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The effect of intraumbilical fetal nutrition via a subcutaneously implanted port system on amino acid concentration by severe IUGR human fetuses

DOI 10.1515/jpm-2016-0155
Received February 19, 2016. Accepted July 4, 2016. Previously published online August 17, 2016.

Abstract

Objective: To determine if intrauterine intraumbilical supplementation with amino acids (AA) and glucose can improve neonatal outcome of severe growth restricted human fetuses (IUGR).

Methods: Prospective pilot study of intrauterine treatment of severe IUGR fetuses [n=14, 27 weeks of gestation (range 23–31)] with cerebroplacental ratio <1, with long-term intraumbilical AA and glucose supplementation (10% of feto-placental blood volume/day) using a perinatal port system alone (n=5) or combined with hyperbaric oxygenation (n=1, HBO) vs. control group (n=8).

Results: The duration of continuous intraumbilical AA/glucose supplementation was 11 (6–13) days. Daily intravascular fetal nutrition significantly prolonged the brain sparing to delivery interval by 24 (14–33) days vs. 5.6 (2–12) days in controls. Fetal nutrition reduced blood flow resistance in the placental circulation but did not affect the Doppler profile of cerebral arteries. Higher weight gain of 113.5 (36–539) g was observed following supplementation compared to 33.3 (8–98) g in the control group (P<0.05). In spite of this, fetuses below 28 weeks of gestation did not sufficiently benefit from infused commercial AA. We found a reduced fetal plasma concentration of the essential AA histidine, threonine, lysine and arginine, and non-essential AA taurine, in severe IUGR fetuses in both groups. Long-term supplementation with a commercial AA formula led to a slight, but not significant, reduction of histidine, threonine, lysine, arginine, asparagine and glutamine. However, the concentration of tryptophan and glutamic acid slightly increased. HBO can be combined with AA supplementation via a port system. In one case, the port system was also successfully used for fetal blood transfusion.

Conclusions: Intravascular treatment of IUGR with fetal nutrition can prolong pregnancy with severe placental insufficiency and brain sparing for many weeks. However, rather than normalizing AA concentrations, an enhanced AA imbalance was observed in IUGR fetuses following supplementation. These deviations in AA concentrations prevent the recommendation for use of commercial AA solutions for prenatal treatment of extreme preterm IUGR fetuses.

Keywords: Amino acids; cordocentesis; fetal growth restriction; HBO; hyperbaric oxygenation; intraumbilical infusion; intrauterine treatment; IUGR; port.

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Introduction

Placental insufficiency, the main contributor to the development of intrauterine growth restriction (IUGR), is caused by numerous factors including chronic placental infections, many maternal diseases, abnormal genome and intravascular trophoblast invasion impairment [1]. Placental insufficiency is responsible for fetal loss in about 40% of all stillbirths and long-term neurological deficits [2–5]. The reduction of blood flow resistance in cerebral

arteries in severe IUGR conditions, and reduced pulsatility index (PI) in the medial cerebral artery, predicts the 11-fold increased risk of intraventricular hemorrhage, periventricular leukomalacia, hypoxic ischemic encephalopathy, necrotizing enterocolitis, bronchopulmonary dysplasia, sepsis, and death [6]. The mean interval from diagnosis of brain sparing to delivery in severe IUGR fetuses was recently identified as only 7 days (ranging 2–15 days) [6].

The concentration of amino acids (AA) in fetal plasma is many times higher than maternal levels because of active transplacental transport of AA and additional AA synthesis in the placenta [1, 7].

The critical placental player in the active AA transport from the mother to the fetus is the trophoblast, which is irreversibly changed in severe IUGR fetuses caused by placental insufficiency [1]. Thus, a logical partial solution of IUGR could be the direct supply of AA and glucose to the fetus, in order to improve fetal growth, normalize fetal programming altered by IUGR and prolong the pregnancy [8, 9]. Additional oxygen supply of fetal tissues could also improve the uptake of injected nutritional supplements and may avoid the development of lactate acidosis in IUGR fetuses [10].

The aim of this prospective pilot study was to further test the efficacy of the administration of AA and glucose via a subcutaneously implanted intraumbilical perinatal port system, as a treatment option for severe IUGR human fetuses with brain sparing effect.

Materials and methods

Study design

IUGR was defined in our study as an estimated fetal weight of <5%, (Hadlock-4) combined with increased resistance in both uterine

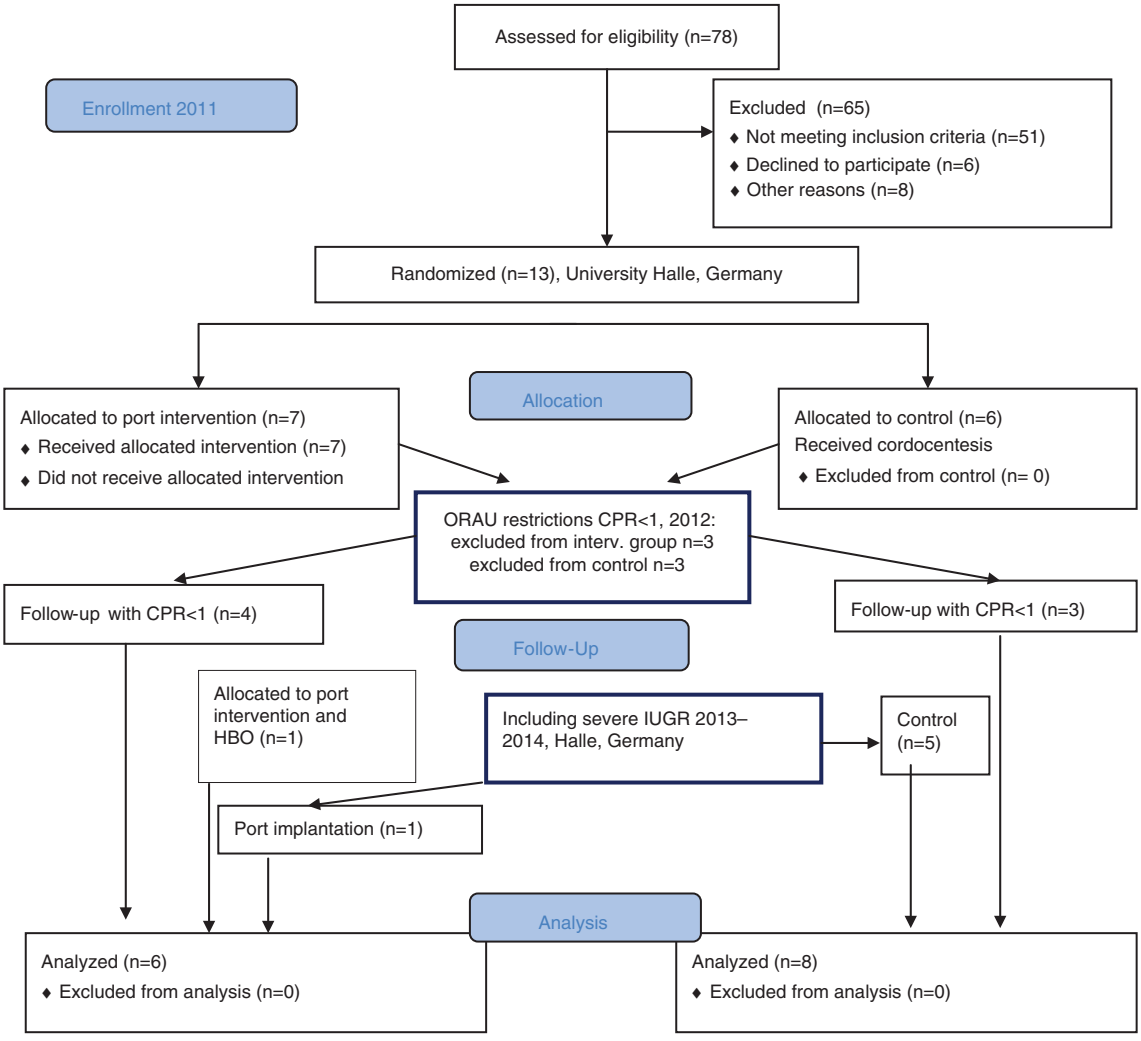

Figure 1: Flow diagram.

arteries (Ut.A.) with PI >95% [11]. Fetuses with morphological and/or chromosomal abnormalities were not included in the final analysis.

The study commenced in 2010 as a prospective randomized clinical trial of patients with intrauterine growth restricted fetuses undergoing intraumbilical amino acid and glucose supplementation, using a subcutaneously implanted port system vs. IUGR control patients (NCT02596594, ClinicalTrials.gov). Seventy-eight patients were referred to the medical center with IUGR. Fifty-one patients did not meet the inclusion criteria of the study. Six patients declined participation in the study after explanation of the study design and risks of port implantation. Thirteen out of 65 patients met the study criteria and gave the permission (Figure 1). The randomisation was performed by institute of Mathematics and Statistics of Martin-Luther University, Halle-Wittenberg, Germany using the SPSS statistics program (IBM SPSS Statistics v 20, Ehningen, Germany). The study design was restricted to severe IUGR cases below 32 weeks' gestation (WG) with estimated fetal weight <3rd percentile, pathologic PI>95% in both Ut.A. and cerebroplacental ratio <1 [CPR=PI MCA/PI umbilical artery (UA)], in response to recommendations of Oak Ridge Associated Universities reviewers (ORAU, Oak Ridge, TN, USA). Due to these restrictions, three patients from each group were excluded from final analysis (Figure 1). We decided to include only patients with severe IUGR and CPR<1 meeting the inclusion criteria from Halle-University, Germany to complete the study in 2014. Thus, six IUGR patients with CPR<1 between 24/0 and 32/0 WG received AA and glucose supplementation via a subcutaneously implanted intraumbilical perinatal port system and eight patients with IUGR and brain sparing belong to the control group. In response to these restrictions, the study became a pilot feasibility study.

The protocol for the port implantation was approved by the institutional review board (Ethics Committee of National Medical Center of the Mother and Child, Nazarbayev University, Astana, N1, 2, 5 and 6 and Clinical Ethics committee for individual treatment of Martin-Luther University Halle-Wittenberg, 4th July, 2012). The invasive procedures were performed with written informed patient consent.

The ultrasound examinations were performed with Voluson E8 Expert (GE, Milwaukee, WI, USA) and (Philips iU22, Philips Medical, Hamburg, Germany). The monitoring protocol of the study included evaluation of amniotic fluid volume, multi-vessel Doppler examination of the PI in the UA, middle cerebral artery (MCA), Ut.A. and ductus venosus at every subsequent contact with research sonographers until birth. The fetal weight was estimated weekly. A report of all sonographic findings was recorded and readily available to the clinical physicians using ultrasound software system (Viewpoint, GE, USA). The timing of delivery by cesarean section was decided by the lead clinician managing each case.

In the control group, fetal blood sampling was performed by ultrasound-guided cordocentesis using a 22-gage needle. The blood was immediately centrifuged and the serum was frozen in liquid nitrogen for AA investigation (high performance liquid chromatography, Hewlett Packard Series 1100, Waldbronn, Germany).

Daily hyperbaric oxygenation (100% O_2) with a pressure of 1.4 atmosphere absolute for 50 min (Baromed, Perry Baromedical Corporation, FL, USA) was used in one case, in an attempt to improve intraumbilical nutrient supplementation. Additional criteria for preterm delivery by C-section were stable pathologic Doppler profiles in the DV and/or CTG pathologic findings (variable deep decelerations, shot time variations <2.9 ms) [12].

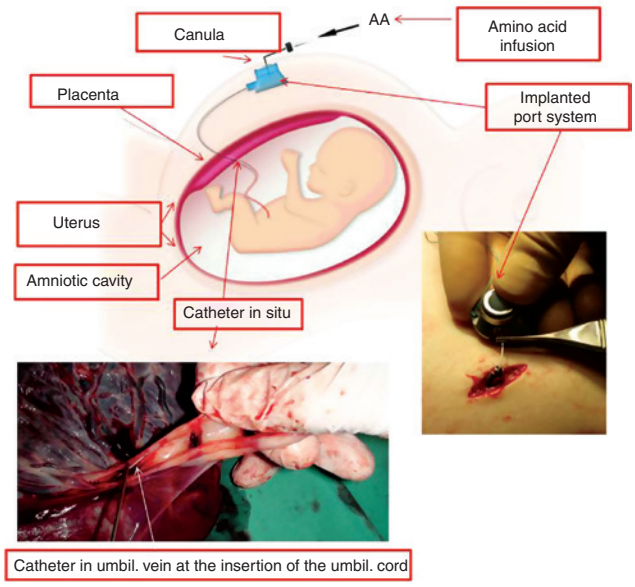

Figure 2: The amino acid and glucose supplementation via a subcutaneously implanted intraumbilical port system.
After sonographic localization of the placenta, a small incision into the skin with a scalpel was undertaken under local anesthesia with 20 mL non-adrenalized 1% Xylocaine and a subcutaneous bag for the port capsule was prepared using a pair of scissors at the side of placental insertion of the cord. The vascular access port capsule was flushed with heparinized saline (10 units/mL). The umbilical vein was punctured with an 18-gage needle (Echotip® Disposable Trocar Needle, COOK Medical, Spencer, IN, USA) under ultrasound control through the prepared bag and anterior wall placenta. After fetal blood sampling the catheter was inserted through the needle into the umbilical vein with a removable 1-French stylet. The port was inserted into the prepared bag, where it was fixed with 3-0 Vicryl stitches to the subcutaneous fat tissue and the skin was closed with Monocryl 4-0 (ETHICON, Cincinnati, OH, USA). Then, the port system was connected to the pump with amino acid and glucose solutions. The tip of the 18G needle points on the anker system. The treatment course included daily infusions of AA and glucose. Note the amniotic cavity remained intact.

Implantation of the port system

Under local anesthesia with 20 mL non-adrenalized 1% xylocaine, a small skin incision was made using a scalpel blade. The subcutaneous pouch for the port capsule was prepared using a pair of scissors at the side of the placental insertion of the cord. The umbilical vein was then punctured with an 18-gage needle (Echotip® Disposable Trocar Needle, COOK Medical, Spencer, IN, USA) under ultrasound control through the prepared pouch and anterior wall placenta (Figure 2).

The amniotic cavity remained intact during this procedure. After fetal blood sampling the catheter was inserted through the needle into the umbilical vein and connected to the vascular access port capsule [8].

The port capsule was fixed with 3-0 Vicryl stitches to the subcutaneous fat tissue and the skin was closed with Monocryl 4-0 (ETHICON, Cincinnati, OH, USA). A 25-gage port needle was used to enter the port system. The treatment course included continuous daily infusions of AA solution, Aminoven infant, (n=4, Fresenius

Table 1A: Patient's data and neonatal outcome: port group.

Patient (n=6)	H1	A1 (HBO)	A2	A3	A4	A5	Median (range)
Age (years)	24	22	20	21	23	31	24 (20; 31)
Gestational age at time of DS brain sparing (days)	149	203	183	216	191	176	187 (149; 216)
Gestational age at time of port implantation (days)	160	211	189	224	211	189	200 (160; 224)
Duration of the AA/glucose supplementations (days)	12	9	12	6	13	10	11 (6; 13)
AA/glucose supplementations mL/h and mL/kg/day	Aminoven Infant 0.62 mL/h, 49.6 mL/kg/day (4 days) and 0.3 mL/h, 24 mL/kg/day (8 days) O_2 2 L/min	Aminoven Infant 1 mL/h, 24.5 mL/kg/day, 1 U/4 g glucose, 1.4 bar HBO 7 days	Aminoplasmal 1.5 mL/h 12 h/day; 23.1 mL/kg/day 1 U/4 g glucose	Aminoplasmal 0.7 mL/h; 16.3 mL/kg/day, 1 U/4 g glucose	Aminoplasmal 2 mL/h 46.5 mL/kg/day	Aminoven Infant 1.5 mL/h 51.4 mL/kg/day	
pH (FBS)	7.43	7.41	–	7.35	7.32	7.4	7.4 (7.32; 7.43)
Weight at port implantation	300	1022	739	1029	1033	697	805 (300; 1033)
Weight gain (g)	36	356	41	539	157	70	113.5 (36; 539)
Weight gain/week (g)	18	131	24	629	85	49	67.0 (18; 629)
Weight at delivery (g)	336	1378	780	1568	1190	767	985 (336; 1378)
Gestational age at delivery (days)	174	230	201	230	224	199	212.5 (174; 230)
Brain sparing/delivery interval (days)	25	27	18	14	33	23	24 (14; 33)
APGAR 1st min	0	6	2	8	1	3	3 (0; 8)
APGAR 5th min	0	7	4	9	2	4	4 (0; 9)
pH arterial	–	7.33	7.28	7.28	7.37	6.89	7.28 (6.89; 7.37)
28 days outcome	IUFD, placental weight 126 g	Survived, healthy state	Deceased: prematurity, IVH IV°, multiple organ failure, sepsis	Survived, healthy state	Survived, healthy state	Deceased: prematurity, RDS IV, multiple organ failure	

IVH=Interventricular hemorrhage, RDS=respiratory distress syndrome, DS=diagnosis.

Table 1B: Patient's data and neonatal outcome: control group.

Patient (n=8)	A1	A2	A3	H4	H5	H6	H7	H8	Median (range)
Age (years)	26	39	30	30	36	42	28	25	32 (25; 42)
Gestational age at time of DS brain sparing (days)	222	204	196	207	196	187	184	159	187 (159; 222)
Estimated weight	1160	715	854	632	900	703	538	313	709 (1160; 313)
Weight at delivery (g)	1169	740	930	640	910	801	550	341	770.5 (1169; 341)
Weight gain (g)	9	25	76	8	10	98	12	28	33.3 (8; 98)
Gestational age at delivery (days)	223	206	199	212	201	191	187	171	200 (223; 171)
Brain sparing/delivery interval (days)	11	2	3	5	5	4	3	12	5.6 (2; 12)
APGAR 1st min	6	5	4	5	6	9	4	1	5 (1; 9)
APGAR 5th min	7	5	5	8	6	9	6	1	6 (1; 9)
pH arterial	–	7.3	–	7.23	7.19	7.31	7.24	7.37	7.27 (7.19; 7.37)
28 days outcome	Survived	Survived	IVH-III/IV°	RDS IV	IVH-IV°, RDS-IV°, early onset sepsis	Survived	IVH-I°, RDS-IV°, pneumonia	Sepsis deceased	

IVH=Interventricular hemorrhage, RDS=respiratory distress syndrome, DS=diagnosis.

Kabi, Bad Homburg, Germany) or Aminoplasmal B. Braun 10% (n=2, B. Braun, Melsungen, Germany) with a 10% glucose solution. We limited the volume of the intraumbilical infusion to 10% of the estimated feto-placental blood volume per day [13]. On average, the AA/glucose-infusion was below 50 mL/kg. In addition to glucose and AAs, fetal supplementation via the port system included insulin (1 unit/4 g glucose).

Statistical analysis

The values of the core parameters for the groups were compared using an unpaired *t*-test and multivariate ANOVA. The Doppler parameters before and after the fetal nutrition were compared using a paired *t*-test. Data are presented as mean±SD and median (range). P-values of <0.05 were considered significant. Calculations were performed using Statistica® software Version 8.1 (Statsoft, Tulsa, OK, USA).

Results

The port system could be implanted in all patients without any complications. The brain sparing to delivery interval could be prolonged in the port group to 3 weeks (Table 1A and B). The duration of the intraumbilical amino acid supplementation was 11 days on average. Fetal nutrition appeared to reduce the resistance to blood flow in the placental circulation but did not affect the Doppler profile of cerebral arteries (Figure 3A–C).

We found a reduced fetal plasma concentration of essential AAs, histidine, threonine, lysine and arginine, and non-essential AA taurine, in severe IUGR fetuses in both groups [14, 15]. Phenylalanine, glycine, alanine, aspartic acid were slightly increased compared to the normal values. The intraumbilical administration of commercial AAs via the port system led to a slight but not significant reduction of histidine, threonine, lysine and arginine, asparagine and glutamine. However, the concentration of tryptophan and glutamic acid slightly increased (Table 2).

Intraumbilical AA/glucose supplementation increased fetal weight gain (Table 1). The mean weight gain remained under the 3rd percentile until delivery, except in fetuses below 28 WG, which despite receiving sufficient supplementation of 41.3 mL/kg/day on average (Table 1A), just failed to gain weight.

In one fetus with 23/3 WG, direct blood supplementation with 6 mL erythrocyte concentrate at 1 mL/h via the port system was required 4 days post-port implantation due to suspected fetal anemia in the Doppler ultrasound of the MCA Vmax [16]. Feto-maternal hemorrhage could be excluded by extremely low concentration of fetal Hb in the maternal circulation. The amino acid and glucose infusion

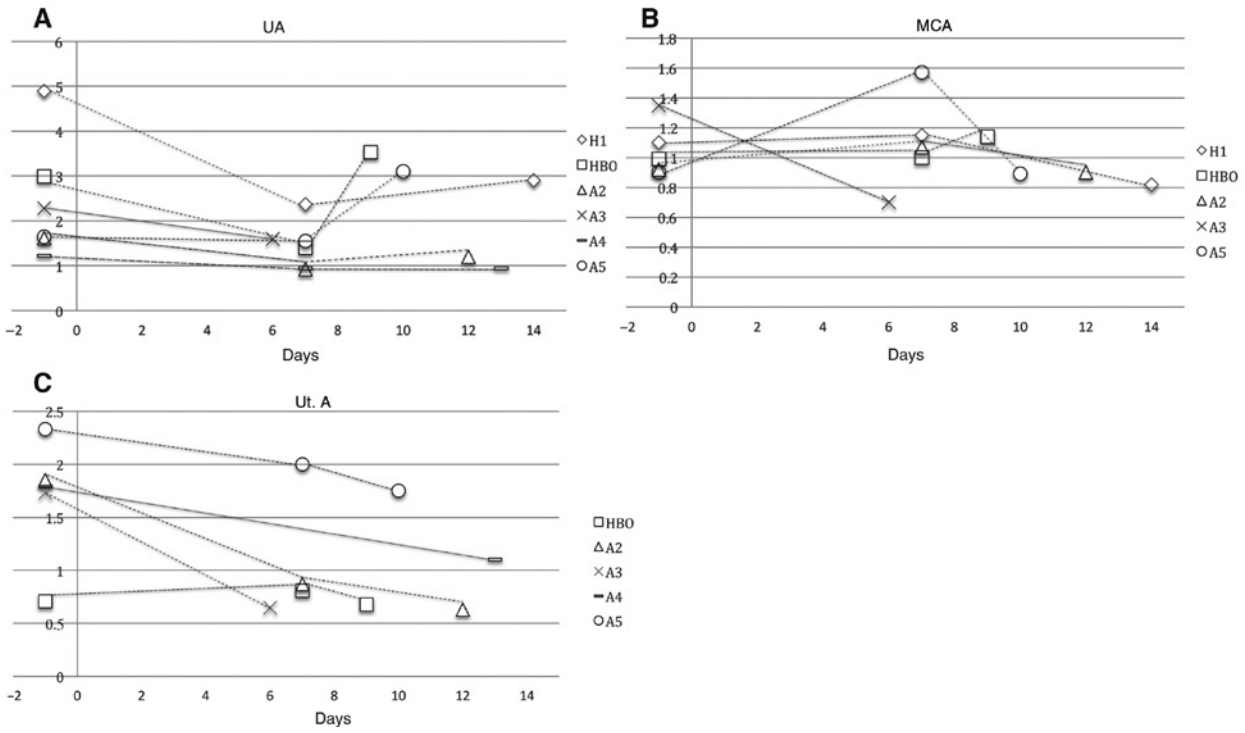

Figure 3: (A–C) Dynamic of the pulsatility index in the umbilical artery (UA), middle cerebral artery (MCA) and non-placental uterine artery (Ut. A) under intraumbilical amino acid and glucose supplementation through the subcutaneously implanted port system.

Table 2: Amino acid concentration of severe growth restricted fetuses with brain sparing.

Amino acid (µmol/L)	AA/Glucose suplementation via port (mean±SD)		Control (mean±SD)	
	Port implantation	C-section	FBS	C-section
Aspartic acid	18.9±2.9	31.3±11.3	27.3±0.9	26.5±0.5
Glutaminic acid	234.9±176.9	376.0±48.9	372.3±44.3	490.7±50.7
Asparagine	41.0±5.0	35.7±0.9	67.3±5.2	69.3±1.3
Serine	136.2±12.2	140.90±17.9	147.7±0.9	148.3±2.3
Glutamine	355.6±431.7	195.5±9.9	262.0±45.3	218.3±30.7
Histidine	93.4±14.7	77.7±16.5	110.3±7.0	100.3±0.7
Glycine	286.2±71.9	363.0±64.4	290.0±45.2	374.3±23.3
Threonine	284.4±56.7	162.0±10.1	263.3±42.9	315.0±17.0
Citrulline	21.4±2.7	10.7±1.8	23.7±5.2	19.7±0.3
Arginine	56.2±26.2	30.7±22.7	55.7±24.6	42.3±17.7
Alanine	508.9±16.2	471.7±79.2	538.0±60.7	610.0±16.1
Taurine	95.6±9.5	109.7±21.8	64.0±14.2	82.0±0.0
Tyrosine	74.0±3.0	67.3±14.2	63.0±9.3	70±12.0
Aminobutyric acid	25.3±7.5	22.0±5.0	15.0±4.2	16.3±3.3
Valine	218.9±24.9	247.7±40.5	222.0±21.0	214.0±11.0
Methionine	34.0±16.0	20.0±6.1	25.7±9	17.3±9.3
Tryptophane	60.0±1.0	118.7±64.3	66.7±3.8	61.7±1.7
Isoleucine	64±7	71.0±14.7	79.0±3.5	78.3±3.3
Phenylalanine	85.4±4.7	106.7±24.5	91.3±6.9	93.7±6.7
Leucine	106.9±19.9	139.7±31.5	129.7±8.3	132.7±8.7
Lysine	243.7±33.7	222.7±25.7	315.3±40.7	357.3±18.3
Hydroxyproline	52.9±5.2	34.0±2.9	43.0±12.5	37.7±5.7
Proline	236.85±16.2	259.0±41.0	203.3±20.3	202.0±19.0

C-section=Cesarean section, FBS=fetal blood sampling; AA=amino acids. The fetal blood sampling was obtained from the umbilical vein during port implantation in the port group or by means of cordocentesis in the control group. The second blood probe was obtained from the umbilical vein during the cesarean section in both groups.

was reduced to 0.1 mL/h after the blood transfusion. The Vmax in MCA was normalized to 35.9 cm/s with PI 1.05, UA 3.3.1 reversed flow, DV PI 1.42. Intraumbilical nutrition was increased to 0.3 mL/h the following day. Ten days later, a second transfusion of 6 mL erythrocyte concentrate was performed via the port system. The fetus, with an estimated weight of 334 g, developed reversed blood flow in the DV after 12 days of continuous AA/glucose supplementations (Table 1A). The patient declined an immediate delivery by cesarean section, which was recommended by our pediatric staff. As expected, an intrauterine fetal death occurred the following day and the mother delivered spontaneously. Pathological examination reported an insufficient placenta, measuring only 11×7×3 cm, with a weight of 126 g and infarcts present in about 10% of the total placental parenchyma. The position of the catheter was found to be optimal without any local hemorrhage. The increased Vmax in the CMA was likely a dilution effect.

We lost one neonate in the control group from sepsis 1 week after delivery at 24/4 weeks of gestation. A second control group neonate survived sepsis with severe interventricular hemorrhage grade III/IV. Two extremely preterm neonates born at <28 weeks gestation from the port group, died unexpectedly, 1 week post-delivery (Table 1A). The low-weight adopted standard care perinatal AA nutrition (2.5 g/kg) was given at the intensive care pediatric station. Signs of infection were identified in one of these very preterm newborns 4 days after the delivery. One newborn in the control group, which was delivered at 32 WG developed severe cerebral palsy (Table 1B). Importantly, we tried to improve the AA/glucose supplementation of IUGR fetuses by hyperbaric oxygenation (1.4 Barr absolute for 50 min). We did not find any adverse effects of this combined therapy for placental insufficiency on the mother nor her baby.

Discussion

In this study, we have demonstrated that a perinatal port system for long-term nutrient administration directly into the umbilical vein could be successfully implanted into all IUGR fetuses with anterior placental location without any surgical complications. Flood et al. presented in IUGR PORTO study that the mean interval from diagnosis of abnormal cerebroplacental ratio <1 to delivery was 7 days (ranging 2–15 days) [6]. In our study, this interval was prolonged by continued nutrient administration via a prenatal port system at least three times compared to the control group. To our knowledge, this is the first prospective pilot study for the treatment of human IUGR fetuses with severe placental insufficiency and brain sparing, using continuous AA and glucose supplementation via the subcutaneously implanted intraumbilical port system. HBO combined with intraumbilical AA/glucose supplementation was also tested for the first time (n=1). The intrauterine application of AA and glucose into amniotic fluid for the treatment of IUGR human fetuses was first developed and introduced in the 1970s [17]. Application of intra-amniotic AA increased the concentration of AA in fetal plasma. Unfortunately, the researchers were faced with an increased rate of amnion infection syndrome which thwarted this method.

Under physiological conditions, the concentration of AA is significantly higher in fetal plasma, compared to maternal levels due to active transplacental transport of AA and additional AA synthesis in the placenta [1, 7, 14, 15]. We found unbalanced AA concentrations in the plasma of severe IUGR fetuses. Surprisingly, the level of glutamic acid was increased but glutamine was dramatically decreased. This phenomenon needs further investigation. Rather than normalizing the AA concentration an enhanced AA imbalance was observed in IUGR fetuses following supplementation.

Our protocol was hindered by the proportion of AA in commercially available solutions, which differ from physiological concentrations. We also encountered every low weigh gain in IUGR fetuses below 28 weeks' gestational age in spite of daily intraumbilical AA/glucose supplementation of 41.3 mL/kg/day on average (Table 1A). Chronic hypoxia of IUGR fetuses leads to the ductus venosus sparing with severe reduction of umbilical blood perfusion of the fetal liver [18]. It is generally accepted that the fetal liver produces proteins, lipids and carbohydrates involved in their metabolism [19–21]. In addition, the liver synthetizes growth factors, such as insulin growth factor (IGF) and its binding proteins (IGFBPs), as well as epithelial growth factor, and hepatocyte growth factor [20, 21]. The reduction of hepatic blood supply is also associated with low IGF-I and -II mRNA expression in the fetal liver and significantly reduced cell proliferation in fetal organs, such as skeletal muscles, heart, liver and kidneys [20]. We speculate that the IUGR occurring in extremely preterm fetuses, below 28 weeks of gestation is harder to overcome with our protocol due to the functional limitations of the fetal liver, combined with blood flow by-pass through the DV. The ductus venosus sparing would result in significantly reduced umbilical blood perfusion of the fetal liver and could be an additional key towards the understanding of some methodological

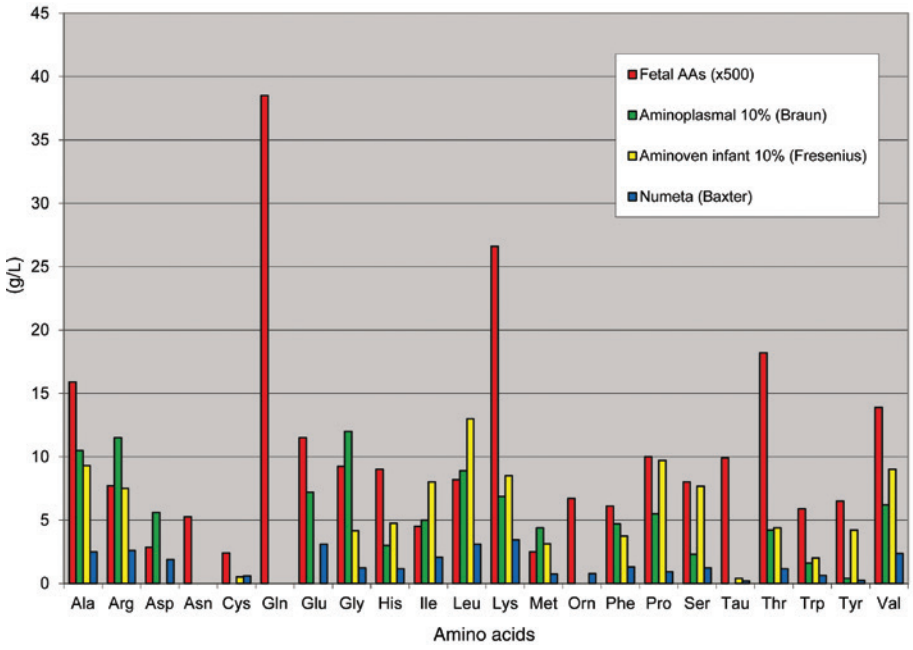

Figure 4: The commercial amino acid formula concentrations compared with calculated AA concentrations related to very preterm fetuses under physiologic conditions.
The commercial AA solutions Aminoven Infant 10% (Fresenius Kabi, Bad Homburg, Germany) and Numeta G 16% (Baxter GmbH, Unterschleisshein, Germany) were recommended to pediatricians in Germany by "Rote Liste 2013" [21]. Required fetal AA concentrations were estimated using physiological AA-means of very preterm fetuses multiplied by 500 [7, 14, 15].

restrictions of AA/glucose intraumbilical supplementations [18].

Additionally, we discovered commercially available AA solutions have enormous deviations from the AA proportions observed in the plasma of preterm fetuses (Figure 4) [7, 22, 23]. The commercial AA solutions used in our protocol, did not contain asparic acid, glutamic acid, glutamine, ornitine (Fresenius), and cysteine, ornitine, glutamine and taurine (B. Braun). Furthermore, these solutions had four-fold lower relative concentrations of lysine, and threonine, and 25-fold lower relative concentration of taurine, compared to the physiologic AA proportions in the plasma of extreme preterm fetuses (Figure 4).

The AA are not only nutrients, but are also regulators of gene expression, the protein phosphorylation cascade, cell signaling molecules and hormone synthesis [24]. It is highly probable that in extremely preterm infants, long-term parenteral supplementation with commercial AA solutions, which deviate from physiological fetal plasma AA concentrations, causes unbalanced plasma AAs in the preterm newborn and is partially responsible for worse short and long-term outcomes. Norwegian pediatricians observed a higher occurrence of septicemia in the very-low-birth-weight infants (63% vs. 29%) that received an enhanced feeding protocol (AA=3.5 g/kg/day) within 24 h after birth [25]. In connection with these critically important functions of AAs, we assume that long-term supplementation with poorly balanced AAs from commercial AA-infusions (in utero and/or postpartum) could have led to worse outcomes of extremely preterm infants in our pilot study. Lastly, Embleton et al. underlined an extremely limited evidence base of parenteral nutrition formulas as the standard-of-care for preterm infants despite its widespread use [26]. Clearly, commercial AA solutions need to have improved formulation prior to use in in fetuses and very preterm neonates.

The intraumbilical infusion appeared to reduce the PI in the UA. This could be explained by passive dilatation of placental pre-capillaries by increased plasma volume. The dilution effect of AA/glucose intraumbilical infusion reducing the concentration of norepinephrine and epinephrine may also take part in this pathway. The reaction of Ut.A., e.g. spiral arteries, to intraumbilical infusion could have other pathways, which need detailed examinations. The advantage of combining HBO with intraumbilical amino acid supplementation for IUGR treatment via a port system must be proved in future studies. In our single participant, we used a low hyperbaric oxygen pressure to avoid up-regulation of the activity of an antioxidant enzyme in the plasma, placenta and fetal brain, and to avoid possible oxygen toxicity to the placenta and the brain [27].

Substitution of insufficient placental function with supplementary AAs, glucose and oxygen, using a port system combined with HBO, could be a therapeutic option for placental insufficiency. This would prolong the pregnancy, avoid complications associated with very preterm birth, reduce under-nutrition, and change the methylation processes, which induce reprogramming of the fetal genome, leading to metabolic syndromes, increased risk of coronary heart disease, strokes and diabetes mellitus type II [9]. Newborns with IUGR are frequently referred to an intensive care department that dramatically increases the cost of neonatal treatment [4]. Therefore, prolongation of the pregnancy with fetal nutrition via a port system could also provide long-term economic benefit. As a matter of course, perinatal specialists must been adequately trained in cordocentesis and port implantation to avoid complications related to this method.

We have to address to some limitations of this study. We were unable to finish the study as a prospective randomized trial following ORAU recommendations to restrict participation to severe IUGR. Due to our limited sample size, the study design became a pilot feasibility study. HBO was included in our final case after the fetuses <28 weeks' gestation failed to respond to AA supplementation. It would be interesting to investigate if AA supplementation contributes to changes in growth factors, insulin and metabolic processes in fetal plasma. The large deviation of the AA concentrations in fetal plasma reduced the power of statistic calculation.

In conclusion, the subcutaneously implanted intraumbilical prenatal port system provides long-term access to the fetal circulation. This method was successfully used in our study for AA/glucose supplementation and intrauterine blood transfusion. Combination of HBO with AA/glucose supplementation could assist intraumbilical nutrient infusion via a port system in cases with severe placental insufficiency. Our data suggest that commercial AA formulas, which lack some AA and have large deviations from physiologic proportions, are not recommended for the prenatal supplementation of extreme preterm IUGR fetuses. Newly formulated AA solutions with physiologic plasma AA concentrations, HBO, vitamins, trace elements and growth factors are evidently required for successful substitution of insufficient placenta in IUGR treatment using the subcutaneous port system.

Acknowledgments: We thank Dr. Jessica Bell, University Halle-Wittenberg, Germany who assisted in the manuscript preparation. Center for Life Science, Nazarbayev University, Astana, (Grant/Award Number: "Grant number 110; Center for Life Sciences, Nazarb").

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The authors stated that there are no conflicts of interest regarding the publication of this article.