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Maternal amino acid supplementation for intrauterine growth restriction

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1. ABSTRACT

Maternal dietary protein supplementation to improve fetal growth has been considered as an option to prevent or treat intrauterine growth restriction. However, in contrast to balanced dietary supplementation, adverse perinatal outcomes in pregnant women who received high amounts of dietary protein supplementation have been observed. The responsible mechanisms for these adverse outcomes are unknown. This review will discuss relevant human and animal data to provide the background necessary for the development of explanatory hypotheses and ultimately for the development therapeutic interventions during pregnancy to improve fetal growth. Relevant aspects of fetal amino acid metabolism during normal pregnancy and those pregnancies affected by IUGR will be discussed. In addition, data from animal experiments which have attempted to determine mechanisms to explain the adverse responses identified in the human trials will be presented. Finally, we will suggest new avenues for investigation into how amino acid supplementation might be used safely to treat and/or prevent IUGR.

2. INTRODUCTION

The clinical questions to be addressed in this review relate to the potential role of protein supplementation to improve fetal growth during pregnancy. Accretion of amino acids into proteins is an essential component of fetal growth. Therefore, maternal protein supplementation to improve fetal growth is an attractive therapeutic option, especially when fetal growth is failing. However, perinatal outcomes in pregnant women who received high amounts of protein supplementation are worse than in women who receive standard care or balanced energy supplementation. In fact, high protein supplementation increased small for gestational age (SGA) birth. Mechanisms responsible for this are unexplained and future experiments are required to fully understand this observation.

Maternal dietary supplementation with large amounts of protein results in an increased risk for preterm and SGA delivery, and increased perinatal mortality rates.(1) Prior to publication of these concerning clinical

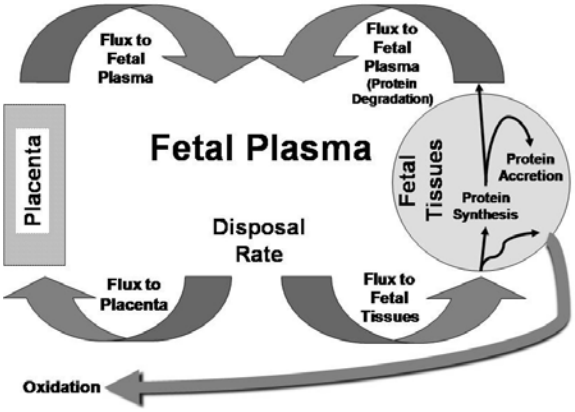

Figure 1. Amino acid fluxes within fetal compartments.

findings, a concept promoted in the literature was that the mother and fetus were competing for certain amino acids. Expansion of maternal tissues during pregnancy, development of the placenta, and growth of the fetus all require amino acids for protein accretion. One can view these various tissues as "competitors" for the same pool of amino acids which are considered to be a "scarce resource." In the context of intrauterine growth restriction (IUGR), the fetus is the "loser" in this competition.(2) Based on this line of reasoning, it seemed obvious that providing supplemental dietary protein to pregnant women, especially those at risk for having an IUGR infant, would improve fetal growth. This was supported by rodent data in which experimental maternal dietary protein restriction reduced fetal growth.(3-5) When these approaches apparently failed, the emphasis on maternal dietary protein intake as a regulator of fetal growth shifted to an evaluation of placental transport of amino acids to the fetus.(6; 7) Also it was realized that decreased fetal growth during protein malnutrition is not due to pure protein deficiency but is more likely due to other confounding variables including micronutrient deficiencies and the psychosocial environment.(8) In contrast, maternal caloric malnutrition is clearly associated with IUGR. This is supported by the finding that balanced maternal energy supplementation without excessive amounts of dietary protein increased fetal weight, though not necessarily lean mass.(1)

This review will discuss relevant human and animal model data to generate explanatory hypotheses that could test therapeutic interventions using amino acids during pregnancy to improve fetal growth. First, we will consider some relevant aspects of fetal amino acid metabolism during normal pregnancy and those pregnancies affected by IUGR. Then, we will review data from animal experiments which have attempted to determine mechanisms to explain the adverse responses identified in the human trials. Finally, we will suggest new avenues for investigation into how amino acid supplementation might be used safely to treat and/or prevent IUGR.

3. AMINO ACID TRANSFER AND METABOLISM

Most amino acids are supplied from the maternal circulation to the fetus via active transport across the placenta.(9; 10) Energy-dependent amino acid transporters are present on both the maternal-facing (apical) and fetal-facing (basal) surfaces of the trophoblast in the human placenta. Several different transport systems exist to transfer particular groups of amino acids based on their charge and structure.(9-11) For example, one of the amino acid transport systems can transfer all of the branched chain amino acids (BCAA, leucine, isoleucine and valine), threonine, tryptophan, phenylalanine, and methionine. Conversely an individual amino acid can be transferred by multiple systems. The final rate of transfer for an individual amino acid depends upon the relative concentrations of amino acids in the maternal plasma and the abundance and activity of transport systems.(11; 12) Additionally, there are amino acid shuttles between the fetal liver and placenta which exchange serine for glycine and glutamate for glutamine. These exchanges result in net uptake of serine and glutamate from the fetus by the placenta.(13; 14) However, with the exceptions of serine and glutamate, under normal conditions there is net fetal amino acid uptake from the placenta.(15)

Because amino acids also are released into the fetal circulation from fetal tissues, overall rates of amino acid appearance in the fetal plasma (which are equal to fetal amino acid disposal rates at steady state) are greater than net fetal uptake rates from the placenta. Fetal amino acid disposal is divided into direct flux back into the placenta and flux into fetal tissues. For most amino acids this flux is further divided into protein synthesis and amino acid oxidation. Synthesized proteins can then be degraded and the difference between the rates of protein synthesis and degradation is the net protein accretion rate (Figure 1). The relative contribution of each of these rates (flux from fetal plasma into the placenta, protein synthesis, and oxidation) to total fetal amino acid disposal varies for each particular amino acid.(16-25) However, overall protein accretion rates at the end of gestation are estimated to be between 2-4 gm/day.(24)

4. INTRAUTERINE GROWTH RESTRICTION

Experimental evidence from humans and animal models indicate that amino acid transport from mother to fetus and fetal amino acid metabolism are disturbed during IUGR. IUGR represents a pathophysiological condition in which a fetus is restricted from reaching its genetically determined size. This distinguishes IUGR patients from those that are simply SGA based on their genetic make-up. Identifiable causes of IUGR include intrauterine infections and maternal illnesses, but most cases are idiopathic. In the majority of cases, excluding intrauterine infections but including idiopathic cases, placental insufficiency and decreased nutrient transfer to the fetus are hallmark pathophysiological features (Figure 2). Although the incidence of IUGR depends on the specific definition used to make the diagnosis, estimates place it between 4-8% in developed countries.(26) IUGR fetuses have significantly elevated risks of intrauterine fetal demise, neonatal mortality, and short and long term complications.(27) Current clinical management consists of close monitoring of fetal growth rates and well being and indicated preterm delivery when fetal growth or well being

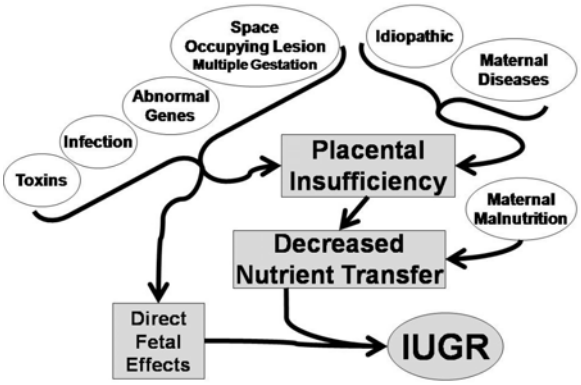

Figure 2. Causes of intrauterine growth restriction.

become so poor that the risks of intrauterine fetal demise are greater than the risks of prematurity.(28) Currently, there are no standard prenatal therapies which are designed to specifically improve fetal growth or reverse the complications of IUGR. It is therefore evident that any successful prenatal therapies have the potential to improve mortality and reduce short and long term complications of both IUGR and prematurity.

Despite worrisome outcomes in the human trials, interest in using protein and amino acid supplementation to prevent or treat IUGR remains an attractive potential therapeutic option. Human studies measuring fetal amino acid concentrations in IUGR pregnancies provide conflicting data. Some studies have documented decreased concentrations of certain amino acids including the BCAA, threonine, and arginine,(29-31) while others have not found differences.(32) Animal models indicate that this variability is likely due to differences in the severity of placental dysfunction.(33) While amino acid concentrations in IUGR fetuses are variable, a consistent feature in both human and animal studies is reduced placental transfer of certain essential amino acids.(16; 23; 32; 34-36) Furthermore, the severity of IUGR correlates with the severity of decreased amino acid transfer.(33; 37; 38) Decreased placental transfer of essential amino acids in cases of placental insufficiency might account for a lack of improved fetal growth when mothers were given a high dietary protein intake. Less clear are the mechanisms responsible for *decreased* fetal growth and worse overall mortality rates in these pregnancies. Prior to addressing the potential mechanisms we will review the pertinent human clinical trials.

5. PROTEIN SUPPLEMENTATION TO PREVENT OR TREAT HUMAN IUGR

Human trials generally show that increased maternal energy intake, without high amounts of dietary protein, improve fetal weight (though not necessarily lean mass) without significant adverse effects.(1) When increasing amounts of dietary protein are used to supply this energy, poor fetal weight gain and adverse perinatal outcomes occur.(1; 39) Therefore, high dietary protein supplementation can be viewed as toxic to the fetus. Nutritional intervention trials during pregnancy are challenging to interpret and often preclude specific mechanistic insight into the observed outcomes. Inclusion criteria of patients were variable, thus normal and IUGR pregnancies as well as other high risk pregnancies were often included. Supplements vary by more than just energy and protein; fat, vitamin, and mineral contents were also different between studies. The timing of introduction of the supplement during gestation varied between studies as did the source of protein (and thus the amino acid profile) within the supplement. Finally, long term clinical nutrient supplementation might have replaced nutrients in the normal diet if the mother decreased intake from other sources, or the supplement might have been shared among family members. These last two problems are more likely to occur in subjects with limited resources, but this population was more often targeted in these studies because they exhibit a higher incidence of IUGR.(40)

Despite these limitations the human trials of high maternal dietary protein supplementation provide important observations. Human trials using maternal intravenous amino acid mixtures showed promise, but suffered from several methodological shortcomings including small sample size and poor patient selection.(6; 41; 42) Oral dietary protein supplementation during pregnancy has been evaluated in several studies. Mardones-Santander *et. al.* selected low-income pregnant women at risk for having an IUGR pregnancy by including only underweight subjects. Women were randomized to receive one of two supplements; one provided approximately 330 kilocalories and 19 grams of protein per day and the other provided 310 kilocalories and 10 grams of protein per day. Subjects began supplementation in the 14th week of gestation and continued until delivery, which occurred at the same time in both groups (39 weeks). Birth weights in the group that received higher protein supplement were statistically lower (3.105 vs. 3.178 kilograms) as were the percentages of births with a weight less than three kilograms.(43) Similarly, a series of studies by Viegas *et. al.* found concerning results for high dietary protein supplementation during pregnancy. In their first study pregnant women were included regardless of their risk for an IUGR pregnancy and were allocated to one of three daily supplements. The first group received a high protein supplement providing 273 kilocalories with 26 grams of protein per day. The second group received a protein free supplement that provided 273 kilocalories per day from carbohydrates only. A third control group received vitamins only, which were also included in the other two supplements. Supplementation began in the 18th week of gestation and continued until delivery, which occurred at ~38 weeks in all groups. There were no differences in birth weights in any of the groups.(44) However, in a study published by the same group women were divided into two populations; those at risk for IUGR and those that were not. Both groups were further subdivided to receive one of three daily supplements: a high protein supplement that provided 425 kilocalories with 40 grams of protein per day, a carbohydrate supplement that provided 425 kilocalories per day, and a vitamin only group. These supplements were not started until the 28th week of pregnancy and continued until

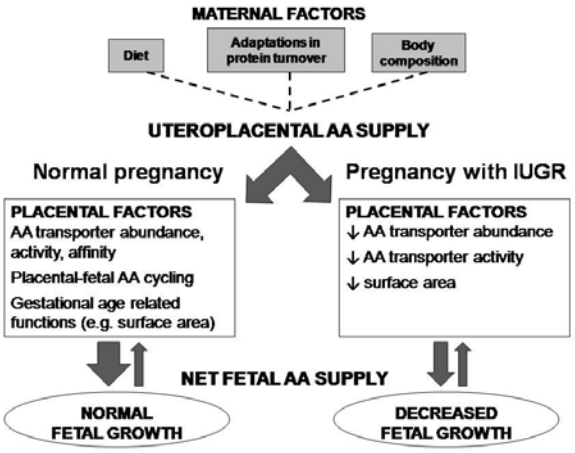

Figure 3. Determinants of net fetal amino acid (AA) supply.

delivery, which occurred at 38 weeks in all groups. In the at risk group of women, protein supplementation increased birthweight compared to carbohydrate supplementation (3.335 vs. 2.900 kilograms). However, in the group not at risk for IUGR, birth weights in the high protein supplementation group were marginally lower than in the carbohydrate group (2.940 vs. 3.080 kilograms, $p<0.06$).(45) Possible explanations for the differences observed in birthweight between these two trials include dose of protein (40 vs. 26 grams), gestational age when the intervention began (28 vs. 18 weeks), and likelihood of IUGR pregnancy. Finally, the most concerning outcomes of increased maternal dietary protein supplementation come from a large study by Rush *et. al.* This study included over 700 women at risk for having an IUGR pregnancy. Women were allocated to one of three groups prior to 30 weeks gestation. The high protein group received a supplement containing 470 kilocalories and 40 grams of protein per day, the second supplement group received 322 kilocalories and six grams of protein per day, and a third group received only vitamins. Mothers receiving a high protein supplement tended to deliver prematurely compared to the other groups and those women who delivered prematurely reported taking more of the supplement. Overall birth weights in the three groups were not different, however, when analysis was restricted to premature births there was a higher incidence of SGA in the high protein supplement group. Most concerning was that fetal and neonatal death rates in the high protein group were increased compared to the other two groups.(46)

6. POSTULATED MECHANISMS TO EXPLAIN FETAL OUTCOMES FROM INCREASED PROTEIN INTAKE

The mechanisms responsible for adverse fetal outcomes as a result of maternal high protein supplementation are unknown. Elucidation of these mechanisms has the potential to allow for the rational design of interventions which can safely promote intrauterine growth, decrease the incidence of indicated preterm delivery for IUGR, and prevent short and long term complications of this disease. We will review three potential mechanisms for fetal amino acid toxicity that have been explored in animal models of normal human fetal growth and metabolism and animal models of IUGR: 1) competitive inhibition of transport among essential amino acids across the placenta, 2) mismatch of increased fetal amino acid supply with persistently low fetal anabolic hormone concentrations, and 3) preferential utilization of increased fetal amino acids for oxidative metabolism rather than protein synthesis and accretion. It should be emphasized that these potential mechanisms are not mutually exclusive and most likely interact to explain the observations made in the human clinical trials.

6.1. Placental transfer of amino acids to the fetus

Amino acid flux across the trophoblast depends on several factors (Figure 3). Transporter abundance, activity, and affinity, as well as villous and microvillous surface area all affect transport capacity. Transport properties change as gestation advances to augment total amino acid exchange capacity and support exponential fetal growth.(47-49) These properties are affected during an IUGR pregnancy. Examination of isolated human and animal placentas from IUGR pregnancies demonstrate reduced expression and/or activity in several specific amino acid transport systems.(34; 37; 50-53) Reduced placental surface area has been reported for the IUGR placenta, indicating that morphometric changes in addition to reduced transporter activity contribute to the overall reduction in placental amino acid transport capacity.(54-57) In human IUGR pregnancies and animal models the severity of IUGR correlates with both reduced amino acid transport and fetal oxygenation.(33; 37; 38; 58) Because amino acid transport is an energy dependent process, further studies are warranted to determine if amino acid transport is regulated by low fetal oxygen concentrations in IUGR.

The maternal plasma amino acid profile is a major factor in determining protein delivery to the fetus. While maternal diet plays a key role in determining concentrations of maternal amino acids, maternal body composition (lean body mass) and maternal protein turnover and metabolism also affect circulating amino acids.(59-61) Experimental manipulation of the maternal plasma amino acid profile during pregnancy has highlighted the major effect of maternal amino acid concentrations on the fetal plasma amino acid profile. Balanced mixtures of essential and nonessential amino acids infused into pregnant sheep for two, three, and 12 hours toward the end of gestation consistently resulted in increased fetal concentrations of the BCAA, phenylalanine, and methionine, and decreased fetal concentrations of threonine and serine.(62-64) When maternal amino acid infusion enriched with essential amino acids was extended out to four days, fetal BCAA remained elevated and threonine concentrations remained low.(15) Acute maternal mixed amino acid infusions during human pregnancy just prior to delivery yielded similar results with failure to increase threonine concentrations.(65; 66) When threonine is individually infused into the pregnant ewe, fetal concentrations increase indicating that it is the presence of other amino acids, specifically the BCAA, that inhibit

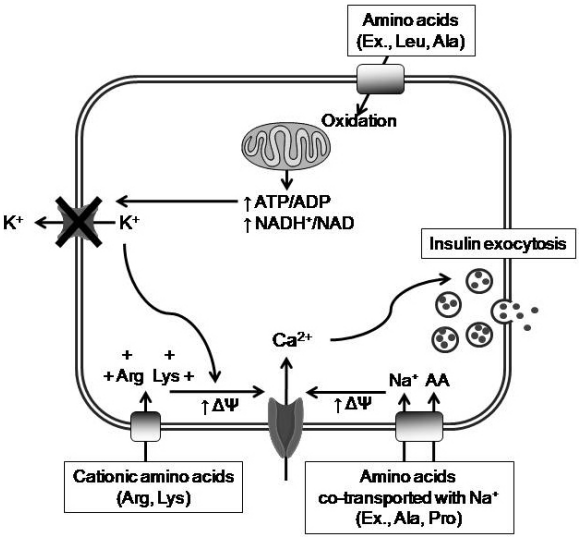

Figure 4. Mechanism of amino acid-stimulated insulin release. Three mechanisms for amino acid-stimulated insulin release have been identified: 1) Amino acids that are partially oxidized generate ATP and $NADH^+$, leading to closure of K_{ATP} channels and membrane depolarization; 2) Cationic amino acids such as arginine and lysine directly depolarize the cell membrane upon entry into the β-cell, causing the voltage-gated Ca^{2+} channels to open; and 3) Amino acids co-transported with Na^+ indirectly depolarize the cell membrane, also causing the voltage-gated Ca^{2+} channels to open. In all three cases, Ca^{2+} influx into β-cell results in insulin exocytosis. ↑ Δ Ψ, membrane depolarization.

threonine transfer to the fetus during a maternal infusion of mixed amino acids.(67-69) Collectively, these results indicate that fetal amino acid concentrations are affected by changes in maternal amino acid concentrations. However, competitive inhibition among co-infused amino acids that share affinity to specific transporter systems leads to unbalanced transport of amino acids across the placenta to the fetus. The limitation of supply of essential amino acids such as threonine or the transport of amino acids in inappropriate ratios are likely to limit fetal protein accretion and growth.(15) In summary, composition and content of protein supplements, maternal amino acid profile, timing of protein supplementation during a pregnancy, and sufficiency of the placenta will all have critical implications for placental transport and amino acid delivery to the fetus.

6.2. Mismatch of increased amino acids with persistently low fetal anabolic hormone concentrations

Insulin is a primary fetal growth factor.(70) Its anabolic effects are mediated by direct actions on fetal tissues as well as by increasing fetal IGF-1, which also increases fetal growth.(71-74) Fetal insulin concentrations are decreased in IUGR.(75-77) Many amino acids act as insulin secretagogues or potentiate glucose-stimulated insulin secretion in the pancreatic β-cell. But if supplying additional amino acids to the fetus in a more chronic fashion fails to increase insulin fetal growth might not be enhanced. During fetal development, β-cell sensitivity to amino acids for insulin secretion occurs earlier in gestation compared to sensitivity to glucose.(78; 79) Therefore, amino acid supply might act as a more potent stimulus for insulin secretion and growth than glucose during early gestation. Amino acids have a bidirectional endocrine effect by stimulating both insulin and glucagon release,(15; 80) such that some of the metabolic effects of insulin might be counteracted by glucagon.

Several mechanisms for amino acid stimulated insulin secretion have been identified and are not mutually exclusive (Figure 4). Amino acids are oxidized as fuels and generate ATP and $NADH^+$ which stimulate exocytosis of insulin granules;(81) leucine and alanine are two examples.(82; 83) Entry of arginine, lysine and other positively charged amino acids directly depolarize the β-cell membrane to cause the voltage-gated calcium channels to open. The increase in cytosolic calcium concentrations stimulates exocytosis of insulin granules.(84) Several other amino acids, like proline for example, are co-transported into the β-cell with positively charged sodium ions and cause membrane depolarization and activation of Ca^{2+} channels.(85; 86) In addition, some amino acids also have been shown to influence gene expression in the β-cell to promote insulin secretion.(87)

Individual amino acids vary in their ability to stimulate insulin secretion. Direct fetal infusions of leucine, lysine,(88) arginine,(89-91) and alanine(92) all have been shown to stimulate insulin secretion in the fetal sheep. Maternal infusions of BCAA alone or threonine did not affect fetal insulin concentrations in late gestation fetal sheep, despite significant uptake across the placenta and increased fetal amino acid concentrations.(62; 68) However, the timing of fetal blood sampling might have missed acute changes in insulin concentrations in the first several minutes of the infusion. In human full-term fetuses, a maternal leucine infusion alone did not increase fetal insulin but did potentiate acute fetal glucose stimulated insulin secretion.(78) In human neonates at 3 days and 3 weeks of age, a combined infusion of leucine, phenylalanine, and tyrosine caused no change in insulin or glucagon secretion. Again, the timing of blood sampling in this trial might have missed an acute increase in insulin.(93) In isolated fetal rat or sheep islets, taurine, leucine, lysine, methionine, and arginine have been shown to increase insulin secretion,(88; 94-96) while cysteine does not.(94)

Several experiments have measured *in vivo* fetal insulin secretion following an acute infusion of a full complement of amino acids. Insulin secretion in the ovine fetus has been demonstrated following either direct fetal infusions or maternal infusions that increased most fetal amino acid concentrations.(62; 89; 97) These findings have been replicated in human preterm infants.(78; 98) Some studies have included infusions of amino acids in combination with glucose and have found this mixture even more effective at stimulating insulin secretion than either alone. This demonstrates that amino acids can potentiate fetal glucose-stimulated insulin secretion.(90; 98) Consistent with *in vivo* findings, fetal rat pancreas incubated in essential amino acids plus glycine and alanine show

amino acid stimulated insulin secretion, which is further enhanced when glucose is included in the media.(99) The potentiating effect of amino acids on glucose stimulated insulin secretion may be important to the fetuses' ability to increase insulin concentrations following small changes in blood glucose.

The above data show that in general, a short term infusion of amino acids acutely stimulates insulin secretion, particularly when a full complement of amino acids is provided. However, very little work has been done to determine the effects of a chronic infusion of amino acids on fetal insulin secretion. In one study, the catheterized fetal sheep preparation was used to determine the effects of a four-day maternal infusion of the full complement of amino acids.(15) Despite increases in most fetal plasma amino acids, there was no change in fetal plasma insulin or IGF-1 concentrations. On the other hand, fetal glucagon concentrations progressively increased. Thus, the chronic amino acid infusion caused an increase in glucagon, a catabolic hormone, but no increase in anabolic hormones. Similar results have been found after a 24-hour fetal infusion of mixed amino acids following 48 hours of maternal starvation in the sheep – neither insulin nor IGF-1 were increased.(100) Therefore, treatment with chronic amino acid infusion is unlikely to be conducive to improved growth without a strategy that also increases the anabolic signals of insulin and IGF-1.

6.3. Increased oxidative metabolism of extra amino acids

The rate of fetal protein synthesis must exceed the sum of fetal protein breakdown and amino acid oxidation rates for net protein accretion and growth to occur. Understanding the balance between anabolic and catabolic pathways when additional amino acids are supplied to the fetus is critical in assessing the potential growth promoting effects of the substrate versus the potential for fetal toxicity. When amino acids were infused into the pregnant ewe for four days, fetal leucine oxidation increased without a concurrent increase in fetal protein accretion. Decreased oxygen saturation and content were observed with a trend towards increased fetal oxygen consumption, suggesting that overall fetal substrate oxidation rates were increased to handle the increase in protein load.(15) These findings indicate that the balance in fetal substrate utilization in the face of chronic, excess substrate might be tipped towards catabolic pathways.

There are several possibilities to consider when evaluating the balance between anabolic and catabolic pathway activation by substrates in the fetus. First, as already discussed, understanding the interactive effects of amino acids and insulin are important when considering amino acid therapy to improve fetal growth. Many *in vivo* studies in postnatal animals and humans have demonstrated that an acute infusion of amino acids (and particularly leucine) promotes translation initiation and muscle protein synthesis independently of any changes in insulin concentrations.(101-107) Other studies indicate that insulin is required for this effect, especially in fetal life.(97; 108-112) The independent and interactive effects of insulin and amino acids on muscle protein synthesis have been well described in the neonatal pig,(104; 113; 114) but fetal studies addressing this issue have been more limited. In the unique hormonal and nutrient environment during fetal life when insulin concentrations are relatively low compared to postnatal concentrations,(115; 116) additional amino acids without concurrent increases in insulin concentrations might favor oxidation as opposed to net protein accretion. A second possibility is that oxidation might represent the preferred pathway for exogenously increased amino acids in the fetus. Studies in pregnant sheep have shown that oxidation rates for both glucose and lactate increase with an increase in their respective plasma concentrations.(117; 118) Likewise, increases in fetal plasma amino acid concentrations such as leucine and phenylalanine promote their oxidation.(21; 89; 119) Studies in human preterm neonates also demonstrate stepwise increases in leucine and phenylalanine oxidation in response to intravenous amino acids.(120) Finally, there is evidence that fetal skeletal muscle is relatively more resistant to increasing protein accretion in response to additional amino acid supply compared to postnatal muscle. In a study by DeBoo *et al.*, phenylalanine kinetics across the ovine hindlimb were performed to measure skeletal muscle specific protein metabolism in response to an acute mixed amino acid infusion in the IUGR fetus. The amino acid infusion failed to promote hindlimb-specific protein accretion.(89) Additionally, in normally grown fetal sheep a two hour mixed amino acid infusion failed to activate signal transduction proteins that upregulate mRNA translation in skeletal muscle, independently of physiologic increases in insulin.(97) Since the fetus receives an uninterrupted supply of amino acids from the placenta resulting in fetal amino acid concentrations higher than those of the mother,(20) additional amino acid supplementation might simply drive oxidative pathways.

7. GROWTH PROMOTING EFFECTS OF SPECIFIC AMINO ACIDS

In addition to the need for future research to define the mechanisms of toxicity seen in the human trials of maternal dietary protein supplementation, there also is a need to investigate the unique growth promoting properties of individual amino acids during fetal life. The mechanisms by which individual amino acids might promote fetal growth are varied, and the examples given below serve to highlight the type of ongoing research in this area.

7.1. Arginine

Arginine has been evaluated for the treatment of IUGR. Two studies reported improved fetal weight gain and/or increased birthweight with arginine supplementation in IUGR pregnancies. Sieroszerski *et. al.* started women on an oral arginine supplement of three grams per day for 20 days or a placebo around the 32nd week of gestation. Fetal growth, as measured by ultrasound, was higher in the arginine group compared to the placebo group. Furthermore, at delivery, which occurred shortly after the treatment period and at the same gestational age in both groups, mean birth weight was increased in the arginine supplemented group (2.823 vs. 2.495

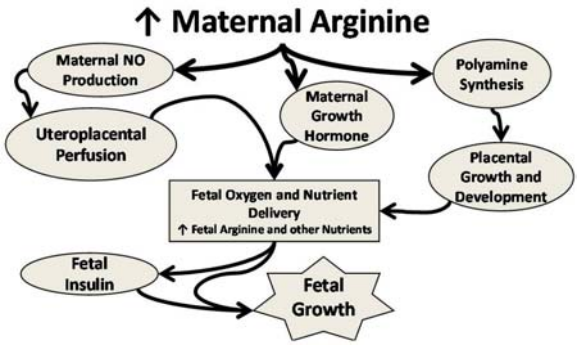

Figure 5. Mechanisms by which arginine might improve fetal growth.

kilograms).(121) A second trial by Xiao *et. al.* also reported improved mean birth weight with intravenous arginine administration of 20 grams per day for seven days (2.972 vs. 2.794 kilograms). In this trial the treatment began at an average gestational age of 33 weeks and also included other supplemental amino acids provided to both groups. Gestational age at delivery was the same in both groups (39 weeks), demonstrating that the benefits of arginine supplementation might persist beyond the immediate treatment period for IUGR pregnancies.(122) However, a more recent study by Winer *et. al.* failed to replicate these positive results. In this study pregnant women diagnosed with IUGR were enrolled at 28 weeks and randomized to receive either 14 grams per day of oral arginine or a placebo for the duration of pregnancy. Delivery occurred at 31 weeks gestation for both groups. Average birth weight in the arginine supplemented group was 1.042 kilograms, not different from the placebo group (1.068 kilograms).(123) The major difference between these three studies, which might explain the variable results, was the inclusion criteria. In the Xiao trial, mothers were only enrolled if fetal growth parameters were less than the third percentile, which selected for severely growth restricted fetuses. The other two trials included women if fetal growth was less than the tenth percentile. Other differences included a more thorough analysis of subjects lost to follow up in the Winer trial and the use of general amino acid supplementation in both the placebo and arginine group in the Xiao trial.

Despite these conflicting results, interest in arginine supplementation for IUGR remains high.(124-127) There are several proposed mechanisms by which arginine supplementation might improve fetal growth (Figure 5). Most experimental evidence suggests that arginine improves fetal growth in IUGR by increasing uteroplacental perfusion and fetal nutrient delivery by increasing local nitric oxide (NO) concentrations. NO is a potent vasodilator regulating uteroplacental blood flow and NO production in the placenta is decreased in IUGR.(122; 128) NO is synthesized by nitric oxide synthase (NOS), and arginine serves as the NO donor. In pregnant rodents, NOS inhibitors and genetic endothelial NOS inhibition both cause IUGR.(129; 130) Similarly, an arginine deficient diet in the rodent leads to IUGR.(130) Arginine supplementation has been shown to reverse the IUGR caused by NOS inhibition.(131) In one study of pregnant women with IUGR fetuses, 30 grams of intravenous arginine acutely increased uteroplacental perfusion.(132) However, of the three human studies that measured fetal growth response to arginine, only the study by Winer *et. al.* measured uterine artery blood flow, and there was no difference between the arginine or placebo group.(123) A second mechanism that would tend to increase fetal nutrient delivery is arginine stimulation of maternal growth hormone secretion.(132) Growth hormone alters maternal nutrient partitioning to favor delivery to the fetus,(133-135) although a recent study in undernourished pregnant ewes demonstrated improved fetal weight following maternal arginine supplementation independent of changes in maternal growth hormone concentrations.(124) A third potential mechanism is by enhancement of placental growth and development via the promotion of polyamine synthesis.(181) Arginine (among other amino acids such as proline) is an important substrate for polyamine production. Polyamines are synthesized from the conversion of arginine to ornithine and then to putrescine by ornithine decarboxylase (ODC). In ovine pregnancy, polyamine synthesis peaks early in gestation when placental growth is rapid. (182) Maternal nutrient restriction during the first half of ovine pregnancy decreases arginine and polyamine concentrations in fetal fluids and results in IUGR. (183) Furthermore, inhibition of ODC in rats results in IUGR.(184) A fourth potential mechanism is that arginine can stimulate insulin secretion. Arginine, in modest to high amounts, is a potent fetal insulin secretagogue,(91; 133) and insulin is a major anabolic hormone in the fetus.(70) Finally, arginine has been shown to stimulate skeletal muscle protein synthesis, though as discussed earlier this effect might be dependent on simultaneously increased insulin concentrations.(133; 136; 137)

7.2. Taurine

Taurine has many physiological and developmental functions. It is considered an essential amino acid for the fetus and neonate, as de novo fetal synthesis is inadequate at these ages.(138-140) Specific effects of taurine on the developing pancreas have been demonstrated by a series of studies using one particular rat model of IUGR characterized by progressive β-cell loss and dysfunction. Dams fed a low protein (LP) isocaloric diet (8% vs. 20% dietary protein) throughout gestation gave birth to pups with lower birth weight and reduced β-cell mass and function compared to controls.(3; 141) Plasma taurine was lower in LP dams and their fetuses, and taurine supplementation to the LP dams during pregnancy normalized β-cell mass and insulin secretion.(95; 141; 142) However, fetal and pup body weights were not corrected.(141) Despite the persistently low body weights in the LP fetuses and pups, which in this particular model is almost certainly due to deficiency of other amino acids, the improvement in β-cell mass and function with taurine supplementation has important potential implications for the design of future therapeutic interventions. This is supported by work in a second rat model of IUGR in which uterine artery blood flow is decreased at the end of gestation. In this model prenatal maternal taurine supplementation increased postnatal growth and weight in both IUGR and control groups.(143) Thus, coupled with the appropriate provision

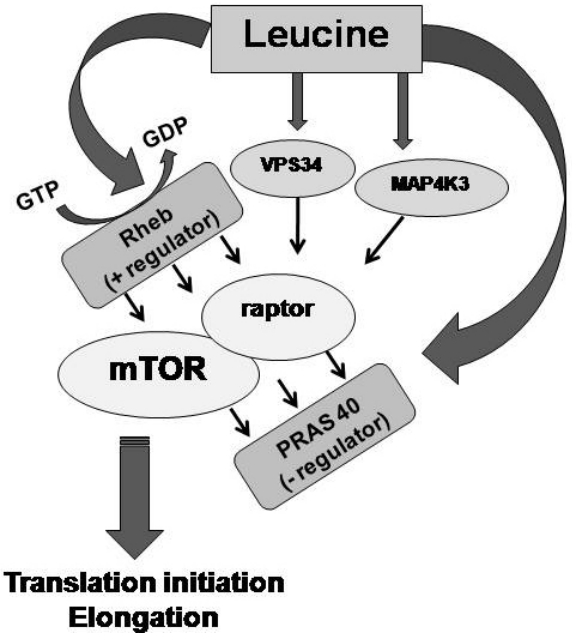

Figure 6. Mechanisms by which leucine activates protein synthesis. Through exact mechanisms remain unclear, leucine has been proposed to increase m TOR activity by 1) increasing the binding of Rheb-GTP to mTOR, 2) increasing the activity of MAPAK3, 3) mediating VPS34 action, and 4) decreasing the binding of PRAS40, a repressor of MTOR signaling.

of nutrients, fetal taurine supplementation has the potential to improve β-cell function and insulin secretion allowing for the necessary increase in fetal anabolic hormones to improve fetal growth.

7.3. Leucine

Leucine has a stimulatory effect on muscle protein synthesis during fetal and postnatal life by serving as a substrate for synthesis of new proteins, stimulating concurrent increases in insulin concentrations, and acting to directly stimulate translation initiation pathways. Studies using *in vitro* myocyte cultures and *ex vivo* muscle explants were the first to demonstrate the potent effects of BCAA in stimulating muscle protein synthesis.(144; 145) They do so to a similar degree as a full complement of mixed amino acids and more so than mixtures of amino acids that lack BCAA.(146-148) Of the BCAA, leucine has the greatest capacity to increase muscle protein synthesis through signaling pathways involving the mammalian target of rapamycin (mTOR). mTOR regulates the initiation of mRNA translation by increasing the phosphorylation of p70S6 kinase and 4E-BP1. When myocytes in culture were exposed to individual amino acids, leucine had the greatest capacity to upregulate mTOR and phosphorylate 4E-BP1 and p70S6 kinase.(149) *In vivo* studies in postnatal animals and adult humans have shown the potent effects of leucine, whether administrated intravenously or orally, in upregulating mTOR signal transduction and promoting muscle protein synthesis.(101; 111; 150-154) Exactly how leucine functions to upregulate mTOR activity is unknown, though recent work has improved our understanding of these mechanisms.(155; 156) Briefly, TORC1 (TOR complex 1) is a nutrient regulator comprised of several subunits including but not limited to mTOR (protein kinase), Rheb (RAS homolog), raptor (regulatory associated protein), and PRAS40 (repressor of mTOR activity). Amino acids might function to increase the binding of Rheb-GTP to mTOR, increasing the activity of mTOR.(157) Most recently, Vps34 (a class 3 PI3K), and MAP4K3 (a unique MAP kinase) and PRAS40 have been shown to sense amino acids and signal to mTOR (Figure 6).(158-160)

Given its ability to independently stimulate muscle protein synthesis, leucine may be considered as a nutritional therapy to promote lean mass growth in the IUGR fetus during pregnancy. The human IUGR fetus and neonate are characterized by reduced lean mass.(161-163) Adults who were born with low birth weight for gestational age have persistent reductions in muscle mass, reduced muscle to fat ratios, and reduced muscle strength.(164-170) Postnatal growth rates and body composition after growth restriction *in utero* have been explored in sheep models that create placental insufficiency and IUGR. These studies have shown that reduced lean body mass persists beyond the fetal and neonatal period despite a period of accelerated postnatal growth from increased insulin sensitivity, indicating preferential adipose tissue growth and limited lean mass growth.(171-175) Similarly, rapid postnatal growth in infants during the first 3 months of life is a risk factor for developing obesity as early as three years of age.(176) Future studies are imperative to fully understand optimal tissue-specific growth rates during and after fetal exposure to IUGR and, importantly, how nutritional therapies with leucine or other amino acids might maximize lean mass growth early in life.

Not only can leucine stimulate acute fetal insulin secretion and muscle protein synthesis, it also regulates β-cell mass (Figure 7). Like in muscle, mTOR mediated signaling is also critical in regulating growth and proliferation in the β-cell. Leucine activates mTOR in the β-cell via its own oxidative metabolism and by stimulating glutamate metabolism.(177; 178) β-cell proliferation and establishment of normal β-cell mass and size is dependent on mTOR signaling.(179; 180) As we consider prenatal and/or postnatal therapy with leucine to improve lean mass, β-cell function, and overall fetal growth, future studies are needed to define the adaptations that evolve in the fetal skeletal muscle and β-cells in response to nutrient restriction and the responsiveness of those adaptations to increased fetal leucine concentrations.

8. CONCLUDING REMARKS AND FUTURE DIRECTIONS FOR AMINO ACID SUPPLEMENTATION

Due to the potent growth promoting effects of amino acids discussed in this review, maternal protein supplementation during IUGR pregnancy to improve fetal growth remains an attractive option. However, adverse fetal outcomes observed in several clinical studies highlight the need to fully understand the mechanisms by which

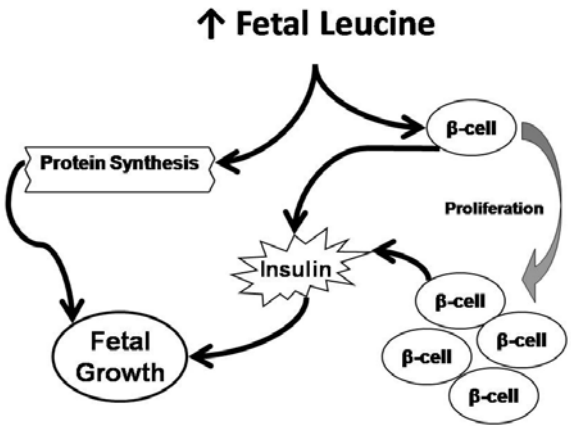

Figure 7. Mechanisms by which leucine might improve fetal growth.

additional amino acids in the maternal diet are transferred to the fetus and how the fetus handles the protein load. Initially, we need to understand how an external supply of amino acids provided directly to the fetus affects fetal protein metabolism, hormonal profiles, and growth. This can be done with chronic, direct intravenous fetal infusions using animal models of normal and IUGR pregnancy. This method allows for detailed analyses of the *in vivo* fetal metabolic, physiological, and endocrine responses to amino acid infusion. Cellular and molecular responses to the infusion at the level of individual tissues and organs can also be evaluated. Intra-amniotic amino acid supplementation should be considered, as this delivery method bypasses a poorly functioning placenta in IUGR and circumvents the problem of reciprocal inhibition of amino acid transport to the fetus.(71) Only after beneficial growth responses as a result of direct fetal amino acid supplementation have been documented can strategies for maternal supplementation be undertaken. Maternal studies in normal and IUGR pregnancies will require experimental designs that can evaluate the effects of a maternal protein supplement on placental amino acid metabolism. Furthermore, a thorough understanding of the mechanisms by which amino acids are transferred from the mother to the fetus and how the fetus uses amino acids for proper growth is necessary. These studies are critical to effectively provide a balanced complement and a safe dose of amino acids to the fetus that will improve fetal growth during an IUGR pregnancy. Finally, individual amino acid supplementation during pregnancy might be beneficial. Taurine, arginine, and leucine all have the potential to promote favorable fetal growth by promoting improved placental and fetal perfusion, tissue-specific growth and metabolism, or through mechanisms yet to be discovered. Due to the lack of currently accepted therapies to improve the outcomes for IUGR pregnancies, nutritional therapies to maximize fetal growth and well-being during IUGR pregnancy deserve further study.

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