decreased expression and/or reduced activity of certain transport proteins could lead to Intrauterine Restricted Growth (IUGR), which refers to a small-for-gestational-age fetus that exhibits a growth rate lower than usual in the womb. Following birth, IURG could lead to complications, such as hypoglycemia, neurodevelopmental handicaps, and growth restrictions.[2] On the other hand, increased expression and/or higher activity of certain transport proteins could lead to macrosomia, which refers to excessive fetal growth. At birth, macrosomia could increase the risk of stillbirth and brachial plexus injuries. After birth, macrosomia predisposes the child to developing obesity and type 2 diabetes.[3,4]

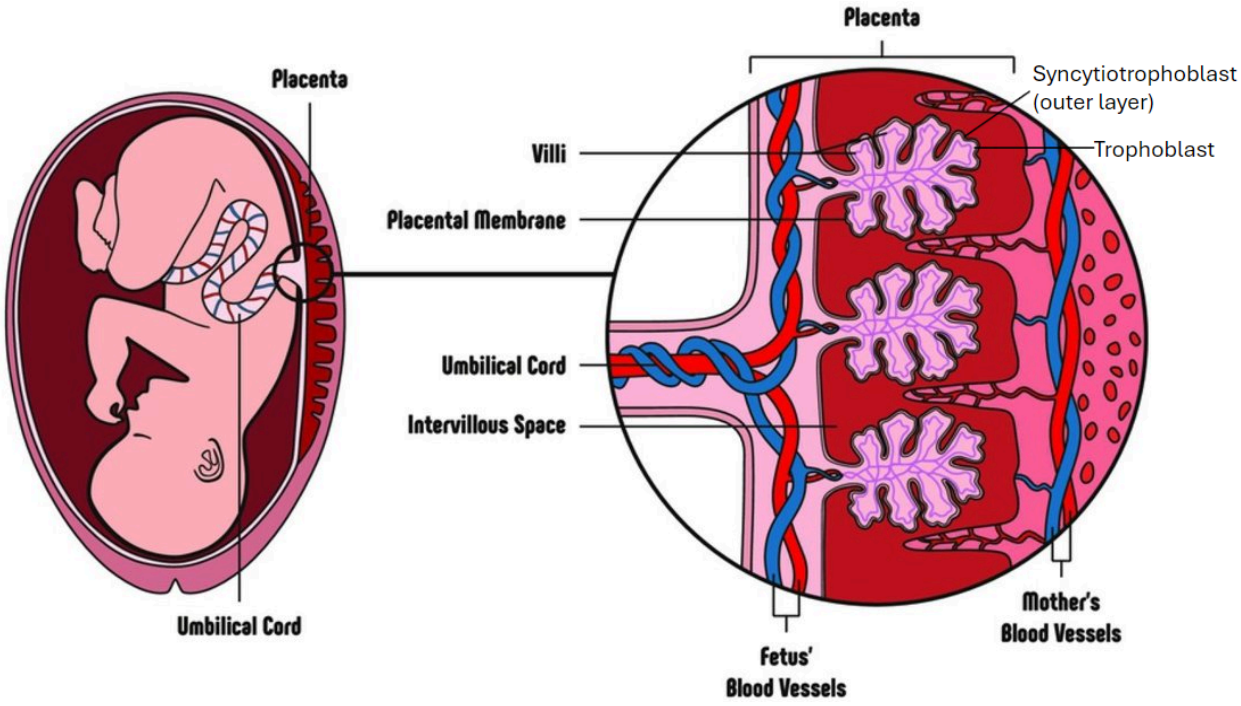

Figure 1. Placental structure and location of transport proteins. Adapted from Reference 5. Transport of substrates occurs at the trophoblast cell layer. The syncytiotrophoblast, the outer layer of the trophoblast in contact with the maternal blood supply, controls the movement of substrates.[5]

Although some mechanisms of placental transport proteins are understood, some still remain unknown, and their connection to fetal growth needs further analysis. The goals of this paper are to focus

on the mechanisms of placental transport proteins, their regulation and dysregulation, and how they affect fetal growth and development. Among the many different types of placental transport proteins, the types addressed in this paper are Glucose Transporter Proteins (GLUTS), Amino Acid Transporters, Fatty Acid Transporter Proteins (FATP), and Organic Anion/Cation Transporters (OATs/OCTs), as they are central for nutrient transport. To study their mechanisms, localizations, and expression levels, a variety of biochemical techniques could be used, including, but not limited to, Western blots and radiolabeled substrates. Taken together, these transport proteins demonstrate the importance of biochemical regulations and clinical implications for fetal growth.

Section 2.1: Glucose Transporter Proteins (GLUTs)

Glucose transporters (GLUTs) are essential for proper fetal growth and development during pregnancy. Since the transfer of glucose to the fetus is proportional to and relies on maternal glucose concentrations, maternal nutrition before and during pregnancy plays a large role in determining pregnancy outcomes. Based on a concentration gradient, glucose is transported from the maternal blood supply (higher glucose concentration) across the syncytiotrophoblast towards the fetal blood supply (lower glucose concentration) through facilitated diffusion. Facilitated diffusion uses a transporter, but not adenosine triphosphate (ATP), an energy source. Alterations in transporter densities can lead to pregnancy complications through varying levels of expression and activity. Glucose transporter proteins localized in the MVM control glucose uptake, while GLUTs localized in the BM control glucose delivery. These proteins contain approximately 500 amino acids in transmembrane alpha helices and a single N-linked oligosaccharide. GLUT isoforms are divided into 3 classes based on differences in their sequences, structures, and substrates. Class I contains GLUT1 through GLUT4 and includes high-glucose-affinity transporters. Class II contains GLUT5, GLUT7, GLUT9, and GLUT11 and includes fructose-specific transporters. Class III contains GLUT6, GLUT8, GLUT10, and GLUT12 and includes transporters without a glycosylation site in the first extracellular linker domain. However, the only known isoforms that are expressed in the human placenta are GLUT1, GLUT3, GLUT4, GLUT8, GLUT9, GLUT10, and

GLUT12. Many of these isoforms are scattered throughout the syncytiotrophoblast symmetrically. Notably, GLUT1 is localized to the MVM, GLUT9a to the BM, and GLUT9b to the MVM. Expression levels vary throughout pregnancy.[6] The function of all GLUT isoforms will not be addressed in this paper due to length and available literature.

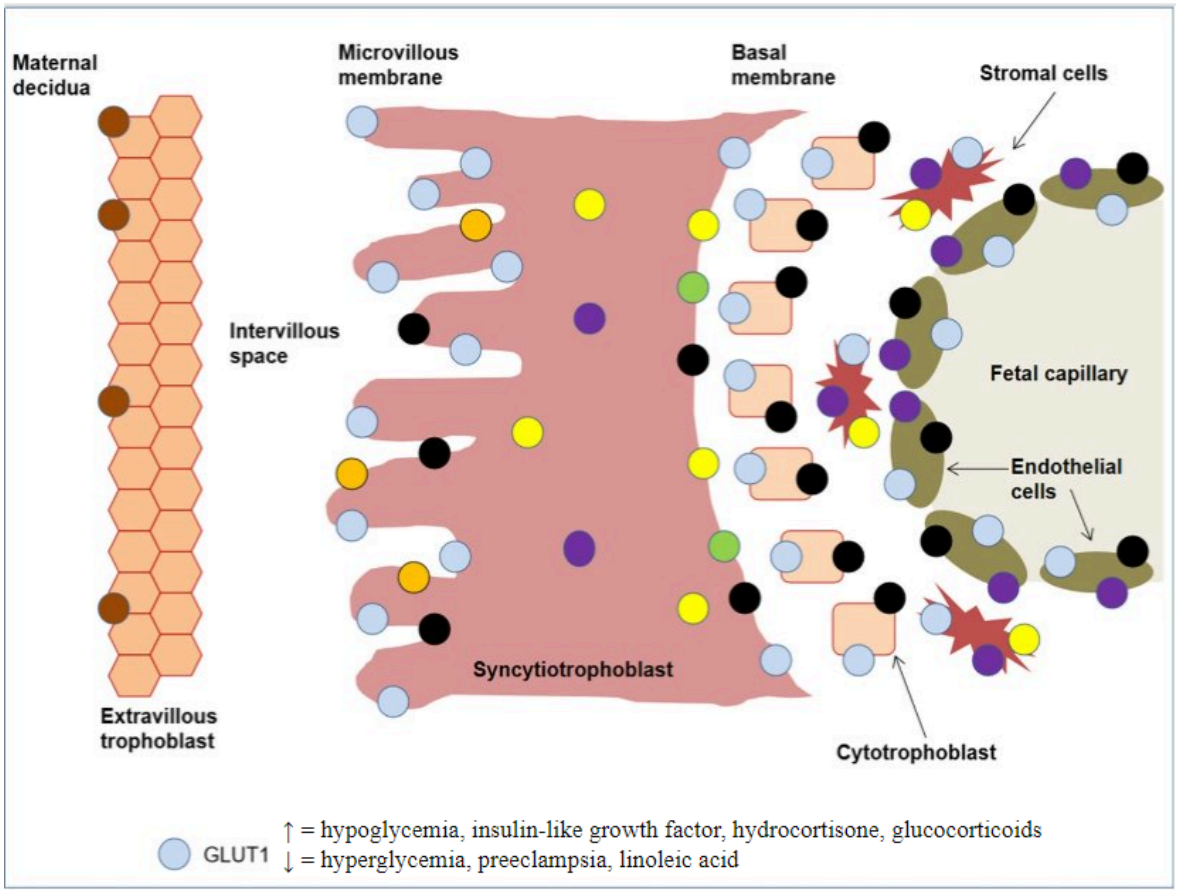

Figure 2. Localization of Glucose Transporter Proteins (GLUTs) in the syncytiotrophoblast, with a focus on GLUT1. Adapted from Reference 6. GLUT1, one of the many isoforms, is asymmetrically localized at the syncytiotrophoblast membranes, with higher expression on the microvillous membrane (MVM). Expression at the basolateral membrane (BM) increases throughout pregnancy. However, GLUT1 expression can be regulated by a variety of factors. Increased expression can be caused by maternal hypoglycemia, insulin-like growth factor, hydrocortisone, and glucocorticoids, while decreased expression can be caused by maternal hyperglycemia, preeclampsia, and linoleic acid. A sufficient amount of GLUT1 is required to transport the necessary nutrients for fetal development. Abnormal amounts can lead to either intrauterine growth restriction or macrosomia.[6]

Section 2.2: Glucose Transporter Type 1 (GLUT1)

The most significant isoform is GLUT1. As seen in Figure 2, it is highly expressed in both the MVM and BM; however, expression in the MVM is three times greater than in the BM. In this figure, there are 11 representative transporters in the MVM and 2 representative transporters in the BM. Since GLUT1 is expressed at much lower levels in the BM than in the MVM, it has a lower transport capacity. By limiting the transport capacity in the BM, the movement of glucose from the trophoblast into fetal circulation is slowed down, and BM GLUT1 becomes the rate-limiting factor in overall placental glucose transport. However, the expression of BM GLUT1 increases during the second trimester and is maintained until birth to support the energy needs of the growing fetus, while MVM GLUT1 expression remains constant.[6]

Section 2.3: Relevant Studies and Effects on Fetal Growth

A study used isolated MVM and BM membranes along with Western Blots to determine the positive correlation between GLUT1 expression and birthweight.[7] In addition to insulin-like growth factor 1 (IGF1), a variety of substrates and factors have been shown to upregulate GLUT1 expression. For instance, maternal hypoglycemia, hydrocortisone, and glucocorticoids are all correlated with an increase in GLUT1 expression. In contrast, maternal hyperglycemia, linoleic acid, and preeclampsia have been correlated with a decrease in GLUT1 expression.[8] Studies have shown that there is a positive association between maternal fasting glucose concentration and neonatal weight, head circumference, and ponderal index, a measurement often used to evaluate infants for IUGR. Glucose provides the necessary nutrients and energy for tissue growth and cell proliferation. Therefore, a lower glucose concentration or maternal undernutrition is associated with decreased fetal weight, a lower ponderal index, and a higher chance for developing IUGR, whereas a higher glucose concentration or maternal overnutrition is associated with increased fetal weight, a higher ponderal index, and a higher chance for developing macrosomia. Increased glucose transport, due to maternal diabetes, can also contribute to the risk of the fetus developing macrosomia.[6] To study glucose uptake, radiolabeled glucose can be used. After mixing

membrane vesicles with radiolabeled glucose, filtering them, and placing the filters in liquid scintillation fluid, a counter was used to quantify glucose concentrations.[7]

Section 3.1: Amino Acid Transporters

Amino acid transporters play an essential role in the movement of amino acids through the placenta for fetal protein synthesis. Amino acid transfer can be directed towards the maternal blood supply or taken from maternal circulation to supply the fetus. Since there is a higher concentration of amino acids in the fetal plasma than in the maternal plasma, active transport, which requires ATP to move substrates against a concentration gradient, is suggested to occur. To facilitate this transfer, there are over 20 different amino acid transporters, localized on both the MVM and the BM of the syncytiotrophoblast. First, an ATP-powered sodium-potassium pump increases the sodium concentration outside of the cells on the maternal side. Then, accumulative transporters, using secondary active transport, couple the movement of amino acids into the trophoblast using the concentration gradient of sodium, resulting in an increased amino acid concentration within the cells. Sodium moves into the cells and “pulls” amino acids with it. Using its concentration gradient, amino acids that accumulate within the trophoblast are then transported towards the fetus through facilitated transport using an efflux transporter.

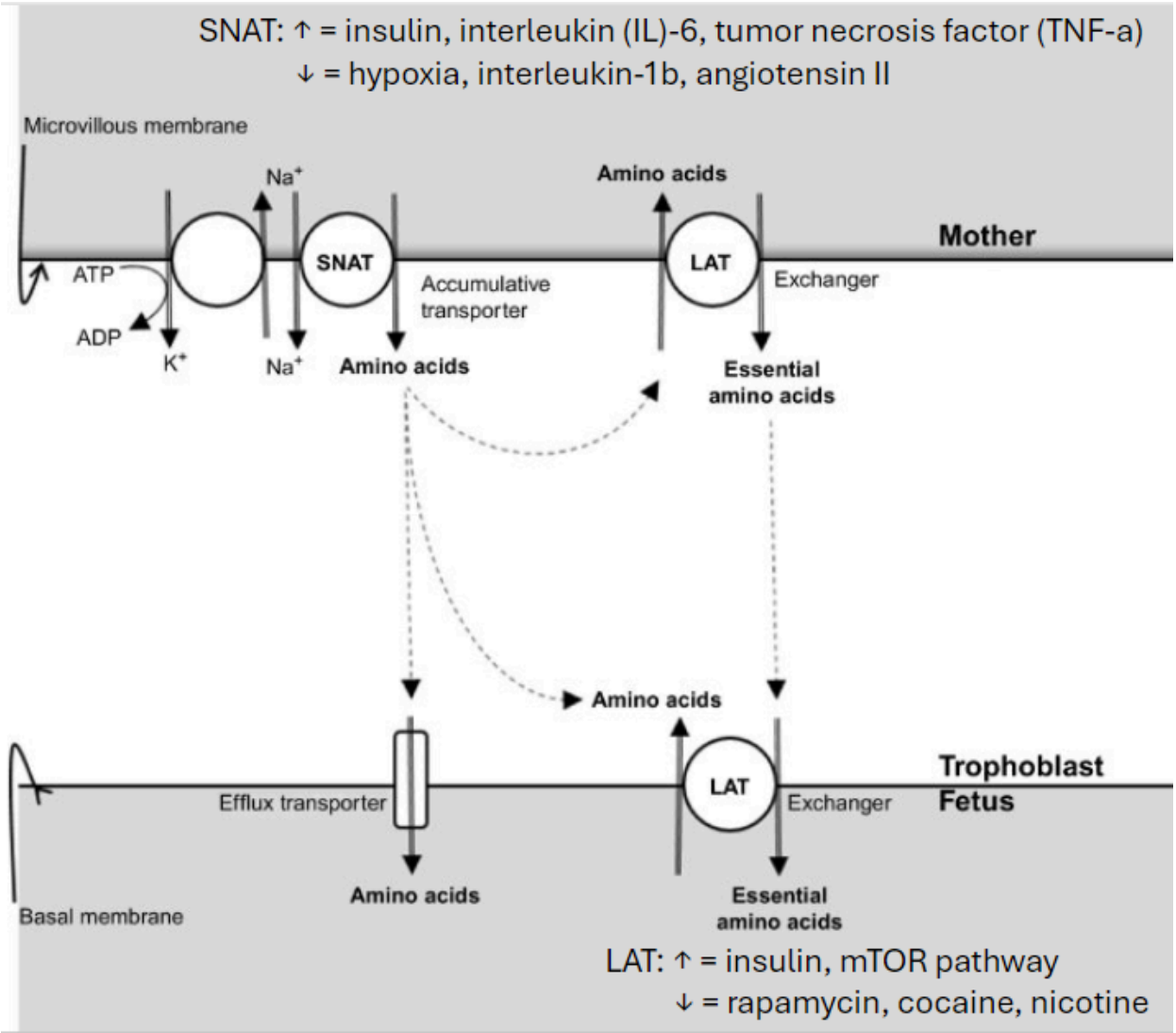

Figure 3. Localization of amino acid transporters, SNAT (System A) and LAT (System L). Adapted from Reference 8. SNAT is localized to the microvillous membrane (MVM). Expression can be increased by insulin, interleukin (IL)-6, and tumor necrosis factor (TNF-α), and decreased by hypoxia, interleukin-1β, and angiotensin II. LAT is localized to both the MVM and the basolateral membrane (BM). Expression can be increased by insulin and the mTOR pathway, and decreased by rapamycin, cocaine, and nicotine. A sufficient amount of SNAT and LAT is needed to control the movement of essential and nonessential amino acids. Decreased amounts can lead to intrauterine growth restriction.[8]

Section 3.2: SNAT - System A

SNAT (system A) is a sodium-dependent transporter that drives the active transport of amino acids from the mother into the trophoblast using an electrochemical gradient resulting from a sodium-potassium pump, as illustrated in Figure 3.[8] Commonly transported amino acids include glycine, alanine, and serine, among other small, neutral amino acids. Although it is expressed in both the MVM and the BM, it is more concentrated in the MVM. Isoforms include SNAT1, SNAT2, and SNAT4. Activity levels of each isoform differ throughout pregnancy, with SNAT1 being the major contributor to system A in full-term placentas. Factors like maternal insulin, interleukin (IL)-6, and tumor necrosis factor (TNF-α) have been shown to increase SNAT activity, while hypoxia, interleukin-1b, and angiotensin II have been shown to decrease SNAT activity. In cases of IUGR, lower system A transporter activity, and thereby decreased amino acid uptake from maternal circulation, can be seen.[9]

Section 3.3: LAT - System L

LAT (system L) is a sodium-independent transporter that directs essential amino acids from the mother towards the fetus and amino acids from the fetus towards the mother using an antiport mechanism. This mechanism is illustrated in Figure 3. By coupling with system A activity, essential amino acids from maternal circulation are exchanged for nonessential amino acids that accumulate within the trophoblast or from fetal circulation, especially glycine. Similar to SNAT, LAT is also expressed in both the MVM and BM, but is expressed at a higher level in the MVM. Isoforms include LAT1 and LAT2, with LAT1 primarily localized to the MVM and LAT2 primarily localized to the BM. By regulating the rates of gene transcription and translation, the mTOR pathway helps regulate cell growth and protein synthesis. The mTOR pathway works as a positive regulator of LAT activity.[8-10] Insulin can also increase LAT activity. Rapamycin inhibits the mTOR pathway and can consequently decrease LAT activity. Cocaine and nicotine can also decrease system L activity. Lower birth weights have been seen after cocaine and/or nicotine use during pregnancy.[9]

Section 3.4: Relevant Studies and Effects on Fetal Growth

The activity of these amino acid transporters can be assessed using radiolabeled substrates. For example, transporter activities were studied in baboons by measuring the uptake of radiolabeled methylaminoisobutryric acid (MeAIB), an amino acid analog, through system A, and radiolabeled L-leucine through system L. Baboons were chosen as they have a similar placentation to humans. MVM and BM vesicles were isolated and mixed with either MeAIB or leucine before performing incubation, termination at various time points, and rapid filtration. The concentrations of amino acids in maternal and fetal plasma were determined using mass spectrometry.[11] The same study also hypothesized that placental amino acid transporters are down-regulated before the onset of IUGR. To study this, baboons were either fed a normal diet or a maternal nutrient restriction (MNR) diet. Halfway through gestation at day 90 (GD 90), the maternal and fetal plasma amino acid concentrations were the same between the control and MNR populations. On day 120 (GD 120), which corresponds to 60% of gestation, the concentration of leucine and isoleucine within the fetal plasma was significantly lower than that of the maternal plasma. This observation occurred before the development of IUGR. On day 165 (GD 65), which corresponds to 90% of gestation, the concentrations of leucine and isoleucine, and other amino acids, decreased in the MNR population. The fetal plasma concentrations were also reduced. Additionally, these findings highlight how decreased fetal availability of essential amino acids, especially leucine, plays an important role in the development of IUGR. It has also been found that lower fetomaternal enrichment ratios of leucine have been associated with IUGR severity, and leucine supplements have the capacity to prevent fetal growth restrictions, such as IUGR.[11] Leucine is the main amino acid that activates the mammalian target of rapamycin (mTOR) pathway. Therefore, maintaining appropriate concentrations of leucine within the maternal blood supply is important for proper fetal growth and development.[10]

Section 4.1: Fatty Acid Transport Proteins (FATPs)

Fatty acid transport proteins (FATPs) influence the delivery of fatty acids (FAs) to the fetus. As gestation progresses throughout pregnancy, the maternal concentration of triglycerides, phospholipids,

cholesterol esters, and non-esterified FA shows a pronounced increase to support maternal energy use. These lipids are the primary source of FA for placental transfer to the fetus. Due to being too large in size, some lipids, like triglycerides, have to be hydrolyzed into non-esterified ("free") FA by lipases in the MVM before entering the syncytiotrophoblast. Other lipids that are already non-esterified can enter through simple diffusion or receptor-mediated endocytosis. Simple diffusion does not require transporters or ATP, and receptor-mediated endocytosis requires the recognition of receptors on the cell membrane by the substrates to initiate the engulfment process, powered by ATP. Isoforms of FATPs include: FATP2, FATP4, and FATP6. It is important to note that the specific roles of the different isoforms are not well understood.[12]

Section 4.2: Fatty Acid Binding Proteins (FABPs)

Within the syncytiotrophoblast, FAs are bound to FA-binding proteins (FABPs) and are transferred to either the mitochondria or the endoplasmic reticulum. After rapidly converting to acyl-CoA, FAs in the mitochondria are oxidized. In the endoplasmic reticulum, FAs are re-resterified and integrated into lipid droplets. FAs must be converted to acyl-CoA to prevent efflux through transporters or diffusion. It is theorized that the release of FAs from syncytiotrophoblasts occurs through dissociation from FABPs, thioesterases, and/or hydrolases; however, the exact mechanism remains unknown due to the limited depth of the available literature. Isoforms for the FABPs include: FABP1, FABP3, FABP4, and FABP5.[13]

Section 4.3: Relevant Studies and Effects on Fetal Growth

Using baboon placentas, which have a similar placentation to humans, an experiment studied the expression levels of FATPs and FABPs in baboons on an MNR diet compared to a normal diet. It was found that restrictive maternal diets contributed to increases in FATP and FABP expression in late gestation. Western blotting was used to determine protein expression levels. Although FA concentration decreased following maternal MNR, the plasma FA concentration of the fetus remained relatively unchanged. FA content in plasma was determined by using gas chromatography-mass spectrometry. This

relationship suggests that expression of FATPs and FABPs increases to compensate for the decreased FA supply and to maintain standard placental FA transfer.[12] An important fatty acid to mention is docosahexaenoic acid (DHA). DHA plays an important role in proper fetal neurodevelopment, which could affect postnatal intelligence, behavior, and risk for psychiatric illnesses. DHA also plays a role in lipid transfer, and DHA levels are positively correlated with insulin sensitivity, which could be a predisposing factor for childhood obesity if negatively affected. Fetal plasma shows a higher concentration of DHA than maternal plasma; therefore, decreased maternal DHA levels, like in the case of a maternal MNR diet, would result in decreased DHA being transported into the fetal blood supply. Decreased fetal DHA levels would then eventually lead to defects in neural function, behavior, and insulin response.[13] Additionally, maternal gestational diabetes or diabetes mellitus has also been reported to affect placental FA transfer. Since the fetal plasma FA levels rely on maternal FA levels, diabetes would result in an increase in fetal plasma FA levels. The combination of hyperglycemia and insulin resistance would result in higher levels of plasma FAs. This larger concentration gradient accelerates the transfer of FAs into fetal circulation, which further results in higher fetal fat depositions that increase the risk of developing macrosomia.[14]

Section 5.1: Organic Anion/Cation Transporters (OATs/OCTs)

Organic Anion/Cation Transporters are important proteins in the placenta that enable the movement of charged compounds. Examples of charged compounds include amino acids for protein synthesis, hormones for signaling, waste products, drugs, and many others that are important for fetal growth and development. Movement of these charged compounds can occur towards the maternal or fetal blood supply.[15]

Section 5.2: Organic Anion Transporters (OATs)

Organic Anion Transporters (OATs) mediate the transport of negatively charged compounds. OAT4 is the primary and most expressed OAT isoform in the human placenta. This isoform is mainly

localized to the BM, where it mediates transfer of materials out of fetal circulation. OAT4-mediated transport occurs through coupling to a glutamate gradient. Glutamate moves down its concentration gradient as it moves from the syncytiotrophoblast into fetal circulation. Through coupling, OAT4 can transport compounds out of the plasma in exchange for glutamate into the plasma. Substrates for OAT4 include select steroid sulfates, such as dehydroepiandrosterone sulfate (DHEAS). DHEAS is important for fetal development as it is believed to be used for estrogen biosynthesis in the placenta. Estrogen can drive placental angiogenesis and vasodilation. Placental blood vessels that are bigger in size and quantity allow for increased oxygen delivery and nutrient transfer to the fetus, which directly impacts fetal growth.[15] However, excess DHEAS has been linked to slower and/or interruptions in intrauterine growth, which increase the risk of developing IUGR. OAT4 expression can be increased by cAMP-dependent protein kinase A (PKA) activators forskolin and 8-bromo-cAMP, a cAMP analog, which would lead to an increase in DHEAS uptake. In contrast, OAT4 expression can also be decreased. Protein kinase C (PKA) activators, phorbol 12-myristate 13-acetate and phorbol 12,13-dibutyrate, can decrease OAT4 expression. Decreased DHEAS, from a decrease in OAT4 activity, can decrease estrogen levels, which can contribute to the risk of miscarriage or premature birth.[16] OAT4 also mediates the transport of some drugs and toxins, like perfluorinated alkyl acids (PFAS). Often referred to as “forever chemicals,” PFAS are man-made chemicals with particularly strong carbon-fluorine bonds. They break down very slowly and can be found in materials such as drinking water, food, household products, and dust.[17,18] Since the toxins exposed to by pregnant women can be found within the umbilical cord, fetal exposure to the same toxins is suggested. However, these toxins are also substrates for OAT4, which allows for fetal detoxification.[16,19]

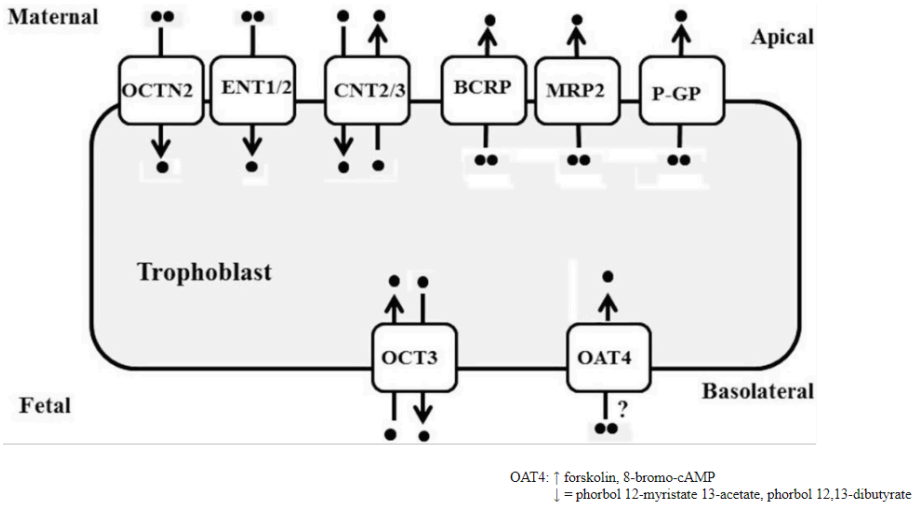

Figure 4. Localization of Organic Anion Transporters in the syncytiotrophoblast, with a focus on OAT4. Adapted from Reference 16. OAT4, one of the isoforms, is localized to the basolateral membrane (BM). Expression can be increased by forskolin and 8-bromo-cAMP, and decreased by phorbol 12-myristate 13-acetate and phorbol 12,13-dibutyrate. The "?" represents uncertainty in the bidirectional function of the transporter. However, this is outdated, and OAT4 can facilitate bidirectional transport.[16]

Section 5.3: Organic Cation Transporters (OCTs)

Organic Cation Transporters (OCTs) mediate the transport of positively charged compounds. OCT3 is the only OCT isoform in the human placenta. This isoform is mainly localized to the BM, where it mediates transfer of materials out of fetal circulation. OCT3 can also mediate transport towards fetal circulation, but it tends to transport in the direction favored by the electrochemical potential gradient. Cations transported include monoamine neurotransmitters, like serotonin, which is essential for fetal growth and brain development.[20,21] Among others, metformin is another notable substrate for OCT3. Metformin is given to patients with type 2 diabetes, including mothers with gestational diabetes, to increase their insulin sensitivity. Since metformin given to women can be found within the umbilical cord, fetal exposure to metformin is suggested. Fetal exposure to metformin does not result in any major congenital abnormalities, but it can lead to an increased risk for low birth weights. Although more

research is needed to clarify the safety of metformin's use during pregnancy, it is generally considered a safer alternative to insulin. Since OCT3 can facilitate bidirectional transfer, excess metformin in fetal plasma can be transported back out. Animal studies have shown regulation of metformin transport to be dependent on transporter saturation. However, due to the limited number of human studies, the exact mechanisms behind the regulation and dysregulation of metformin transfer require further research.[22]

Section 6.1 - Summary and Conclusions

The placenta is a precise and selective barrier that helps regulate the fetal environment. The syncytiotrophoblast, the outermost layer of the placental villous trees, is where the transport of nutrients and essential substrates takes place. Transport is localized to the MVM and/or the BM. Different placental transport proteins facilitate the transport of different types of molecules. Glucose Transporters (GLUTs) control the uptake and delivery of glucose through facilitated diffusion based on a concentration gradient. Amino acid transporters control the movement of essential and non-essential amino acids through a multi-step system involving secondary active transport, facilitated transport, and an exchanger. Fatty Acid Transport Proteins (FATPs) contribute to the multi-step delivery of fatty acids to the fetus through lipid processing, simple diffusion, endocytosis, and intracellular trafficking. Organic Anion/Cation Transporters (OATs/OCTs) control the movement of charged molecules. OATs transport negatively charged molecules by coupling to a concentration gradient, while OCTs transport positively charged molecules through an electrochemical potential gradient. Transport through all of these transport proteins is tightly regulated and coordinated, and dysregulation could lead to abnormal fetal growth and development.

Although there is significant research on placental transport proteins, some aspects still remain unknown. Currently, many of the published studies were done on animal models. Therefore, relevance to human placental cells can only be inferred. Additionally, many aspects of the exact roles, mechanisms, and regulations of transporters are still being investigated. For example, the understanding of how FAs

leave the syncytiotrophoblast is incomplete. The mechanisms of OCT3 regulation are also not fully understood.

By analyzing the activity level of certain placental transport proteins, real-time diagnostic tests during pregnancy could potentially be developed. These tests could then be used as an early indicator for developmental outcomes like IUGR and macrosomia. Additionally, these tests could be used in conjunction with targeted therapeutics to improve the efficiency of specific transporters. A better understanding of transporter kinetics can lead to a better understanding of placental drug transport, further improving the delivery of personalized medicine during pregnancy. Since fetal outcomes can be affected by maternal nutrition and other environmental factors, additional research in this field can help address health disparities in pregnancy outcomes.

Since efficient placental transport is the foundation of healthy fetal growth and development, continued research is essential. A deeper understanding of these placental transport proteins can play a large role in reducing pregnancy complications and maximizing positive health outcomes. The placenta does more than just support fetal life; it shapes their future.

References

(1) Zhao, S. Cell Types of the Placenta. In *Vascular Biology of the Placenta*; Morgan & Claypool: San Rafael, California, 2017.

(2) Sharma, D.; Shastri, S.; Sharma, P. Intrauterine Growth Restriction: Antenatal and Postnatal Aspects. *Clinical Medicine Insights: Pediatrics* **2016**, *10* (10), CMPed.S40070. https://doi.org/10.4137/cmped.s40070.

(3) Akanmode, A. M.; Mahdy, H. *Macrosomia*. PubMed. https://www.ncbi.nlm.nih.gov/books/NBK557577/.

(4) Gu, S.; An, X.; Liang, F.; Zhang, X.; Zhang, C.; Wang, J.; Liu, Q.; Zhang, Y.; Wei, Y.; Hu, Z.; Chen, F.; Shen, H. Risk Factors and Long-Term Health Consequences of Macrosomia: A Prospective Study in Jiangsu Province, China. *Journal of Biomedical Research* **2012**, *26* (4), 235–240. https://doi.org/10.7555/jbr.26.20120037.

(5) Barta, E. Lactate Transport at the Uteroplacental Unit- a Theoretical Study. *bioRxiv (Cold Spring Harbor Laboratory)* **2020**. https://doi.org/10.1101/2020.10.23.351841.

(6) Joshi, N. P.; Mane, A. R.; Sahay, A. S.; Sundrani, D. P.; Joshi, S. R.; Yajnik, C. S. Role of Placental Glucose Transporters in Determining Fetal Growth. *Reproductive Sciences* **2021**. https://doi.org/10.1007/s43032-021-00699-9.

(7) Acosta, O.; Ramirez, V. I.; Lager, S.; Gaccioli, F.; Dudley, D. J.; Powell, T. L.; Jansson, T. Increased Glucose and Placental GLUT-1 in Large Infants of Obese Nondiabetic Mothers. *American Journal of Obstetrics and Gynecology* **2015**, *212* (2), 227.e1–227.e7. https://doi.org/10.1016/j.ajog.2014.08.009.

(8) Paweł Jan Stanirowski; Lipa, M.; Dorota Bomba-Opoń; Mirosław Wielgoś. Expression of Placental Glucose Transporter Proteins in Pregnancies Complicated by Fetal Growth Disorders. *Advances in protein chemistry and structural biology* **2020**, *123*, 95–131. https://doi.org/10.1016/bs.apcsb.2019.12.003.

(9) Lager, S.; Powell, T. L. Regulation of Nutrient Transport across the Placenta. *Journal of Pregnancy* **2012**, *2012*, 1–14. https://doi.org/10.1155/2012/179827.

(10) Teodoro, G. F. R.; Vianna, D.; Torres-Leal, F. L.; Pantaleão, L. C.; Matos-Neto, E. M.; Donato, J.; Tirapegui, J. Leucine Is Essential for Attenuating Fetal Growth Restriction Caused by a Protein-Restricted Diet in Rats. *The Journal of Nutrition* **2012**, *142* (5), 924–930. https://doi.org/10.3945/jn.111.146266.

(11) Pantham, P.; Rosario, F. J.; Weintraub, S. T.; Nathanielsz, P. W.; Powell, T. L.; Li, C.; Jansson, T. Down-Regulation of Placental Transport of Amino Acids Precedes the Development of Intrauterine Growth Restriction in Maternal Nutrient Restricted Baboons. *Biology of Reproduction* **2016**, *95* (5), 98–98. https://doi.org/10.1095/biolreprod.116.141085.

(12) Chassen, S. S.; Ferchaud‐Roucher, V.; Palmer, C.; Li, C.; Jansson, T.; Nathanielsz, P. W.; Powell, T. L. Placental Fatty Acid Transport across Late Gestation in a Baboon Model of Intrauterine Growth Restriction. *The Journal of Physiology* **2020**, *598* (12), 2469–2489. https://doi.org/10.1113/jp279398.

(13) Lewis, R. M.; Wadsack, C.; Desoye, G. Placental Fatty Acid Transfer. *Current Opinion in Clinical Nutrition & Metabolic Care* **2018**, *21* (2), 78–82. https://doi.org/10.1097/mco.0000000000000443.

(14) Larqué, E.; Pagán, A.; Prieto, M. T.; Blanco, J. E.; Gil-Sánchez, A.; Zornoza-Moreno, M.; Ruiz-Palacios, M.; Gázquez, A.; Demmelmair, H.; Parrilla, J. J.; Koletzko, B. Placental Fatty Acid Transfer: A Key Factor in Fetal Growth. *Annals of Nutrition and Metabolism* **2014**, *64* (3-4), 247–253. https://doi.org/10.1159/000365028.

(15) Parisi, F.; Fenizia, C.; Introini, A.; Zavatta, A.; Chiara Scaccabarozzi; Biasin, M.; Savasi, V. The Pathophysiological Role of Estrogens in the Initial Stages of Pregnancy: Molecular Mechanisms and Clinical Implications for Pregnancy Outcome from the Periconceptional Period to End of the First Trimester. *Human Reproduction Update* **2023**, *29* (6).

https://doi.org/10.1093/humupd/dmad016.

(16) Liu, L.; Liu, X. Contributions of Drug Transporters to Blood-Placental Barrier. In *Advances in Experimental Medicine and Biology*; Springer, Singapore, 2019; Vol. 1141, pp. 505–548. https://doi.org/10.1007/978-981-13-7647-4_11.

(17) United States Environmental Protection Agency. *Our Current Understanding of the Human Health and Environmental Risks of PFAS*. www.epa.gov. https://www.epa.gov/pfas/our-current-understanding-human-health-and-environmental-risks-pfas.

(18) *Yale Experts Explain PFAS "Forever Chemicals" | Yale Sustainability*. Yale.edu. https://sustainability.yale.edu/explainers/yale-experts-explain-pfas-forever-chemicals.

(19) Fokina, V. M.; Patrikeeva, S.; Wang, X.; Noguchi, S.; Tomi, M.; König, J.; Ahmed, M. S.; Nanovskaya, T. Role of Uptake Transporters OAT4, OATP2A1, and OATP1A2 in Human Placental Bio-Disposition of Pravastatin. *Journal of Pharmaceutical Sciences* **2022**, *111* (2), 505–516. https://doi.org/10.1016/j.xphs.2021.09.035.

(20) Karahoda, R.; Horackova, H.; Kastner, P.; Matthios, A.; Cerveny, L.; Kucera, R.; Kacerovsky, M.; Duintjer Tebbens, J.; Bonnin, A.; Abad, C.; Staud, F. Serotonin Homeostasis in the Materno‐Foetal Interface at Term: Role of Transporters (SERT/SLC6A4 and OCT3/SLC22A3) and Monoamine Oxidase a (MAO‐A) in Uptake and Degradation of Serotonin by Human and Rat Term Placenta. *Acta Physiologica* **2020**, *229* (4). https://doi.org/10.1111/apha.13478.

(21) Wixey, J.; Beecher, K. Could Serotonin Play a Role in Abnormal Brain Outcomes in Fetal Growth Restriction? *Neural Regeneration Research* **2023**, *18* (3), 543. https://doi.org/10.4103/1673-5374.346481.

(22) Lofthouse, E. M.; Cleal, J.; Lewis, R. M.; Sengers, B. G. Computational Modelling of Paracellular Diffusion and OCT3 Mediated Transport of Metformin in the Perfused Human Placenta. *Journal of Pharmaceutical Sciences* **2023**, *112* (9), 2570–2580. https://doi.org/10.1016/j.xphs.2023.05.008.