

Dissertation

**THE IMPACT OF NUTRIENT TIMING AND STRATEGY ON GROWTH  
OF PRETERM INFANTS FROM BIRTH TO DISCHARGE:  
PROSPECTIVE ANALYSIS OF A LARGE CLINICAL SAMPLE  
ACCORDING TO ESPGHAN-GUIDELINES**

Submitted by

**Zahra KHAN**

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Institute of Pathophysiology and Immunology

&

Division of Neonatology, University Children's Hospital, Graz

under the supervision of

**Assoc. Prof. Dr. Sandra WALLNER-LIEBMANN**

**Univ. Prof. Dr. Berndt URLESBERGER**

**Univ. Prof. Dr. Marguerite DUNITZ-SCHEER**

**Dr. Nicholas MORRIS**

*(September, 2016)*

## **Declaration**

*I hereby declare that this thesis is my own original work and that I fully acknowledged the names of all those individuals and organizations that have contributed to the research of this thesis. Due acknowledgement has been made in the text to all other material used. Throughout this thesis and in all related publications I followed the guidelines of “Good Scientific Practices”*

*Date: September, 2016*

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## List of Abbreviations

WHO	The World Health Organization
AAP	American Academy of Pediatrics
ESPGHAN	The European Society of Pediatric Gastroenterology, Hepatology & Nutrition
PDA	Patent Ductus Arteriosus
IVH	Intraventricular Hemorrhage
NEC	Necrotizing Enterocolitis
RDS	Respiratory Distress Syndrome
BPD	Bronchopulmonary Dysplasia
LBW	Low Birth Weight
VLBW	Very Low Birth Weight
ELBW	Extremely Low Birth Weight
GA	Gestational Age
AGA	Appropriate for Gestational Age
SGA	Small for Gestational Age
LGA	Large for Gestational Age
VPI	Very Preterm Infants
EPI	Extremely Preterm Infants
IUGR	Intrauterine Growth Restriction
EUGR	Extra-uterine Growth Restriction
PUFA	Polyunsaturated Fatty Acids
EFA	Essential Fatty Acids
CHOP	European Childhood Obesity project
IGF 1	Insulin like Growth Factor 1
BMI	Body Mass Index
NCDs	Non-Communicable Diseases
WAZ	Weight-for-Age Z score
HAZ	Height-for-Age Z score
Hc-Z	Head circumference-for-Age Z score
MUAC	Mid Upper Arm Circumference
SD	Standard Deviation
EDD	Expected Date of Delivery

PN	Parenteral Nutrition
TPN	Total Parenteral Nutrition
EN	Enteral Nutrition
FEN	Full Enteral Nutrition
EQ	Energy Quotient
RGO	Rate of Glucose Oxidation
LBM	Lean Body Mass
FM	Fat Mass
FFM	Fat Free Mass
BF%	Body Fat percentage
ECF	Extra Cellular Fluid
IVF	Intra-Ventricular Fluid
OMM	Own Mother Milk
HM	Human Milk
BM	Breastmilk
PMA	Postmenstrual Age
NICU	Neonatal Intensive Care Unit
EMR	Electronic Medical Record

## Abstract (German)

**Hintergrund:** Die Frühgeburt ist ein medizinischer Notfall und bedarf einer besonderen medizinischen Fachexpertise mit entsprechender Infrastruktur für ein mögliches Überleben unter spezifischen extrauterinen Bedingungen. Eine adäquate Ernährung beginnend in den ersten Lebensstunden scheint großen kurzfristigen, aber auch langfristigen Einfluß auf die Gesundheit des Frühgeborenen zu haben. Mediziner sind daher aufgefordert, spezifische Ernährungsstrategien mit einer Kombination von enteraler und parenteraler nutritiver Unterstützung zu entwickeln, um den täglichen Nährstoffbedarf zu erreichen.

**Ziel:** Die Analyse der effektiven individuellen Nährstoffzufuhr und Wachstumsprofile von Frühgeborenen in intensivmedizinischer Betreuung mit einem standardisierten Ernährungsprogramm.

**Methoden:** In einer prospektiven Kohortenstudie wurden über den Zeitraum von einem Jahr Frühgeborene (<32 Wochen) in intensivmedizinischer Betreuung rekrutiert. Die Zufuhr von Hauptnährstoffen, Flüssigkeit und Energie wurde täglich protokolliert. Das Körpergewicht wurde täglich gemessen; der Kopfumfang und die Körpergröße wurden wöchentlich gemessen. Die Säuglinge wurden in zwei Gruppen nach dem Gestationsalter eingeteilt: <28 Wochen (extrem frühgeborene Kinder; EPI) und  $\geq 28$  Wochen (sehr frühgeborene Kinder; VPI).

**Ergebnisse:** Es wurden 83 Säuglinge in die Analyse eingeschlossen. EPI (n = 27) wurden im Vergleich zu VPI (n = 56) länger parenteral ernährt, erreichten später ausschließlich enterale Ernährung, brauchten mehr Tage an Beatmung und wiesen bei der Entlassung ein höheres korrigiertes Alter auf. Darüber hinaus wurde die Anreicherung enteraler Nahrung bei EPI später begonnen, als bei VPI ( $p < 0.001$ ). Als Folge daraus war die kumulative Aufnahme an Protein, Fett und Energie signifikant niedriger in EPI während der ersten fünf Lebenswochen. Beide Gruppen überstiegen aber die ESPGHAN-Empfehlungen für Glukose in der Woche 5. Bei Entlassung sahen wir signifikante Gruppenunterschiede in allen Wachstumsparametern und der Körperzusammensetzung (weight Z scores: EPI=-1.19 vs VPI=-0.71;  $p < 0.001$ , length Z scores: EPI=-1.62 vs VPI=-0.84;  $p < 0.01$ ; HC Z scores: EPI=-1.19 vs VPI=-0.46;  $p < 0.01$ , BF%: EPI=18.6 Vs VPI=14.1;  $p < 0.01$ ).

Sehr frühgeborene Kinder waren bei der Entlassung leichter und kleiner als extrem frühgeborene Kinder, hatten aber einen höheren Körperfettanteil.

**Konklusion:** Die Implementierung eines standardisierten evidenzbasierten Ernährungsprotokolls ergab eine variable Nährstoffaufnahme und Wachstumsrate in beiden Gruppen während des Beobachtungszeitraumes. Sehr früh geborene Kinder konnten bessere Z-scores bei Entlassung mit einer Körperzusammensetzung vergleichbar Normalgeborener erzielen. Bei extrem frühgeborenen Kindern aber, hatten die komplizierten Bedingungen bei der Ernährungszufuhr Einfluß auf Wachstum und Körperzusammensetzung bei Entlassung.

Gerade bei extrem frühgeborenen Kindern scheint die verstärkte parenterale Ernährung wichtig zu sein. Wenn die enterale Ernährung gut vertragen wird, sollte die Anreicherung bei einem enteralen Volumen von 100ml/kg/d oder sogar früher begonnen werden. Zu beachten ist dabei aber, dass die Muttermilchanreicherung später zu einer Glukosezufuhr über den ESPGHAN-Empfehlung geführt hat – dies bedarf noch weiterer Evaluierung in zukünftigen Studien.

## Abstract

**Background:** Preterm birth is a medical emergency and an advanced medical proficiency with suitable infrastructure is required for survival against the numerous odds of the extrauterine environment. It is becoming evident that adequate nutrition starting in the first hours of life is of major importance for short and even more so for long-term health outcomes of the premature newborn. There are separate guidelines for parenteral nutrition and enteral nutrition for preterm infants. Clinicians need to develop unit-specific feeding protocols combining parenteral and enteral nutritional support to meet the daily nutrient requirements.

**Aim:** To analyze the effective individual postnatal nutrient supply and growth patterns in response to standardized feeding protocol provided to preterm infants during their stay at NICU.

**Methods:** A prospective cohort study was conducted including preterm infants born <32 weeks entering the NICU during a 1-year-time period. Macronutrient, fluid and energy supply was recorded daily during their stay at NICU. Weight was recorded daily; head circumference and length were recorded weekly. Infants were divided in two groups on the basis of Gestational Age (GA): <28 weeks (Extremely preterm infants (EPI); and  $\geq$ 28 weeks (very preterm infants (VPI)).

**Results:** 83 infants were included in analysis. EPI (n = 27) compared to VPI (n = 56) stayed longer on parenteral nutrition, needed more time to reach FEN, required more days on ventilation and had a higher corrected age at discharge. Moreover, fortification of enteral feeds was initiated later in EPI vs VPI ( $p < 0.001$ ). As a consequence, cumulative supply of protein, fat and energy was significantly lower in EPI during the first five weeks of life. However, both groups exceeded the ESPGHAN recommended glucose intake in week 5. At discharge, we found significant differences in all growth parameters and body composition between both groups (weight Z scores: EPI=-1.19 vs VPI= -0.71;  $p < 0.001$ , length Z scores: EPI=-1.62 vs VPI=-0.84;  $p < 0.01$ ; HC Z scores: EPI=-1.19 vs VPI=-0.46;  $p < 0.01$ , BF%: EPI=18.6 Vs VPI=14.1;  $p < 0.01$ ). At discharge EPI were lighter and shorter than VPI but exhibited higher body fat percentage.

**Conclusion:** After implementation of a standardized evidence-based nutritional support protocol, nutrient supply and growth rate was variable in both groups during the observation period.

Very preterm infants could achieve better Z-scores at discharge accompanied by body composition comparable to full-term new born infants. However, in extremely preterm infants, the complicated course of nutritional support during their stay at NICU influenced growth and body composition at discharge.

In extremely preterm infants at risk of not making significant progress in escalating enteral intake, it seems prudent to practice aggressive parenteral nutritional support.

If enteral feeds are tolerated well, fortification should be initiated at enteral volumes of 100ml/kg/d or even earlier. Moreover, the use of mother milk fortifier resulted in glucose intake above the ESPGHAN recommendations in later weeks – this need to be evaluated in future studies.

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# 1. INTRODUCTION

Over the last few decades improved standards of care have led to markedly increased survival rates of medically fragile children, particularly of very preterm infants and extremely preterm infants.<sup>1-3</sup> The World Health Organization (WHO) has recently published global mortality estimates and reported that the infant mortality rate has dropped by 50% between 1990 and 2015.<sup>4</sup> Therefore, increased attention is now directed to improve long-term health outcomes and quality of life. Adequate nutrition during the early years of life is of prime importance for survival, growth, development, and long-term health through adulthood. Infancy and early childhood are considered critical periods with a high risk of irreversible faltering in linear growth and cognitive deficits.<sup>5,6</sup> Inappropriate nutrition (either over or under) during this critical period might contribute to significant morbidity and mortality.<sup>7</sup> Studies have shown that higher supplies of nutrients during the first month of life contribute to a significantly higher intelligence quotient and reduced risk of cerebral palsy.<sup>8</sup>

Neonatologists and other health professionals involved in neonatal care rely mainly on the assessment of growth characteristics to determine whether or not nutrition support is adequate. Normal fetal growth for example, comprises a doubling in weight, between 30 and 36 weeks of gestation, alongside with remarkable tissue differentiation.<sup>9</sup> It remains a big challenge to match this quality and quantity of growth and development in infants whose nutrient supply via the umbilical cord has been prematurely interrupted. Major progress has been achieved in our understanding of meeting their nutritional needs, but surprisingly still a great deal of variability exists in clinical practice around the world, within countries and even between neighboring centers.

Evidence based standardized feeding guidelines have been published by different organizations such as The American Academy of Pediatrics<sup>10</sup>, the Canadian Pediatric Society<sup>11</sup>, the European Society of Pediatric Gastroenterology, Hepatology and Nutrition<sup>12,13</sup> and the Life Sciences Research office<sup>14</sup>. These guidelines cover either parenteral or enteral nutrition, requiring centers to define their own local combined parenteral/enteral nutritional support protocols.

Nevertheless, implementation of these guidelines may reduce practice variation within a center. It is to be expected that development and implementation of a standard feeding protocol based on these guidelines could improve postnatal growth and clinical outcomes.<sup>15</sup>

Keeping this in view, we planned a prospective follow up study to evaluate nutrient supply and growth outcomes of preterm infants born <32 weeks in response to a standard feeding protocol based on the ESPGHAN guidelines.

## 1.1 Preterm Birth

Preterm birth is defined as live-birth before 37 weeks of completed gestation.<sup>16</sup>

These preterm babies are categorized based on their gestational age at birth:

- extremely preterm (<28 weeks)
- very preterm (28 to <32 weeks)
- moderate to late preterm (32 to <37 weeks).

It is not recommended to medically induce labour or plan caesareansection before 39 completed weeks of gestation without any significant medical indication.<sup>16</sup>

Worldwide 15 million babies are born prematurely every year.<sup>16</sup> Due to complications of prematurity death rates are significantly higher in this population.<sup>17</sup> Survivors of prematurity are at increased risk of a wide range of developmental issues, including learning disabilities and visual and hearing problems.<sup>18</sup> A large proportion of preterm infants need respiratory support during the first days of life because of lung immaturity. Sequelae of prematurity can be reduced by nutritional optimization and to some extent by using drugs to accelerate maturation of the lungs, but the most important goal is to prevent preterm birth.

Preterm birth is a recognized public health problem<sup>16</sup>, with Africa and South Asia carrying the burden of over 60% of all preterm births per year. Globally the preterm birth rate is between 8-18%.<sup>16</sup> In developed countries the preterm birth rate is estimated to be around 9% whereas in developing countries it is much higher i.e.(12%)<sup>16,17</sup> Global trends show an increase in preterm birth rates over

the past 20 years. Possible causes for this include improved data recording, increased maternal age and underlying maternal health problems such as diabetes and high blood pressure, greater use of infertility treatments leading to increased rates of multiple pregnancies, and changes in obstetric practices such as more caesarean births before term.<sup>16</sup>

## **Short-term & long-term complications of preterm birth**

### **Short-term**

There is a great variability in preterm survival around the globe. In developing countries, the majority of extremely preterm infants (90%) do not survive their first week of life, whereas in developed countries the mortality rate for the same gestational age is around or less than 10%. Similarly, due to lack of sophisticated health care facilities in many developing countries, 50% of the babies born at 32 weeks die. In high-income countries, survival rate for these infants is almost 100%.<sup>16</sup>

Preterm birth is the direct cause of neonatal death in more than 27% of the cases born globally.<sup>18</sup> It is also a major determinant of short- and long-term morbidity in infants and children. The burden of preterm birth includes neonatal morbidity and long-term sequelae, including neurodevelopmental deficits (eg, cerebral palsy, impaired learning, visual disorders) and an increased risk of a spectrum of diseases in adulthood.<sup>19</sup>

Preterm neonates develop short term complications due to functional immaturity; for example hypothermia, respiratory abnormalities, cardiovascular abnormalities (patent ductus arteriosus, systemic hypotension), glucose abnormalities, necrotizing enterocolitis (NEC) and infections.<sup>20</sup>

A report from the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network was published on morbidity and mortality rates of very low birth weight (VLBW) infants, according to gestational age (GA). They concluded, over the last 20 years the survival rate increased most markedly among extremely preterm infants.<sup>21</sup> The risk of developing complications gets higher with decreasing GA and birth weight.

They reported the prevalence of bronchopulmonary dysplasia (BPD) 45%, severe intraventricular hemorrhage (IVH) 15%, necrotizing enterocolitis (NEC) 9% and late-onset sepsis 24%. At discharge, 56% of the extremely preterm infants had retinopathy status.<sup>21</sup>

## **Long-term**

Infants born  $\leq 25$  weeks of gestation, have low chances of survival and the greatest risk of long-term morbidity.<sup>22</sup> Among the survivors of this vulnerable group, long-term neurodevelopmental disability is the major form of morbidity. In addition, preterm children require frequent hospitalization due to other medical problems including respiratory abnormalities and postnatal growth retardation.<sup>20</sup>

Poor neurodevelopmental outcome consists of compromised cognitive and motor development, including (mild fine/gross motor delay, and cerebral palsy), sensory impairment and a range of behavioral problems.<sup>22</sup>

Prematurity appears to have an impact on health later in life often promoting insulin resistance and high blood pressure.<sup>23,24</sup> These children also require additional healthcare and educational services beyond those routinely required by children because of poor neurodevelopmental outcomes that result in functional limitations.<sup>25</sup>

Studies conducted on the issues of economic consequences of preterm birth for the health services, for families and ultimately for society, suggest that prevention of premature birth can contribute towards tremendous savings to society by reducing the expenditures from medical costs for care in the neonatal period and additional health and educational costs in childhood, and increased productivity in adulthood.<sup>26-28</sup>

## **1.2 Fetal Nutrition**

Fetal nutrition is closely related to maternal nutrition. There is a well established association between maternal pre-pregnancy weight, pregnancy weight gain and birth weight. With higher maternal pregnancy weight gain there is improved

birth weight.<sup>29</sup> Similarly poor maternal weight gain in all trimesters of pregnancy has been associated with lower birth weight.<sup>30,31</sup>

Nutritional factors during the early phase of development determine the rate of fetal growth for the rest of gestation, and may also determine the length of gestation.<sup>32</sup>

Recently a study demonstrated that the growth trajectory of fetuses can be set very early in gestation. Reduced fetal growth rate in sheep has been reported after modest nutrient restriction around the time of conception.<sup>33</sup> Furthermore, the brief and relatively minor period of under-nutrition resulted in preterm birth in half of the undernourished ewes.<sup>34</sup>

Glucose is the main energy substrate in utero and it passes the placenta by facilitated diffusion down a concentration gradient from mother to fetus. For this reason fetal blood glucose concentration is always directly proportional to but lower than that of the mother. In humans, fetal blood glucose concentrations are 60-70% of maternal levels.<sup>32</sup>

Amino acids are transported across the placenta by active transport. Total amino acid concentrations in the fetus are higher than that in the mother.<sup>35</sup> Placental amino acid transporters are very important and have been reported to show reduced activity in rats following maternal under-nutrition and intrauterine growth restriction (IUGR).<sup>35,36</sup> Amino acids stimulate insulin release which enhances glucose and amino acid uptake into fetal tissue.<sup>37,38</sup>

In general term infants are born with large stores of fat due to exponentially increasing fat deposition with increasing gestational age. During the last trimester the accretion rate is about 2.4g/kg/day.<sup>39</sup> Early in gestation, the fetus derives most of the fatty acids from the mother, but as gestation progresses there is an increase de novo synthesis.<sup>39</sup> Fatty acids are crucial for many fundamental processes of fetal development for example; membrane formation, as precursor of prostaglandins, and as a source of energy. For this reason sufficient supply of polyunsaturated fatty acids (PUFA) is important. PUFA are also considered essential fatty acids because of the inability for them to be synthesized in humans and therefore, exogenous sources must be provided either by the mother or postnatally by parenteral/enteral nutrition. Intrauterine requirements of essential fatty acids (EFA) during the last trimester have been calculated to be approximately 400 mg/kg/d for  $\Omega$ 3 and 50 mg/kg/d for  $\Omega$ 6.<sup>40,41</sup>

## **IUGR and SGA**

IUGR and small for gestational age (SGA) are terms describing growth deficit during pregnancy. Newborns are classified as having normal birth weight (2500-4000g), low birth weight (<2500grams) (LBW), very low birth weight (<1500 grams) (VLBW) or extremely low birth weight (<1000grams) (ELBW).<sup>42</sup> Cross-sectional growth curves have been derived from anthropometric measurements of newborns at different gestational ages to define growth status of the fetus. The fetus and new born infant are considered appropriate for gestational age (AGA) if his/ her weight is between 10<sup>th</sup> and 90<sup>th</sup> percentile. Those who are <10<sup>th</sup> percentile are considered as small for gestational age (SGA) and those who are >90<sup>th</sup> percentile are considered large for gestational date (LGA).<sup>42</sup>

Intrauterine weight gain is estimated to be about 12-15g/kg/day.<sup>42,43</sup> Fetal weight gain appears to be linear during early gestation, but reaches a plateau between 37-42 weeks. Peak growth velocity of a fetus is generally observed at about 32 weeks when a fetus is depositing approximately 200grams per week. While specific IUGR growth data is scarce, a smaller fetus on the 10<sup>th</sup> percentile is estimated to accumulate weight at approximately 80% of the normal rate (160 grams per week) and as gestation advances, an IUGR fetus can be expected to accumulate only 640 grams between 37-41 weeks in contrast to the average fetus that will deposit over 800grams during the same period.<sup>44</sup>

### **1.3 Nutritional Environment and its role in Metabolic programming**

The quality and quantity of nutrition during fetal/neonatal development plays a critical role. The genome and the environment are universally recognized factors that influence growth and development.<sup>45</sup>

Acquisition of metabolic capacities and their adaptations in early life are influenced by three critical periods: 1. Fetal development during gestation 2. An abrupt fetal-neonatal transition occurring at birth 3. A gradual postnatal-weaning transition.<sup>46</sup>

Rapid changes in enzyme activities occur in response to the nature of the available nutrients during these periods. These metabolic adaptations are considered part of the process of maturation during early periods of life. An altered nutritional environment during such periods induces abnormal metabolic adaptations which can lead to negative outcomes later in life.<sup>45</sup>

“Metabolic programming is the phenomenon by which nutritional stress/stimuli experienced during early periods of life overlapping with the critical window of organogenesis of target tissues permanently alters the physiology and metabolism of the organism.”<sup>47</sup>

Consequences of this brief period of dietary manipulation can leave significant impact on the health of an individual later in life. In this section metabolic programming due to malnutrition during immediate postnatal period will be discussed.

It is evident that intrauterine undernutrition and subsequent low birth weight result in many health problems later in adulthood e. g. obesity, cardiovascular diseases, hypertension and type 2 diabetes.<sup>48,49</sup> However there are many questions still unanswered. These questions include mechanisms through which metabolic activities are altered, determination of sensitive time periods for a certain programming effect and identification of nutrients and dietary interventions. There is also increasing interest in investigating the role of nutrients in epigenetics. Further research is needed to understand which programming effects can be reversible and to what extent.<sup>49</sup>

VLBW infants and large for gestational age infants have a higher risk of cardiovascular mortality in adulthood.<sup>50</sup> Small size at birth has also been associated with an increased risk of adolescent hypertension<sup>51</sup> and glucose intolerance.<sup>52</sup> However, the ingestion of breast milk appears to have a protective effect on childhood obesity, adolescent hypertension and type 2 diabetes.<sup>53-55</sup>

Thrifty phenotype hypothesis was studied by Barker and colleagues suggesting that the developing fetus makes adaptations in response to nutrient restriction during pregnancy in order to allow near-normal development of the brain by

compromising growth of other organs like liver, muscle, pancreas and kidneys etc.<sup>56</sup> This phenomenon seems appropriate and sensible when food supply is poor to permit adequate brain growth but becomes detrimental later in life when aggressive nutritional support is provided<sup>57,58</sup>

Metabolic programming can critically influence the developing organs by making permanent alterations in the physiology and metabolism. For example it has been reported that hyperinsulinemia can influence brain development resulting in disorganization of the hypothalamus, which may lead to other pathological conditions.<sup>59</sup> Results of a study with high carbohydrate dietary intervention (high carbohydrate milk formula) during the suckling period in 12 days old rats showed adaptation in pancreatic islets (hyperinsulinemia), gut (increased glucagon-like peptide1 levels) and hypothalamus (alterations in the level of neuropeptides). It is important to note that all these organs are responsible for maintaining glucose levels.<sup>45</sup>

A high protein diet during the early postnatal days of life has been associated with increased risk of obesity generating a new “early protein hypothesis” concept. Researchers from European Childhood Obesity Project (CHOP) have reported that high protein intake stimulates an increased release of insulin and insulin like growth factor 1 (IGF1) and as a consequence causes faster weight gain and higher body mass index (BMI) in the first years of life.<sup>60</sup>

## **Non-communicable diseases**

In the USA and Europe obesity has been recognised as a public health problem. Its prevalence has increased exponentially in the last two decades. Obesity has a causal association with various physical disabilities and psychological issues, however there is additional risk of developing a number of noncommunicable diseases (NCDs), including cardiovascular disease, cancer and diabetes.<sup>61</sup>

The recent demography report of the Commission of European communities' anticipates a large shift in population demographics by the year 2060 due to an increase in life expectancy and a decrease in birth rate. Furthermore, it predicts a significant reduction in the productive workforce creating challenges to increase

the individuals' number of healthy and productive years and also to limit age-related NCDs.<sup>62</sup>

Effective nutritional interventions during the perinatal period may reduce the life time burden of NCDs, which are therefore an important element of health promotion with large impact. Early growth acceleration is a major risk factor for obesity later on in life.<sup>63</sup> Studies suggest an impact of early dietary interventions on the development of white adipocytes, and therefore a major role in the development of the metabolic syndrome, insulin resistance and also hypertension.<sup>64,65</sup> For the above reasons one can conclude that finding the ideal nutritional strategy to regulate growth and optimize development is an important avenue leading to healthier adults.

#### **1.4 Postnatal growth in preterm infants**

Preterm birth is a medical emergency and advanced medical expertise with suitable infrastructure are required for survival against numerous odds in extrauterine life. Recently we published that the prevalence of malnutrition was 25% in medically fragile children during infancy and early childhood.<sup>66</sup>

In the early postnatal period rapid growth is characterized by cellular hyperplasia and hypertrophy making it important for the individual's health later in life. Hack and colleagues reported that VLBW infants were at risk of stunting (<3rd percentile) at 20 years of age.<sup>67</sup>

There is complex interaction between numerous factors influencing normal growth and development up until infancy. Growth is influenced by the fetal genome and its environment.<sup>68</sup> Factors responsible for postnatal growth failure include multiple morbidities, difficulties in suck and swallow coordination and various drugs that interfere with nutrient metabolism.<sup>69</sup>

Gestational age at birth is another important determinant of postnatal growth outcomes,<sup>70</sup> but nutritional and medical care during hospitalisation is considered mainly responsible for weight gain velocity.<sup>71-73</sup> Different morbidities in postnatal

life for example early onset of SEPSIS, IRDS, BPD, IVH etc<sup>74</sup> can lead to extra-uterine retardation.(EUGR).

### **1.4.1 Growth Monitoring**

The American Academy of Pediatrics (AAP) and the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) have defined a nutritional management goal: to achieve a rate of growth with a body composition similar to a normal fetus of the same postmenstrual age.<sup>10,13</sup> Infants who experience respiratory problems, infections and many other medical complications may suffer growth failure.<sup>75</sup> Growth curves which are used in clinical practice to monitor postnatal weight gain of preterm infants are not appropriate. These charts are based on either estimated intrauterine growth or on estimates of preterm infants growth pattern. However, these curves may not be the ideal way to measure optimal growth.

Current practice is to follow the goals based upon estimated intrauterine growth from historical cohort studies of live births of varying gestational age.<sup>76</sup>

- Weight – 15 g/kg per day
- Length – 1 cm/week
- Head circumference – 0.7 cm/week

Observational data reports following intrauterine growth rate can be a misleading practice, because most low birth weight infants are SGA at the time of discharge.<sup>77,78</sup> It also appears that weight gain velocity at different stages of postnatal life can impact on later health outcomes. In extremely preterm infants rapid catch up growth/ weight acceleration is required to support brain development and is associated with better neurodevelopmental outcomes at later stages in life.<sup>79</sup> Further studies are needed to establish appropriate growth targets in preterm infants.

## 1.4.2 Growth Charts

Growth assessment is done with focus on size for age comparing with intrauterine size for age growth charts. The anthropometric measurements (weight, length and head circumference (HC)) are plotted against gestational age on growth charts. For example nutritional status at birth is defined by plotting birth weight against gestational age to classify infants to SGA, AGA or LGA.

During the last decade growth charts from Olsen, Bertino and Fenton have been used in NICUs for growth monitoring in preterm neonates.<sup>80-82</sup> Currently, the selection of growth charts used depends on local practice and preference. Mostly Fenton growth charts are used because these charts give better estimates of AGA, SGA, or LGA status at lower gestational ages and for growth monitoring of preterm infants up to 50 weeks corrected age (10 weeks post-term).<sup>83</sup>

Sakurai and Colleagues published the incidence of EUGR in a large population of preterm infants in Japan. According to their results the incidence of EUGR was 57%, 48% and 6% for weight, length and head circumference respectively.<sup>84</sup> In another study of very preterm infants, the prevalence of EUGR was reported to be 37%, 27% and 32% for weight, length and head circumference respectively.<sup>85</sup>

In the last couple of years, an increased number of studies have been published reporting about disproportionate growth in preterm infants. Altered body composition cannot be detected by size for age growth charts. To assess the quantity and quality of postnatal growth, it is important to monitor not only weight for age but also weight for length. It is quite possible that an infant has reduced weight for age but appropriate weight for length.<sup>86</sup> For this purpose Olsen and colleagues have developed BMI curves for preterm infants helping to define quality of postnatal growth.<sup>86</sup> The importance of BMI assessment in predicting obesity in adolescence and early adulthood has recently been demonstrated in several studies<sup>87-88</sup>

### 1.4.3 Body Composition

Body composition is becoming an important measure of postnatal growth especially in VPI and EPI. Optimal nutrition is a goal which has gained importance aiming to achieve near-intrauterine growth in terms of weight gain and body composition. Some studies report that preterm infants have higher body fat percentage and reduced lean body mass at term corrected age.<sup>89-92</sup> Although it is still unclear whether higher body fat is good or bad, body composition measurements are considered important in the assessment of nutritional status at term corrected age. This information can help to direct all nutritional support in the early postnatal days to achieve ideal growth in terms of weight gain as well as balanced composition of lean mass and fat mass.

It is a complex issue to understand the process of weight gain in preterm infants and interpret normal composition of postnatal weight gain. As mentioned earlier, it is a misleading concept to compare postnatal growth with in utero growth. Griffin IJ and Cooke RJ wrote a comprehensive review about the development of whole body adiposity in preterm infants. They reported that postnatal growth curves can not be in continuation with prenatal ones. They report a sudden, brief acceleration in fat mass.<sup>93</sup> In the late 60s it was estimated that fat mass accounted for around 11% of total body mass before birth increased to 41% until the age of 4 months and then decreased to 20% by the age of 12 months.<sup>94</sup>

Research indicates that rapid growth in the first year of life is associated with an increased risk of adiposity and metabolic syndrome in preterm infants. There is a strong argument suggesting that increased deposition of fat during early postnatal life is associated with metabolic programming leading to various health issues. But on the other hand sufficient growth is essential in preterm infants enabling normal development of sensitive organs during early postnatal period e.g. brain, lungs and kidneys. Long-term studies showed that preterm infants are not heavier than their term peers.

It is important to define nutritional strategies to help achieve optimal growth outcomes.

#### 1.4.4 Z-Scores

Traditionally three anthropometric parameters (weight, length and head circumference) are measured during early postnatal life and growth status is assessed by various methods for example by using intrauterine growth charts or Z-Scores. For population-based surveys and nutritional surveillance programs the Z-score is widely recognized as the best system for analysis and presentation of anthropometric data.<sup>95</sup> The Z-score system expresses the anthropometric value as a number of standard deviations or Z-scores below or above the reference mean or median value.<sup>96</sup>

There are weight-for-age (WAZ), height-for-age (HAZ), and headcircumference-for-age (HcAZ) parameters which can be calculated to see if individual cases are growing age appropriately or not. There are some other parameters like weight-for-height and Mid upper arm circumference (MUAC) measurements which can be used to classify malnutrition.

Common cut-off values are  $<-2$  SD and  $>+ 2$ SD Z-score which means that 95% of the population fall into the normal range .<sup>96</sup>

There is different software available online which can provide exact Z-score measurements for different age groups. A Fenton Z-score calculator is available online for free download on (<http://ucalgary.ca/fenton>).<sup>97</sup>

Interpreting results in terms of Z scores has certain advantages. For example the whole sample population Z-scores can be summarized and presented in mean and standard deviation (SD). Significantly lower values of mean Z-score of a population reflect that the majority of the population is malnourished. Mean Z score can be used as an index of severity for growth and nutritional problems and may help to plan intervention for whole risk group.<sup>96</sup>

The majority of extremely premature and VLBW infants experience some degree of intrauterine and/or neonatal growth failure.<sup>69,98,99</sup> Despite advances in neonatal care and nutrition, many infants fail to grow adequately during the initial neonatal period with the result that the rates of subnormal weight, length and head circumference are much higher at the time of discharge than at birth. Poor neonatal growth is most prevalent among the least mature and/or smallest infants,

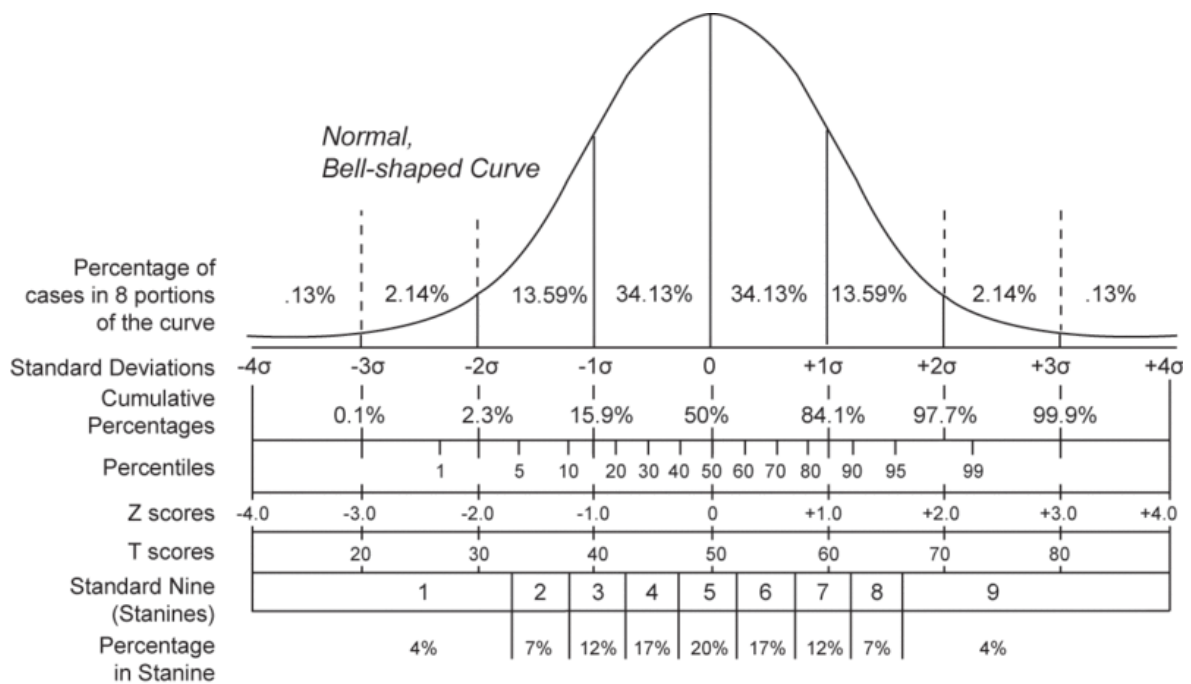
the majority of whom have high rates of neonatal morbidity and also tolerate feeds poorly.<sup>100</sup>

The Epicure study of growth and associated problems in children born extremely premature (<25weeks) showed that this vulnerable group of infants was at risk of postnatal growth failure. Feeding problems, neurodevelopmental disability, chronic lung disease requiring long-term ventilation and postnatal steroid therapy were among the significant factors which influenced postnatal growth. A total of 271 preterm infants born in 1995 were followed until 30 months corrected age. At birth their mean z-scores for weight were 0.27, these mean z-scores dropped to -1.72 at the expected date of delivery (EDD) due to neonatal growth failure, and then increased to -1.19 by 30 months corrected age. The mean z-scores for head circumference were 0.09 at birth, -0.86 at the EDD and -1.40 at 30 months corrected age, and the mean z scores for length/height were -2.49 at the EDD and -0.70 SD at 30 months corrected age. At 30 months corrected age 25% of the children had subnormal weight (<-2 SD), 37% subnormal head circumference, 13% subnormal height. Eight percent of the children had a weight <-3 SD and 16% a head circumference<-3SD. Of note is the fact that the head circumference z-score decreased between the EDD and 30 months corrected age.<sup>101</sup>

Another study reported growth outcomes of Swedish preterm infants born  $\geq 23$  weeks gestation during 1990-1992. According to their results 24% of infants had reduced WAZ scores, 17% had reduced HAZ scores and 10% had reduced HcAZ scores at term corrected age.<sup>102</sup>

Weight for age Z scores  $\pm 2$  SD from the mean are considered outside the normal range yet the corresponding percentile approximating to -2SD from mean is the 3rd percentile (-2SD = 2.3 percentile).<sup>103</sup>

Therefore, Z-scores provide a wide range where more infants with postnatal growth failure could be accommodated and considered normal, whereas percentiles are very strict criteria to define postnatal growth failure.



103  
( source: Wikipedia)

## 1.5 Nutrient Requirements

### (Importance of optimal nutrition in NICU)

In this section health consequences of early postnatal nutritional support and possible mechanisms by which early nutrition can influence individual long-term outcomes will be discussed.

In 1962 McCance was the first to show a very convincing impact of early nutrition on growth outcomes. Malnutrition during the suckling period was associated with postnatal growth retardation in rats whereas malnutrition after weaning showed a short-term impact on growth.<sup>104</sup> Other studies also showed similar findings<sup>105,106</sup> confirming that the immediate postnatal period is a critical window for nutritional manipulation in order to create better outcomes.

Research on this topic is encouraged to understand the factors that alter growth trajectories for children who are born extremely preterm and to develop targeted interventions that can improve overall growth.

To date, we have separate guidelines for parenteral nutrition and enteral nutrition. Clinicians need to plan unit-specific mixed feeding protocols according to parenteral and enteral guidelines.

### **1.5.1 Parenteral Nutrition**

The first report of safe and long-term use of parenteral nutrition (PN) in pediatric patients was in 1972.<sup>107</sup> Since that time significant improvements in survival rates of infants have been achieved.

Various studies have shown that postnatal growth failure is a consequence of inadequate nutritional support during the first weeks of life which is difficult to recover from and may lead to long-term detrimental effects.<sup>98,108</sup> In these critical conditions PN can help to provide an adequate amounts of required nutrients and prevent weight loss. Improved nutrient supply is associated with improvement in growth and neurodevelopmental outcomes and reduced risk of mortality and other medical complications such as necrotizing enterocolitis and bronchopulmonary dysplasia.<sup>109</sup>

Extremely preterm birth should be considered a nutritional emergency. It is recommended to start PN in preterm born infants because of limitations to start enteral nutritional support due to immature gastrointestinal tract.<sup>110</sup> PN is more frequently served with the purpose to „bridge“ where nutritional intake is managed with PN until sufficient enteral intake can be achieved.<sup>111</sup>

#### **1.5.1.1 Energy**

In adults energy is mainly used to maintain normal functioning of the human body. But a preterm infant has specially increased energy needs for both maintenance of normal functioning (resting metabolic rate, activity, and thermoregulation) and for growth.

There are many factors which are considered important for the calculation of the preterm infant's total energy requirements during the first few days of life. These factors include:<sup>112</sup>

- Postnatal age
- Activity level (e.g respiratory distress)
- Environmental temperature
- Energy losses (particularly in stool)
- Medical conditions (e.g. sepsis, lung disease)
- Mechanical ventilation (which reduces the energy cost of breathing)
- Dietary induced thermogenesis (energy costs of digesting & metabolizing food)
- Body size and composition
- Catch up growth

Energy intake also has an impact on nitrogen balance. The minimum energy needs of new born infants can be met by providing 50-60Kcal/kg/day but 100-120 Kcal/kg/ day are required for maximal protein accretion.<sup>12,110</sup> It is estimated that small preterm infants with respiratory disease requiring mechanical ventilation exhibit higher energy expenditure during early postnatal days of life i.e. 85 kcal/kg/day.<sup>113</sup> During the last trimester of pregnancy energy accretion is approx. 24kcal/kg/day.<sup>114</sup> Keeping these facts in consideration Guidelines on Pediatric Parenteral Nutrition from ESPGHAN suggest that a growing preterm infant with higher energy needs would require at least 110 kcal/kg/day.<sup>12</sup>

### **1.5.1.2 Glucose**

Glucose serves as a source of energy to preterm neonates who are receiving total parenteral nutrition. Maintenance of glucose levels is a challenging task with the risk of hypo- and hyperglycemic episodes during early postnatal days. Extremely low birth weight infants (ELBW) are at risk of developing hyperglycemia, due to decreased insulin production, insulin resistance, absence of enteral nutrition which can stimulate insulin production and high glucose infusion rate.

While providing nutritional support via parenteral passage, glucose should be supplied at a constant rate without exceeding the maximum rate of glucose

oxidation (RGO). It has been suggested the glucose infusion rate should be 4-8mg/kg/minute.<sup>12</sup>

### **1.5.1.3 Amino Acid and Protein Requirements**

Amino acid requirements are generally estimated to achieve positive nitrogen balance. Provision of insufficient dietary amino acids leads to a catabolic state and a negative nitrogen balance. Clinically, this translates to extrauterine growth restriction (EUGR), also known as growth faltering. To avoid negative nitrogen balance and support growth, amino acids should be prescribed in preterm infants on the first day of life. Although the optimal composition mix of amino acids for very preterm infants has yet to be determined.<sup>115</sup> It is important to provide sufficient energy in combination with protein to prevent protein breakdown covering energy requirements.<sup>116</sup>

With an average energy supply of 90 Kcal/kg/d, 3.2g/kg/d of protein supply lead to a positive protein balance.<sup>12</sup> A systematic review concluded that even higher intakes such as 3.5g/kg/d are well tolerated during the first week of life.<sup>13</sup> A minimum amino acid intake of 1.5g/kg/d is necessary to prevent a negative nitrogen balance and a maximum of 4g/kg/d is recommended.<sup>12</sup>

### **1.5.1.4 Lipids**

Lipids serve as a major source of energy for preterm infants. Preterm infants, especially extremely preterm infants, who miss the opportunity to acquire normal fat stores during late gestation, are born with very low fat stores. For example, a 1000g infant has a total body fat content of only about 20g (2%).<sup>117</sup> Lipids have been shown to have a fundamental important role in growth, development and long term health.<sup>118</sup> Administration of lipids through PN is necessary to fulfill the requirements for essential fatty acids of the growing infant.<sup>12</sup> In preterm infants continuous infusion of lipid emulsion is well tolerated.<sup>119,120</sup>

Parenteral lipids supply should be initiated within few hours after birth and should be limited to a maximum of 3-4g/kg/d in infants.<sup>12</sup>

### **1.5.1.5 Fluid and Electrolyte Requirements**

#### **Neonatal period:**

The amount of total body water changes immensely from intrauterine life through childhood to adulthood. Total body water accounts for as much as 90% of body weight in a 24 week old foetus which is then around 75% in the term infant and only 50% in adults.<sup>121</sup>

Water turn over is related to lean body mass (LBM) but has no close relation to body fat mass. Extremely preterm infants and very preterm infants have relatively low body fat content and a higher percentage of LBM which reflects they have more body water than older infants.<sup>12</sup>

After birth, with the interruption of continuous placental exchange, the infant goes through an immediate adaptation process affecting metabolism of water and electrolytes leading to considerable losses of insensible water also affecting thermoregulation. This process of adaptation is divided into three phases:

#### **Phase I: (Transition)**

Immediately after birth rearrangement of fluid compartments takes place. It continues from birth to the point of maximum weight loss, which can be up to 10% of birth weight.

During this phase the following occurs:

- Relative oliguria due to decreased glomerular filtration rate;( lasting from hours to days)
- Followed by increased diuresis which leads to contraction of the extra cellular fluid (ECF)

First phase is completed in 3-5 days in otherwise healthy preterm infants but in very low birth weight (VLBW) infants it can take up to 8 days.<sup>122</sup>

### **Phase I fluid and electrolyte management clinical goals<sup>122</sup>**

Allow the physiological ECF contraction, although aiming not to compromise intraventricular fluid (IVF) volume and cardiovascular function.

- Allow a negative Na<sup>+</sup> balance (2-5 mmol Na /kg/day).
- Maintenance of normal serum electrolytes.
- Allow sufficient urine loss to excrete waste (urea, acid equivalents, etc).
- Avoid oliguria (less than 0.5-1.0 ml of urine/kg/h) for longer than 12h.
- Provide enough fluids to allow transepidermal evaporation facilitating the body thermoregulation,
- Administration of sufficient calories to keep maintenance needs (around 40-60 kcal/kg/day).

### **Phase II: (The intermediate phase)**

This phase is characterised by diminished insensible water loss and:

- Increased cornification of the epidermis
- Fall in urine volume (less than 1 to 2 ml/kg/day)
- Low Na<sup>+</sup> excretion

### **Phase II fluid and electrolyte management clinical goals: <sup>122</sup>**

- Electrolyte replenishment for losses that might have occurred during the Phase I, due to ECF contraction;
- Electrolyte and fluid replenishment for losses occurring in this phase to secure homeostasis;
- Increase of enteral feedings.

### **Phase III: (Stable growth)**

Characterized by:

- Epidermis totally cornified
- Mature kidney function, completely adapted to the extrauterine conditions

- Low Na<sup>+</sup> excretion

**Phase III fluid and electrolyte management clinical goals:** <sup>122</sup>

- Full enteral intake of fluids and electrolytes
- It must be ensured that water and electrolytes are given in sufficient amounts to both maintain homeostasis and support tissue accretion - ideally at a rate comparable to the intrauterine growth (around 15-20 g/kg/day).

## **1.5.2 Enteral Nutrition**

The most recent recommendations regarding enteral nutritional support for preterm infants by ESPGHAN were published in 2010.<sup>13</sup> These recommendations are mainly based on available data for a stable growing preterm infants with body weight up to 1800g. Unfortunately scientific basis to estimate nutrient needs according to specific developmental stages/disease categories is lacking in most cases.<sup>13</sup>

### **1.5.2.1 Minimal Enteral feeds**

In the past years it has become a universally accepted clinical practice that minimal enteral nutrition should be started from the very first day of life which is known as “trophic feeding”. Trophic feeding represents administration of small feeds i.e.  $\leq 24\text{ml/kg/d}$  to promote postnatal gut maturation and avoid mucosal atrophy.<sup>123,124</sup> Depending on body weight and clinical condition, enteral feeds are generally progressively advanced by 10-35ml/kg/day.<sup>72</sup>

### **1.5.2.2 Energy**

Growth model for preterm infants is the developing fetus of the same postmenstrual age both in terms of weight gain as well as body composition. Based on clinical evidence, it is suggested that protein to energy ratio should be adequately maintained i. e. >3-3.5g/100kcal which may help to attain fat mass percentage closer to both intrauterine reference and normal term infants.<sup>125</sup>

For a healthy growing preterm infant recommended energy supply is 110-135Kcal/kg/d.<sup>13</sup> Furthermore, it is suggested that energy supply beyond 135kcal/kg/d may not be of benefit for infants whose growth is inadequate because it is more likely that other nutrients are limiting.<sup>13</sup>

### **1.5.2.3 Glucose**

For enteral nutrition glucose requirements are estimated by calculating the total energy needs of the growing infant. Keeping in view that protein (amino acids) has specific functions energy needs should be mainly fulfilled by glucose and fats. The ideal ratio of glucose/lipids is still not determined.<sup>111</sup>

For a healthy growing preterm infant the recommended range for glucose is 11.6-13.2g/kg/d.<sup>13</sup>

### **1.5.2.4 Proteins**

Protein requirements estimated by empirical formula suggest that a continuation of in utero weight gain can be achieved by protein supply of approximately 3 g/kg/day. There is a linear relationship between weight gain and protein intake up to amounts of 4.5g/kg/day of protein<sup>13</sup>

It is important to supply protein in an ideal composition of amino acids including all essential amino acids to promote lean body mass accretion. With protein supply of <3-3.5g/kg/day accompanied by high energy supply, intrauterine similar weight gain can be achieved but it will lead to higher body fat than in the normal foetus.<sup>13</sup>

Studies have reported reduced production of IGF-1 due to under-nutrition. Low concentrations of IGF-1 are associated with lower rates of growth parameters.<sup>126</sup> Therefore, protein intake of 4.0-4.5 g/kg/day is recommended for infants weighing up to 1000g and 3.5-4.0 g/kg/day for infants weighing 1000-1800g.<sup>13</sup>

### **1.5.2.5 Lipids**

Approximately 40 to 55% of the total energy from human milk is provided by fats, with an average fat content of about 3.8 g/100 ml. However, fat content is highly variable in human milk, depending on gestational age, location, maternal diet, etc. Triglycerides are the major fat component in human milk (98%), followed by phospholipids (0.7%) and cholesterol (0.5%).<sup>118</sup> In preterm infants, absorption of enterally-provided lipids is not very efficient, due to low levels of digestive enzymes and low concentrations of bile salts.<sup>118</sup> During the last trimester of pregnancy essential fatty acids (alpha-linolenic acid, linoleic acid) and their products (docosahexaenoic acid/ omega 3 and arachidonic acid/ Omega 6) are provided to growing fetus in ample amounts for brain growth.<sup>118</sup> Long chain polyunsaturated fatty acids (LC-PUFA) are essential for brain and visual development. Studies reported significant benefits in regard to visual acuity<sup>127</sup> and some developmental scores<sup>128</sup> in patients who received higher doses of dietary essential fatty acids.

Considering this information, ESPGHAN recommends 4.8-6.6g/kg/day fat intake for preterm infants.<sup>13</sup>

### 1.5.2.6 Human/Breast Milk

Human milk is considered to be the standard diet for preterm infants, however it should be fortified to meet requirements.<sup>13</sup>

The American Academy of Pediatrics and World Health Organization recommendations suggest that if mother milk is not available, pasturized donor breast milk should be provided as first alternative.<sup>129</sup>

In many developed countries, human milk banks have been established to provide pasturized breast milk to preterm infants. ESPGHAN committee on nutrition has recently published that the presence of human milk banks does not influence breastfeeding rate but it certainly reduces the use of formula milk during the early weeks of life.<sup>129</sup>

There are several studies reporting nutritional, gastrointestinal, immunological and developmental benefits of breast milk and there is evidence that it may influence later health outcomes in preterm infants.<sup>130-132</sup> Human/Breast milk feeds deliver unique structural proteins and a special patterns of fatty acids which provide further nutritional benefits for the growing infant.<sup>133</sup> Protein from human milk is easily digestible as compared to bovine milk. Moreover, human milk also contains numerous bioactive factors e.g. lactoferrin, lasozyme and immunoglobulins that enhance the immune system and facilitate gastrointestinal maturation processes.<sup>134</sup> Long chain fatty acids present in breast milk play an important role in cognition, growth and visual development in preterm infants.<sup>135,136</sup> In addition to that, oligosacchrides and lactose from human milk also provide additional protection to the host mucosa by preventing bacterial attachment.<sup>137</sup>

Studies have documented that the incidence of necrotizing enterocolitis (NEC)<sup>138</sup> and bronchopulmonary dysplasia (BPD)<sup>139</sup> was significantly reduced in infants receiving human milk compared to infants who received formula milk. Breast milk is also associated with higher scores for cognitive development than is formula milk.<sup>131</sup> Surprisingly these benefits are more prominent in low birth weight infants.

Low birth weight infants exhibited a 5.2 point advantage in IQ when fed breast milk.<sup>131</sup>

## **Human/Breast milk composition**

Preterm infants and particularly extremely preterm infants require higher amounts of protein, energy and minerals. Breast milk alone, is unable to provide sufficient nutrients to fulfill their needs.

It is important to determine breast milk composition so that increased needs of these vulnerable infants can be met by providing fortified breast/human milk feeds. The composition of breast/human milk varies with length of gestation, time during each lactation episode and other maternal factors. Mothers with preterm deliveries have to face many challenges. Fortunately there are higher levels of protein, sodium, chloride, calcium, zinc, copper and folate in preterm milk than term breast milk.<sup>140</sup>

A study in 2013 assessed the composition of human milk samples by using a device called mid-infrared analyzer Milkoscan Minor.<sup>141</sup> Their results included mean values of protein, fat, carbohydrates and energy content of Own mother milk (OMM) (n=428), single-donor pooled HM (n=138), multiple-donor pooled HM (n=224), and pooled colostrum (n=14). Interestingly OMM contained significantly higher levels of protein, fat and energy compared to single-donor pooled HM and multiple-donor pooled HM. In pooled colostrum only the protein content was higher. However, multiple-donor pooled HM showed higher levels of protein compared to single donor. So this study concluded that the macronutrient content of human milk is widely variable. Another study reported similar findings. Protein content of human milk decreases within weeks after preterm birth. These inter-individual differences in human milk content can influence the nutritional support provided to preterm infants during hospital stay and raise the question of the need for improvement in fortifying human milk for preterm infants.<sup>142</sup>

## **Fortification**

Human milk does not meet the high nutrient demands of a growing preterm infant. Especially the protein and fat content in human milk are highly variable and protein content is inversely related to the duration of lactation. Standard multicomponent human milk fortifiers are designed to enhance the nutritional composition of breast/ human milk.<sup>143</sup> Bovine milk based fortifiers are commercially available and are being used in routine clinical practice for preterm infants born under 32 weeks gestation<sup>144</sup>, however recently human milk based fortifiers have also been introduced in some developed countries.<sup>143</sup>

Fortification is conducted either as per standardized protocol according to manufacturers' instructions or can be individualized as mentioned in ESPGHAN recommendations on enteral nutrition. Individualized fortification can be performed using either "adjustable fortification" where blood urea nitrogen (BUN) is measured in patients or by "targeted fortification" where milk samples are analysed to achieve optimized fortification.<sup>129</sup>

The current nutritional management goal for preterm infants is to avoid dropping percentiles during their stay at NICU and being small for gestational age (SGA) at discharge.<sup>129</sup> Further research is needed to explore how improved fortification of human milk for preterm infants can help to achieve this goal. There is an urgent need for randomized clinical trials in this vulnerable preterm population.

### **1.5.2.7 Attainment of full oral/suck feeds**

In preterm infants, attainment of safe oral feeding skills is an important milestone. AAP has provided guidelines for hospital discharge of high risk neonates. According to these guidelines, "prior to discharge, it is recommended that preterm infants establish competent oral feeding by breast or bottle, without cardiorespiratory compromise."<sup>145</sup> Therefore these infants need to gradually transit

from gavage feeding to oral/suck feeds. The ideal time to introduce oral feeds has yet to be established. Clinicians have to keep many factors in consideration before initiating oral feeds. Research is needed to clarify multiple issues surrounding the topic of the initiation of oral feeding in the NICU.

Attainment of earlier oral feeds can offer many benefits such as improved maternal-infant relationship, greater maternal satisfaction, shorter hospital stay and reduction in healthcare costs.<sup>146</sup>

It is a big challenge to recognise the readiness of an infant to start oral feeding. Naturally an infant's ability to develop the skill of sucking depends on multiple variables such as postmenstrual age (PMA), neurodevelopmental maturity, behavioural state organization, and likely the NICU environment.<sup>147,148</sup> A full term new born infant can receive oral feeds because he/she is born with skills to coordinate sucking, swallowing and respiration.<sup>149</sup> This coordination is also necessary to achieve successful oral feeds in preterm infants. Suck-swallow coordination is positively associated with advancing PMA.<sup>149</sup> Sometimes poor fluid management or aspiration, behavioural distress, unstable heart rate, hypoxia during feedings, increased energy expenditure and poor weight gain are the consequences of initiation of oral feeds at the wrong time.<sup>148</sup>

It is important to evaluate feeding performance and provide support towards development of sucking skills in order to achieve successful oral feeding.

## **2. METHODS**

### **2.1 Hypotheses and Aims**

There are guidelines available for preterm nutritional support during hospitalization by the Nutrition committee of ESPGHAN. For clinical use unit-specific mixed feeding protocols are planned keeping in view the parenteral nutrition and enteral nutrition guidelines to meet the daily nutrient requirements. A standardized feeding protocol was implemented in NICU- Graz and we hypothesized that:

- The daily amount of each nutrient (protein, glucose, fat) energy and fluid does not differ from current ESPGHAN guidelines for preterm infants
- Growth and body composition does not vary with gestational age in preterm infants

#### **Aims:**

- To measure the actual daily amount of each nutrient (protein, glucose, fat) energy and fluid and compare it with current ESPGHAN guidelines for preterm infants
- To analyse the role of gestational age on growth and body composition of preterm infants

A prospective cohort study was conducted including all preterm infants born <32 weeks entering the NICU during a 1-year period. Inclusion criteria was preterm birth (<32 weeks), and exclusion criteria were congenital malformations, metabolic disorders and conditions requiring surgical interventions.

### **2.2 Ethical Considerations**

The parents of the study population gave consent for participation in the study and for the use of anonymized data for scientific research. The study was approved by

the Ethics Committee of The Medical University of Graz. Approval letter notification number: 26-208 ex 13/14

## **2.3 Data Collection**

Data related to the actual daily nutrient intake was electively collected from routine nutrition monitoring system **Centricity**® Electronic Medical Record (EMR) from GE Healthcare at bedside of the patients by a single observer. Anthropometric parameters (weight, length, and head circumference (HC) were recorded from birth to discharge. Length and HC were measured weekly and weight was measured daily. Whole body composition was assessed only before discharge. Data regarding any perinatal diagnosis and medical treatment which could have had an impact on postnatal growth were also recorded.

### **2.3.1 Growth Parameters**

All anthropometric parameters were measured with standardized techniques. All measurements were performed twice. An average of two values was recorded. If the measurements differed by more than 5%, additional measurements were performed and the median value was recorded.

#### **Weight**

The infants were weighed naked daily on an electronic scale (Soehnle Scale CWB 7726, Made in Germany) during their stay in hospital. The scales were calibrated with a maximum weight deviation of +/- 2g. The data on body weight was collected daily and analyzed each week until discharge. All measurements were performed at a same time each day.

#### **Length**

Recumbent length was measured weekly with SECA 210 Mobile measuring mat for babies and toddlers (Vogel & Halk GmbH & Co Hamburg, Germany). The infant was measured lying in a supine position with one examiner holding the

infant's head in a midline position with the top of the head touching the fixed head board, while a second examiner extended the legs and firmly placed the moveable foot board against the infant's heels.<sup>150</sup>

### **Head Circumference**

Head circumference was measured weekly. It was determined by applying a non-flexible measurement tape firmly around the head above the supraorbital ridges, at the most prominent part of the frontal bulge anteriorly and over the part of the occiput that gave the maximum circumference.<sup>151</sup> (sasanow SR 1986).

### **Body Composition**

Body composition was assessed by using an infant sized air-displacement plethysmography instrument known as PEA POD; (Life Measurement Inc, Concord CA). Studies have proven the PEAPOD device as a reliable and accurate tool for determining body fat percentage in infants.<sup>152,153</sup> This system measures weight and volume of infants weighing between 1 and 8kg. The naked infant is placed in a closed chamber. Air displacement is measured using pressure and volume changes. The calculated body volume and body mass are used to determine body density. Age and gender specific values for fat free mass density are used to calculate the body fat percentage.<sup>154</sup>

## **2.3.2 Nutrient Intakes**

Daily amounts of parenteral nutrition and enteral nutrition are routinely recorded in our nutrition monitoring system Centricity® Electronic Medical Record (EMR) from GE Healthcare. We adapted our software to calculate the total macronutrient, energy and fluid intake derived from enteral and parenteral nutrition over every 24hour period.

### **Protein**

We recorded daily amounts of protein from enteral and parenteral nutrition and calculated every infant's total protein intake per day and daily averages per individual week until the infant was discharged.

### **Glucose**

We recorded daily amounts of glucose from enteral and parenteral nutrition and calculated the total glucose intake per day and daily averages per individual week until the infant was discharged.

### **Fat**

We recorded daily amounts of lipids from enteral and parenteral nutrition and calculated the total lipid intake per day and daily averages per individual week until the infant was discharged.

### **Energy**

We recorded daily amounts of calories from enteral and parenteral nutrition and calculated the total caloric intake per day and daily averages per individual week until the infant was discharged.

### **Fluid**

We recorded daily amounts of fluid from enteral and parenteral nutrition and calculated the total fluid intake per day and daily averages per individual week until the infant was discharged.

## **2.3.3 Perinatal Diagnoses**

All data is regularly updated electronically (MEDOCs software system) by the responsible doctor. A comprehensive data sheet (Arzt brief) including records on perinatal diagnosis, birth events, postnatal diagnosis and medications is available for each patient.

Data regarding perinatal diagnosis and medical treatment which were considered relevant to postnatal growth was recorded; this included intrauterine growth

restriction (IUGR), postnatal steroid treatment and the number of days on ventilation.

## **2.4 Standard Feeding Protocol Graz, 2011**

In 2011, a new enteral feeding and TPN protocol was introduced at the Department of Neonatology, Children Hospital Graz, based on ESPGHAN 2005 and 2010 Guidelines<sup>12,13</sup> focusing on the achievement of optimum growth in very preterm infants. This protocol is shown in table 1. All infants were treated according to a standard protocol that was uniformly applied. The aim of this protocol was to establish full enteral feeds by the 10th day of life whilst supplying parenteral nutrition from the first day of life to meet the recommended daily requirements for protein, lipids, carbohydrates and fluids.

### **Total Parenteral Nutrition (TPN) Protocol**

Protein was given parenterally at a dose of 2g/kg/day starting in the first 1-2 hours post-partum and continued at this dose until no longer possible due to the total amount of parenteral fluids diminishing as enteral fluids increased over the first 2 to 3 weeks of life. Lipids were started at 1g/kg/day on day 1 and then increased to 2g/kg/day from day 2 onwards until they were removed from TPN as TPN volumes diminished between week 2 and 3 of life. Carbohydrates were prescribed to fulfill several aims, on the one hand to fulfill the recommendations regarding total energy needs (in combination with parenteral lipid and protein and enteral nutrition) on the other hand to gauge TPN osmolality (max 800 mosmol for peripheral TPN or 1200 mosmol for central line TPN) and of course occasionally adaptations were made in response to blood glucose levels. Total fluids were commenced and increased very much in accordance with the ESPGHAN recommendations and adjusted as per clinical situation.

## Enteral Nutrition Protocol

Enteral feeds were commenced at a volume of approximately 1ml/kg 3 hourly and increased daily by 15 to 20ml/kg/day. Full enteral nutrition was defined as enteral feeding volumes reaching 140-150ml/kg/day. Milk feeds were fortified, once a daily volume of 150ml/kg/day had been achieved.

**Table 1. Standard Feeding Protocol Graz, 2011**

Ernährung bei Neu- und Frühgeborenen in den ersten Lebenstagen												
Lebenstage	1	2	3	4	5	6	7	8	9	10	11	12
<b>Fluessigk. ml/kg/d -GESAMT</b>												
							ca 2/3				voll	voll
NG<1000g = ELBW	100	110	120	130	140	150	160	170	180	180	180	180
NG 1000-1499g = VLBW	90	100	110	120	130	140	150	160	170	180	180	180
NG 1500g-2499g = LBW	80	90	100	110	120	130	140	150	160	170	180	180
NG >2500g	70	80	90	100	110	120	130	140	150	160	160	160
<b>Strategie:</b>	Erwarte und akzeptiere ein Gewichtsverlust in den ersten 5-7 Tagen											
	halte Elektrolyte im Normbereich:											
	in ersten 2-3 Tagen bei Hyponatriämie: durch Flüssigkeitsrestriktion mit aliquote Anpassung der Glukosezufuhr											
	in ersten 2-3 Tagen bei Hypernatriämie: durch Erhöhung der Flüssigkeitsmenge event. mit Anpassung der Glukosezufuhr											
<b>Enterale Ernährung (siehe Schema für FG&lt;1500g )</b>												
enteral: ml/kg/Tag	8	24	40	56	80	96	112	128	144	160	180	180
<b>PARENTERALE ERNAEHRUNG in den ersten Lebenstagen - Teil 1</b>												
Glukose g/kg/d -parenteral	5-6	7-8	9-10	11-12	13-14	15-16	16-18	16-18	16-18	16-18	16-18	16-18
mg/kg/min	4-8	bis max 13mg/kg/min										
Protein g/kg/d -parenteral												
<1500g	2	2	2	2	2	2	1	1	1	1	0	0
>1500g	2	2	2	2	2	2	1	1	1	1	0	0
Fett g/kg/d -parenteral												
<1500g	1	2	2	2	2	2	1	1	1	1	0	0
>1500g	1	2	2	2	2	2	1	1	1	1	0	0
Glukose: Zahlen gelten für rein parenterale Ernährung; bei normale enteraler Aufbau sollte Glukosezufuhr je nach EQ und BZ angepasst werden.						<b>Protein:</b>	<b>Fett:</b> SMOF-Lipide bei schwere Sepsis parenterale Lipide auf 1g/kg begrenzen;					

## Breast Milk

For enteral nutrition, the majority of mothers chose to provide breast-milk. However, the volumes of own mother milk varied and therefore most infants were fed a combination of breastmilk, fortified breast-milk, pasteurized fortified and unfortified donor breast-milk and preterm formula. Mother milk samples (n =200) were analyzed using Miris Human Milk analyzer. Average nutrient content was 1.3g of protein/100ml, 7.3g of Lactose/100ml, 3.5g of fat/100ml and 70Kcal/100ml.

## **Donor Breast Milk**

In selected cases where mother`s milk was not available, donor breast milk was offered. A large milk bank was established at The Children`s Hospital of Graz in the beginning of 19<sup>th</sup> century. Mothers of preterm babies can donate excess amounts of breast milk on a daily basis. This milk is pasteurized and then stored at suitable temperature and is fed to infants according to following criteria:

Depending on the availability of sufficient donor milk, we prioritize the smallest and most vulnerable infants to receive donor milk if their mother`s milk is not available in adequate amounts. We aim to provide all infants under 1800g with either mother`s milk or donor milk.

## **Mother Milk Fortifier**

In VLBW infants Breast milk fortifier is added to enrich the mother's milk. Current literature seems conclusive on the idea that unfortified breast milk can neither support normal ossification of bones in preterm infants nor meet their energy and protein needs.

## **Fortification Protocol**

When 50% of total enteral feeding volume has been achieved, 1% fortification is started with a subsequent increase to 2% in the following few days if this is well tolerated. Full (4%) fortification is only aimed for after reaching full enteral feeds.

Fortification is usually stopped 1 week prior to discharge. In infants receiving only mother`s milk, we recommend additional Ca and P for prophylaxis of Osteopenia post discharge

As alternative to mother`s milk, we prescribe a post-discharge formula (BEBA FG 2, Aptamil PDF) until reaching a weight of 5kg.

Currently, there is only one product available as human milk fortifier ("Aptamil FMS") in Graz. It dissolves in cold milk. Following fortification-concentrations are available: 1%, 2%, 3%, and 4%.

Nutrient Composition:

Fortification with 2% means an increase in energy quotient (EQ) to 77kcal / 100ml, and fortification with 4% increases the EQ to 85kcal / 100ml.

In addition, the protein content is raised up to 2.6g/100ml with 4% fortification. (table 2).

**Table 2: Nutrient Composition of mother milk fortifier**

<b>Milupa Aptamil FMS</b>		FrauenMilchSupplement				
Standard- auflösung	100 ml Frauenmilch + 4,2 g Milupa Aptamil FMS					
Zusammen- setzung		100 g Pulver	1 g Pulver	4,2 g Pulver	100 ml Frauenmilch <sup>1)</sup>	100 ml Frauenmilch <sup>1)</sup> + 4,2 g Pulver
Energie	kJ	1535	15,5	65	293	356
	kcal	361	3,6	15	70	85
Eiweiß	g	19	0,19	0,8	1,8	2,6
Kohlenhydrate	g	71,5	0,71	3	7	10
davon:						
Glucose	g	1,0	0,01	0,04	0	0,04
Lactose	g	0,7	0,01	0,03	7	7,03
Maltose	g	3,1	0,02	0,1	0	0,1
Polysaccharide	g	62,4	0,61	2,6	0	2,6
Fett	g	0	0	0	4	4
<b>Mineralstoffe</b>						
Natrium	g	0,235	0,002	0,01	0,03	0,04
Kalium	mg	184	1,9	8	60	68
Calcium	mg	1545	15,5	65	22	87
Magnesium	mg	143	1,4	6	2,5	8,5
Phosphor	mg	1070	10,7	45	14	59
Chlorid	mg	166	1,7	7	59	66
Eisen	mg	0	0	0	0,09	0,09
Zink	mg	9,5	0,1	0,4	0,39	0,79
Kupfer	mg	0,62	0,001	0,03	0,063	0,093
Jod	µg	261	2,6	11	18	29
Mangan	µg	190	1,9	8	0,36	8,36
<b>Vitamine</b>						
Vitamin A	mg	3,1	0,03	0,13	0,014	0,144
Vitamin B <sub>1</sub>	mg	3,1	0,03	0,13	0,009	0,14
Vitamin B <sub>2</sub>	mg	4,0	0,04	0,17	0,03	0,2
Vitamin B <sub>6</sub>	mg	2,6	0,03	0,11	0,006	0,116
Pantothensäure	mg	18	0,18	0,75	0,23	0,98
Folsäure	µg	1188	11,9	50	3,1	53,1
Niacin	mg	58	0,57	2,4	0,21	2,61
Vitamin B <sub>12</sub>	µg	4,8	0,05	0,2	0,02	0,22
Vitamin C	mg	285	2,86	12	4,5	16,5
Vitamin D	µg	119	1,19	5,0	0,2	5,2
Vitamin E	mg	62	0,62	2,6	0,3	2,9
Biotin	µg	59	0,59	2,5	0,5	3
Vitamin K	µg	150	1,5	6,3	2,0	8,3
Osmolarität <sub>2)</sub>	mosmol/l			84	248	355
<sup>1)</sup> Daten nach Wharton, B.A.: Nutrition and feeding of preterm infants. Blackwell, Oxford 1987 - Werte der Frauenmilch sind Durchschnittswerte und unterliegen beträchtlichen Schwankungen Tsang RC et. al. (eds.) Nutritional needs of the preterm infants. Table A.4. Williams & Williams: Baltimore, 1992; Pages 300-301 <sup>2)</sup> Osmolarität von 4,2 g Aptamil FMS in 100 ml entmineralisiertem Wasser: 93 mosmol/kg H <sub>2</sub> O						
Packungen	Klink: 200 g		Apotheker: 200 g		PZN: 2731966	

In addition to milk feeds, Multivitamin is given to all preterm infants from 2nd week of life until the end of 1<sup>st</sup> year. Vitamin D3 is given from 1<sup>st</sup> week of life until the end of 1<sup>st</sup> year and Iron supplementation is started from 3<sup>rd</sup> week until 6 to 12 month corrected age.

## **2.5 Data management and Statistical Analysis**

### **Excel Sheets**

For primary data collection, excel sheets were designed to record individual patient's details. These sheets included demographic information, birth record details, maternal age, daily nutrient intake and anthropometric measurements from birth to discharge. This data was collected by reviewing online/ offline patients' records and doctors' notes in daily charts.

After completion of data collection, averages per week were calculated for all parameters and one combined data set was created containing a complete record of all patients for further analysis.

### **Z-Scores**

The subsequent anthropometric measurements (weight, length, and HC) were converted into Z-scores according to age and gender specific growth standards developed by Fenton in 2013.<sup>82</sup> The Z-score calculator was downloaded from (<http://ucalgary.ca/fenton>).<sup>97</sup>

The Z-score calculator consists of gender specific excel sheets. Data regarding gestational age in weeks, current weight, length and HC was entered in separate sheets for boys and girls. This calculator can calculate Z scores i.e. Weight-for-age, length-for-age, HC-for-age. Additionally it also provides information regarding percentiles. Based on Fenton growth charts, we can define if infant is small for gestation age in that specific week for which calculations have been made.

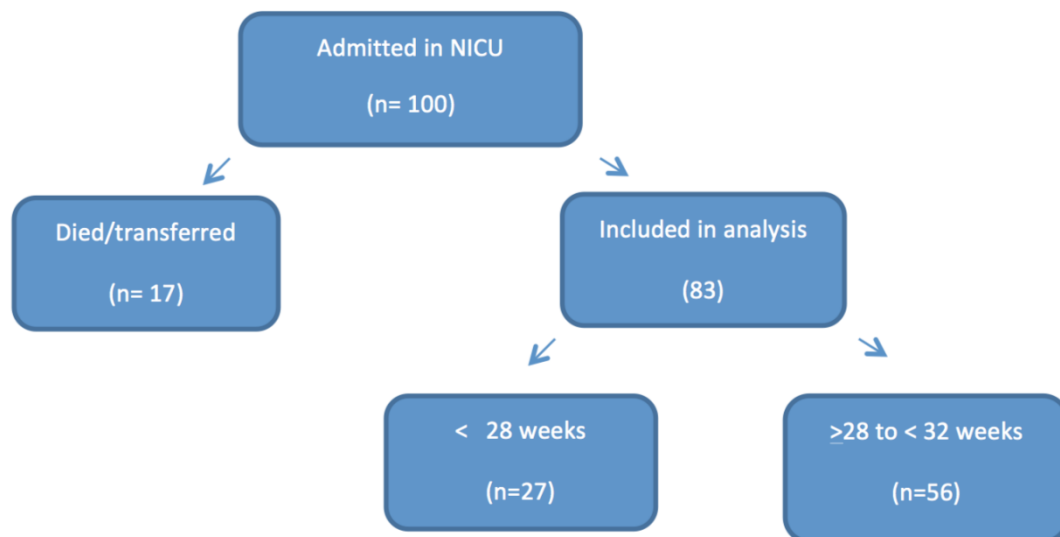
## **Statistical Analysis**

The whole data set was analyzed by using a Statistical Package for Social Sciences (SPSS, Inc, Chicago, IL) version 21 for Windows. To specify our population, mean/median and standard deviation were calculated. Differences in descriptive, nutritive and clinical characteristics between very preterm and extremely preterm infants were calculated by means of independent groups' t-tests or chi<sup>2</sup>-tests. The alpha-level for statistical significance was set at  $p < 0.05$ .

### 3. RESULTS

During the one year of observation period a total of 100 preterm infants (<32 weeks GA) were admitted to NICU-Children Hospital Graz. Infants were divided into two groups on the basis of gestational age: Extremely Preterm Infants (EPI) born < 28 weeks gestational age (GA) and Very Preterm Infants (VPI) born  $\geq$  28 to <32 weeks GA. Seventeen infants had to be excluded [transferred to surgery (n=10); died (n=7)] leaving 83 infants to be included in analysis. (see Fig. 1)

**Figure 1: Sample distribution**



#### 3.1 Basic Characteristics

Basic characteristics of the total sample and the two groups of EPI and VPI are shown separately in table 3. EPI compared to VPI stayed longer on parenteral nutrition (28.8 vs 9.3 days,  $p<0.001$ ), needed more time to reach full enteral nutrition (21.0 vs 8.7days,  $p<0.001$ ), more frequently received postnatal steroids (37% vs 1.8%,  $p<0.001$ ), were ventilated for a longer duration (19.1 vs 1.4 days  $p<0.001$ ), had less often the diagnosis IUGR (0% vs 17.9%,  $p<0.001$ ) and had a higher corrected age at discharge ( $p<0.001$ ).

**Table 3. Perinatal characteristics of preterm infants**

Characteristics	EPI (Mean±SD)	VPI (Mean±SD)	Total Sample (Mean±SD)	p-value
Gestational age (weeks)	25.48 ± 1.25	29.84 ± 1.14	28 ± 2	<0.001
Birth weight (kg)	0.76 ± 0.15	1.38 ± 0.36	1.18 ± 0.42	<0.001
Gender (n;% male/female)	M:16 (59.3%) F: 11 (40.7%)	M:26 (46.4%) F: 30 (53.5%)	M:42 (50.6%) F: 41 (49.3%)	ns
Total days on PN	28.85±23.93	9.38±4.66	15.71± 16.75	<0.001
Days to reach FEN	21.04±9.97	8.73±3.70	12.73± 8.62	<0.001
Days to initiate oral feeds	41.5 ± 16.18	10.7 ± 7.56	20.9 ± 18.3	<0.05
Antenatal Steroids (n)	23 (85.2%)	42 (75%)	65 (78.3%)	ns
Postnatal Steroids (n)	10 (37%)	1 (1.8%)	11 (13.2%)	<0.001
Maternal Age (years)	30.11±6.78	29.54± 6.01	29.72± 6.24	0.34 (ns)
Ventilation status (days of ventilation)	19.11±21.05	1.48± 3.05	7.22± 14.69	<0.001
IUGR (n)	0 (0%)	10 (17.90%)	10 (12%)	<0.001
Multiple birth (n twins)	8 (29.6%)	19 (33.9%)	27 (32.5%)	ns
Corrected age at discharge(weeks)	39.41±3.5	36.07±1.5	37.16±2.8	<0.001

## **3.2 Nutrients Supply**

The standard feeding protocol described in the methods was followed in both groups. Nutrient supply was significantly different between the two groups, during the first 5 weeks of admission. Comparison beyond 5 weeks was not possible because mean length of stay in hospital for VPI was only 6.5 weeks.

### **3.2.1 Protein**

#### **Parenteral Nutrition**

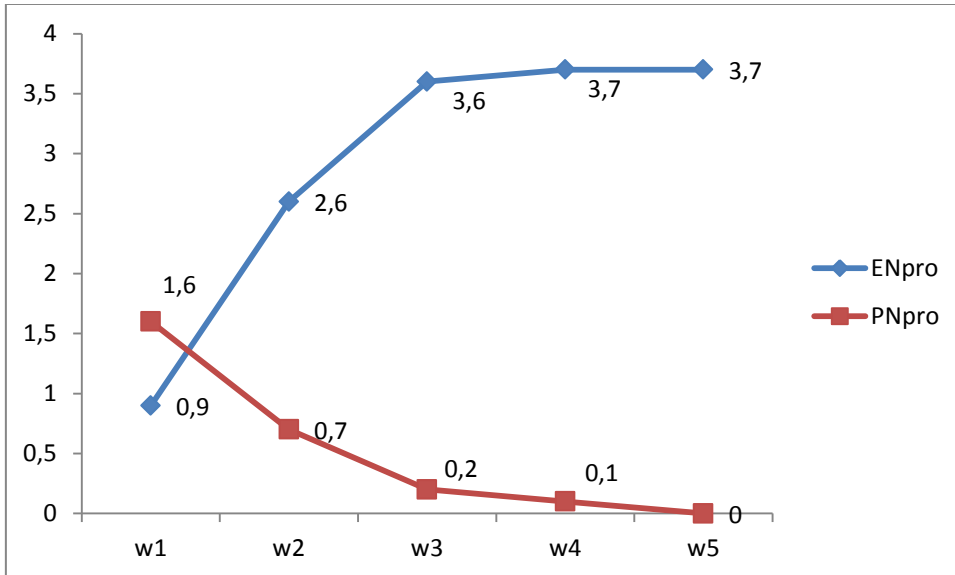
According to the protocol protein was supposed to be given parenterally at a dose of 2g/kg/day starting in the first 1-2 hours post-partum but results revealed that protein was not always administered at this dose to all preterm infants. On average, VPI received 1.5 g/kg/day and EPI received 1.75g/kg/day during the first week of life. From 2<sup>nd</sup> week of life parenteral protein intake was reduced in both groups. EPI received 47%, 27%, 19% and 13% of their total protein intake parenterally in their 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> week of life respectively. VPI received 20% and 4,7% of total protein intake parenterally during their 2<sup>nd</sup> and 3<sup>rd</sup> week of life. Parenteral nutrition was discontinued by the end of the third week in VPI.

#### **Enteral Nutrition**

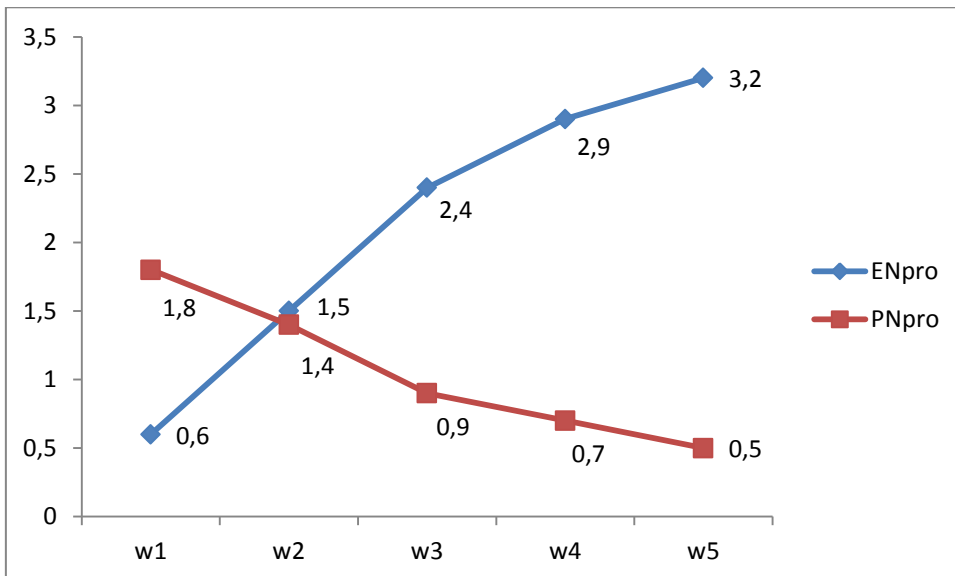
Enteral feeds were commenced at a volume of approximately 1ml/kg 3 hourly and increased daily by 15 to 20ml/kg/day. On average protein supply via enteral feeds was 0,5 g/kg/d in EPI and 0,86 g/kg/day in VPI during the first week of life. Although, according to protocol, enteral intake is gradually increased during the first 5 weeks of life, but during the 2<sup>nd</sup> and 3<sup>rd</sup> week of life enteral feeds were not able to be satisfactorily increased in EPI. Therefore protein supply was significantly affected during this critical period.

The average protein supply in both groups via parenteral nutrition and enteral nutrition is shown in figure 2 & 3.

**Figure 2. Protein supply (g/kg/d) in VPI**



**Figure 3. Protein supply (g/kg/d) in EPI**



**Total**

Total protein intake via parenteral and enteral nutrition was calculated and analyzed. EPI received significantly less protein than VPI during W2 ( $p < 0.001$ ) and

W3 ( $p < 0.001$ ), whereas there was no difference between the groups in W1, W4 and W5. Thus the cumulative supply of 5 weeks ( $p < 0.05$ ) was lower in EPI compared to VPI. A maximum protein supply of 3.73g/kg/d was recorded in both groups during first 5 weeks. Table 4

**Table 4. Average total Protein supply during first 5 weeks of life**

weeks	Protein intake (g/kg/d)				
	EPI	VPI	P-Value	(95% CI)	
				Lower	Upper
Week1	2.40	2.46	0.39	-0,18	0.07
Week2	2.95	3.39	0.00*	-0.65	-0.22
Week3	3.40	3.80	0.00*	-0.66	-0.12
Week4	3.68	3.77	0.49	-0.08	0.12
Week5	3.73	3.73	0.97	-0.24	0.25
True Cumulative	113.1	120.0	0.02*	-12.76	-1.11

### 3.2.2 Glucose

#### Parenteral Nutrition

The decision regarding the amount of glucose to be administered parenterally is based on total energy needs and limitations due to TPN osmolality. On average EPI received 4.2 g/kg/d and VPI received 3.5 g/kg/d of parenteral glucose during the first week of life.

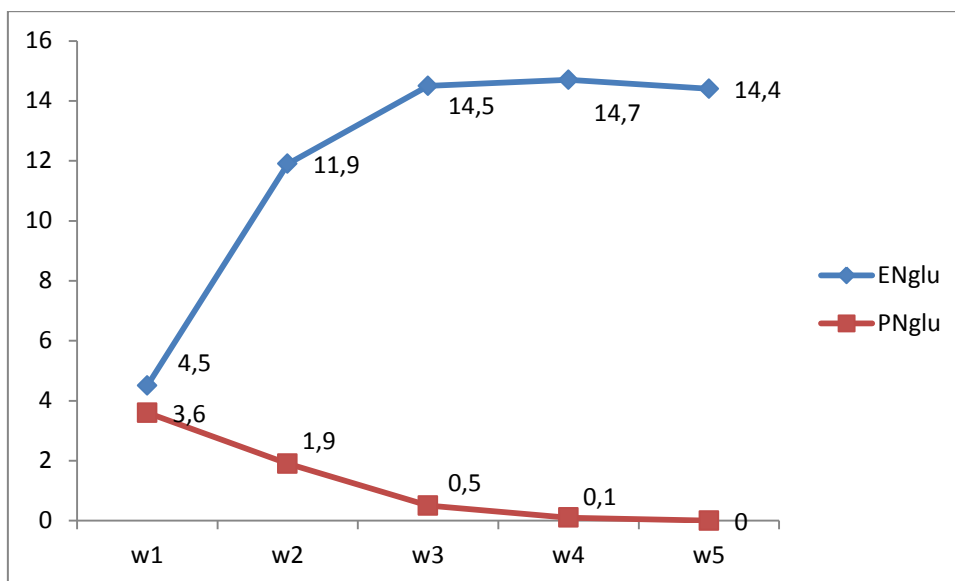
Parenteral administration of glucose to EPI reached 31%, 18%, 16% and 15% of total glucose intake in 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> week of life respectively. VPI received minor amounts of parenteral glucose in the 3<sup>rd</sup> week.

## Enteral Nutrition

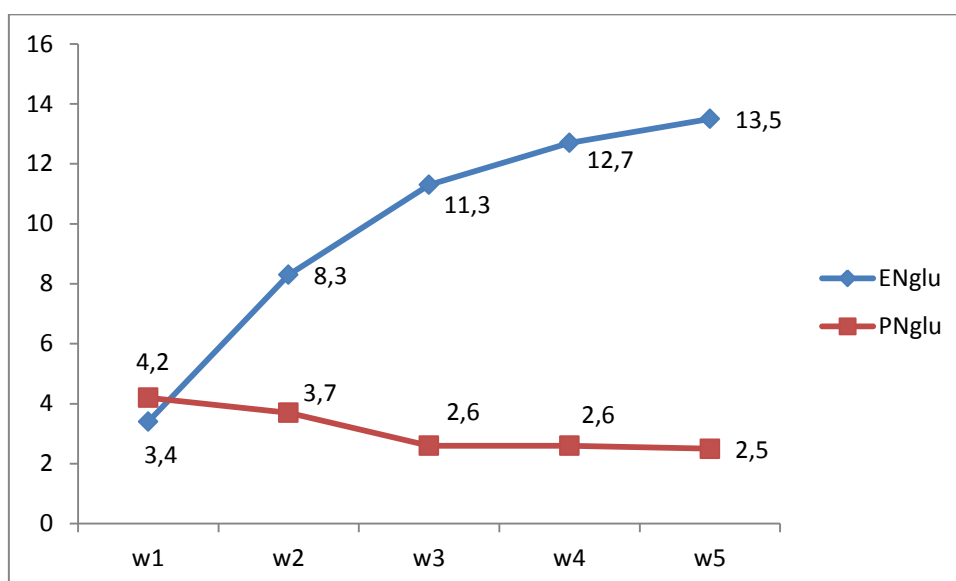
Enteral feeds were commenced on the first day of life. On average during the first week of life glucose was supplied via enteral nutrition at rates of 3.3 g/kg/d in EPI and 4.4 g/kg/day in VPI. According to protocol, there were gradual increases in enteral feeds during the 5 weeks observation period. Fortification of enteral feeds led to increased glucose supply in both groups.

The average glucose supply in both groups via parenteral nutrition and enteral nutrition is shown in figure 4 & 5.

**Figure 4. Glucose supply (g/kg/d) in VPI**



**Figure 5. Glucose supply (g/kg/d) in EPI**



**Total**

EPI received less glucose (g/kg/d) in W2, W3, whereas there was no difference between groups in W1, W4. However, during W5 EPI received significantly more glucose than VPI. We did not find any significant difference in the cumulative supply of 5 weeks between the both groups. (Table 5)

**Table 5. Average total glucose supply during first 5 weeks of life**

weeks	Glucose intake (g/kg/d)				
	EPI	VPI	P-Value	(95%CI)	
				Lower	Upper
Week1	7.66	8.12	0.06	-0.95	0.02
Week2	12.01	13.80	0.00*	-2.51	-1.07
Week3	13.92	15.03	0.01*	-2.00	-0.23
Week4	15.31	14.81	0.37	-0.61	1.60
Week5	16.0	14.4	0.03*	0.09	3.09
True Cumulative	454.4	463.8	0.43	-33.02	14.35

### 3.2.3 Fat

#### Parenteral Nutrition

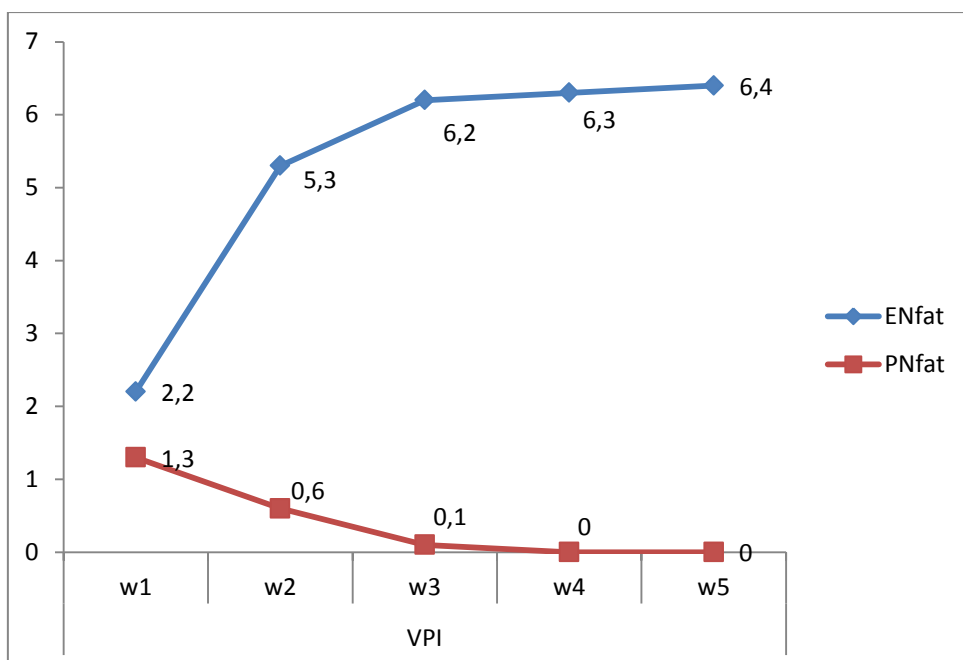
Fat/Lipids were started at 1g/kg/day on day 1 and then increased to 2g/kg/day from day 2 onwards until they were removed from TPN as TPN volumes diminished between week 2 and 3 of life. VPI received lipids parenterally only until the 2<sup>nd</sup> week whereas the EPI group continued to receive parenteral lipids until the 5<sup>th</sup> week of life.

#### Enteral Nutrition

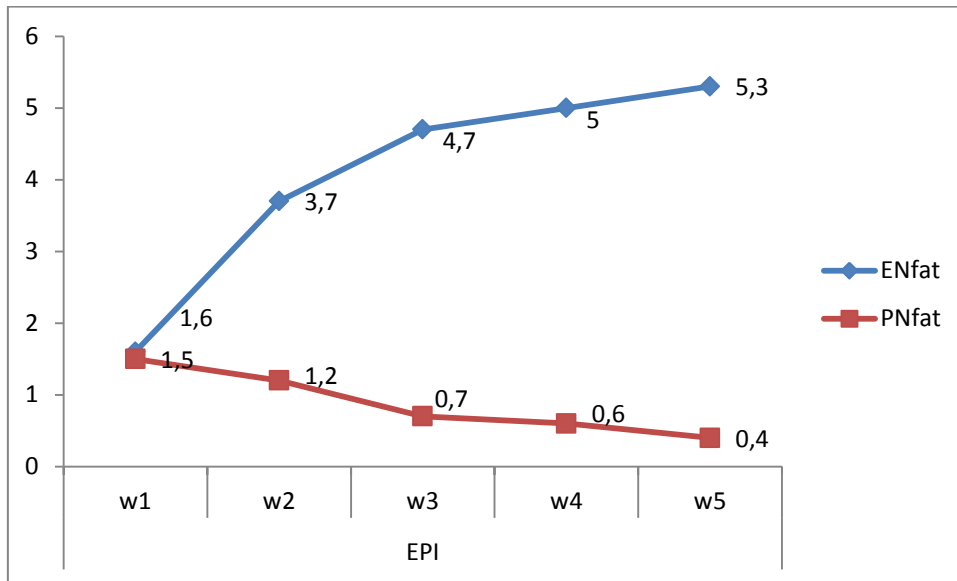
Lipid supply through enteral nutrition increased gradually during the first weeks of life. There was significant increase in lipids supply in VPI group whereas EPI group was receiving less lipids.

The average fat supply in both groups via parenteral nutrition and enteral nutrition is shown in figure 6 & 7.

**Figure 6. Fat supply (g/kg/d) in VPI**



**Figure 7. Fat supply (g/kg/d) in EPI**



**Total**

EPI received significantly less fat (g/kg/d) compared to VPI in all 5 weeks. Thus the cumulative supply in 5 weeks was lower in EPI compared to VPI. (table 6)

**Table 6. Average total Fat supply during first 5 weeks of life**

weeks	Fat intake (g/kg/d)				
	EPI	VPI	P-value	(95%CI)	
				Lower	Upper
Week1	3.15	3.50	0.01*	-0.60	-0.08
Week2	4.93	5.91	0.00*	-1.44	-0.51
Week3	5.41	6.31	0.00*	-1.32	-0.47
Week4	5.65	6.39	0.00*	-1.15	-0.34
Week5	5.82	6.40	0.01*	-1.01	-0.14
True Cumulative	174.7	198.1	0.00*	-34.7	-12.1

### 3.2.4 Energy

#### Parenteral Nutrition

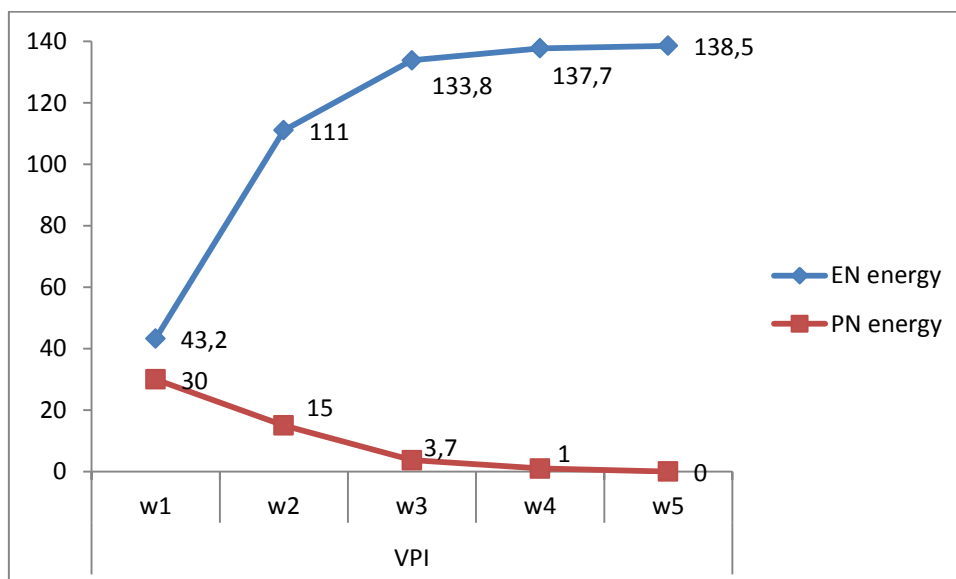
Carbohydrates and Fats are considered energy sources for growing preterm infants, sparing proteins for tissue build up. As mentioned earlier, EPI received parenteral fats and glucose longer than VPI. Therefore in the EPI group parenteral supply was continued until the 5<sup>th</sup> week whereas VPI received only during 1<sup>st</sup> & 2<sup>nd</sup> week of life.

#### Enteral Nutrition

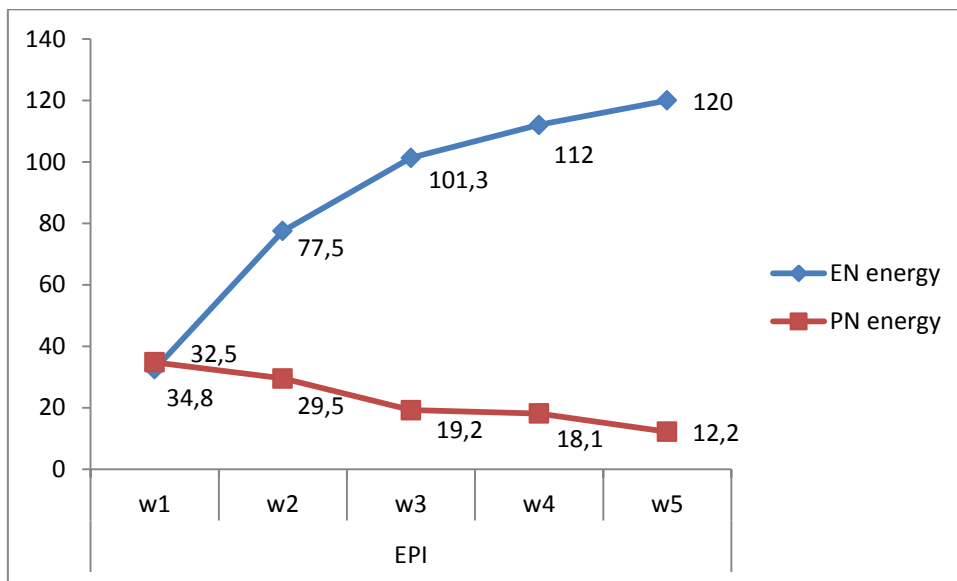
Enteral intake progressed faster in VPI and they were receiving sufficient energy from FEN by the 3<sup>rd</sup> week of life whereas the EPI group could not achieve FEN by the 3<sup>rd</sup> week. Energy supply through enteral feeds was less than the requirements and therefore needed to be supplemented by parenteral means.

Figure 8 & 9 show the average energy supply in both groups via parenteral nutrition and enteral nutrition.

**Figure 8. Energy supply (Kcal/kg/d) in VPI**



**Figure 9. Energy supply (Kcal/kg/d) in EPI**



### **Total**

As a consequence of the above results EPI received significantly less energy (Kcal/kg/d) in comparison to the VPI in single weeks (W1 ( $p<0.001$ ), W2 ( $p<0.001$ ), W3 ( $p<0.001$ ), W4 ( $p<0.05$ ) and in the 5 week cumulative calculation ( $p<0.001$ ). Table (7)

### **3.2.5 Fluid**

#### **Parenteral Nutrition**

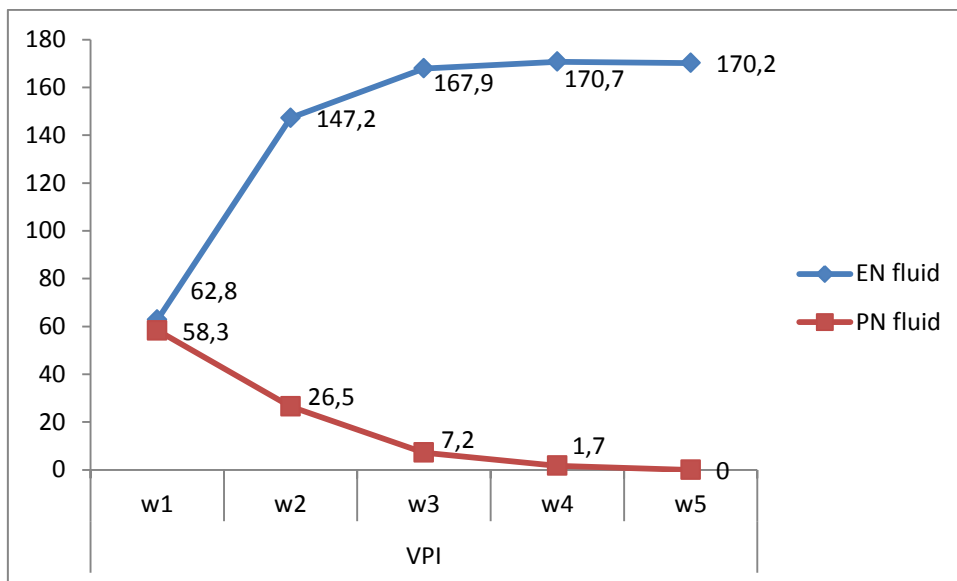
Total fluids were commenced and increased very much in accordance with ESPGHAN recommendations and adjusted as per clinical situation. The majority of the VPI group received parenteral fluids until 10<sup>th</sup> day of life whereas the EPI group continued to receive parenteral fluids until the 5<sup>th</sup> week of life.

## Enteral Nutrition

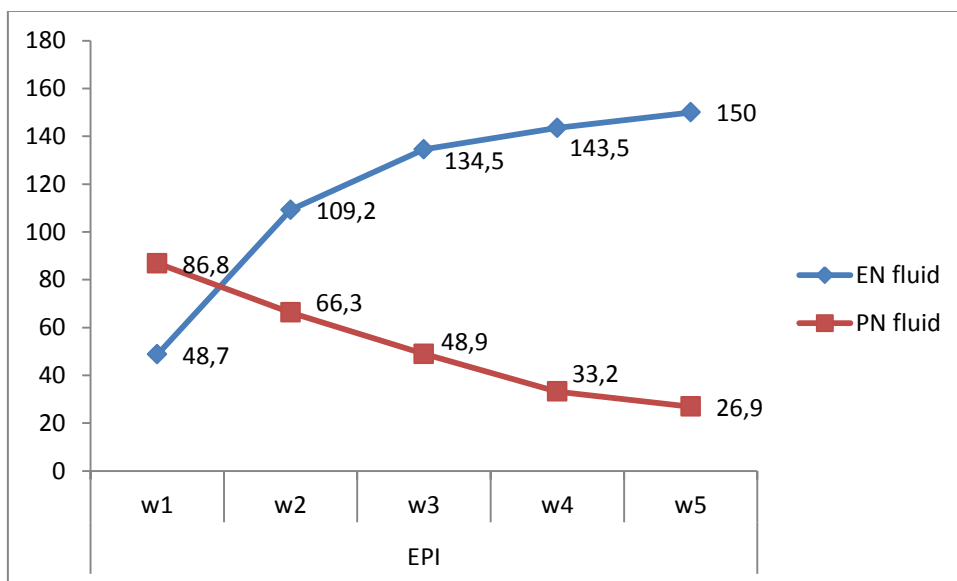
Enteral intake of VPI group increased significantly during 2<sup>nd</sup> week of life. EPI group received mixed feeds to meet ESPGHAN fluid recommendations.

Figure 10 & 11 show the average fluid supply in both groups via parenteral nutrition and enteral nutrition.

**Figure 10. Fluid supply (ml/kg/d) in VPI**



**Figure 11. Fluid supply (ml/kg/d) in EPI**



## Total

There was a significant difference in fluid supply (ml/kg/d) between both groups in W1 (EPI: 135.8 vs. VPI: 121.3 ml/kg/d;  $p < 0.001$ ) and in the cumulative 5 weeks supply (EPI: 5941.3 vs. VPI: 5677.5 ml/kg/d;  $p < 0.001$ ). (Table 7)

**Table 7: Average Energy and Fluid supply during first 5 weeks of life**

Energy & Fluid  weeks	Calories intake (Kcal/kg/d)					Fluid intake (ml/kg/d)				
	EPI	VPI	P- Value	(95% CI)		EPI	VPI	P-Value	(95% CI)	
				Lower	Upper				Lower	Upper
Week1	67.6	73.4	0.00*	-9.72	-1.94	135.8	121.3	0.00*	8.99	20.08
Week2	106.7	125.9	0.00*	-26.91	-11.49	175.4	173.3	0.60	-5.82	9.96
Week3	120.8	137.7	0.00*	-24.21	-9.61	183.4	174.9	0.10	-1.70	18.86
Week4	130	138.8	0.02*	-16.68	-0.91	177.3	172.4	0.18	-2.37	12.18
Week5	135.16	138.54	0.42	-11.72	4.96	176.2	170.2	0.13	-1.98	14.98
True Cumulative	3051.96	3365.22	0.00*	-467.2	-159.24	5941.3	5677.5	0.04*	88.8	437.7

### 3.3 Enteral Nutrition and Fortification

By the 5th week of life 100% of VPI and 54% of EPI were receiving full enteral nutrition (FEN). The remaining 46% of EPI were receiving a combination of enteral and parenteral feeds. 61% of VPI and 88% of EPI were exclusively receiving human milk. Fortified milk feeds were started at 2% fortification and progressed to achieve 4% fortification. Table 8 shows day of life when fortification was initiated. As mentioned earlier EPI reached full enteral nutrition later than VPI (table 3), as a consequence of this fortification was also started significantly later which resulted in lower nutrient supply in weeks 2 & 3.

**Table 8. Day of life to start fortified feeds**

Days to reach fortified feeds						
groups	2% fortified	p-value	4% fortified	p-value	Enteral volume @ start of fortification	p-value
VPI	10.6	<0.001	13.4	<0.001	149.9	<0.05
EPI	15.4		20.2		136.8	

### 3.4 Growth Parameters

#### 3.4.1 Weight

Mean birth weights of EPI and VPI were 0.76 kg and 1.38 kg respectively. To analyze growth patterns of preterm infants' weight was measured daily and analyzed each week until discharge. Weekly increments in body weight (kg) during the first 5 weeks of life are shown in figure 12. Weight gain velocity during average weeks of stay in both groups is shown in figure 13 and 14.

#### Weight-for-age Z Scores

Weight-for-age Z scores (WAZ) did not differ significantly between groups at birth, week 1 and week 5. However, at discharge there were significant differences in WAZ scores. (Table 8)

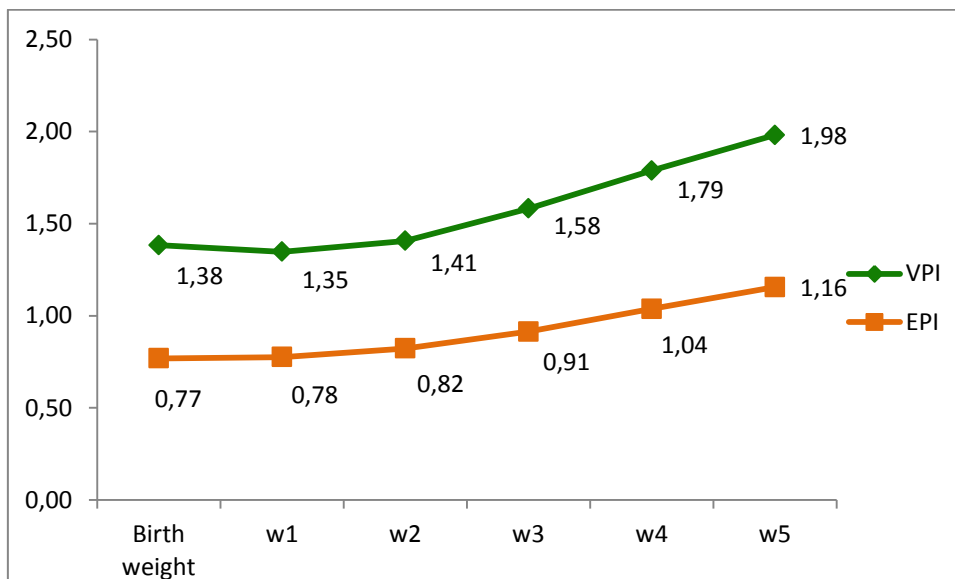
WAZ scores of both groups were below zero at birth and remained below zero throughout hospital stay. At discharge VPI could regain week 1 WAZ scores whereas EPI WAZ scores dropped further until discharge and 18.5% had Z scores less than -2SD.

## Percentiles

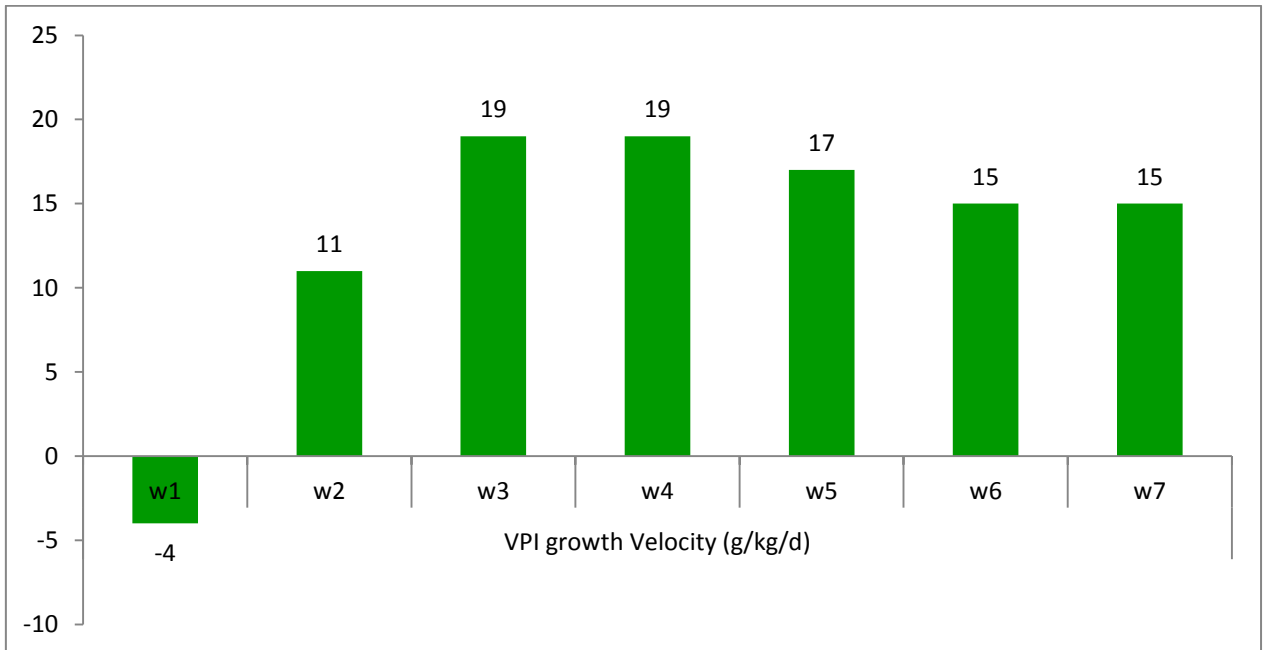
At birth the majority of our study sample was appropriate for gestational age (AGA). Only 2 infants were SGA and 1 infant was LGA in the EPI group and similarly, 2 infants were SGA and 3 infants were LGA in the VPI group. But, at discharge, 19% of VPI and 37% of EPI were SGA.

Mean time of stay at NICU from W5 to discharge was 2 weeks in VPI and 9 weeks in EPI.

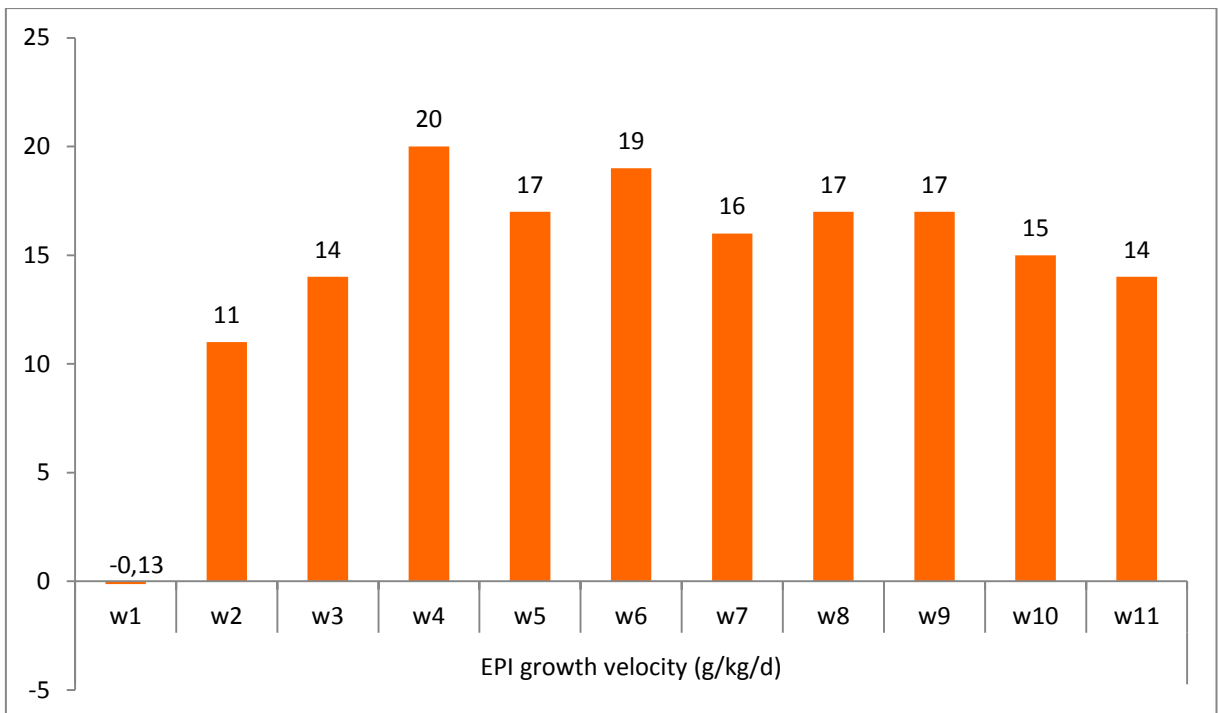
**Figure 12: Average weight (kg) in VPI and EPI during first 5 weeks of life**



**Figure 13: Average growth velocity of VPI during stay at NICU (g/kg/d)**



**Figure 14: Average growth velocity of EPI during stay at NICU (g/kg/d)**



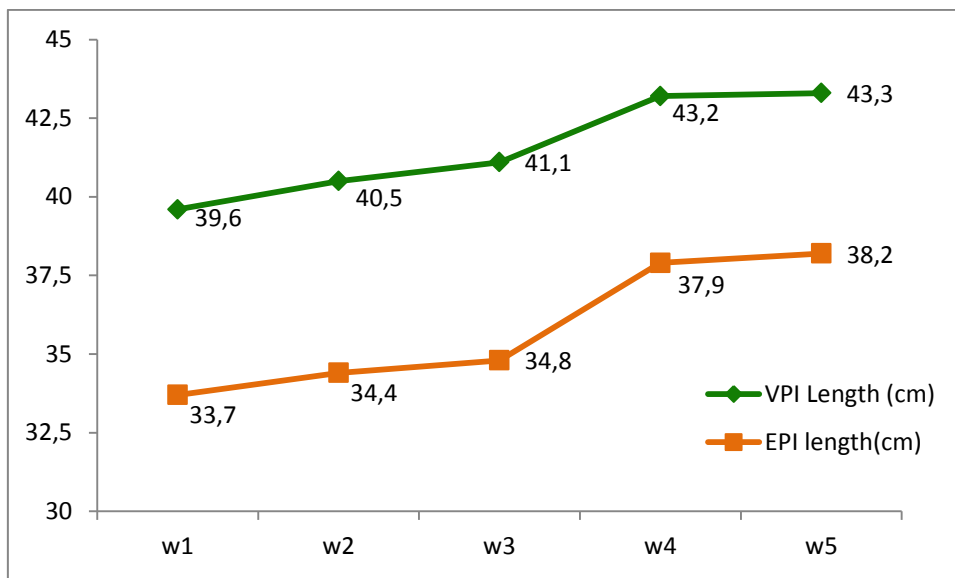
### 3.4-2 Length

Length was recorded weekly and Figure 15 shows weekly increments in length (cm) during the first 5 weeks of life in both groups.

#### Length-for-age Z scores

There were no significant differences between the groups at birth, week1 and at week 5. However, we found significant differences at discharge. VPI gained higher LAZ scores compared to EPI and 29% of EPI had LAZ less than -2SD. (table 9)

**Figure 15. Average Length (cm) in VPI and EPI during first 5 weeks of life**



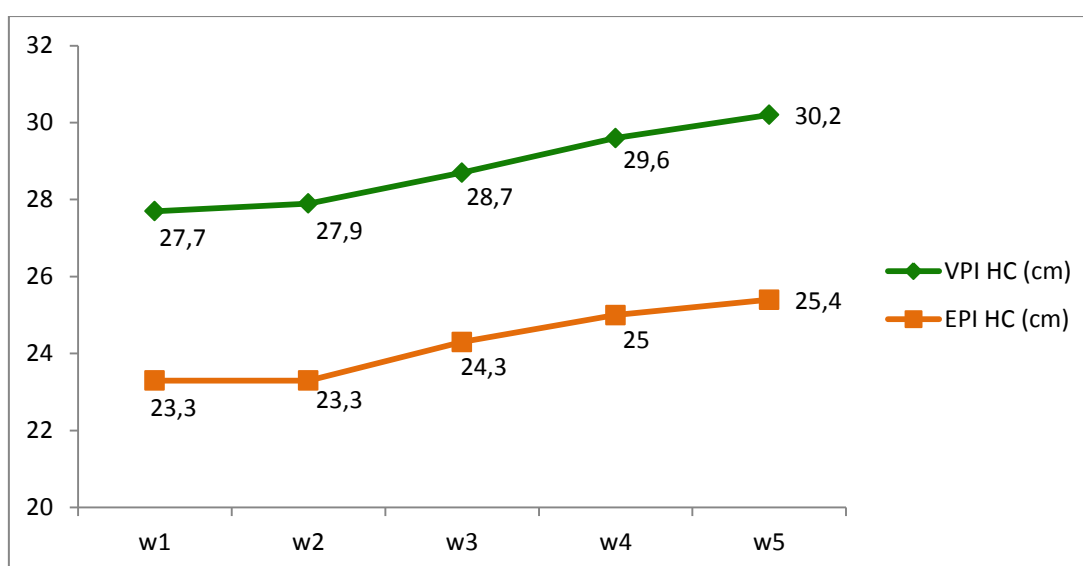
### 3.4-3 Head Circumference

Head circumference (HC) measurements were taken weekly. Head growth is critically important for preterm infants because it reflects brain growth. Figure 16 shows weekly increments in HC (cm) during the first 5 weeks of life in both groups.

## HC-for-age Z Scores

HC z scores were calculated and there were no significant differences at birth and week1 between both groups. However, at week 5, and at discharge, VPI had significantly higher HC z scores and 37% of EPI had Hc Z scores less than -2SD. (table 9)

**Figure 16. Average HC (cm) in VPI and EPI during first 5 weeks of life**



**Table 9. Anthropometric outcomes from birth to discharge in EPI and VPI**

	EPI <i>mean±SD</i>	VPI <i>mean±SD</i>	<i>p</i> -value
<b>At birth:</b>			
Birth weight z score	-0.28±0.69	-0.11±0.97	ns
WAZ < -2 Z (n, %)	0	0	
Birth length z score	0.43±0.71	0.13±1.05	ns
LAZ < -2 Z (n, %)	0	2(3%)	
Birth HC z score	-0.15±0.75	0.07±0.94	ns
HcZ < -2 Z (n,%)	0	1(1.7%)	
<b>At week 1:</b>			

Weight z score	-0.77±0.54	-0.71±0.81	ns
WAZ < -2 Z (n, %)	0	0	
Length z score	-0.34±0.59	-0.36±1.01	ns
LAZ < -2 Z (n, %)	0	2(3%)	
HC z score	-0.68±0.64	-0.53±0.93	ns
HcZ < -2 Z (n,%)	0	2(3%)	
<b>At week 5:</b>			
Weight z score	-1.09±0.45	-1.07±0.85	ns
WAZ < -2 Z (n, %)	0	5(8%)	
Length z score	-1.12±0.93	-1.06±0.87	ns
LAZ < -2 Z (n, %)	1(3%)	4(7%)	
HC z score	-1.88±0.82	-0.95±0.76	<0.001
HcZ < -2 Z (n,%)	10(37%)	2(3%)	
<b>At discharge:</b>			
Weight z score	-1.19±0.78	-0.71±0.73	<0.001
WAZ < -2 Z (n, %)	5(18.5%)	1(1.7%)	
Length z score	-1.62±1.20	-0.84±1.03	<0.01
LAZ < -2 Z (n, %)	8(29%)	6(10%)	
HC z score	-1.19±1.24	-0.46±0.64	<0.01
HcZ < -2 Z (n,%)	8 (29%)	0	
BF %	18.68±4.11	14.12±3.80	<0.001

### 3.4-4 Body Composition

At discharge, body fat percentage was significantly higher in the EPI group compared to the VPI group ( $p < 0.001$ ). There was also a significant difference in BF% and FFM% between groups. (table 10)

We further analyzed gender differences within the groups and results did not show any significant difference in BF% and FFM% between the genders within groups. ( $p > 0.05$ )

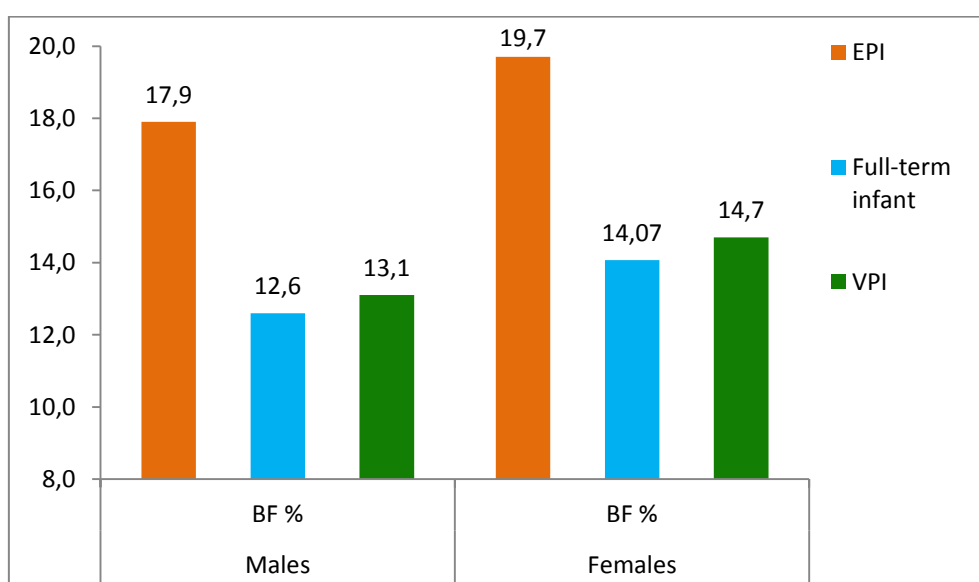
**Table 10. Body composition differences between the groups**

Body composition	genders	VPI	EPI	p-Value (between groups)
<b>Mean BF%</b>	males	13.1	17.9	0,005
	females	14.7	19.7	0,000
<b>Mean FFM %</b>	males	86.8	82	0.004
	Females	85	80	0.000

**Comparison Group (Full-term new born Infant)**

The Growth model for preterm infants is the developing fetus of the same postmenstrual age both in terms of weight gain and body composition. Figure 16 below shows data of our study group and reference values of full-term human infants at 2 weeks age.<sup>155</sup>

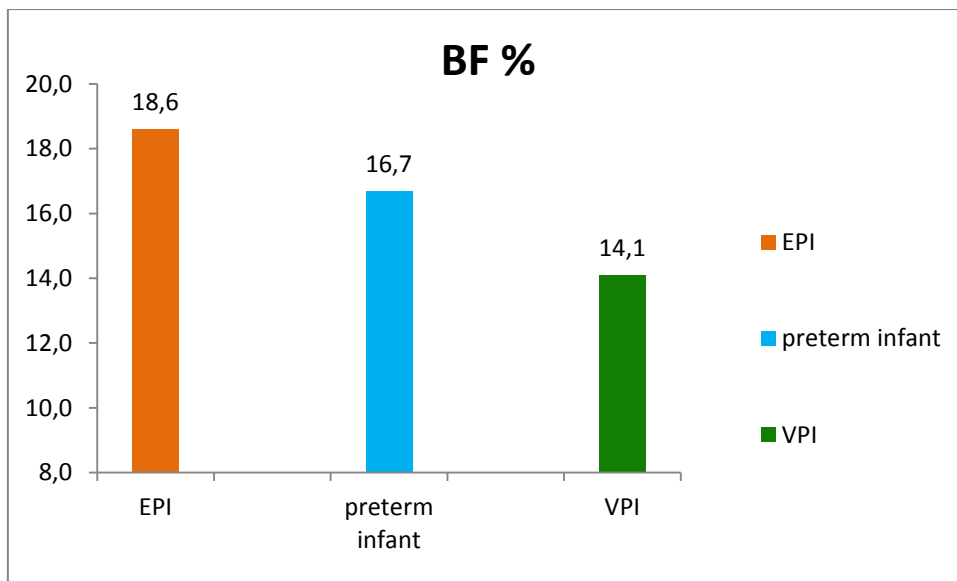
**Figure 17. Body fat % in Comparison to Full-term newborn Infant**



### Comparison Group (Preterm infant at term corrected age)

In order to compare our results with already published studies on preterm infants' results of body fat mass were pooled up for both genders.<sup>156</sup> Figure 17 shows data from our study group in comparison to reference values of preterm infants at term corrected age.<sup>156</sup>

**Figure 18. Body fat % in Comparison to Preterm infant at term corrected age**



## 4. DISCUSSION

In the population of extremely preterm infants (EPI) and very preterm infants (VPI), following a standardized unit specific feeding protocol led to significant differences in various growth parameters and actual nutrient supply between the 2 groups. This was no surprise since one would expect these very diverse groups to have different needs due to different phases of development and of course co-existing morbidities. Similar to our findings, other authors have also reported on nutritional support of LBW and VLBW infants according to nutritional recommendations but ELBW infants are being still prescribed smaller amounts of nutrients.<sup>157</sup>

Postnatal growth deficits resulting from insufficient nutrient supply may have serious long-term consequences.<sup>115,158</sup> The concept of “developmental origin of health and disease” raises several questions regarding nutritional deprivation during sensitive/critical periods in life.<sup>47</sup> Widdowson and McCance had previously reported on animal models showing the impact of undernutrition immediately after birth can permanently damage size, structure and function of the central nervous system<sup>159</sup>

Embleton et al (2001) conducted a study to analyze factors related to postnatal malnutrition and growth retardation. They showed that 45% of growth variation was related to nutritional intake and 7% was related to birth weight but the remaining 45% of growth variation was unexplainable.<sup>98</sup> This raises the question whether nutritional support alone is sufficient to produce optimized growth outcomes or not. Overall care practices might also need to be adjusted according to individual cases. Gestational age at birth is one of the important contributors towards achievement of better growth outcomes,<sup>70</sup> but nutritional and medical care during hospital stay can also play a vital role.<sup>71-73</sup> Very Preterm Infants (VPI) and Extremely Preterm Infants (EPI) experience a variety of challenges in the extra uterine environment. Postnatal morbidities like early onset of SEPSIS, IRDS, BPD, IVH etc<sup>74</sup>, influence their nutritional requirements and make them different from healthy neonates.

Due to neurological immaturity, oral feeding is delayed in preterm infants which often leads to prolonged periods of tube feeding.<sup>160</sup> The results of the presented study show that the investigated groups exhibited different feeding performances during their stay at NICU. EPI received parenteral nutrition longer than VPI, and they reached FEN later. Our results are consistent with those of Mcleod and colleagues.<sup>73</sup> They reported that in infants born under 28 weeks gestation, parenteral nutrition was administered until the third and fourth week of life and it took an additional 12 days to reach FEN<sup>73</sup> in comparison to more mature neonates. Recently a study reported that parenteral nutrition can have an additive effect on prolonged oxidative stress and increase the risk of BPD in EPI.<sup>161</sup> Thus progress in enteral feeding has to be regarded as extremely important. When we analyzed the reasons for the differences in nutrient supply in our study population, it became evident, that it was most pronounced in W2 and W3 and that the main difference was in the amount of fortification of enteral feeding. There were no differences in the volume intake between both groups, but the EPI group reached FEN later and this led to late initiation of fortification of feeds resulting in compromised protein and energy supply.

#### **4.1 Nutrient Supply in Comparison to ESPGHAN recommendations**

Nutritional supply during early infancy can either help to sustain the infant's metabolic capacity or can develop metabolic load. It is a widely accepted principle, that nutrient supply should be started promptly in the amounts that allow the preterm infant to grow like the fetus regarding both weight gain velocity as well as body composition.<sup>10</sup> Unfortunately, many neonatal intensive care units fail to comply with the recommended nutritional guidelines, especially during the first week after birth.<sup>162</sup> Interpreting our results in light of the amounts of nutrient intake currently recommended by ESPGHAN for preterm infants,<sup>12-13</sup> overall initiation of macronutrients fell just below the guidelines in the first week of life, but EPI received significantly lower amounts of nutrients than VPI during the following 5 weeks.

## Protein and Energy Supply

The ESPGHAN- recommendations generally recommend starting protein supply within 24 hours after birth at 1.5-3.0g/kg/day with daily advancement to reach the goal of 3.5-4.0g/kg/day for parenteral supply.<sup>12</sup> For enteral supply the recommended range of protein intake is 3.5-4.0g/kg/d for infants with a birth weight of 1000-1800g and 4.0-4.6g/kg/d for infants with a birth weight under 1000g.<sup>13</sup> In our cohort, mean protein supply was 2.4g/kg/d in both groups in W1 (table 3) and reached a maximum protein supply of 3.73g/kg/d during first 5 weeks (table 3). Mean protein supply via parenteral nutrition was 1.5g/kg/d in VPI and 1.75g/kg/day in EPI during the first week of life. Recommended intakes of protein for EPI (with mean birth weight <1000g) were not achieved following standard feeding protocol throughout the observation period. Energy supply was also significantly lower in EPI. This shortfall of protein and energy leads to invincible deficits by term gestation.<sup>115</sup> Studies related to nutritional management of preterm infants indicate that optimal growth rates cannot be achieved with inadequate supplies of protein and energy.<sup>163-164</sup> To prevent protein and energy deficits, it is suggested during this critical period, on the one hand prompt provision of parenteral amino acids at a rate of 3g/kg/d should be ensured and on the other hand fortification should be initiated early i.e. when an enteral feeding volume of 100ml/kg/d has been achieved.<sup>162,165-166</sup>

Senterre and Rigo prospectively observed growth outcomes of VLBW preterm infants after application of a nutritional strategy with early aggressive parenteral nutrition. They reported on a protein supply of 3.8 g/kg/d and energy supply of 120 kcal/kg/d by the end of the first week of life to be achievable.<sup>167</sup> Early high dose amino acid infusions after birth are well tolerated<sup>168-170</sup> and improve short-term growth outcomes.<sup>171-173</sup>

Senterre T has recently published “reasonable recommendations for optimizing enteral feeding practices”.<sup>72</sup> According to these recommendations fortification of enteral feeds can be started as early as when enteral feeding volumes have reached 50 ml/kg/d and full strength fortification can be given at an enteral feeding volume of 100 ml/kg/d. Furthermore, in infants where FEN is only achieved later ,

a combined nutritional strategy using parenteral nutrition should be adapted to cover the nutritional deficits otherwise arising.<sup>72</sup>

## **Glucose Supply**

Mean glucose supply during the first week of life mostly reflects intravenous glucose infusions for all preterm infants but EPI may receive parenteral nutrition for longer duration. Glucose infusion rate is recommended to start with 4-8mg/kg/min with maximum increase up to 10mg/kg/m (14.4g/kg/d).<sup>12</sup> For enteral nutrition 11.6-13.2 g/kg/d glucose supply is recommended.<sup>13</sup> In our cohort, mean glucose supply was initially 7.66g/kg/d to EPI and 8.12g/kg/d to VPI which increased progressively to 16g/kg/d for EPI and 14.4g/kg/d for VPI at week 5. EPI received significantly less glucose during w2 and w3, but in W 5 EPI was receiving significantly higher glucose supply. Both groups exceeded the upper limits of ESPGHAN recommendations in regard to glucose supply in w5. This increased supply of glucose was continued during w6, w7, w8 and w9 for EPI (data is not shown here). Increased supply of glucose could be the consequence of two clinical practices. Most preterm infants who are unstable and stressed are fed too aggressively with intravenous dextrose<sup>174</sup> or breast milk fortification can end up providing very high glucose concentrations. In our cohort 88% of EPI received fortified breast milk, which may have led to higher glucose supply. Hence our results suggest that glucose intake should be closely observed during FEN phase. There is need to monitor breast milk fortification very carefully. This point needs to be evaluated in future studies to answer the question whether the composition of breast milk fortifiers needs to be modified in order to stay within ESPGHAN recommendations. It is commonly observed that high rates of glucose are supplied to preterm infants in order to promote protein growth but these high amounts of glucose can contribute towards increased production of fat in organs like heart and liver as well as adipose tissue.<sup>175</sup> Moreover, perinatal hyperinsulinemia, due to early postnatal over-feeding leads to mal-programming of the neuroendocrine system putting the infant on risk of metabolic syndromes later in life.<sup>176</sup>

## Fat Supply

Mean fat supply is recommended at 0.5-1.0g/kg/d on the first day with incremental advancement to a goal of 3.0-4.0g/kg/d at the end of week 1 for parenteral nutrition,<sup>12</sup> whereas for enteral nutrition fat intake is recommended to be 4.8-6.6g/kg/d.<sup>13</sup> Results of our study showed that in both groups the mean fat supply (g/kg/d) was between the range of ESPGHAN recommendations, however EPI were receiving significantly less than VPI during first 5 weeks of life. Early and aggressive initiation of intravenous lipids seems to be safe and effective. Extremely preterm infants can tolerate higher amounts of fats, but it is necessary to determine whether higher fat supply is required or not, because provision of additional energy in form of fat promotes more fat mass. Although the rate of weight gain during the stay at NICU is directly proportional to the absolute intake of protein and energy, the relative composition of the weight gained depends on protein-energy ratio of the diet.<sup>158</sup>

## 4.2 Growth Outcomes

Postnatal growth failure remains a universal complication of extreme prematurity.<sup>177</sup> Altered perinatal growth pattern is linked to the risk of developing diseases of metabolic origin and cardiovascular diseases later in life.<sup>178</sup> Variation in nutrient supply of our cohort during the first weeks of life reflected clearly on growth outcomes of both groups at discharge.

Postnatal growth failure can result in continuous reduction in weight Z scores during hospital stay and it may lead to altered body composition at discharge.<sup>90</sup> Anthropometric parameters are often used to define growth in preterm infants. However, there is a growing body of literature suggesting that the assessment of composition of weight gain is also important for growth monitoring.<sup>179</sup> Therefore, estimation of BF% and FFM% can play a useful role in taking steps towards optimizing the nutritional management of preterm infants.

According to the presented data we conclude that, a standardized protocol, based on evidence-based recommendations can facilitate adequate nutrient delivery and growth outcomes in very preterm infants. In this cohort very preterm infants could receive nutrients in amounts close to recommendations<sup>12-13</sup>. Maximum growth velocity of 19g/kg/d was achieved during their stay in hospital. Moreover the duration of stay at hospital was also significantly shorter. Benefits of implementation of a standardized feeding protocol have been reported in several studies in the past. It certainly improves feeding administration and growth velocity.<sup>15</sup>

VPI could achieve WAZ scores of week 1 at discharge. LAZ scores and HcZ scores were also improved at discharge. However, despite better nutrient supply, 19% of these infants were below 10<sup>th</sup> percentile at discharge. As mentioned earlier, our results suggest that extra uterine growth retardation (EUGR) may be unavoidable in some infants. Similar to our results, it has been reported earlier on postnatal growth retardation to be an inevitable outcome that cannot be prevented with current recommended nutrient supplies for preterm infants.<sup>98</sup>

Body composition of very preterm infants at discharge was comparable to body composition of a reference new born infant published by Fomon SJ and colleagues in 1982.<sup>121</sup> Body fat % in regard to gender was also similar to already published references.<sup>121</sup> Although the method of estimation of body fat % was different in the work of Fomon SJ and colleagues but our results were comparable to another study published in 2011 using the same air-displacement plethysmography method (PEAPOD).<sup>155</sup> Hence it can be concluded that improved nutritional management may facilitate catch up growth and normal development of lean mass in preterm infants born between 28-32 weeks gestation. Effective management of preterm infants during hospitalization and implementation of evidence-based dietary regimes can facilitate achieving optimized growth outcomes.

Growth outcomes of EPI were significantly lower than VPI group at discharge. The complicated courses of nutritional support during stay at NICU influenced head

growth at week 5 and at discharge. WAZ, LAZ and HcZ scores were below 2 SD in 18.5%, 29% and 29% infants respectively. Previously studies reported the prevalence of extra-uterine growth restriction as high as 28% for weight, 34% for length and 16% for head circumference in preterm infants with GA of 23-34 weeks.<sup>21</sup> Another study reported the incidence of EUGR was 57%, 48% and 6% for weight, length and head circumference respectively in Japanese preterm infants population.<sup>84</sup> Although nutritional support in NICU and postdischarge has been improved, still extremely immature survivors continue to suffer postnatal growth deficits.<sup>70,158</sup> Protein and energy deficits during the first weeks of life are responsible for growth faltering at discharge and may have long-term consequences.<sup>115,177</sup> Postnatal growth failure is reported to be associated with poor neurodevelopmental outcomes at 18-24 months corrected age.<sup>177</sup> Many studies have reported the role of early nutrition on postnatal head growth and later neurodevelopmental outcomes.<sup>8,71,180-184</sup> Lucas and colleagues studied patients with cerebral palsy and reported that suboptimal nutritional management during a critical period of rapid brain growth could impair functional compensation. Moreover, suboptimal nutrition during the neonatal period can permanently damage cognitive functions in males.<sup>8</sup>

We observed that the extent of prematurity seems to have a strong impact on postnatal growth. In our study population extremely preterm infants were less tolerant of escalating feeds in general which resulted in postnatal growth retardation. However, beside the fact of not being able to receive optimized nutrition, EPI had higher body fat stores at discharge. Similar to our findings, reduced weight Z scores and increased body fat at discharge have been published by different researchers in the past.<sup>15,185</sup>

Our results showed significantly altered body composition compared to full-term new born<sup>121,155</sup> and preterm infants.<sup>156</sup> To our knowledge, there are unfortunately not many studies with large data sets to define cut-off points for extremely preterm infants' body composition. Therefore, further studies need to be planned to establish separate standards for this vulnerable group and EPI should not be expected to follow fetal growth patterns.

This may be a result of insufficient provision of protein and energy on the one hand and excessive glucose supply on the other hand. This overfeeding of

glucose can result in early hyperinsulinism. Hyperinsulinism leads to 'Malprogramming' of neuroendocrine systems regulating body weight, food intake and metabolism. This whole process can put an individual at increased risk of becoming obese or developing diabetes later in life.<sup>176</sup>

It is very important to define which growth patterns should be considered "normal" and should be "aimed for" by the clinicians during the hospital stay of extremely preterm infants. Risk factors associated with postnatal growth outcomes characterized by (decreased Z-scores and high body fat stores) have been repeatedly highlighted by different researchers.<sup>15,185</sup> At the present time, the theories behind "developmental origins of health and disease" still remain controversial but it is also difficult to reject the evidence that early exposure to nutritional deprivation may program long-term health outcomes.<sup>178, 105-106</sup>

To avoid these rates of postnatal growth failure, unit-specific, evidence based feeding protocols should be developed. Ideally a multidisciplinary team with expertise in neonatal nutrition should be consulted to develop practical clinically friendly plans.<sup>111,177</sup> Active involvement of a neonatal nutritionist can help to monitor compliance with standard recommendations, ensure individualized nutritional support to infants with complications, facilitate smooth transition from parenteral nutrition to enteral nutrition and suggest adjustments to nutritional support aimed at maintaining steady growth.<sup>111,177</sup>

## 5. CONCLUSION

After implementation of evidence based standardized nutritional support protocol, nutrient supply and growth rate of both groups was variable during the observation period.

Very preterm infants could achieve better Z-scores at discharge accompanied by body composition comparable to full-term new born infants. However, in extremely preterm infants the complicated course of nutritional support during their stay at NICU influenced growth and body composition at discharge.

Of interest is the fact that nutritional care practices for these diverse groups need more attention and particularly extremely preterm infants should be provided aggressive parenteral nutrition during the first 3 weeks of life if significant progress in enteral intake is not achieved. If enteral feeds are tolerated well, fortification should be initiated at enteral volume of 100ml/kg/d or even earlier. Moreover, the use of breast milk fortifier resulted in glucose intakes above the ESPGHAN recommendations in later weeks – this needs to be evaluated in future studies.

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