Optimizing Individual Nutrition in Preterm Very Low Birth Weight Infants:

Randomized Clinical Trial

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1. Introduction and Purpose:

Background: Insufficient growth before or after birth and insufficient nutrient intake in preterm infants in the neonatal intensive care unit (NICU) are associated with growth insufficiency and neurodevelopmental impairment at follow-up. Both extremely low gestational age (GA) neonates (ELGANs) and small for gestational age (SGA) (weight < 10^{th} percentile for GA) neonates may develop signs suggestive of metabolic syndrome during the first 3 years of life. Low birth weight (BW), prematurity and rapid or disproportionate weight gain (with increased weight for length) during the first months after birth are associated with signs of metabolic syndrome in adults.

In preterm infants fed human milk, milk needs to be fortified to meet nutrient recommendations. Fortification can be 1) standard, 2) individualized (adjusted based on daily human milk nutrient analysis and milk volume), or 3) optimized (adjusted based on growth rate and serum analyses).

The first specific aim will determine whether individualized and optimized nutrition during hospitalization results in improved growth in the neonatal intensive care unit (NICU) in extremely low gestational age (GA) neonates (ELGANs, <29 weeks) and in small for GA (SGA, birth weight <10th percentile for GA) preterm infants compared with optimized nutrition.

The second specific aim will determine whether individualized and optimized nutrition in the NICU improves neurodevelopmental outcomes (acquisition of development milestones) and reduces the risk of disproportionate growth (i.e., excess fat) in the NICU and findings suggestive of metabolic syndrome in the first 3 years of life.

Hypotheses:

1. Primary hypothesis: In preterm infants (GA <29 weeks or GA <35 weeks and SGA) individualized and optimized nutrition will increase velocity of growth (weight gain velocity by 2 g x kg^{-1} x day^{-1} and length velocity by 0.2 cm per week) from birth to 36 weeks of postmenstrual age (GA plus postnatal age) in comparison with optimized nutrition.

2. Secondary hypotheses: Individualized and optimized nutrition will improve neurodevelopmental outcome and reduce the risk of disproportionate growth (excess fat) in the NICU and findings suggestive of metabolic syndrome in the first 3 years of life.

Primary study endpoint (Aim 1):

- Growth velocity (rate of weight gain [g x kg^{-1} x day^{-1}] and length velocity [cm x $week^{-1}$]) from birth to 36 weeks of PMA (or discharge if patient is discharged earlier than 36 weeks PMA) will be calculated using Patel's geometric method.

Secondary endpoints (Aim 2):

In the NICU:

- Disproportionate growth (abdominal circumference, subscapular skinfold and BMI greater than their respective 90^{th} percentiles for age) at 36 weeks of PMA or discharge if patient is discharged earlier than 36 weeks PMA
- Body composition analysis using air displacement plethysmography (Pea Pod)
- Blood pressure (systolic and mean, calm or sleeping) at 36 weeks PMA and discharge
- Hypertension, defined as systolic blood pressure >99th percentile for PMA for at least 24 hours while sleeping or calm
- Blood sample for measurements of cholesterol, fats, cystatin C, adipokines and some blood markers that may indicate a higher risk for development of diabetes or metabolic syndrome at discharge or 36 weeks of PMA, whatever comes first;
- Bacterial diversity and ratio of Firmicutes to Bacteroidetes (F:B) in stool samples (1) at 28 days of age and (2) at 36 weeks or discharge if earlier than 36 weeks
- Body composition analysis using air displacement plethysmography (Pea Pod) at 36 weeks of PMA or discharge if patient is discharged earlier than 36 weeks PMA

At the Follow-up Clinic (Thrive Clinic, Dr. Roy Heyne), a state-of-the-art facility with >95% follow-up rate, at 6, 12, & 36 months chronological age & 18-26 months adjusted age (PNA corrected for prematurity):

- Neurodevelopment (Bayley III) scores (cognitive and language composite) at 18-26 months adjusted age
- Findings suggestive of metabolic syndrome: disproportionate growth (excessive fat mass) and high systolic blood pressure at each visit at 6 months, 1, 2 and 3 years of age
- Serum levels of cholesterol, fats, cystatin C, adipokines and some blood markers that may indicate a higher risk for development of diabetes or metabolic syndrome at 1 and 3 years of age
- Body composition analysis using air displacement plethysmography (Pea Pod) at 6 months of age
- Body composition analysis using Dexascan (Hologic Horizon A) at 1 and 3 years of age Relationship between stool bacterial diversity and F:B ratio, growth and obesity Stopping Rules:

    - Less than 10 patients recruited in 12 months
    - Mortality in enrolled patients significantly higher than in non-enrolled patients (P<0.01)
    - More frequent necrotizing enterocolitis in enrolled patients (P<0.01) **2. Background:**

Purpose: To provide preliminary data for a multicenter randomized trial to test whether individualized and optimized nutrition in ELGANS and SGA preterm neonates improves neurodevelopmental outcomes and reduces the risk of metabolic syndrome. This could be of major public health significance.

Standard of care: Feeding human milk rather than formula has several advantages for preterm infants including decreasing the frequency of necrotizing enterocolitis and infections. However, human milk does not provide enough calories or proteins, leading to less growth; therefore, human must be supplemented with human milk fortifier, and for some babies, additional protein or fat. The amount of protein and fat in mother's breast milk and donor milk is highly variable, often leading to insufficient growth velocity.[1] There are three ways to fortifying human milk for preterm infants: (1) Standard, fixed dosage fortification; (2) Adjustable (optimized) fortification using growth velocity to modify caloric supplementation and a surrogate for protein sufficiency to modify the dosage of protein fortification, and (3) Targeted (customized, individualized) fortification or fortification triggered by poor growth and results from human milk analysis. One small randomized trial showed that growth of preterm infants in the NICU improved using adjustable supplementation of human milk based on weekly BUN levels.[2] Other authors have used this approach and observed better growth without any toxicity.[3-5] At Parkland we have used this approach for more than a decade: adjustment of human milk fortification is based on growth velocity and weekly serum BUN and albumin levels; protein intake is increased weekly until BUN is greater than 10 mg/dl and albumin is > 3 g/dl.

Targeted individualized supplementation of human milk with fat has been done based on creamatocrit (measurement of fat layer after centrifugation); this method was shown inferior to near infrared spectrophotomotry,[6] which has become the gold standard for human, dairy or animal milk analysis.[1] Hair showed in a small RCT that targeted supplementation of human milk with fat (human milk cream) based on serial measurements of human milk nutrients using near infrared spectrophotometry, increased weight gain and linear growth in preterm infants with birth weight 750-1250 grams.[7] Several centers have used targeted individualized nutrient supplementation successfully. Individualized supplementation was associated with improved growth and less gastroesophageal reflux compared to routine supplementation based on assumed nutrient contents.[8-16] Analysis of donor milk nutrient is standard of care in donor milk banks in the US.[17]

Rationale for the RCT: There are three ways to fortifying human milk for preterm infants: (1) Standard, fixed dosage fortification; (2) Adjustable (optimized) fortification using growth velocity to modify caloric supplementation and a surrogate for protein sufficiency to modify the dosage of protein fortification, and (3) Targeted (customized, individualized) fortification or fortification triggered by poor growth and results from human milk analysis.[1] Methods 2 and 3 have been shown by multiple observational studies and each by one randomized controlled trial (RCT) to be superior to the first method, but methods 2 and 3 have never been compared in an RCT.

We plan a RCT to compare standard of care at Parkland (optimized supplementation) with individualized supplementation in high-risk infants. This RCT will determine whether growth of preterm at high risk for growth insufficiency can be improved by targeted daily supplementation based on (1) serial milk analyses in addition to standard of care and (2) the amount of enteral feeding given daily. Patients randomized to the control arm will receive standard of care at Parkland, which involves adjustment of human milk fortification based on growth velocity and weekly serum BUN and albumin levels; protein intake will be increased weekly until BUN (adjusted for creatinine) is greater than 10 mg/dl and albumin is > 3 g/dl. Patients in the treatment arm be part of a quality improvement including targeted daily supplementation of human milk based on serial milk analyses using near infrared spectrophotometry to meet expected requirements based on postmenstrual age and physiologic status; milk intake and fortification will be further adjusted based on growth velocity and weekly serum levels of BUN and albumin levels, which will be used as gold standard.

Status of the device: Measurement of nutrients in human milk has been used by several investigators and has been shown to improve growth compared with standard fortification. Standard for human milk bank is to analyze pooled human milk samples and to provide this information on each milk bottle to the neonatal intensive care unit (NICU). However, devices for analyzing human milk samples have not been approved by the FDA for analyzing individual mother's milk for making individual decisions in the neonatal intensive care unit (NICU). Three devices are currently available in the US for human milk analysis: The Calais Human Milk Analyzer, the SprectraStarTM 2500 XL Neonatal Analyzer (which has now replaced the previous 2400 version) and the Foss FT120. We plan to use the SprectraStarTM 2500 XL. This device has been validated in one study.[18] It is the only device available in the US that was used in a randomized clinical trial, which showed improved growth (weight gain and linear growth) in the group in which nutrition was adjusted based on readings of human milk composition using this device.[7] We believe this device is a non-significant risk device because

1. Measurement of nutrients in human milk has been used in several centers to adjust fortification of human milk for preterm infants, and has been shown in one randomized trial to improve growth without any risk to the infants.
2. Infants randomized to the treatment arm will have growth velocity and BUN and serum electrolytes measured regularly, as is done for standard of care, to make sure that adjustment of human milk fortification is not exceeding needs for each infant.
3. The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals and the University of Texas Health Science Center at San Antonio have approved a randomized trial that used this device and was conducted at Texas Childrens and San Antonio without an IDE (Hair et al, attached).

Status of relevant research:

Several studies have shown major variability in human milk nutrients. [7-18] The best method for measuring nutrients in human milk is near infrared spectrophotometry.[1,6] Supplementing human milk based on its measured composition has been standard of care in several NICUs for years. Measuring nutrients in human milk to provide individualize milk fortification was shown to improve growth (weight gain and linear growth) without side effects in a randomized trial at Texas Children's.[7] However, no RCT has been conducted to compare individualized targeted supplementation with adjustable optimized supplementation.

The bowel utilizes a high amount of enterally derived threonine and glutamine, two essential amino acids, for its own metabolism and production of mucin;[19,20] suggesting that growth might improve by discounting enteral contribution to protein intake while providing less than 75 ml x kg^{-1} x day^{-1} of enteral feeding; such an approach has been used routinely in the Netherlands but has not been tested in a RCT; no evidence has been published at this point to show whether this approach improves any of the clinical outcomes.[21]

Prenatal and postnatal growth as precursors of metabolic syndrome:

Metabolic syndrome is defined as the presence of at least three of the following conditions: abdominal obesity, a high triglyceride level, a low high-density lipoprotein, high blood pressure and high fasting blood sugar. Cardiovascular risk in adults (including signs of metabolic syndrome) is associated with low BW, small size for GA and prematurity[22-25] and increases with weight gain by 3 years of age.[26,27] Some authors found no independent association between weight or length at term age and blood pressure in adulthood.[28-31] In contrast, rapid postnatal weight gain during the first 1.5-3 postnatal months is associated with high systolic blood pressure, obesity, or insulin resistance in adults.[32-34] Weight gain in excess of linear growth before term corrected age and during the next 3 months is associated with abnormal blood levels of cholesterol, body fat percentage, waist circumference and acute insulin response in young adults.[35] Breastfeeding is associated with better brachial artery endothelial function in adult men. [36] Exclusive breastfeeding for the first 2 months is associated with less frequent non-insulin-dependent diabetes in adult Pima Indians.[37]

In preterm infants, body composition at term age is different than in infants born at term, with a greater proportion of fat mass to lean body weight.[38] In a population study of ELGANs, the amounts of macronutrients (protein, fat and calories) provided in the NICU were independent predictors of growth even after adjusting for severity of illness.[39] Duncan et al have conducted a cross-sectional study including 3 sets of 40 VLBW infants born at Parkland Hospital in 2003-2006 and analyzed at either 1, 2 or 3 years of age.[40,41] They observed low height and weight z scores for age but increased weight for height, increased ratio of subcutaneous skinfold thickness to abdominal circumference, elevated systolic blood pressure and dysfunctional renal regulation and hyperfiltration, and elevated leptin level, suggesting leptin resistance. Further studies are needed to determine when after birth these occur and whether they predict adult metabolic syndrome.

Microbiome is associated with health and disease in animals and humans. High ratio of Firmicutes to Bacteroidetes (F:B) in stools is associated with obesity and metabolic syndrome in adults.[42,43] Developmental pattern of the microbiome in infancy is associated with growth rate.[44,45] Infants who acquire a profile high in Bifidobacterium and Collinsella after 6 months of age have lower adiposity at 18 months of age.[45] Addition of lactobacillus acidophilus to formula increases weight gain in neonates.[46] Several factors affect neonatal microbiome. Mother's own breast milk promotes bacterial diversity in preterm infants compared with formula; pasteurized donor human milk moderately increased bacterial diversity.[47] Antibiotic exposure in neonates reduces stool bacterial diversity and increases risk for obesity.[48] However, no data are available about any effect of individualization of macronutrient intake affects neonatal microbiome.

Importance of adequate nutrition for growth and neurodevelopment:

In SGA and preterm infants, insufficient weight gain and linear growth and insufficient macronutrient intake in the NICU are associated with growth insufficiency and neurodevelopmental impairment, even after adjustment for other morbidities.[49-53] However, systematic administration of higher protein intake in the NICU than is recommended may not improve, and very high protein intake (6.0–7.2 gm x kg^{-1} x day^{-1} of protein), well beyond the upper maximum of current practice (5.5), may harm neurodevelopment.[54-55]

Gap of knowledge:

Limited information is available regarding the impact of higher protein intake on long-term neurodevelopment than is currently recommended.[21,56] Further studies are needed to evaluate (1) whether or individualizing macronutrient intake to nutrient concentration in human milk is better than adjusting

fortification to serum levels of BUN, and (2) whether protein in enteral feeding should be counted in the total protein intake when only small amount of enteral feed is provided, to improve growth and neurodevelopmental outcome while minimizing the risk for metabolic syndrome in very preterm and SGA infants. No data are available about any effect of individualization of macronutrient intake affects neonatal microbiome.

Preliminary Retrospective Data

Infants <29 weeks GA or < 1000 grams BW, 2008-2012: Parkland Hospital vs. NRN (**Table 1**):
At birth, infants at Parkland Hospital had similar weight, greater length and fronto-occipital circumference (FOC), and lower ponderal index (PI) [weight in grams x (length in cm)$^{-3}$ x 100] vs. those in other NRN centers. At 36 weeks PMA, they had greater weight, length and PI, suggesting more weight gain but disproportionate fat deposition.

Table 1

	Parkland Hospital		Other NRN Centers	
Gestational age (weeks)	25.3±2.0 (485)**		25.7±1.9 (8704)	
Age of measurement	At Birth	At 36 weeks PMA	At Birth	At 36 weeks PMA
Weight (grams)	843±283 (485)	2323±394 (303)**	834±256 (8699)	2122±409 (5537)
Length (cm)	34.2±2.9 (391)**	43.5±2.3 (261)**	33.4±3.5 (8033)	42.6±3.1 (5135)
FOC (cm)	24.1±1.9 (429)**	30.9±1.5 (283)	23.7±2.2 (7861)	30.9±2.0 (5261)
Ponderal Index	2.08±0.7 (434)*	2.83±0.48 (279)**	2.23±0.69 (8352)	2.74±0.53 (5328)

Table 1. Numbers are mean ± SD (n). * P< 0.01; *** P < 0.001 vs. other NRN Centers, Student t-test. Abbreviations: NRN, Neonatal Research Network; PMA, postmenstrual age; FOC, fronto-occipital circumference

Retrospective data from Parkland Hospital NICU 2004-2013 (vs. Olsen & Holston curves[57,58]):

- Term infants: A PI > 90th percentile was observed in 19% of the neonates, twice the expected proportion. PI was greater in Hispanics and non-Hispanic Whites vs. other groups (**Table 2**).

Table 2

Race/ethnicity	Hispanics	Non-Hispanic Whites	African Americans	Asians
Ponderal index	2.68±0.39* (2326)	2.67±0.39* (1710)	2.61±0.40 (791)	2.56±0.33 (128)

Numbers are mean ±SD (n); *P<0.05 vs. African-Americans and Asians, ANOVA followed by Tukey test

- Preterm infants < 35 weeks GA born 2004-2013 and discharged home (**Table 3**):

Weight gain velocity[59] but not *linear growth* was lower in 29-34 weeks GA with appropriate BW for GA (AGA) infants than in SGA or 23-28 weeks GA infants. *Growth insufficiency* or *small FOC at discharge* was more frequent in SGA vs. AGA infants and in 23-28 weeks GA vs. 29-34 weeks GA infants. *Disproportionate growth* was more frequent in 23-28 weeks GA vs. 29-34 weeks GA infants.

Table 3

GA (weeks) and Size for Age	<29 weeks AGA	<29 weeks SGA	29-34 weeks AGA	29-34 weeks SGA	P values
Growth insufficiency at D/C	28% (305)	94% (17)	8% (2784)	69% (304)	***, ***
Small FOC at D/C	5% (325)	53% (17)	2% (2880)	17% (319)	***, ***
Disproportionate growth at D/C	36% (354)	41% (22)	5% (2886)	5% (320)	0.96. ***

Weight gain velocity ($gxkg^{-1}xd^{-1}$)	13.3±1.9 (470)	13.5±2.4 (48)	7.4±6.0 (2989)	13.1±3.3 (355)	***,***,***
Linear growth velocity (cm/week)	1.0±0.2 (459)	1.0±0.2 (47)	0.8±1.0 (2949)	0.9±0.6(354)	0.46,0.06,0.24
Change in weight z score#	-0.42±0.67 (326)	+0.52±2.31 (17)	-0.43±0.43 (2893)	+0.14±0.38 (319)	***,**, **
Change in length z score #	-1.03±0.79 (325)	-0.84±0.96 (17)	-0.32±0.76 (2862)	-0.23±0.79 (319)	0.15,***, 0.60
PI at birth	2.21±0.29 (470)	2.14±0.21 (48)	2.36±0.31 (2988)	2.17±0.30 (356)	***,**,*
PI at D/C	2.90±0.35 (459)	3.15±0.80 (47)	2.45±0.29 (2952)	2.52±0.33 (354)	***,***,***
Change in PI (birth to D/C)	0.69±0.41 (459)	1.01±0.76 (47)	0.09±0.40 (2949)	0.35±0.42 (354)	***,***, 0.34

Abbreviations: GA, gestational age; AGA, appropriate for gestational age; SGA, small for gestational age; #, from birth to discharge; D/C, discharge; d, days: FOC, fronto-occipital circumference. Continuous variables are presented as mean±SD (number); comparisons include 2-way ANOVA; P values are shown as: ***, < 0.001, ** < 0.01, * < 0.05 for SGA vs. AGA; <29 weeks vs. 29-34 weeks; and interaction GA vs. size for age, respectively. Categorical variables are presented as % (total N); comparisons were done using logistic regression; p values are shown as ***, < 0.001 for comparisons SGA vs. AGA and <29 weeks vs. 29-34 weeks.

In multivariate analyses (n=3809) taking into account GA and BW z score, the presence of a morbidity* affecting growth (n=464) was significantly (P<0.001) associated with greater increase in PI from birth to discharge and greater odds of growth insufficiency at discharge.

* chronic lung disease, intestinal obstruction, gastrointestinal surgery, necrotizing enterocolitis, heart surgery, shunt for hydrocephalus

 VLBW infants : BW ≤ 1500 grams born in 2004-2013 and discharged home (**Table 4**):

Table 4 Many patients had growth insufficiency, small FOC and disproportionate growth at discharge.

GA	Growth insufficiency	Small FOC	Disproportionate growth	Weight gain	Linear growth	PI at birth	PI at D/C	Change in PI
28.8±2.7	26% (999)	7% (1062)	18% (1113)	13.5±3.2	1.0±0.4	2.19±0.30	2.75±0.39	0.56±0.44

3. Concise Summary of the Project:

Study design: Randomized trial: Following parental consent, infants will be double blind randomized with 1:1 allocation using a stratified permuted block design by computer, using opaque envelopes. Stratification will be by GA: 23-$28^{6/7}$ and 29-$34^{6/7}$ weeks.

Hypotheses: This study is designed to test the hypothesis that, in preterm infants with gestational age < 29 weeks or with gestational age < 35 weeks and small for gestational age, weight gain velocity from birth to 36 weeks of postmenstrual age will increase by 2 g x kg^{-1} x day^{-1} and length gain will increase by 0.2 cm per week with individualized nutrient supplementation. Secondary hypotheses are that individualized nutrition will improve growth and neurodevelopmental outcome and reduce the risk of disproportionate growth and metabolic syndrome.

Primary study endpoint (Aim 1):

- Growth velocity (rate of weight gain [g x kg^{-1} x day^{-1}] and length velocity [cm x $week^{-1}$]) from birth to 36 weeks of PMA or discharge (if discharge is earlier than 36 weeks) will be calculated using Patel's geometric method.[59]

Secondary endpoints (Aim 2):

In the NICU:

- Disproportionate growth (abdominal circumference, subscapular skinfold and BMI[60] greater than their respective 90th percentiles for age) at discharge or 36 weeks of PMA (whatever comes first)
- Air displacement plethysmography at discharge: this will be done using the Pea Pod, a noninvasive instrument that is FDA approved for measuring fat in neonates and infants. This apparatus measures body volume; taking into account body weight and accepted assumptions, the analysis generates the percentage of fat and lean body mass. This is the gold standard method for body composition in neonates and young infants, for which normative data are available.[61,62]
- Blood pressure (systolic and mean, calm or sleeping) at 36 weeks PMA and discharge
- Hypertension, defined as systolic blood pressure >99th percentile for PMA[63] for at least 24 hours while sleeping or calm
- Serum levels of cholesterol, fats, cystatin C, adipokines and some blood markers that may indicate a higher risk for development of diabetes or metabolic syndrome at discharge or 36 weeks of PMA, whatever comes first; these blood samples will be obtained at the same time as other blood samples for clinical indication

At the Follow-up Clinic (Thrive Clinic, Dr. Roy Heyne), a state-of-the-art facility with >95% follow-up rate, at 6 months, 12 & 36 months chronological age & 18-26 months adjusted age (PNA corrected for prematurity):

- Body composition using Pea Pod at 6 months of age
- Neurodevelopment (Bayley III) scores (cognitive and language composite) at 18-26 months adjusted age
- Findings suggestive of metabolic syndrome: disproportionate growth (excessive fat mass) and high systolic blood pressure at each visit;
- Serum levels of cholesterol, fats, cystatin C, adipokines and some blood markers that may indicate a higher risk for development of diabetes or metabolic syndrome at 1 and 3 years of age (half a teaspoon of blood); these blood samples will be obtained at the same time as other blood samples for clinical indication

Measurement of nutrients in human milk: At present there are no Food and Drug Administration (FDA) approved devices for the analysis of nutrient content in human milk samples obtained in the NICU. The SprectraStar™ Neonatal Analyzer has been validated[18] and has been used in a RCT.[7] Twice a year, the device will be calibrated against control human milk samples purchased from the Ohio Milk Bank and analyzed using state-of-the art chemical analysis, and variability will be assessed. The UT Southwestern Institutional Review Board (IRB) has classified this device as non-significant risk device.

Other measurements:

- Maternal pre-pregnancy weight, height, body mass index (BMI), weight gain during pregnancy, placenta weight, antenatal and perinatal maternal diseases (e.g., diabetes mellitus, preeclampsia), antenatal steroids, intrauterine growth restriction
- GA: best obstetrical estimate (if no prenatal care, new Ballard score)[64]
- Race/ethnicity, insurance, socioeconomic status, maternal education
- Birth & 36 weeks measurements: weight, FOC, length (obtained on stadiometer within 3 days of birth; up to 7 days of life in patients who are initially critical and unstable), BMI (using BW and length on stadiometer to improve accuracy), abdominal circumference, mid-arm circumference

- Comparison of relative changes [(36-week measurement – birth measurement)/birth measurement] in weight, length and FOC from birth to 36 weeks PMA; change in z score in weight, length and FOC
- Serial measurements of weight, length (using stadiometer for calculating BMI), linear growth estimated from the percent change of heel-knee height measurements[65] in the critically ill unstable neonate, FOC, arm and thigh muscle circumference, abdominal circumference
- At discharge: triceps and subscapular skinfold thickness
- Nutritional tests: weekly BUN adjusted if necessary for renal dysfunction (see study intervention), albumin, electrolytes, phosphate, alkaline phosphatase; if the latter two tests suggest osteopenia: bone radiogram; vitamin D levels will be considered in severe cases.
- Days without feeding, human milk vs. formula, days to full feeds, days of central line & parenteral nutrition, refeeding syndrome, metabolic acidosis, osteopenia of prematurity, micronutrient deficiency (e.g. zinc, essential fatty acids, carnitine), mineral deficiency (e.g., magnesium), type and composition of feeding at discharge
- Morbidities affecting growth or neurodevelopment, e.g., congenital infection, genetic anomaly, organ failure, bronchopulmonary dysplasia, antibiotics[66]/sepsis, major surgery, intestinal perforation, necrotizing enterocolitis, severe intraventricular hemorrhage, periventricular leukomalacia, congenital anomalies, systemic steroids
- Cumulative percentage of enteral intake as breast milk in the NICU, which may influence the effect of the intervention and glucose, lipid and apolipoprotein metabolism.[19]
- Stools will be collected and frozen for later analysis of microbiome diversity, Firmicutes:Bacteroidetes ratio and metagenomics. Samples will be collected every other week from randomization to discharge.
- Follow-up at 12 and 36 months chronological age and at 18-26 months adjusted age:
- Weight, length, FOC, abdominal circumference, skinfold thickness, two calm blood pressure measurements using Doppler amplification with the exact same protocol as previously described,[67,68] history of steroids and antibiotics at each visit[66]
- Neurodevelopment (Bayley III) cognitive and language composite scores at 18-26 months
- Serum levels of cystatin C, creatinine, cholesterol (total, LDL, HDL), triglycerides, Leptin, Adiponectin, Resistin, IL-6 and TNF alpha at 1 and at 3 years of age (half a teaspoon of blood)
- Whole-body Dexascan to analyze body composition including percentage of fat at 1 and 3 years of age

Procedures: Patients will be randomized to either (a) Optimized and individualized nutrition by adjusting milk fortification based on daily measurements of macronutrients in mother's own milk[33,47] or information on milk from the milk bank or (b) optimized (standard of care at Parkland), i.e., assuming macronutrient concentration in human milk based on available literature and summing enteral and intravenous protein intake. Patients will be followed for growth parameters in the neonatal intensive care unit and for growth, neurodevelopment and signs suggestive of metabolic syndrome in the low birth weight clinic until 3 years of age.

Maximum number of consented patients; 200; this will allow attrition for comfort care, deaths before 36 weeks PMA and withdrawal from the study; we expect to recruit approximately 150 patients into the study with a goal of 100 patients reaching endpoint at 36 weeks PMA

Duration of the study: 6 years: recruitment for 2 years, follow-up for 3 years, analysis for 1 year

Reason for removing from the study: comfort care, withdrawal by the parents

4. Study Procedures:

Screening:

Screening for potential candidates for the RCT will be done by the investigators who will run the quality improvement project that started in May 2015: Optimizing Individual Nutrition in Preterm Very Low Birth Weight Infants (STU 102014-045).

Nutrient intake is decided by the clinical care team with recommendations by dietitians, using published data on breast milk composition, published recommendations, weekly rate of growth, feeding tolerance and measurements of serum BUN (> 10 mg/dl), albumin (> 3 g/dl), electrolytes, phosphate and alkaline phosphatase and bone radiograms as needed. Weight and length at birth were previously obtained by nurses and classified using 1963 Lubchenco's growth curve.[69] Size after birth was assessed using the 2003 Fenton's growth curve,[70], length curve including crown-heel measurements (obtained by nurses using paper tape or dietitians using a stadiometer) and height calculated from heel-knee height, mid-arm circumference and abdominal circumference.

New curves have become available on EPIC: weight, length and FOC[57] in 2015 and BMI[60] in 2016. The research team have implemented 2010 Olsen's curve[57] for birth and subsequent measurements up to 39-40 weeks, 2013 Fenton's curves[71] up to 50 weeks PMA, and WHO curves[72] starting at 40-41 weeks), 2015 Holston's (BMI)),[60] and serial anthropometric measurements to compare growth parameters with expected values at each stage. Weight gain velocity will be calculated as described by Patel.[59] Linear growth in critical unstable infants will be followed using heel-knee height.[65] BMI will be calculated using only lengths measured on a stadiometer or an infantometer. Nutrition will be optimized using serum BUN goals with adjustment for serum creatinine levels compared to normal values for age.[4] This approach will be standard care, which will be the intervention in any patient entered in the RCT.

Randomization:

Following parental consent, infants will be double blind randomized with 1:1 allocation using a stratified permuted block design by computer, using opaque envelopes in 3 strata: AGA 23-28$^{6/7}$ weeks, SGA 23-28$^{6/7}$ weeks and SGA 29-34$^{6/7}$ weeks. Twins and multiples will be randomized to the same arm of the study.

Blinding: Only the dietitian, infant formula technician and a statistician will be aware of the allocation. The other members of the research team, the care givers and the parents will not know the allocation.

Study intervention:

Patients will be randomized to either

(1) Optimized and individualized nutrition by adjusting milk fortification based on daily measurements of macronutrients in mother's own milk or information on milk from the milk bank
(2) Or optimized (standard of care at Parkland), i.e., assuming macronutrient concentration in human milk based on available literature and summing enteral and intravenous protein intake

Data collection in the NICU will be identical to that for other patients who are part of the QI protocol submitted to the IRB: STU 102014-045: **Optimizing Individual Nutrition in Preterm Very Low Birth Weight Infants.**

Procedures done for research during follow up until 3 years of age include:

- skinfold thickness measurements
- Neurodevelopment exam (Bayley III cognitive and language composite scores) for patients who are not enrolled in the NICHD follow-up study (<27 weeks GA)
- (only if parent checks the box on the consent form): half a teaspoon of blood sample; this blood will be kept in the freezer at -80 degrees for 10 years for measurement of cholesterol, fats, and

some blood markers that may indicate a higher risk for development of diabetes or metabolic syndrome.

- (only if parent checks the box on the consent form): whole-body Dexascan to analyze body composition including percentage of fat at 1 and 3 years of age. The radiation dose is approximately 1% of the Annual US Avg. Natural Background.

Due to COVID-19 restrictions, the 18-26 months Adjusted Age Follow-up extended up to one year from the end of the study window.

5. Criteria for Inclusion of Subjects:

Patients born at Parkland Hospital & Health System
Gestational age < 29 weeks or gestational age < 35 weeks and small for gestational age
Maternal plan to breastfeed or to use milk from the milk donor bank From
birth to 1 week of life

6. Criteria for Exclusion of Subjects:

Comfort care only
Patients with major congenital abnormalities
Patients who are too unstable for the first 7 days to have an accurate length measurement

7. Sources of Research Material:

- NICU database
- Duncan's database
- Medical records from Parkland NICU on EPIC
- Medical records from the low birth weight clinic (paper records at the clinic or EPIC)

8. Recruitment Methods and Consenting Process:

Potential subjects may be patients of the investigators.

The researchers will check every day whether a baby is born either very preterm (born at less than 29 weeks of gestational age, that is about three months before expected due dates) or small for gestational age (weight less than the majority [90%] of babies born at same gestational age as your baby) and less than 35 weeks of gestational age. They will ask the mother whether she plans to breastfeed her baby or to have her baby receive milk from the donor's milk bank.

Non-English speaking subjects will be consented using informed consent; a Spanish consent will be available.

All research personnel involved in recruitment has been trained in research ethics and is used to openly communicate with families in the NICU.

Privacy will be assured by talking with the parents in a private room or using soft voice.

All research personnel involved in recruitment has been trained in research ethics and is used to openly communicate with families in the NICU.

If parents are not available, the patient will not be eligible for consenting.

Parents will be paid $40.00 at each research visit at the low birth weight clinic to reimburse them for parking or travel cost. If they stop taking part in this study or are withdrawn by the research team, they will receive payment for only the visits they have completed. For example, if they complete 3 study visits they will be paid $120.00.

9. Potential Risks:

Possible side effects include feeding intolerance, high BUN and metabolic acidosis.

Excessive fortification of milk can cause feeding intolerance (full stomach, more residuals [milk aspirated by the nurse] in the stomach before next feeding). Nurses check the baby before each feed and inform the doctor about any sign of feeding intolerance; the doctor may decide to adjust the amount of volume of milk and the amount of fortification if necessary.

Excessive protein intake may raise blood urea nitrogen or BUN, and decrease serum bicarbonate concentration. Blood urea nitrogen (BUN), creatinine and serum electrolytes are measured weekly. If a high BUN: creatinine ratio or a low serum bicarbonate concentration is observed the doctor may decide to decrease the amount of protein supplementation.

Being randomized to the standard arm may, in comparison with the other arm, increase the risk of insufficient nutrition, decreased growth and neurodevelopment. If the baby does not grow well enough, the doctor may decide to give more calories; this may cause the baby to gain fat.
Being randomized to individualized nutrition may, in comparison with the other arm, cause excessive weight gain.

If the parent consents to the Dexascan, the additional radiation dose is approximately 1% of the Annual US Avg. Natural Background.

SprectraStarTM 2500 XL Neonatal Analyzer

Upon submission of the original protocol, this device was considered by the IRB as a non-significant risk device. We believe this device is a non-significant risk device because
1. Measurement of nutrients in human milk has been used in several centers to adjust fortification of human milk for preterm infants, and has been shown in one randomized trial to improve growth without any risk to the infants.
2. Infants randomized to the treatment arm will have growth velocity and BUN and serum electrolytes measured regularly, as is done for standard of care, to make sure that adjustment of human milk fortification is not exceeding needs for each infant.
3. The Institutional Review Board of Baylor College of Medicine and Affiliated Hospitals and the University of Texas Health Science Center at San Antonio have approved a randomized trial that used this device and was conducted at Texas Childrens and San Antonio without an IDE (Hair et al, attached).

Near infrared (NIR) spectrophotometry has been used to analyze material of solid and liquid states uses the wavelength spectrum 1200 to 2400 nm. Commercially available NIR spectrophotometers have been used to

measure carbohydrate, fat and protein content in animal milk (cow, sheep, donkey and goat) and human milk. These analyzers are used routinely to analyze nutrients in donor's milk provided to NICUs for preterm infants.[7,17]

The SprectraStarTM 2500 XL Neonatal Analyzer is not approved by the FDA. The SpectraStar 2400 Near Infrared Analyzer (Unity Scientific, Columbia, MD, USA) has been validated against reference chemical analysis of protein, carbohydrate and fat (University of Guelph Laboratory Services, Guelph, Canada) in 42 samples (32 morning, 5 evening, 3 hind milk and 2 foremilk samples) of human milk by Sauer and Kim.[18] Using these samples, the authors generated a calibration curve, which was tested against a second set of 10 samples (6 morning, 0 evening, 1 hind milk and 3 foremilk samples) and their reference values. After logistic regression analysis, the validation set had a correlation (r^2) of 0.91 for carbohydrates, 0.95 for fat and 0.95 for protein.

Hair et al have used the SpectraStar 2400 to measure fat content on human milk in a randomized trial involving 78 preterm infants with birth weight 750-1250 grams.[7] In this trial, they found that the addition of milk cream based on results of milk nutrients to calculate individualized supplementation improved weight and length velocity. There was no difference in necrotizing enterocolitis, sepsis or death.

Several observational studies have assessed individualized fortification of milk using similar devices using near infrared spectroscopy to measure nutrients in human milk for preterm infants in the NICU. These studies have shown improved growth without side effects.[8-16]

Loss of Confidentiality

Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep your information confidential; however, this cannot be guaranteed.

Risks of Blood Drawing

If the parent agrees, the child will have half a teaspoon of blood collected at 3 years of age for the study. Many children at this age have blood drawn for routine care; we will try to obtain the research blood sample at the same time as blood is drawn for clinical care. Risks associated with drawing blood from your child's arm include minimal discomfort and/or bruising. Infection, excess bleeding, clotting, and/or fainting also are possible, although unlikely.

Other Risks

There may possibly be other side effects that are unknown at this time.

10. Subject Safety and Data Monitoring:

How will risks be minimized or prevented?

Potential risks and discomforts will be minimized to the greatest extent possible by using procedures such as appropriate training of personnel, monitoring, withdrawal of the subject upon evidence of difficulty or adverse event; referral for treatment, counseling or other necessary follow-up.

Nurses check the baby before each feed and inform the doctor about any sign of feeding intolerance; the doctor may decide to adjust the amount of volume of milk and the amount of fortification if necessary.

Blood urea nitrogen (BUN) and serum electrolytes are measured weekly. If a high BUN (or BUN: creatinine ratio) or a low serum bicarbonate concentration is observed the doctor may decide to decrease the amount of protein supplementation.

Many children at 3 years of age have blood drawn for routine care; we will try to obtain the research blood sample at the same time as blood is drawn for clinical care.

Type of Research Data or Events to be Monitored:

Study accruals
Protocol deviations: errors in allocation to study arm
Protocol violations: error in amount of fortifier or protein supplementation
Unanticipated problems: NEC, death, sepsis
Adverse events: feeding intolerance, high BUN (or high BUN: creatinine ratio), low serum bicarbonate concentration

Methods and Frequency of Analysis:

Method: review of
report from the database entry
yearly results of the control samples analyses
adverse events and unanticipated adverse events changes in risk/benefits

Frequency: meetings every 6 months after report from the database

Data collection form is attached (spreadsheet)

Person(s) Responsible for Data Monitoring:

Internal DSMC:
The randomized trial has an internal data safety monitoring committee, which meets every 6 mon and reviews the accumulated data. The closed portion of the committee, including Julide Sisman, MD and Larry Brown, MS, has access to all data including blinded data, and reviews any potential chart that may raise concern, and makes recommendations to the full committee to either stop or continue the study. The rest of the committee, including CR Rosenfeld, MD, R Heyne, MD and Maria Caraig, RN, research coordinator, and LP Brion, MD, do not have access to blinded data.

Name of the person responsible for submitting reports of unanticipated problems, adverse events, protocol deviations and protocol violations to the IRB and sponsor: PI

The PI will send the data safety reports to the IRB.

Reporting Unanticipated Problems, Adverse Events, Protocol Deviations and Protocol Violations:

- PI will report adverse events to the IRB immediately
- PI will report safety reports and DSMC reports to the IRB.

Stopping Rules:

1. Specific stopping rules:
    - Less than 10 patients recruited in 12 months
    - Mortality in enrolled patients significantly higher than in non-enrolled patients ($P<0.01$)
    - Necrotizing enterocolitis in enrolled patients more frequent than in non-enrolled patients ($p<0.01$)

1. Action indicated by the specific stopping rule:
   PI will notify the IRB, all research team members and the funding agency.

Procedures and Time Frames for Communicating Outcomes:

An open report of the accrual and demographics will be provided to the DSMC by the research team. A closed report of mortality and necrotizing enterocolitis will be provided to the DSMC by a statistician.

The report of the DSMC will be provided to the PI and to the IRB.

Precautions for Maintaining Data Integrity:

Random selection of data will be checked by the PI and the DSMC for completeness and integrity.

11. Procedures to Maintain Confidentiality:

Identified data are entered by a dedicated Research Nurse (STU 102010-064) or a dietitian into a secure computer database. The computer used for this data entry and the database itself are both password-protected and can only be accessed by the research nurse and the physicians listed on the protocol. The database will be kept on a password-protected subdirectory of a designated UTSW network drive. All the data collection sheets are stored in a secure cabinet. Data for study analysis will be de-identified with a link kept on a secure database.

Only members of the research team will have access to the data. Confidentiality will be assured by using encrypted electronic devices for transmission of identified information.

Identifiers will be deleted once data on the same patients have been merged across databases. Data for study analysis will be de-identified with a link kept on a secure database.

Privacy will be maintained by speaking with the parents in quiet tones with only authorized persons present in a private room or behind a screen. Identification of the study subject is done in EPIC.

12. Potential Benefits:

Benefits to the patient:

There may or may not be direct benefits to the patient. The researchers cannot guarantee that he or she will benefit from participation in this research. We do not know which group the baby will be in; it is possible that he or she may grow faster if he or she is randomized to individualized nutrition and if measurements of milk nutrients inform the nutritionists and infant formula personnel suggest more fortifier may be helpful. It is

possible that he or she may have less feeding problems if he or she is randomized to standard nutrition. It is possible that the researchers may identify a problem with your baby's growth or blood pressure; it that is the case they will let the mother know. It is possible that being in either arm of the study may reduce the risk for neurodevelopmental delay or metabolic syndrome as an adult.

Benefits to the society:
We hope the information learned from this study will benefit others with prematurity or small size for gestational age in the future. Information gained from this research could lead to better growth and neurodevelopment and less metabolic syndrome.

14. Biostatistics:

The primary outcome variable (growth) will be analyzed by intention to treat (i.e., as randomized). Continuous variables including the primary outcome will be analyzed using Student's t-test or analysis of variance and a generalized linear model (to determine the effect of the intervention while taking into account co-variates and confounding variables). Dichotomous variables will be analyzed using chi-square analysis and by estimating relative risk using robust Poisson regression in a generalized estimating equation model. Subgroup analyses will be done to compare the 3 strata (AGA 23-28 weeks, SGA 23-28 weeks and SGA 29-34 weeks) and to compare SGAs due to insufficient calories (e.g., preeclampsia, low placental insertion) vs. constitutionally small neonates (e.g., congenital infection, genetic anomaly, short parents). A probability value <0.05 will be considered statistically significant. Secondary outcome variables will be analyzed according to appropriate statistical techniques.

Sample size analysis: Based on the number of patients delivered at Parkland during the last 10 years, we expect that 342 (1140x3/10) eligible neonates will be delivered over 3 years. We anticipate enrolling half in the study and expect that 2/3 will survive to 36 weeks PMA. Thus we will enroll 150 patients to yield a 100 patients available for the primary outcome and to have 80% power using 2-tailed Student t-test (alpha error 0.025) to detect an increase in weight gain velocity in the NICU by 2 g x kg^{-1} x day^{-1} (standard deviation 3 g x kg^{-1} x day^{-1}) and 95% power (alpha error 0.001) to detect an increase in length velocity by 0.2 cm per week (standard deviation 0.2 cm per week), taking into account clustering due to twins and multiples. We will have >80% power for both outcomes (alpha error 0.05).

References

1. Adamkin DH, Radmacher PG. Fortification of human milk in very low birth weight infants (VLBW < 1500 g birth weight). Clin Perinatol 2014 Jun;41(2):405-21. PMID: 24873840
2. Arslanoglu S, Moro GE, Ziegler EE. Adjustable fortification of human milk fed to preterm infants: does it make a difference? J Perinatol. 2006 Oct;26(10):614-21. PMID: 16885989
3. Alan S, Atasay B, Cakir U, Yildiz D, Kilic A, Kahvecioglu D, Erdeve O, Arsan S An intention to achieve better postnatal in-hospital-growth for preterm infants: Adjustable protein fortification of human milk. Early Hum Dev. 2013 Dec;89(12):1017-2 PMID: 24035039
4. Moro GE, Minoli I, Ostrom M, Jacobs JR, Picone TA, Räihä NC, Ziegler EE. Fortification of human milk: evaluation of a novel fortification scheme and of a new fortifier. J Pediatr Gastroenterol Nutr. 1995 Feb;20(2):162-72. PMID: 7714681

5. Arslanoglu S, Moro GE, Ziegler EE, The Wapm Working Group On Nutrition. Optimization of human milk fortification for preterm infants: new concepts and recommendations. J Perinat Med. 2010 May;38(3):233-8. PMID: 20184400
6. O'Neill EF, Radmacher PG, Sparks B, Adamkin DH. Creamatocrit analysis of human milk overestimates fat and energy content when compared to a human milk analyzer using mid-infrared spectroscopy. J Pediatr Gastroenterol Nutr. 2013 May;56(5):569-72. PMID: 23274342
7. Hair AB, Blanco CL, Moreira AG, Hawthorne KM, Lee ML, Rechtman DJ, Abrams SA. Randomized Trial of Human Milk Cream as a Supplement to Standard Fortification of an Exclusive Human Milk-Based Diet in Infants 750-1250 g Birth Weight. J Pediatr. 2014 Aug 13. PMID: 25130571
8. de Halleux V, Rigo J. Variability in human milk composition: benefit of individualized fortification in verylow-birth-weight infants. Am J Clin Nutr. 2013 Aug;98(2):529S-35S. PMID: 23824725
9. Corvaglia L, Aceti A, Paoletti V, Mariani E, Patrono D, Ancora G, Capretti MG, Faldella G. Standard fortification of preterm human milk fails to meet recommended protein intake: Bedside evaluation by NearInfrared-Reflectance-Analysis. Early Hum Dev. 2010 Apr;86(4):237-40. PMID: 20447779
10. Corvaglia L, Battistini B, Paoletti V, Aceti A, Capretti MG, Faldella G. Near-infrared reflectance analysis to evaluate the nitrogen and fat content of human milk in neonatal intensive care units. Arch Dis Child Fetal Neonatal Ed. 2008 Sep;93(5):F372-5. PMID: 18192331
11. Aceti A, Corvaglia L, Paoletti V, Mariani E, Ancora G, Galletti S, Faldella G. Protein content and fortification of human milk influence gastroesophageal reflux in preterm infants. J Pediatr Gastroenterol Nutr. 2009 Nov;49(5):613-8. PMID: 19633575
12. Di Natale C1, Coclite E, Di Ventura L, Di Fabio S. Fortification of maternal milk for preterm infants. J Matern Fetal Neonatal Med. 2011 Oct;24 Suppl 1:41-3. PMID: 21883045
13. Polberger S, Lönnerdal B. Simple and rapid macronutrient analysis of human milk for individualized fortification: basis for improved nutritional management of very-low-birth-weight infants? J Pediatr Gastroenterol Nutr. 1993 Oct;17(3):283-90. PMID: 8271128
14. Polberger S, Räihä NC, Juvonen P, Moro GE, Minoli I, Warm A. Individualized protein fortification of human milk for preterm infants: comparison of ultrafiltrated human milk protein and a bovine whey fortifier. J Pediatr Gastroenterol Nutr. 1999 Sep;29(3):332-8. PMID: 10468001
15. Polberger S, Lönnerdal B. Simple and rapid macronutrient analysis of human milk for individualized fortification: basis for improved nutritional management of very-low-birth-weight infants? J Pediatr Gastroenterol Nutr. 1993 Oct;17(3):283-90. PMID: 8271128
16. Radmacher PG, Lewis SL, Adamkin DH. Individualizing fortification of human milk using real time human milk analysis. J Neonatal Perinatal Med. 2013 Jan 1;6(4):319-23. PMID: 24441088
17. Wojcik KY1, Rechtman DJ, Lee ML, Montoya A, Medo ET. Macronutrient analysis of a nationwide sample of donor breast milk. J Am Diet Assoc. 2009 Jan;109(1):137-40. PMID: 19103335
18. Sauer CW, Kim JH. Human milk macronutrient analysis using point-of-care near-infrared spectrophotometry. Journal of Perinatology (2011) 31, 339–343. PMID: 21052045
19. van der Schoor SR, Wattimena DL, Huijmans J, Vermes A, van Goudoever JB. The gut takes nearly all: threonine kinetics in infants. Am J Clin Nutr. 2007 Oct;86(4):1132-8. PMID: 17921393
20. van der Schoor SR, Schierbeek H, Bet PM, Vermeulen MJ, Lafeber HN, van Goudoever JB, van Elburg RM. Majority of dietary glutamine is utilized in first pass in preterm infants. Pediatr Res. 2010 Feb;67(2):194-9. PMID: 19809373
21. van Goudoever JB, Vlaardingerbroek H, van den Akker CH, de Groof F, van der Schoor SR. Amino acids and proteins. World Rev Nutr Diet. 2014;110:49-63. PMID: 24751621
22. Barker DJP. The Developmental Origins of Adult Disease. Journal of the American College of Nutrition, Vol. 23, No. 6, 588S–595S (2004). PMID: 15640511

23. Parkinson JR, Hyde MJ, Gale C, Santhakumaran S, Modi N. Preterm birth and the metabolic syndrome in adult life: a systematic review and meta-analysis. Pediatrics. 2013 Apr;131(4):e1240-63. PMID: 23509172
24. Skilton MR, Viikari JS, Juonala M, Laitinen T, Lehtimäki T, Taittonen L, Kähönen M, Celermajer DS, Raitakari OT. Fetal growth and preterm birth influence cardiovascular risk factors and arterial health in young adults: the Cardiovascular Risk in Young Finns Study. Arterioscler Thromb Vasc Biol. 2011 Dec;31(12):2975-81. PMID: 21940950
25. Kerkhof GF, Breukhoven PE, Leunissen RW, Willemsen RH, Hokken-Koelega AC. Does preterm birth influence cardiovascular risk in early adulthood? J Pediatr. 2012 Sep;161(3):390-396.e1. PMID: 22578582
26. Belfort MB, Rifas-Shiman SL, Sullivan T, Collins CT, McPhee AJ, Ryan P, Kleinman KP, Gillman MW, Gibson RA, Makrides M. Infant growth before and after term: effects on neurodevelopment in preterm infants. Pediatrics. 2011 Oct;128(4):e899-906. PMID: 21949135
27. Vohr BR, Allan W, Katz KH, Schneider KC, Ment LR. Early predictors of hypertension in prematurely born adolescents. Acta Paediatr. 2010 Dec;99(12):1812-8. PMID: 20586997
28. Irving RJ, Belton NR, Elton RA, Walker BR. Adult cardiovascular risk factors in premature babies. Lancet 2000;355:2135-6. PMID: 10902631
29. Hack M, Schluchter M, Cartar L, Rahman M. Blood pressure among very low birth weight (<1.5 kg) young adults. Pediatr Res 2005;58:677-84. PMID: 16192252
30. Hovi P, Andersson S, Raikkonen K, Strang-Karlsson S, Jarvenpaa AL, Eriksson JG, et al. Ambulatory blood pressure in young adults with very low birth weight. J Pediatr 2010;156:54-9.e1. PMID: 20008427
31. Keijzer-Veen MG, Finken MJ, Nauta J, Dekker FW, Hille ET, Frolich M, et al. Is blood pressure increased 19 years after intrauterine growth restriction and preterm birth? A prospective follow-up study in The Netherlands. Pediatrics 2005;116:725-31. PMID: 16140714
32. Fabricius-Bjerre S, Jensen RB, Færch K, Larsen T, Mølgaard C, Michaelsen KF, Vaag A, Greisen G. Impact of birth weight and early infant weight gain on insulin resistance and associated cardiovascular risk factors in adolescence. PLoS One. 2011;6(6):e20595. PMID: 21655104
33. Finken MJ, Keijzer-Veen MG, Dekker FW, Frölich M, Hille ET, Romijn JA, Wit JM; Dutch POPS-19 Collaborative Study Group. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. Diabetologia. 2006 Mar;49(3):478-85. PMID: 16450090
34. Elks CE, Loos RJ, Sharp SJ, Langenberg C, Ring SM, Timpson NJ, Ness AR, Davey Smith G, Dunger DB, Wareham NJ, Ong KK.Genetic markers of adult obesity risk are associated with greater early infancy weight gain and growth. PLoS Med. 2010 May 25;7(5):e1000284. PMID: 20520848
35. Kerkhof GF, Leunissen RW, Hokken-Koelega AC. Early origins of the metabolic syndrome: role of small size at birth, early postnatal weight gain, and adult IGF-I. J Clin Endocrinol Metab. 2012 Aug;97(8):2637-43. PMID: 22564668
36. Järvisalo MJ, Hutri-Kähönen N, Juonala M, Mikkilä V, Räsänen L, Lehtimäki T, Viikari J, Raitakari OT. Breast feeding in infancy and arterial endothelial function later in life. The Cardiovascular Risk in Young Finns Study. Eur J Clin Nutr. 2009 May;63(5):640-5. PMID: 18285807
37. Pettitt DJ, Forman MR, Hanson RL, Knowler WC, Bennett PH. Breastfeeding and incidence of non-insulindependent diabetes mellitus in Pima Indians. 1. Lancet. 1997 Jul 19;350(9072): 166-8.PMID: 9250183
38. Johnson MJ, Wootton SA, Leaf AA, Jackson AA. Preterm birth and body composition at term equivalent age: a systematic review and meta-analysis. Pediatrics. 2012 Sep;130(3):e640-9. PMID: 22891222
39. Sjöström SE, Öhlund I, Ahlsson F, Engström E, Fellman V, Hellström A, Källén K, Norman M, Olhager E, Serenius F, Domellöf M. Nutrient intakes independently affect growth in extremely preterm infants: results from a population-based study. Acta Paediatr. 2013 Nov;102(11):1067-74. PMID: 23855971

40. Duncan AF, Heyne RJ, Morgan JS, Ahmad N, Rosenfeld CR. Elevated systolic blood pressure in preterm very-low-birth-weight infants ≤3 years of life. Pediatr Nephrol. 2011 Jul;26(7):1115-21. PMID: 21399900
41. Frankfurt JA, Duncan AF, Heyne RJ, Rosenfeld CR. Renal function and systolic blood pressure in verylow-birth-weight infants 1-3 years of age. Pediatr Nephrol. 2012 Dec;27(12):2285-91. PMID: 22832668
42. Remely M, Haslberger AG.The microbial epigenome in metabolic syndrome. Mol Aspects Med. 2017 Apr;54:71-77. PMID: 27670623
43. Koliada A, Syzenko G, Moseiko V, Budovska L, Puchkov K, Perederiy V, Gavalko Y, Dorofeyev A, Romanenko M, Tkach S, Sineok L, Lushchak O, Vaiserman A. Association between body mass index and Firmicutes/Bacteroidetes ratio in an adult Ukrainian population. BMC Microbiol. 2017 May 22;17(1):120. PMID: 28532414
44. White RA1, Bjørnholt JV, Baird DD, Midtvedt T, Harris JR, Pagano M, Hide W, Rudi K, Moen B, Iszatt N, Peddada SD, Eggesbø M. Novel developmental analyses identify longitudinal patterns of early gut microbiota that affect infant growth. PLoS Comput Biol. 2013;9(5):e1003042. PMID: 23671411
45. Dogra S, Sakwinska O, Soh SE, Ngom-Bru C, Brück WM, Berger B, Brüssow H, Lee YS, Yap F, Chong YS, Godfrey KM, Holbrook JD; GUSTO Study Group. Dynamics of infant gut microbiota are influenced by delivery mode and gestational duration and are associated with subsequent adiposity. MBio. 2015 Feb 3;6(1). PMID: 25650398
46. Robinson EL, Thompson WL. Effect on weight gain of the addition of Lactobacillus acidophilus to the formula of newborn infants. J Pediatr. 1952 Oct;41(4):395-8. PMID: 12991171
47. Gregory KE, Samuel BS, Houghteling P, Shan G, Ausubel FM, Sadreyev RI, Walker WA. Influence of maternal breast milk ingestion on acquisition of the intestinal microbiome in preterm infants. Microbiome. 2016 Dec 30;4(1):68. PMID: 28034306
48. Zeissig S, Blumberg RS. Life at the beginning: perturbation of the microbiota by antibiotics in early life and its role in health and disease. Nat Immunol. 2014 Apr;15(4):307-10. PMID: 24646587
49. Stephens BE, Vohr BR. Protein intake and neurodevelopmental outcomes. Clin Perinatol. 2014 Jun;41(2):323-9. PMID: 24873835
50. Ehrenkranz RA, Das A, Wrage LA, Poindexter BB, Higgins RD, Stoll BJ, Oh W; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early nutrition mediates the influence of severity of illness on extremely LBW infants. Pediatr Res. 2011 Jun;69(6):522-9. PMID: 21378596
51. Ramel SE, Demerath EW, Gray HL, Younge N, Boys C, Georgieff MK. The Relationship of Poor Linear Growth Velocity with Neonatal Illness and Two-Year Neurodevelopment in Preterm Infants. Neonatology 2012;102:19-24. PMID: 22441508
52. De Jesus LC, Pappas A, Shankaran S, Li L, Das A, Bell EF, Stoll BJ, Laptook AR, Walsh MC, Hale EC, Newman NS, Bara R, Higgins RD; Eunice Kennedy Shriver National Institute of Health and Human Development Neonatal Research Network. Outcomes of small for gestational age infants born at <27 weeks' gestation. J Pediatr. 2013 Jul;163(1):55-60.e1-3. PMID: 23415614
53. Isaacs EB, Morley R, Lucas A. Early diet and general cognitive outcome at adolescence in children born at or below 30 weeks gestation. J Pediatr. 2009 Aug;155(2):229-34. PMID: 19446846
54. Burattini I, Bellagamba MP, Spagnoli C, D'Ascenzo R, Mazzoni N, Peretti A, Cogo PE, Carnielli VP; Marche Neonatal Network. Targeting 2.5 versus 4 g/kg/day of amino acids for extremely low birth weight infants: a randomized clinical trial. J Pediatr. 2013 Nov;163(5):1278-82.e1. PMID: 23941670
55. Goldman HI, Goldman J, Kaufman I, Liebman OB. Late effects of early dietary protein low-birth-weight infants. J Pediatr. 1974 Dec;85(6):764-9. PMID: 4472449
56. Fenton TR, Premji SS, Al-Wassia H, Sauve RS. Higher versus lower protein intake in formula-fed low birth weight infants (Review). Cochrane Database Syst Rev. 2014 Apr 21;4:CD003959. PMID: 24752987

57. Olsen IE, Groveman SA, Lawson ML, Clark RH, Zemel BS. New intrauterine growth curves based on United States data. Pediatrics. 2010 Feb;125(2):e214-24. PMID: 20100760
58. Holston A, Stokes T, Olsen C, Choi YS, Curtis J, Higginson J, Adimora C, Hunt CE. Novel noninvasive anthropometric measure in preterm and full-term infants: normative values for waist circumference:length ratio at birth. Pediatr Res. 2013 Sep;74(3):299-306. PMID: 23797534
59. Patel AL, Engstrom JL, Meier PP, Jegier BJ, Kimura RE. Calculating postnatal growth velocity in very low birth weight (VLBW) premature infants. J Perinatol. 2009 Sep;29(9):618-22. PMID: 1946159
60. Olsen IE, Lawson ML, Ferguson AN, Cantrell R, Grabich SC, Zemel BS, Clark RH.BMI curves for preterm infants. Pediatrics. 2015 Mar;135(3):e572-81. PMID: 25687149
61. Ellis KJ, Yao M, Shypailo RJ, Urlando A, Wong WW, Heird WC. Body-composition assessment in infancy: airdisplacement plethysmography compared with a reference 4-compartment model. Am J Clin Nutr 2007;85:90–5. PMID: 17209182
62. Roggero P, Giannì ML, Amato O, Orsi A, Piemontese P, Cosma B, Morlacchi L, Mosca F. Postnatal growth failure in preterm infants: recovery of growth and body composition after term. Early Hum Dev. 2008 Aug;84(8):555-9. PMID: 18329828
63. Dionne JM, Abitbol CL, Flynn JT. Hypertension in infancy: diagnosis, management and outcome. Pediatr Nephrol. 2012 Jan;27(1):17-32. PMID: 21258818
64. Donovan EF, Tyson JE, Ehrenkranz RA, Verter J, Wright LL, Korones SB, Bauer CR, Shankaran S, Stoll BJ, Fanaroff AA, Oh W, Lemons JA, Stevenson DK, Papile LA. Inaccuracy of Ballard scores before 28 weeks' gestation. National Institute of Child Health and Human Development Neonatal Research Network. J Pediatr. 1999 Aug;135(2 Pt 1):147-52. PMID: 10431107
65. Griffin IJ, Pang NM, Perring J, Cooke RJ. Knee-heel length measurement in healthy preterm infants. Arch Dis Child Fetal Neonatal Ed. 1999 Jul;81(1):F50-5. PMID: 10375363
66. Bailey LC, Forrest CB, Zhang P, Richards TM, Livshits A, DeRusso PA. Association of Antibiotics in Infancy With Early Childhood Obesity. JAMA Pediatr. doi:10.1001/jamapediatrics 2014.1539 PMID: 25265089
67. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents: The Fourth Report on the diagnosis, evaluation and treatment of high blood pressure in children and adolescents. Pediatrics 144:555–576. PMID: 1528627
68. Duncan AF, Rosenfeld CR, Morgan JS, Ahmad N, Heyne RJ. Interrater reliability and effect of state on blood pressure measurements in infants 1 to 3 years of age. Pediatrics. 2008 Sep;122(3):e590-4. PMID 18762
69. Lubchenco LO, Hansman C, Dressler M, Boyd D. Intrauterine growth as estimated from liveborn birthweight data at 24 to 42 weeks of gestation. Pediatrics. 1963 Nov;32:793-800. PMID: 14075621
70. Fenton TR. A new growth chart for preterm babies: Babson and Benda's chart updated with recent data and a new format. BMC Pediatr. 2003 Dec 16;3:13. PMID: 14678563
71. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the Fenton growth chart for preterm infants. BMC Pediatr. 2013 Apr 20;13:59. PMID: 23601190
72. WHO Child Growth Standards based on length/height, weight and age. Acta Paediatr Suppl. 2006; 450:76–85. PMID: 16817681