The effects of postdischarge nutrition on growth, body composition, and bone development of preterm infants

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General introduction

Adapted from:
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Timing of nutritional interventions in very low birth weight infants: optimal neurodevelopment compared with the onset of the metabolic syndrome

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Of the 177,713 infants born in The Netherlands in 2008, 13,649 (7.7%) newborns were born preterm (gestational age <37 weeks), 2,637 (1.5%) newborns were born very preterm (gestational age <32 weeks), and 2,456 (1.4%) newborns had a very low birth weight (VLBW; birth weight <1,500 gram) [1]. Preterm infants experience the third trimester in an extrauterine environment and have a different postnatal growth pattern compared to the fetus in utero or the term infant after birth. Postnatal growth patterns of preterm infants are, among other factors, related to the availability of nutrients and may be associated with long-term metabolic and cardiovascular consequences.

PRENATAL AND POSTNATAL GROWTH IN PRETERM INFANTS

Endocrine regulation of prenatal and postnatal growth

In contrast to growth regulation during infancy and childhood, the endocrine regulation of prenatal growth is not growth hormone (GH) dependent. Although fetal GH levels are high, GH has little effect on the fetal insulin-like growth factor (IGF) axis [2]. This is supported by studies on congenital GH deficiency in humans [3-4] and rodents [5] as well as by studies on fetal GH overproduction in mice [5]. These studies demonstrate a similar size at birth in subjects with GH deficiency or GH overproduction compared to their healthy peers [3-5].

During the first half of pregnancy, several animal models [6-7] as well as associations between serum insulin-like growth factor type II (IGF-II) and the degree of fetal growth retardation or fetal overgrowth in humans [8-9] indicate that IGF-II is the dominant factor for fetal growth. IGF-II signal transduction leads to mitogenic and anti-apoptotic effects and to some cell differentiation via the IGF type I receptor [2]. However, in a mouse model, deletion of the IGF-II gene results in growth retardation with a weight 40% lower than normal, deletion of the IGF type I receptor gene results in a weight 55% lower than normal, whereas combined deletion of the IGF-II and the IGF type I receptor genes results in a weight 70% lower than normal [7]. This suggests that, at least in a mouse model, IGF-II may also have a growth-promoting effect via another receptor, possibly the insulin receptor [10]. Embryonic and early fetal growth is independent of nutrient supply because the influence of nutrition on IGF-II is limited [11-12]. The role of IGF-II for fetal growth diminishes with advancing gestation and IGF-II is no longer an important growth regulator after birth [2].

During the second half of pregnancy, there is a shift from IGF-II towards insulin-like growth factor type I (IGF-I) as the dominant factor for growth, as supported by animal models [6-7] and genetic abnormalities in humans [13-15]. IGF-I exerts its growth-promoting action by binding to the IGF type I receptor [2, 11] and in part by binding to the insulin receptor [10]. The IGF type I receptor has a higher affinity for IGF-I than for

IGF-II and the insulin receptor binds IGF-I with an affinity of 1% of the affinity for insulin [16]. In contrast to growth regulation by IGF-II during the first half of pregnancy, IGF-I and fetal growth are strongly related to nutrition during the second half of pregnancy [11-12].

Hepatic IGF-I production and secretion are regulated by thyroid hormones, glucocorticoids, nutrient supply, and insulin secretion [11-12]. Thyroid hormones directly affect IGF-I via modulation of the hepatic IGF-I production [5, 12, 16]. Glucocorticoids and nutrient supply influence IGF-I levels, at least to some extent, via insulin. Increased endogenous or exogenous fetal glucocorticoids suppress insulin actions and decrease IGF-I expression [12]. Furthermore, the availability of nutrients and the resultant insulin concentration influence hepatic IGF-I production and secretion. Placental glucose transfer maintains fetal glucose levels, which enhance insulin secretion; insulin stimulates hepatic IGF-I production [12]. Thus, in addition to optimal thyroid hormone and glucocorticoid levels, adequate insulin concentrations and nutrient supply are necessary to maintain fetal growth during the second half of gestation.

Nutrient availability may be compromised by maternal malnutrition, placental dysfunction, impaired uterine blood flow, and impaired fetal nutrient uptake and this may result in intrauterine growth restriction (IUGR). The impact of IUGR developed under these circumstances (i.e. a nutrient deprived intrauterine environment) is that the fetus does not has the ability to grow to its maximal genetic potential [11]. Other factors may also influence intrauterine growth, such as genetic factors, parity, maternal age, maternal smoking, and gender of the fetus [17].

After birth, fetal growth regulatory mechanisms remain dominant during the first six postnatal months [11]. Thereafter, growth regulation shifts from nutrition/insulin/IGF-I dependency towards IGF-I/GH dependency due to an increase in GH receptors [2]. GH acts on the GH receptors in the liver to stimulate the release of IGF-I, a process that is potentiated by insulin [16]. IGF-I acts by binding to the IGF type I receptor and to some extent to the insulin receptor to increase somatic growth [10]. IGF-I/GH dependent growth allows the individual to attain its full genetic growth potential, while nutrition/insulin/IGF-I dependent growth allows the influence of the environment, which may limit growth.

It has been suggested that preterm infants have a fetal growth regulation during the extrauterine third trimester that under optimal circumstances leads to adequate growth [12]. However, many postnatal conditions that preterm infants are exposed to, such as morbidity and insufficient nutrient availability, may adversely influence growth. Moreover, little is known about the timing of the shift from nutrition/insulin/IGF-I dependent growth towards IGF-I/GH dependent growth in preterm infants. As long as growth regulation is nutrition dependent, increased nutrient supply stimulates insulin and IGF-I release and enhances growth in preterm infants. Insulin primarily enhances adipogen-

esis [11-12] and the effect on lean mass is mediated through IGF-I [11]. The quantity and quality of tissue accretion in early infancy is important with regard to metabolic consequences in later life.

Intrauterine growth patterns

The pattern of intrauterine growth determines the size of the infant at birth. Based on weight and length at birth, preterm infants may be born appropriate-for-gestational-age (AGA) or small-for-gestational-age (SGA). AGA defines infants that are born with a birth weight and a birth length within two standard deviations scores (SDS) of the population mean for gestational age (birth weight and birth length ≥-2 SDS and ≤+2 SDS); SGA defines infants that are born with a birth weight and/or a birth length at least two SDS below the population mean for gestational age (birth weight and/or birth length <-2 SDS) [18]. In the general population, between 2.3% and 10% of infants are born SGA [17-18]. In very preterm infants (gestational age <32 weeks), 6.3-26.7% have a birth weight below -2 SDS [19-20] and 7% have a birth weight and/or a birth length below -2 SDS [21].

Being SGA does not reflect the intrauterine growth pattern but the size at birth of the infant compared to the general population [18] because birth weight and birth length of an individual infant are interpreted in relation to birth weight and birth length of the population. In contrast, IUGR refers to a decline in fetal growth as a result of an underlying pathological process that prevents the fetus to achieve its genetic growth potential [17]. Being SGA may be a result of fetal, maternal, or placental causes [18, 22] that lead to IUGR; however, SGA also includes infants that are constitutionally small and have a lower genetic growth potential. This emphasizes that SGA and IUGR are not synonymous [18]: an infant may be born SGA without IUGR and an infant may experience IUGR and be born AGA. This difference suggests that an accurate assessment of the reason for being SGA is needed by relating the size at birth to the fetal growth potential, which is, among other factors, determined by the maternal and parental size, maternal parity, and ethnicity [23]. Although birth weight and birth length are important surrogate markers of the intrauterine condition that the fetus was exposed to, these parameters remain indirect measures of the growth potential of the fetus [22]. To determine if being small (SGA) at birth is a result of a lower genetic growth potential or a consequence of IUGR, fetal growth needs to be evaluated by serial ultrasonographic measurements. An SGA infant that is small throughout pregnancy is likely to have a lower genetic growth potential, whereas an SGA infant that demonstrates a deflection of intrauterine growth at some point may be classified as IUGR. The distinction between being constitutionally small or having IUGR may be important because it has been suggested that IUGR, even with a resultant birth weight within the normal limits, is associated with an increased risk of long-term cardiovascular and metabolic consequences, whereas constitutional smallness is not [22].

Extrauterine growth patterns

Preterm infants experience the third trimester, when most of the fetal growth takes place, in an extrauterine environment. During this extrauterine third trimester, preterm infants direct their nutrient expenditure towards surviving several postnatal complications of preterm birth at the expense of postnatal growth [24]. Postnatal morbidity increases the nutritional demands, which usually are not reached because of limited feeding possibilities. Among these limitations are gut immaturity and limited amounts of total parenteral nutrition that are tolerated without complications. In addition, even higher nutritional intakes are needed to achieve growth during the third trimester.

As a result, the disequilibrium between nutrient requirement and nutrient supply leads to extrauterine growth restriction (EUGR) of preterm infants during the third trimester. Lemons et al. show that up to 97% of VLBW preterm infants have EUGR at discharge [25]. During infancy and childhood, preterm infants with early EUGR (i.e. EUGR before term age) and SGA preterm infants have a similar growth pattern [17, 24]. At 4-5 years of age, approximately 10% of very preterm infants have height <-2 SDS [24] compared to a similar percentage of SGA preterm infants [17-18]. As a consequence of the similar growth patterns in the early postnatal period and during infancy, it has been hypothesized that preterm infants with EUGR and SGA preterm infants have a comparable risk for the development of cardiovascular and metabolic consequences in later life [21, 24].

CONSEQUENCES OF PRETERM BIRTH

Prematurity, IUGR, and EUGR are associated with long-term metabolic and cardiovascular consequences [26-28]. Furthermore, preterm infants are at risk of decreased bone mass [29], deficits in macronutrients and micronutrients, and impaired cognitive development [22, 30].

Long-term cardiovascular and metabolic consequences

The 'developmental origins of adult health and disease' (DoHaD) concept suggests that early life events can alter the risk of diseases in later life, such as diabetes, hypertension, and cardiovascular disease. The DoHaD concept originates from the epidemiological observations that birth weight is inversely related to the risk of cardiovascular disease [31] and type 2 diabetes [32]. These associations imply that fetal growth restriction during middle and late pregnancy results in disproportional growth and permanent changes in body structures and functions that predispose to chronic diseases in adulthood, as stated by the 'fetal origins of disease' hypothesis [31]. However, the associations are not based on a low birth weight per se, as similar relations are found in subjects with a birth weight within the normal range [31]. This suggests that a mismatch between the actual

size at birth, as a proxy of the growth pattern in utero, and the genetic growth potential may explain the increased risk of chronic adult diseases [33].

The changes in body structures and functions may be an adaptive mechanism of the fetus and young infant to a nutrient deprived environment. This concept suggests that if nutrient availability is decreased, the fetus reduces growth and insulin sensitivity and redistributes its blood flow in order to reduce its anabolic demands and to promote short-term survival [22, 33]. Thus, the fetus adapts to a nutrient deprived environment. After this early adaptation, a mismatch between the predicted future nutrient deprived environment and the actual nutrient rich postnatal environment that the infant is exposed to may lead to accelerated growth [34] with increased fat accumulation and the infant may develop insulin resistance and cardiovascular disease, as demonstrated in animal models [35]. This concept is described by the 'thrifty phenotype hypothesis' [36] or 'accelerated growth hypothesis' [37-38].

During the first year of life, increased fat deposition as a result of rapid growth is demonstrated in term infants [39-40] and preterm infants [41]. In addition, rapid growth during infancy is associated with increased fat deposition and an increased risk of adiposity during childhood [42-43] and adulthood [42, 44] in term subjects as well as with increased body fat during adulthood in preterm subjects [45]. This may be a consequence of tracking of fat mass from infancy to childhood and adulthood [46-47], which may be associated with insulin resistance and cardiovascular disease. These findings suggest that increased fat accumulation during an early critical window may be related to the development of long-term metabolic consequences, such as obesity, insulin resistance, increased blood pressure, and cardiovascular disease [48]. Nevertheless, although intrauterine as well as early postnatal growth appear to affect the risk of long-term cardiovascular and metabolic consequences, the exact critical window for the influence of growth and nutrient availability remains unclear [49].

Decreased bone accretion

The risk of suboptimal bone mass is increased in preterm infants because they lack the intrauterine third trimester when 80% of calcium, vitamin D, and bone accumulation takes place [29, 50-51]. Moreover, decreased intrauterine growth may result in even lower bone accretion in utero and may explain the lower bone mass in SGA compared to AGA preterm infants at birth [52]. Thus, prematurity as well as being SGA is related to a bone mass deficit at birth.

Bone accretion can be divided into bone formation and bone mineralization. The bone formation phase requires a sufficient supply of amino acids for the synthesis of collagen type I that results in bone matrix formation [53]; the bone mineralization phase requires a sufficient supply of calcium, phosphorus, and vitamin D to maintain intestinal mineral absorption and to add minerals to the bone matrix [29]. During the extrauterine

third trimester, it is nearly impossible to achieve protein, mineral, and vitamin D intakes that are adequate to reach a bone accretion rate similar to that achieved in utero. As a consequence, the bone deficit of preterm infants is further aggravated after birth. This is highlighted by lower bone accretion during infancy and childhood in preterm infants compared to their term born peers [54-56]. Decreased bone mass in preterm infants is related to lower peak bone mass in adulthood [57-59], which is subsequently associated with a higher risk of osteoporosis [60].

Postnatal deficits in macronutrients and micronutrients

During the extrauterine third trimester, preterm infants have high nutritional requirements in order to survive postnatal morbidity and to achieve adequate postnatal growth. However, preterm infants encounter several feeding difficulties that limit the administration of an adequate amount of nutrients and this results in postnatal deficits of macronutrients and micronutrients. During the first postnatal weeks, no or limited enteral nutrition is tolerated due to immaturity of the gut and an increased risk of necrotizing enterocolitis [61] and, in fact, the nutritional supply in this crucial period depends predominantly on total parenteral nutrition. Unfortunately, the amount of nutrients that can be administered safely via total parenteral nutrition is also limited. As a consequence, the suboptimal early nutritional intake may result in major cumulative deficits of energy and macronutrients, especially of protein, during the first postnatal months. Hence, these deficits lead to severe EUGR [62].

In addition to deficits of macronutrients, the limitations of nutritional supply also result in deficits of various micronutrients during early postnatal life, such as calcium, phosphorus, vitamin D, iron, and long-chain polyunsaturated fatty acids (LC-PUFAs). An adequate nutritional supply of calcium, phosphorus, and vitamin D is necessary for bone accretion in preterm infants but is difficult to attain. Human milk is low in calcium, phosphorus, and vitamin D [63] and, therefore, human milk fortifier is added to increase the intake of these micronutrients in preterm infants. Nevertheless, preterm infants fed human milk often have lower bone accretion compared to those fed preterm formula during the first months of life [64-65], which may be due to persistently lower calcium, phosphorus, and vitamin D intakes.

Furthermore, preterm infants are at risk of iron deficiency because they are deprived of the fetal iron accretion of the third trimester and are born with low iron stores [66-67]. Postnatally, preterm infants have increased iron requirements as a result of a strongly activated erythropoiesis, a shorter life span of fetal and preterm red blood cells, frequent blood sampling, and rapid postnatal growth [68]. However, due to inadequate dietary iron intake, 25-85% of preterm infants develop iron deficiency during the first year of life [68-70]. Prevention of iron deficiency is necessary to maintain erythropoiesis but may also be beneficial for growth and neurodevelopment [71-72]. Iron is a key factor in

the defense against infections and iron deficiency has been associated with impaired cell-mediated immunity and bactericidal function by in vitro studies. However, these findings remain controversial as no significant benefits of iron supplementation on immunity and infection reduction have been demonstrated in vivo [73]. In addition, virtually all pathogens, such as bacteria, protozoa, and viruses, depend on iron for their growth [74] and this might suggest that higher dietary iron intake increases the risk of infection in preterm infants. On the other hand, several studies have demonstrated that an increased dietary iron intake is safe with regards to severe infections [75]. The low iron stores of preterm infants at birth are usually depleted six to eight weeks after birth. Therefore, to prevent iron deficiency in preterm infants, an elemental iron intake of 2-4 mg/kg/d is recommended between 6 weeks and 12 months postnatal age [76], which can be achieved with iron-fortified formulae or with enteral iron supplementation.

Similar to iron, LC-PUFAs are important for central nervous system development [77-78] and possibly for growth [79], although the effect on growth remains controversial [80-81]. In particular, the LC-PUFAs docosahexaenoic acid (DHA) and arachidonic acid (AA) are important. LC-PUFA stores of preterm infants are low at birth because the intrauterine third trimester when most of the LC-PUFA accretion occurs is lacking [67]. To prevent LC-PUFA deficiency, the European Society of Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommends 0.25-0.45 g% DHA and 0.38-0.64 g% AA in preterm formula [82-83]. Nevertheless, the dietary supply of LC-PUFAs during the first postnatal months is usually insufficient, which results in an increasing deficit that may be associated with reduced postnatal growth and impaired neurodevelopmental outcome.

NUTRITIONAL INTERVENTIONS IN PRETERM INFANTS

With regard to the postnatal deficits in macronutrients and micronutrients that contribute to EUGR, decreased bone accretion, and impaired neurological outcome, in-hospital as well as postdischarge nutritional interventions in preterm infants should aim to prevent and correct these postnatal deficits. Furthermore, such interventions should lead to adequate growth during a critical window, while they prevent excessive fat accumulation that is associated with cardiovascular and metabolic disease in later life.

In-hospital nutritional interventions

During the first postnatal weeks, when no or only limited enteral nutrition is tolerated by preterm infants, total parenteral nutrition has a key role in preventing nutritional deficits. To prevent hypoglycemia and to enable energy utilization, intravenous glucose administration is initiated immediately after birth [84]. Controversy exists on the time of

initiation and the dose of intravenous protein and lipids because of fear of complications. Whereas some studies demonstrate that early high parenteral protein administration to preterm infants leads to uraemia [85-86], others advocate that early administration of an improved amino acid composition of total parenteral nutrition prevents postnatal growth restriction in preterm infants [87-88] without the development of hyperammonaemia or uraemia [89-90]. A recent study demonstrates that improved growth is achieved when preterm infants receive total parenteral nutrition with a relatively high protein content directly after birth [62]. With this early high protein administration, cumulative protein deficits are prevented [62]. Similarly, a recent meta-analysis demonstrates that early initiation of intravenous lipids is safe and well-tolerated [91]. After several days, enteral nutrition is initiated and gradually increased, while it is still supported by parenteral nutrition until enteral nutrition is adequate and can be used as the sole source of nutrition [61].

The recommendations for energy and nutrient intake aim to achieve a postnatal weight gain of preterm infants similar to weight gain in utero. This can be achieved with an energy intake of >100 kcal/kg/d, a protein intake of >3 g/kg/d, and a protein-energy ratio of 3 g protein per 100 kcal [82]. In addition, the supply of energy and protein needs to compensate the cumulative energy and protein deficits that are observed in almost all preterm infants, and, therefore, an energy intake of 110-135 kcal/kg/d and a protein intake of 3.5-4.0 g/kg/d are recommended [82]. Carbohydrates are a major source for energy synthesis as well as for de novo synthesis of fatty acids and nonessential amino acids. For preterm infants, a carbohydrate intake of 11.6-13.2 g/kg/d is recommended [82]. Furthermore, adequate administration of lipids is necessary to provide energy, essential LC-PUFAs, and lipid soluble vitamins. The minimum fat intake is estimated at 3.8-4.8 g/kg/d to meet the daily intrauterine fat deposition as well as the fat loss from malabsorption and conversion. To compensate for a cumulative deficit that might already have developed in preterm infants in early life, a lipid intake of 4.8-6.6 g/kg/d is advised with a DHA intake of 12-30 mg/kg/d, an AA intake of 18-42 mg/kg/d, and a DHA/AA ratio between 1.0 and 2.0 [82].

In addition to an adequate macronutrient intake, in-hospital nutritional interventions should aim to achieve sufficient micronutrient administration. During early postnatal life, this is achieved by mineral and vitamin supplementation via total parenteral nutrition. When enteral nutrition is the sole nutritional source, minerals and vitamins are available from either human milk fortifier or preterm formula. Calcium, phosphorus, and vitamin D are important micronutrients for bone accretion in preterm infants. To ensure adequate bone mineralization, a calcium retention of 60-90 mg/kg/d is necessary [29]. This requires a calcium intake of 120-140 mg/kg/d and a phosphorus intake of 60-90 mg/kg/d in preterm infants based on a calcium absorption of 50-65%, a phosphorus absorption of 90%, and a calcium/phosphorus ratio between 1.5 and 2.0 [82]. Several studies

demonstrate that vitamin D improves calcium absorption [92] and, therefore, a vitamin D intake of 800-1000 IU/d is recommended for preterm infants [82]. Furthermore, as stated previously, an elemental iron intake of 2-4 mg/kg/d from iron-fortified formulae or enteral iron supplementation is recommended between 6 weeks and 12 months postnatal age to prevent iron deficiency in preterm infants [76]. To date, however, cumulative deficits of macronutrients and micronutrients as well as EUGR and decreased bone accretion cannot be fully prevented with the in-hospital feeding regimens used [93].

Nutritional interventions after discharge

After hospital discharge, adequate nutrition is important to achieve an optimal body composition and to prevent further deficits in macronutrients and micronutrients that contribute to growth faltering and insufficient bone accretion [93]. Human milk is preferred for preterm infants after discharge because it has several beneficial effects, for example on immune status and on weight gain composition, which results in lower fat mass in later life. However, when human milk is not available, the ESPGHAN recommends the use of specialized postdischarge nutrition in preterm infants until at least 40-52 weeks postconceptional age [94] and advises that this postdischarge nutrition contains 72-74 kcal/100 ml, 1.8-1.9 g protein/100 ml (2.5 g/100 kcal), and 70-80 mg calcium/100 ml [94].

Some studies show that preterm infants fed high-energy, high-protein postdischarge formulae (per 100 ml: energy 72-81 kcal, protein 1.85-2.3 g) have better growth during infancy compared to those fed standard term formulae (per 100 ml: energy 55-68 kcal, protein 1.4-1.7 g) [95-98]. However, this may be attributed to increased fat deposition during the first six months post-term [96, 99], which may be directly related to the higher energy intake [100-101]. In contrast, a recent study [102] as well as a recent Cochrane meta-analysis, which included 15 trials (1128 preterm infants) [103], demonstrate no clear benefit of high-energy, high-protein postdischarge formulae (per 100 ml: energy >72 kcal, protein >1.7 g) on growth and body composition during infancy. These inconsistent results may be due to differences in methodology and patient cohorts between the included studies. In addition, the high-energy, high-protein postdischarge formulae used in the studies did not only differ in energy and protein content but also in the composition of other macronutrients and micronutrients, such as fat, minerals, and vitamins [95-98, 102-103]. The differences in these other nutrients may, at least to some extent, explain the inconsistent effects of postdischarge formulae on growth and body composition.

Postdischarge nutrition that, in addition to energy and protein, is supplemented with calcium, phosphorus, and vitamin D may also improve bone accretion. After discharge, some studies show that preterm infants fed high-energy, protein- and mineral-enriched formulae reach a higher bone mineral content (BMC) compared to preterm infants fed

standard term formulae [96, 104-105], whereas others did not find a positive effect of such formulae on bone accretion [64, 106]. This controversy may be related to differences in formula composition and patient cohorts. Moreover, although a positive effect of an isocaloric, protein- and mineral-enriched formula on bone accretion has been demonstrated before term age [107], it remains to be elucidated if an isocaloric, protein- and mineral-enriched postdischarge formula has a similar beneficial effect on bone accretion after term age.

In addition, postdischarge nutrition is fortified with iron to provide an adequate iron intake to preterm infants. If preterm infants are fed human milk, iron supplementation is advised until 12 months of age according to the current guidelines [76]. The exact iron content of postdischarge formula that is needed to prevent iron deficiency in preterm infants remains controversial, as iron absorption from formula is problematic due to decreased intestinal resorption of iron from formula. A previous study demonstrated that a formula fortified with 0.5-0.9 mg iron/100 ml leads to iron deficiency in 2.5% of the infants at two months post-term and in 14.3% of the infants between two and six months post-term [108]. This might suggest that a postdischarge formula with a higher iron content prevents iron deficiency even further if the solubility of iron within the formula as well as the intestinal absorption of iron from the formula is taken into account, although both are influenced by several other factors.

CONCLUSION

Early optimal nutritional intake may prevent deficits in macronutrients and micronutrients that are related to growth faltering, decreased bone mineral accretion, iron deficiency, and neurological impairment as well as to increased fat accumulation and concomitant long-term cardiovascular and metabolic consequences. Nevertheless, as long as nutrition cannot be optimized in all preterm infants during the first months of life, specialized postdischarge nutrition has a key role in the nutritional management of preterm infants after discharge in order to prevent further EUGR and to limit fat accumulation, which is associated with long-term cardiovascular and metabolic consequences. However, the extent to which specialized postdischarge nutrition compensates the bone and micronutrient deficits of preterm infants remains to be elucidated. Furthermore, a better understanding of the effect of the prenatal and the early postnatal trajectory on growth, bone accretion, and body composition acquisition of preterm infants during the first six months post-term is important because SGA preterm infants and preterm infants with early EUGR may benefit from specialized postdischarge nutrition.

REFERENCES

1. Stichting Perinatale Registratie Nederland. Perinatal Care in the Netherlands 2008 [In Dutch: Perinatal Zorg in Nederland 2008]. 2011.
2. Gicquel C, le Bouc Y. Hormonal regulation of fetal growth. Horm Res. 2006;65:28-33.
3. Karlberg J, Albertsson-Wikland K. Infancy growth pattern related to growth hormone deficiency. Acta Paediatr Scand. 1988;77:385-391.
4. Albertsson-Wikland K, Niklasson A, Karlberg P. Birth data for patients who later develop growth hormone deficiency: preliminary analysis of a national register. The Executive Scientific Committee of the Kabi International Growth Study and the Swedish Paediatric Study Group for Growth Hormone Treatment. Acta Paediatr Scand. 1990;370:115-120.
5. Schwartzbauer G, Menon R. Regulation of growth hormone receptor gene expression. Mol Genet Metabol. 1998;63:243-253.
6. DeChiara T, Efstratiadis A, Robertson E. A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature. 1990;345:78-80.
7. Baker J, Liu J, Robertson E. Role of insulin-like growth factors in embryonic and postnatal growth. Cell. 1993;74:73-82.
8. Murphy R, Baptista J, Holly J, Umpleby A, Ellard A, Harries L, Crolla J, Cundy T, Hattersley A. Severe intrauterine growth retardation and atypical diabetes associated with a translocation breakpoint disrupting regulation of the insulin-like growth factor 2 gene. J Clin Endocrinol Metab. 2008;93:4373-4380.
9. Bentov I, Werner H. IGF, IGF receptor and overgrowth syndromes. Pediatr Endocrinol Rev. 2004;1:352-360.
10. Butler A, LeRoith D. Minireview: tissue-specific versus generalized gene targeting of the igf1 and igf1r genes and their roles in insulin-like growth factor physiology. Endocrinology. 2001;142:1685-1688.
11. Gluckman P. Endocrine and nutritional regulation of prenatal growth. Acta Paediatr Suppl. 1997;423:153-157.
12. Fowden A, Forhead A. Endocrine regulation of feto-placental growth. Horm Res. 2009;72:257-265.
13. Walenkamp M, Karperien M, Pereira A, Hillhorst-Hofstee Y, van Doorn J, Chen J, Mohan S, Denley A, Forbes B, et al. Homozygous and heterozygous expression of a novel insulin-like growth factor-I mutation. J Clin Endocrinol Metab. 2005;90:2855-2864.
14. Vaessen N, Janssen J, Heutink P, Hofman A, Lamberts S, Oostra B, Pols H, Van Duijn C. Association between genetic variation in the gene for insulin-like growth factor-I and low birthweight. Lancet. 2002;359:1036-1037.
15. Woods K, Camacho-Hübner C, Savage M, Clark A. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. N Eng J Med. 1995;335:1363-1367.
16. Thissen J, Ketelslegers J, Underwood L. Nutritional regulation of insulin-like growth factors. Endocr Rev. 1994;15:80-101.
17. Saenger P, Czernichow P, Hughes I, Reiter E. Small for gestational age: short stature and beyond. Endocr Rev. 2007;28:219-251.
18. Lee P, Chernausek S, Hokken-Koelega A, Czernichow P. International small for gestational age advisory board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. Pediatrics. 2003;111:1253-1261.

19. Grisaru-Granovsky S, Reichman B, Lerner-Geva L, Boyko V, Hammerman C, Samuelhoff A, Schimmel M. Mortality and morbidity in preterm small-for-gestational-age infants: a population-based study. Am J Obstet Gynaecol. 2012;206:150.e151-157.
20. Bonamy A, Parikh N, Cnattingius S, Ludvigsson J, Ingelsson E. Birth characteristics and subsequent risks of maternal cardiovascular disease: effects of gestational age and fetal growth. Circulation. 2011;124:2839-2846.
21. Finken M, Dekker F, de Zegher F, Wit J. Long-term height gain of prematurely born children with neonatal growth restraint: parallellism with the growth pattern of short children born small for gestational age. Pediatrics. 2006;118:640-643.
22. Gluckman P, Hanson M. The consequences of being born small-An adaptive perspective. Horm Res. 2006;65:5-14.
23. Gardosi J. New definition of small for gestational age based on fetal growth potential. Horm Res. 2006;65:15-18.
24. Wit J, Finken M, Rijken M, de Zegher F. Preterm growth restraint: a paradigm that unifies intrauterine growth retardation and preterm extrauterine growth retardation and has implications for the small-for-gestational-age indication in growth hormone therapy. Pediatrics. 2006;117:e793-e795.
25. Lemons J, Bauer C, Oh W, Korones S, Papile L, Stoll B, Verter J, Temprosa M, Wright L, et al. Very Low Birth Weight Outcomes of the National Institute of Child Health and Human Development Neonatal Research Network, January 1995 Through December 1996 Pediatrics. 2001;107:E1.
26. Hofman P, Regan F, Harris M, Robinson E, Jackson W, Cutfield W. The metabolic consequences of prematurity. Growth Hormone & IGF Research. 2004;14:S136-S139.
27. Irving R, Belton N, Elton R, Walker B. Adult cardiovascular risk factors in premature babies. Lancet. 2000;355:2135-2136.
28. de Jong F, Monuteaux M, Van Elburg R, Gillman M, Belfort M. Systematic review and meta-analysis of preterm birth and later systolic blood pressure. Hypertension. 2012;59:226-234.
29. Rigo J, De Curtis M, Pieltain C, Picaud J, Salle B, Senterre J. Bone mineral metabolism in the micropremie. Clin Perinatol. 2000;27:147-165.
30. Cooke R, Foulder-Hughes L. Growth impairment in the very preterm and cognitive and motor performance at 7 years. Arch Dis Child. 2003;88:482-487.
31. Barker D. Fetal origins of coronary heart disease. BMJ. 1995;311:171-174.
32. Hales C, Barker D, Clark P, Cox L, Fall C, Osmond C, Winter P. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ. 1991;303:1019-1022.
33. Gluckman P, Hanson M, Beedle A. Early life events and their consequences for later disease: a life history and evolutionary perspective. Am J Hum Biol. 2007;19:1-19.
34. Carver J. Nutrition for preterm infants after hospital discharge. Adv Pediatr. 2005;52:23-47.
35. Vickers M, Breier B, Cutfield W, Gluckman P. Fetal origins of hyperphagia, obesity, and hypertension and postnatal amplification by hypercaloric nutrition. Am J Physiol Endocrinol Metab. 2000;279:E83-87.
36. Hales C, Barker D. The thrifty phenotype hypothesis. Br Med Bull. 2001;60:5-20.
37. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet. 2004;363:1642-1645.
38. Cianfarani S, Germani D, Branca F. Low birth weight and adult insulin resistance: "the catch up growth" hypothesis. Arch Dis Child Fetal Neonatal Ed. 1999;81:F71-F73.

39. Ay L, van Houten V, Steegers E, Hofman A, Witteman J, Jaddoe V, Hokken-Koelega A. Fetal and postnatal growth and body composition at 6 months of age. J Clin Endocrinol Metab. 2009;94:2023-2030.
40. Chandler-Laney P, Gower B, Field D. Gestational and early life influences on infant body composition at 1 year. Obesity. 2013;21:144-148.
41. Griffin I, Cooke R. Development of whole body adiposity in preterm infants. Early Hum Dev. 2012;88:S19-S24.
42. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ. 2005;331:929.
43. Druet C, Stettler N, Sharp S, Simmons R, Cooper C, Smith G, Ekelund U, Lévy-Marchal C, Jarvelin M, et al. Prediction of childhood obesity by infancy weight gain: an individual-level meta-analysis. Paediatr Perinatal Epidemiol. 2012;26:19-26.
44. Monteiro P, Victora C. Rapid growth in infancy and childhood and obesity in later life-a systematic review. Obes Rev. 2005;6:143-154.
45. Euser A, Finken M, Keijzer-Veen M, Hille E, Wit J, Dekker F. Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. Am J Clin Nutr. 2005;81:480-487.
46. Howe L, Tilling K, Benfield L, Logue J, Sattar N, Ness A, Smith G, Lawlor D. Changes in ponderal index and body mass index across childhood and their associations with fat mass and cardiovascular risk factors at age 15. PLoS ONE. 2010;5:e15186.
47. Cheng S, Völgyi E, Tylavsky F, Lyytikäinen A, Törmäkangas T, Xu L, Cheng S, Kröger H, Alèn M, Kujala U. Trait-specific tracking and determinants of body composition: a 7-year follow-up study of pubertal growth in girls. BMC Med. 2009;7:5.
48. Meas T. Fetal origins of insulin resistance and the metabolic syndrome: A key role for adipose tissue? Diabetes & Metabolism. 2010;36:11-20.
49. Wells J, Chomtho S, Fewtrell M. Programming of body composition by early growth and nutrition. Proc Nutr Soc. 2007;66:423-434.
50. Prentice A. Micronutrients and the bone mineral content of the mother, fetus and newborn. J Nutr. 2003;133:1693S-1699S.
51. Ellis K, Shypailo R, Schanler R, Langston C. Body elemental composition of the neonate: new reference data. Am J Hum Biol. 1993;5:323-330.
52. Petersen S, Gotfredsen A, Knudsen F. Total body bone mineral in light-for-gestational-age infants and appropriate-for-gestational-age infants. Acta Paediatr Scand. 1989;78:347-350.
53. Shiff Y, Eliakim A, Shainkin-Kestenbaum R, Arnon S, Lis M, Dolfin T. Measurements of bone turnover markers in premature infants. J Pediatr Endocrinol Metab. 2001;14:389-395.
54. Avila-Díaz M, Flores-Huerta S, Martínez-Muñiz I, Amato D. Increments in whole body bone mineral content associated with weight and length in pre-term and full-term infants during the first 6 months of life. Arch Med Res. 2001;32:288-292.
55. Bowden L, Jones C, Ryan S. Bone mineralisation in ex-preterm infants aged 8 years. Eur J Pediatr. 1999;158:658-661.
56. Fewtrell M, Prentice A, Jones S, Bishop N, Stirling D. Bone mineralization and turnover in preterm infants at 8-12 years of age: the effect of early diet. J Bone Miner Res. 1999;14:810-820.
57. Hovi P, Andersson S, Järvenpää A, Eriksson J, Strang-Karlsson S, Kajantie E, Mäkitie O. Decreased bone mineral density in adults born with very low birth weight: a cohort study. PLoS Med. 2009;6:e.1000135.

58. Fewtrell M, Williams J, Singhal A, Murgatroyd P, Fuller N. Early diet and peak bone mass: 20 year follow up of a randomized trial of early diet in infants born preterm. Bone. 2009;45:142-149.
59. Smith C, Wright N, Wales J, Mackenzie C, Primhak R, Eastell R, Walsh J. Very low birth weight survivors have reduced peak bone mass and reduced insulin sensitivity. Clin Endocrinol. 2011;75:443-449.
60. Hernandez C, Beaupré G, Carter D. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int. 2003;14:843-847.
61. Clark R, Wagner C, Merritt R, Bloom B, Neu J. Nutrition in the neonatal intensive care unit: how do we reduce the incidence of extrauterine growth restriction? J Perinatol. 2003;23:337-344.
62. Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr. 2012;101:64-70.
63. Wauben I, Atkinson S, Grad T, Shah J, Paes B. Moderate nutrient supplementation of mother's milk for preterm infants supports adequate bone mass and short-term growth: a randomized, controlled trial. Am J Clin Nutr. 1998;67:465-472.
64. Pieltain C, De Curtis M, Gérard P, Rigo J. Weight gain composition in preterm infants with dual energy x-ray absorptiometry. Pediatr Res. 2001;49:120-124.
65. Alpay F, Ünay B, Narin Y, Inanc N, Özcan O, Gökcay E. Measurement of bone mineral density by dual energy X-ray absorptiometry in preterm infants fed human milk or formula. Eur J Pediatr. 1998;157:505-507.
66. Rao R, Georgieff M. Iron therapy for preterm infants. Clin Perinatol. 2009;36:27-42.
67. Klein C. Nutrient requirements for preterm infant formulas. J Nutr. 2002;123:1395S-1577S.
68. Hall R, Wheeler R, Benson J, Harris G, Rippetoe L. Feeding iron-fortified premature formula during initial hospitalization to infants less than 1800 grams birth weight. Pediatrics. 1993;92:409-414.
69. Lundström U, Siimes M, Dallman P. At what age does iron supplementation become necessary in low-birth-weight infants? J Pediatr. 1977;91:878-883.
70. Franz A, Mihatsch W, Sander S, Kron M, Pohlandt F. Prospective randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams. Pediatrics. 2000;106:700-706.
71. Mills R, Davies M. Enteral iron supplementation in preterm and low birth weight infants (Review). Cochrane Database of Syst Rev. 2012;14:CD005095.
72. Long H, Yi J, Hu P, Li Z, Qiu W, Wang F, Zhu S. Benefits of iron supplementation for low birth weight infants: a systematic review. BMC Pediatr. 2012;12:99.
73. Yadav D, Chandra J. Iron deficiency: beyond anemia. Indian J Pediatr. 2011;78:65-72.
74. Domellöf M. Benefits and harms of iron supplementation in iron-deficient and iron-sufficient children. Nestlé Nutr Instr Workshop Ser Pediatr Program. 2010;65:153-165.
75. Gera T, Sachdev H. Effect of iron supplementation on incidence of infectious illness in children: systematic review. BMJ. 2002;325:1142.
76. American Academy of Pediatrics. Iron fortification of infant formulas. Pediatrics. 1999;104:119-123.
77. Lauritzen L, Hansen H, Jorgensen M, Michaelsen K. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res. 2001;40:1-94.
78. Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. J Neurochem. 1998;71:2528-2533.

79. Uauy R, Rojas C, Llanos A, Mena P. Dietary essential fatty acids in early postnatal life: long-term outcomes. Nestlé Nutr Workshop Ser Pediatr Program. 2005;55:101-133.
80. Simmer K, Patole S, Rao S. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database of Syst Rev. 2011;Dec 7(12):CD000376.
81. Schulzke S, Patole S, Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev. 2011;Feb 16(2):CD000375.
82. Agostoni C, Buonocore G, Carnielli V, De Curtis M, Darmaun D, Decsi T, Dömellof M, Embleton N, Fusch C, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50:85-91.
83. Koletzko B, Baker S, Cleghorn G, Neto U, Gopalan S, Hernell O, Hock Q, Jirapinyo P, Lönnderdal B, et al. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. J Pediatr Gastroenterol Nutr. 2005;41:584-599.
84. Thureen P, Heird W. Protein and energy requirements of the preterm/low birthweight (LBW) infant. Pediatr Res. 2005;5:95-98.
85. Arslanoglu A, Moro G, Ziegler E. Adjustable fortification of human milk fed to preterm infants: does it make a difference. J Perinatol. 2006;26:614-621.
86. Clark R, Chace D, Spitzer A. Effects of two different doses of amino acid supplementation on growth and blood amino acid levels in premature neonates admitted to the neonatal intensive care unit: a randomized, controlled trial. Pediatrics. 2007;120:1286-1296.
87. Ziegler E, Carlson S. Early nutrition of very low birth weight infants. J Matern Fetal Neonatal Med. 2009;22:191-197.
88. Clark R, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. Pediatrics. 2003;111:986-990.
89. van den Akker C, te Braake F, Schierbeek H, Rietveld T, Wattimena D, Bunt J, van Goudoever J. Albumin synthesis in premature neonates is stimulated by parenterally administered amino acids during the first days of life. J Clin Nutr. 2007;86:1003-1008.
90. Vlaardingerbroek H, van Goudoever J, van den Akker C. Initial nutritional management of the preterm infant. Early Hum Dev. 2009;85:691-695.
91. Vlaardingerbroek H, Veldhorst M, Spronk S, van den Akker C, van Goudoever J. Parenteral lipid administration to very-low-birth-weight infants-early introduction of lipids and use of new lipid emulsions: a systematic review and meta-analysis. Am J Clin Nutr. 2012;96:255-268.
92. Senterre J, Salle B. Calcium and phosphorus economy of the preterm infant and its interaction with vitamin D and its metabolites. Acta Paediatr Scand Suppl. 1982;296:85-92.
93. Lapillonne A, O'Connor D, Wang D, Rigo J. Nutritional recommendations for the late-preterm infant and the preterm infant after hospital discharge. J Pediatr. 2013;162:S90-S100.
94. Aggett P, Agostoni C, Axelsson I, DeCurtis M, Goulet O. Feeding preterm infants after hospital discharge. J Pediatr Gastroenterol Nutr. 2006;42:596-603.
95. Cooke R, Embleton N, Griffin I, Wells J, McCormick K. Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr Res. 2001;49:719-722.
96. Picaud J, Decullier E, Plan O, Pidoux O, Bin-Dorel S, van Egroo L, Chapuis F, Claris O. Growth and bone mineralization in preterm infants fed preterm formula or standard term formula after discharge. J Pediatr. 2008;153:616-621.
97. Lucas A, Fewtrell M, Morley R, Singhal A, Abbott R. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics. 2001;108:703-711.

98. Jeon G, Jung Y, Koh S, Lee Y, Kim K, Shin S, Kim S, Shim J, Chang Y, Park W. Preterm infants fed nutrient-enriched formula until 6 months show improved growth and development. Pediatr Int. 2011;53:683-688.
99. Cooke R, Griffin I, McCormick K. Adiposity is not altered in preterm infants fed a nutrient-enriched formula after hospital discharge. Pediatr Res. 2010;67:660-664.
100. Kashyap S, Schulze K, Forsyth M, Zucker C, Dell R, Ramakrishnan R, Heird W. Growth, nutrient retention, and metabolic response in low birth weight infants fed varying intakes of protein and energy. J Pediatr. 1988;113:713-721.
101. Romera G, Figueras J, Rodríguez-Miquélez J, Ortega J, Jiménez R. Energy intake, metabolic balance and growth in preterm infants fed formulas with different nonprotein energy supplements. J Pediatr Gastroenterol Nutr. 2004;38:407-413.
102. Roggero P, Giannì M, Amato O, Liotto N, Morlacchi L, Orsi A, Piemontese P, Taroni F, Morniroli D, et al. Growth and fat-free mass gain in preterm infants after discharge: a randomized controlled trial. Pediatrics. 2012;130:e1215.
103. Young L, Morgan J, McCormick F, McGuire W. Nutrient-enriched formula versus standard term formula for preterm infants following hospital discharge. Cochrane Database of Syst Rev. 2012;14:CD004696.
104. Bishop N, King F, Lucas A. Increased bone mineral content of preterm infants fed with nutrient enriched formula after discharge from hospital. Arch Dis Child. 1993;68:573-578.
105. Cooke R, McCormick K, Griffin I, Embleton N, Faulkner K. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res. 1999;46:461-465.
106. Chan G. Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. J Pediatr. 1993;123:439-443.
107. Lapillonne A, Salle B, Glorieux F, Claris O. Bone mineralization and growth are enhanced in preterm infants fed an isocaloric, nutrient-enriched preterm formula through term. Am J Clin Nutr. 2004;80:1595-1603.
108. Griffin I, Cooke R, Reid M, McCormick K, Smith J. Iron nutritional status in preterm infants fed formulas fortified with iron. Arch Dis Child Fetal Neonatal Ed. 1999;81:F45-F49.

Chapter 2

Objective and aims

This thesis focuses on the effects of the prenatal growth trajectory, the early postnatal growth trajectory, and postdischarge nutrition on growth, body composition, and bone accretion of preterm infants during the first half of infancy. It is based on a single-blinded randomized controlled trial in preterm infants entitled "Study Towards the Effects of Postdischarge nutrition on growth and body composition of infants born ≤32 weeks gestational age and/or ≤1,500 gram birth weight (STEP)" [1]. This chapter describes the design and main results of the STEP as well as the aims and outline of this thesis.

STUDY TOWARDS THE EFFECTS OF POSTDISCHARGE NUTRITION (STEP)

The hypothesis of the STEP was that a postdischarge formula with a similar energy content and a higher protein, mineral, and vitamin content compared to standard term formula improved the quality of growth by preventing increased fat deposition because protein intake is associated with lean mass accretion [2]. The STEP evaluated the effect of postdischarge formula (PDF), standard term formula (TF), and human milk (HM) on growth and body composition between term age (i.e. 40 weeks postmenstrual age) and six months corrected age (CA) [1].

Study design

Between 2003 and 2006, infants born at a gestational age of 32 weeks or less and/or with a birth weight of 1,500 gram or less were considered eligible for the STEP (Figure 2.1). Exclusion criteria were congenital malformations or conditions known to affect growth or body composition. At term age, 152 preterm infants were included and at six months CA, 139 preterm infants (52 PDF, 41 TF, and 46 HM fed infants) completed the study (Figure 2.1).

Between birth and term age, preterm infants were fed 150 ml/kg/d preterm formula or fortified human milk (Figure 2.1 and Figure 2.2). The type of feeding between birth and term age could not be determined in two infants due to missing data. At term age, 102 formula fed infants were randomized to PDF or TF and were fed the study formulae until six months CA (Figure 2.1 and Figure 2.2). PDF provided a similar quantity of energy and a higher quantity of protein, minerals, vitamins, and LC-PUFAs compared to TF (Appendix A). Fifty preterm infants that were fed predominantly (>80%) HM at term age were considered to be HM fed and were supplemented with 200 IU vitamin D per day. If HM was insufficiently available, HM fed infants were fed TF additionally. Supplements that provided additional energy were limited before term age and were not allowed thereafter. The introduction of complementary feeding was discouraged until five months CA and was limited to fruit and vegetables. Between term age and six months CA, parents recorded their infant's weight (g), volume intake (ml/d), the type of intake (PDF, TF, or

HM), any supplements, and any changes in their infant's daily intake in a diary once per week. In PDF and TF fed infants, total volume intake (ml/kg/d), energy intake (kcal/kg/d), and protein intake (g/kg/d) were calculated based on the infant's weight, volume intake, and the formula composition (Appendix A).

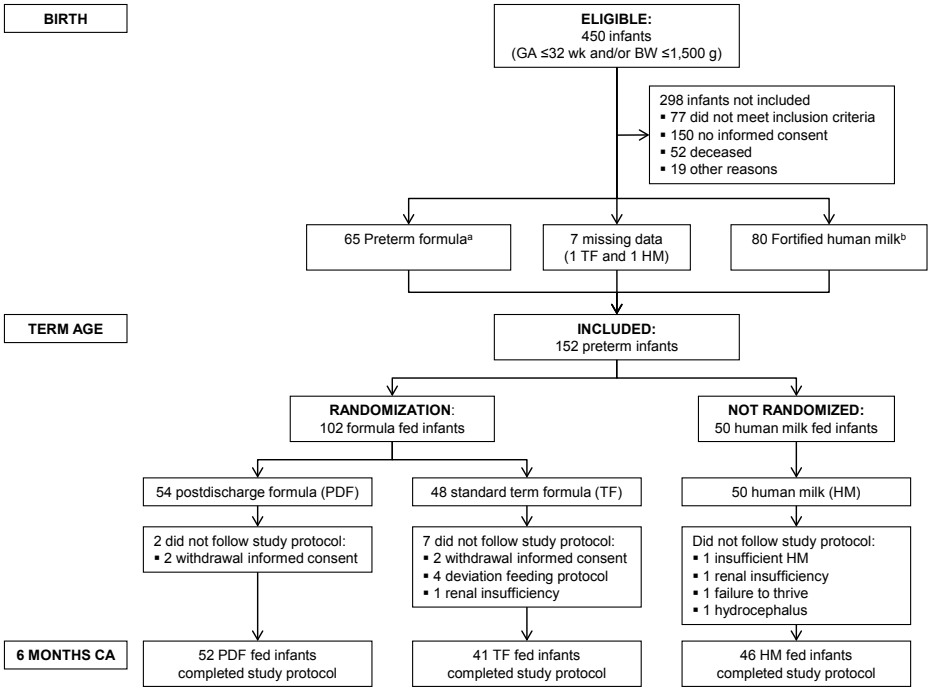


Figure 2.1. STEP subject recruitment and inclusion
BW: birth weight; CA: corrected age; GA: gestational age; HM: human milk; PDF: postdischarge formula; TF: term formula. [a] 39 PDF- and 26 TF-fed infants; [b] 13 PDF-, 18 TF-, and 49 HM-fed infants.

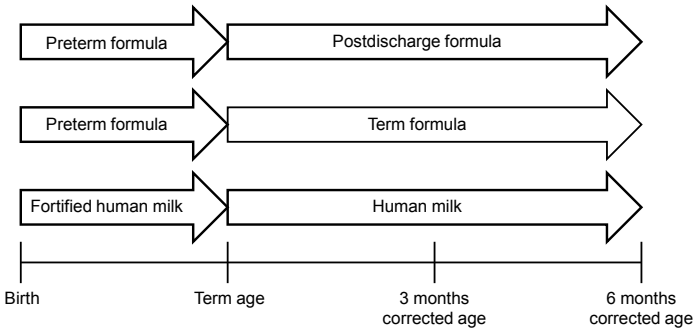


Figure 2.2. Study design of the STEP

Gestational age at birth was extracted from the infant's medical record. At birth, term age, three and six months CA, weight, length, and head circumference were measured and expressed as standard deviation scores (SDS) based on references for preterm infants at birth and term age [3] and based on references for Dutch infants at three and six months CA [4]. At term age and six months CA, lean mass (LM; g) and fat mass (FM; g) were measured by dual-energy x-ray absorptiometry (DXA; Hologic QDR 4500A, Hologic Inc., Bedford, MA, USA) and analyzed by Infant Whole Body Software version 12.3.3.

Main results

At birth and term age, weight, length, head circumference, and body composition were not different between PDF and TF fed infants (Table 2.1 and Table 2.2). Between term age and six months CA, protein intake was higher in PDF compared to TF fed infants (Figure 2.3), whereas volume intake and energy intake were similar. At six months CA, PDF fed infants had similar weight SDS, length SDS, and head circumference SDS compared to TF fed infants (Chapter 8, Figure 8.2). However, PDF fed infants had a lower %FM compared to TF fed infants (Table 2.2). During the first six months post-term, PDF fed infants gained more LM and less FM compared to TF fed infants (Table 2.2). In addition, post-hoc analyses indicated that boys and preterm infants with EUGR benefited from PDF, as they had a lower gain in FM during the first six months post-term when fed PDF compared to TF [1].

Table 2.1. Characteristics of PDF, TF, and HM fed infants at birth and term age

	PDF (n=52)	TF (n=41)	HM (n=46)
Boys[1]	51.9%	56.1%	45.7%
Birth			
Postmenstrual age (wk)	30.7 (1.5)	30.9 (2.3)	30.0 (1.9)
Weight (g)	1344 (483)	1394 (262)	1345 (605)
Length (cm)	38.0 (4.0)	38.3 (3.0)	38.0 (3.5)
Head circumference (cm)	27.9 (2.5)	28.0 (2.1)	28.0 (2.8)
Term age			
Postmenstrual age (wk)	40.6 (1.1)	40.3 (0.9)	40.6 (0.7)
Weight (g)	3173 (699)	3090 (603)	3158 (734)
Length (cm)	49.0 (3.0)	48.5 (3.0)	48.3 (2.6)
Head circumference (cm)	35.9 (1.4)	35.8 (1.6)	35.8 (2.3)

Values expressed as median (interquartile range), unless specified otherwise. Linear regression adjusted for gender and gestational age showed no significant differences between PDF, TF, and HM fed infants. [1] frequency (%); chi-square test showed no significant differences between PDF, TF, and HM fed infants. HM: human milk; PDF: postdischarge formula; TF: term formula.

Table 2.2. Body composition of PDF and TF fed infants between term age and six months CA

	PDF (n=43)	TF (n=34)	PDF vs. TF[1]
Term age			
LM (kg)	3.04 ± 0.43	3.07 ± 0.41	n.s.
FM (kg)	0.24 (0.12;0.45)	0.30 (0.18;0.46)	n.s.
%FM	6.8 (4.5;11.9)	8.3 (5.5;12.4)	n.s.
6 months CA			
LM (kg)	5.78 ± 0.62	5.64 ± 0.62	n.s.
FM (kg)	1.85 (1.32;2.28)	2.01 (1.63;2.42)	n.s.
%FM	23.6 ± 7.1	26.8 ± 6.2	0.043
Term age-6 months CA			
Gain in LM (g/kg/d)	2.00 ± 0.39	1.81 ± 0.31	0.022
Gain in FM (g/kg/d)	1.08 (0.84;1.34)	1.14 (1.02;1.53)	0.050

Values expressed as median ± SD or median (P25;P75). [1] P-value of unpaired t-test. CA: corrected age; FM: fat mass; HM: human milk; LM: lean mass; n.s.: not significant; PDF: postdischarge formula; TF: term formula.

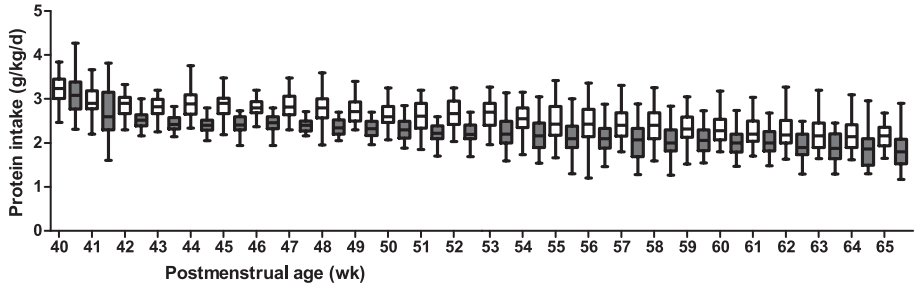


Figure 2.3. Protein intake (g/kg/d) between term age and six months corrected age in postdischarge formula (PDF; white) and term formula (TF; grey) fed infants
Tukey boxplots. PDF: postdischarge formula; TF: term formula. For all weeks, Mann-Whitney U test showed higher protein intake in PDF compared to TF fed infants, P<0.05 [unpublished data].

Conclusion

It was concluded that preterm infants fed the isocaloric, protein- and mineral-enriched PDF used in the STEP have similar growth with less fat mass gain and more lean mass gain during the first six months post-term compared to preterm infants fed TF [1].

AIMS OF THIS THESIS

The first aim of this thesis was to study the effects of the prenatal and early postnatal growth trajectory on growth, body composition, and bone accretion during the first

half of infancy (Part I). The regulation of postnatal growth of preterm infants by the endocrine growth regulators IGF-I and insulin was investigated in relation to nutrient intake during the first six months post-term. In addition, the effect of being SGA (birth weight, birth length, or both <-2 SDS) on growth, body composition, and bone accretion between term age and six months CA was evaluated and it was investigated if this effect was modified by nutritional intake or the type of postdischarge nutrition. The second aim of this thesis was to study the effects of postdischarge nutrition on growth, LC-PUFA status, bone accretion, vitamin D status, and the incidence of iron deficiency of preterm infants between term age and six months CA (Part II).

THESIS OUTLINE

The thesis outline is summarized in Figure 2.4. **Part I (Chapter 3-6)** focuses on the effects of the prenatal and postnatal growth trajectory. **Chapter 3** reports on the role of IGF-I and insulin in growth regulation of preterm infants during the first half of infancy. **Chapter 4** describes the body composition between term age and six months CA of SGA preterm infants and AGA preterm infants with EUGR and without EUGR before term age. **Chapter 5** reports on the bone accretion of SGA compared to AGA preterm infants during the first six months post-term. **Chapter 6** shows the relation between growth, bone accretion, and procollagen type I N-terminal peptide (PINP), a marker of collagen type I synthesis, in SGA and AGA preterm infants between term age and six months CA.

Part II (Chapter 7-10) focuses on the effects of postdischarge nutrition. **Chapter 7** demonstrates the effects of PDF, TF, and HM on bone accretion during the first six months post-term. **Chapter 8** describes LC-PUFAs in relation to growth of preterm infants between term age and six months CA. **Chapter 9** shows the effects of PDF, TF, and HM on vitamin D status of preterm infants during the first six months post-term.

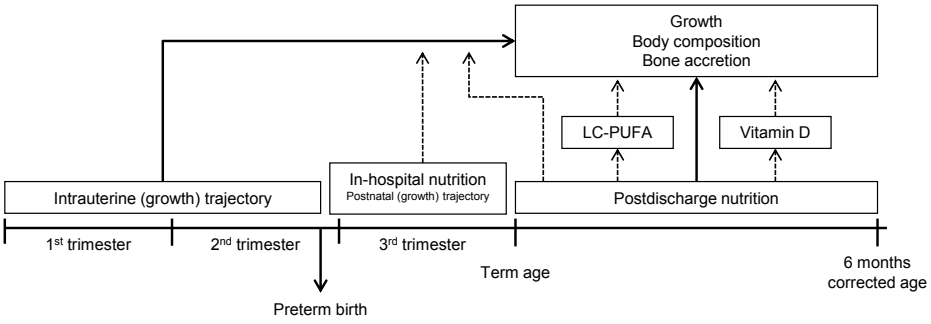


Figure 2.4. Thesis outline
LC-PUFA: long-chain polyunsaturated fatty acid

Chapter 10 reports on the incidence of iron deficiency in PDF, TF, and HM fed preterm infants during the first half of infancy.

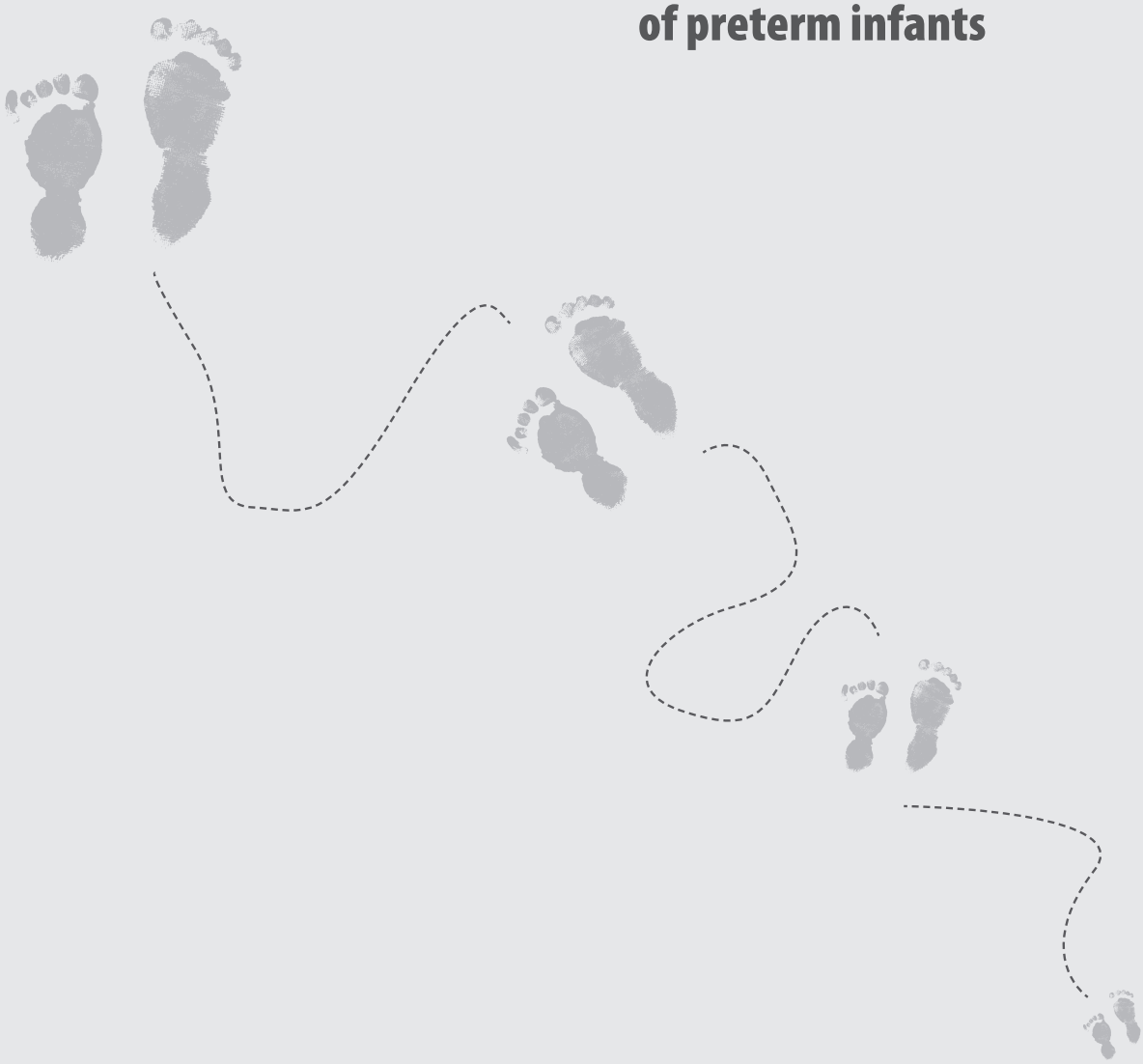
Chapter 11 discusses the findings and implications of this thesis in relation to the current literature and provides suggestions for future research. **Chapter 12** summarizes the findings of this thesis in English and Dutch, respectively.

REFERENCES

1. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
2. Kashyap S, Schulze K, Forsyth M, Zucker C, Dell R, Ramakrishnan R, Heird W. Growth, nutrient retention, and metabolic response in low birth weight infants fed varying intakes of protein and energy. J Pediatr. 1988;113:713-721.
3. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
4. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.

Part I

Prenatal and postnatal growth of preterm infants



Chapter 3

Growth in preterm infants until six months post-term: the role of insulin and IGF-I

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ABSTRACT

Background/Aims

Since insulin-like growth factor type I (IGF-I) and insulin regulate growth in term infants, they were studied in relation to nutrient intake and growth until 6 months corrected age (CA) in preterm infants.

Methods

In 138 preterm infants (51% male, gestational age [expressed as median (IQR)] 30.6 (1.9) weeks, birth weight 1368 (389) g), weight SDS, length SDS, IGF-I, and insulin were measured at term age, 3 and 6 months CA.

Results

IGF-I and insulin at term age were associated with weight SDS and length SDS at term age and 3 months CA. IGF-I and insulin at 3 months CA were associated with weight SDS and length SDS at 3 and 6 months CA. IGF-I and insulin at term age were negatively associated with gain in weight SDS and gain in length SDS between term age and 6 months CA (IGF-I: β=-1.03, 95% CI -1.65;-0.41, p=0.001 and β=-0.78, 95% CI -1.32;-0.23, p=0.005; insulin: β=-0.19, 95% CI -0.37;-0.01, p=0.044 and β=-0.18, 95% CI -0.35;-0.01, p=0.035). Nutrient intake was not associated with IGF-I or insulin.

Conclusions

The present study suggests that IGF-I and insulin are important growth regulators in preterm infants until 6 months CA, independent of nutrient intake.

INTRODUCTION

Postnatal growth restriction is common in preterm infants and is usually explained by postnatal morbidity in combination with insufficient nutrient intake. Postnatal growth is mainly regulated by nutrients, insulin, and insulin-like growth factor type I (IGF-I). Sufficient nutrient supply and adequate insulin action are important for fetal IGF-I production, indicating a linkage between growth and nutrient availability [1-2]. In term infants, IGF-I levels are associated with postnatal growth and protein intake during infancy [3-8].

In preterm infants, IGF-I levels are associated with protein intake until term age (40 weeks postmenstrual age) [9-13] and with postnatal growth until 6 months corrected age (CA) [14]. Little attention has been devoted to IGF-I and insulin in relation to growth and nutrient intake in preterm infants after term age. We hypothesized that IGF-I levels and insulin levels were positively related to nutrient intake and growth until 6 months CA in preterm infants. Therefore, the present study investigated IGF-I levels and insulin levels between term age and 6 months CA in relation to nutrient intake and growth between birth and 6 months CA in preterm infants.

METHODS

Subjects

The present study was an observational analysis within a randomized controlled trial that evaluated the effects of postdischarge formula, term formula, and human milk on growth and body composition of preterm infants, as described in detail elsewhere [15]. In short, 152 infants with a gestational age ≤32 weeks and/or a birth weight ≤1,500 g were included shortly after birth [15]. The present study combined nutritional data with anthropometry, serum IGF-I levels, and serum insulin levels. The infants attended our outpatient clinic at term age (40.3 ± 0.7 weeks postmenstrual age), 3 months CA (53.0 ± 0.5 weeks postmenstrual age), and 6 months CA (66.0 ± 0.5 weeks postmenstrual age). At 6 months CA, 139 preterm infants completed the study. 13 infants did not complete the study for different reasons (withdrawn by parents (n=4), incorrect feeding protocol (n=5), renal disease (n=2), failure to thrive (n=1), and hydrocephalus (n=1)). In 1 infant, length at 6 months CA was not measured due to a body cast for developmental hip dysplasia. Therefore, 138 infants were included in the analyses of the present study (51% boys, gestational age [expressed as median (interquartile range)] 30.6 (1.9) weeks (range 25.3-32.9 weeks), birth weight 1,368 (389) g (range 710-2,065 g)).

The study protocol was approved by the ethics committee of VU University Medical Center, Amsterdam, The Netherlands. All the parents of the participating children gave written informed consent.

Anthropometry and severity of illness

At birth, term age, and 3 and 6 months CA, a single research nurse measured weight (g) with a digital scale to the nearest 1.0 g, length (cm) with a length board to the nearest 0.1 cm, and head circumference (cm) with a non-stretchable measuring tape to the nearest 0.1 cm, as described previously [15]. To adjust for postmenstrual age and gender, weight, length, and head circumference were expressed as standard deviation score (SDS) based on Swedish references for preterm infants at birth and term age [16] and as SDS based on Dutch references at 3 and 6 months CA [17]. Unfortunately, Dutch references for weight, length, and head circumference of preterm infants at birth and term age were not available. Gain, expressed as ΔSDS, in weight, length, and head circumference was calculated for the following intervals: birth-term age, birth-3 months CA, birth-6 months CA, term age-3 months CA, term age-6 months CA, 3-6 months CA. During hospital admission, the Neonatal Therapeutic Intervention Scoring System was used to evaluate severity of illness. The Neonatal Therapeutic Intervention Scoring System has a maximum score of 130 and is a valid indicator of severity of illness in neonates, independent of birth weight [18].

Hormone level analyses

At term age and at 3 and 6 months CA, fasting venous blood samples were collected for IGF-I and insulin level analyses and mean fasting duration was recorded as the interval between blood sampling and the last feed before blood sampling. Mean fasting duration was 3.4 ± 0.7 h at term age, 3.6 ± 0.7 h at 3 months CA, and 3.5 ± 0.7 h at 6 months CA. Serum was stored at -80°C and serum IGF-I and insulin level analyses were performed at two time-points.

IGF-I concentrations (nmol/l) were measured using two different methods as the local assay changed. In 80 infants, IGF-I levels were determined using one immunoassay (Advantage, Nichols Diagnostics, Capistrano, Calif., USA) with an intra-assay variation of <6%, an inter-assay variation of <8% for the whole range, and a lower limit of quantification of 2 nmol/l. In 59 infants, IGF-I levels were measured using another immunoassay (Immulite 2500, Siemens Diagnostics, Deerfield, IL., USA) with an intra-assay variation of <5%, an inter-assay variation of <8% for the whole range, and a lower limit of quantification of 3.2 nmol/l. All measurements were performed at the Endocrine Laboratory of VU University Medical Center, Amsterdam, The Netherlands. Since a comparison of the two methods by regression analysis, as described by Passing and Bablok [19], resulted in the following formula: Immulite=0.99*Advantage+1.2 and showed a correlation coefficient of 0.99, we decided that IGF-I levels measured by both methods were comparable and could be analyzed together in the present study. IGF-I levels were available from 129 preterm infants at term age, 132 preterm infants at 3 months CA, and 126 preterm

infants at 6 months CA. The difference in IGF-I levels was calculated for the following intervals: term age-3 months CA and 3-6 months CA.

Insulin concentration (pmol/l) was measured using an automated immunoassay (Advia Centaur, Siemens Medical Solutions, USA) with an intra-assay variance of <4% and an inter-assay variance of <8% for the whole range. At 3 and 6 months CA, 1 infant was excluded from the insulin level analysis, because blood samples were collected within 2 h after feeding. Insulin levels were available from 135 preterm infants at term age, 134 preterm infants at 3 months CA, and 134 preterm infants at 6 months CA.

Nutritional intake

Between birth and term age, infants were fed preterm formula (n=61) or fortified human milk (n=75) and in 2 infants the type of diet could not be classified due to missing data. Between term age and 6 months CA, infants were fed postdischarge formula (n=51), term formula (n=41), or human milk (n=46) [15]. Complementary feeding was not introduced before 5 months CA. If it was introduced between 5 and 6 months CA, it was limited to fruits and vegetables once a day.

Between birth and discharge, details on enteral and parenteral intake were collected from the daily log in each infant's medical record by one single person. Between discharge and 6 months CA, parents recorded their child's intake in a diary. Between discharge and term age, parents recorded their child's intake daily; between term age and 6 months CA, they recorded their child's intake 1 day per week and indicated if the amount of formula given per day changed at any time during the week. The medical records and parental diaries provided information about type and amount of intake (ml). Before term age, human milk fed infants were predominantly bottle-fed and of these infants the amount of enteral intake was recorded. After term age, human milk fed infants were breast-fed and their exact enteral intake was unknown. The available information on the amount of intake was used to calculate total parenteral and enteral volume intakes (ml/kg/day) between birth and 6 months CA. The mean intakes of energy (kcal/kg/day), carbohydrate (g/kg/day), fat (g/kg/day), and protein (g/kg/day) between birth and 6 months CA were calculated based on the total volume intake (ml/kg/day), the composition of parenteral fluids and infant formulas, an estimated composition of unfortified human milk (per 100 ml: 68 kcal, 1 g protein, 4 g fat, 7 g carbohydrates), and the composition of breast milk fortifier (per 100 g: 361 kcal, 19 g protein, 71.5 g carbohydrates) [20]. Between birth and term age, energy, fat, and protein intakes were available from 135 infants (61 preterm formula fed infants and 74 fortified human milk fed infants) and carbohydrate intake was available from 127 infants (56 preterm formula fed infants and 71 fortified human milk fed infants). Energy, fat, protein, and carbohydrate intakes were available from 90 formula-fed infants between term age and 3 months CA and from 91 formula-fed infants between 3 and 6 months CA.

Statistics

Gestational age, severity of illness, IGF-I levels, insulin levels, and nutritional intake were natural log transformed and described as median with IQR. The other variables were normally distributed and described as mean ± SD. Changes in weight SDS, length SDS, and head circumference SDS were evaluated by generalized estimating equations adjusted for gender and gestational age for the following intervals: birth-term age, term age-3 months CA, 3-6 months CA, term age-6 months CA. Changes in insulin levels and IGF-I levels were evaluated by generalized estimating equations adjusted for gender and gestational age for the following intervals: term age-3 months CA, 3-6 months CA, and term age-6 months CA. Associations between insulin levels, IGF-I levels, growth, and nutrient intake were evaluated by linear regression analyses, adjusted for gender and gestational age. Insulin levels were adjusted for mean fasting duration. $P<0.05$ was considered significant. All statistical analyses were performed in SPSS 17.0 for Windows (SPSS, Inc., Chicago, Ill., USA).

RESULTS

Growth, IGF-I levels, and insulin levels

Weight SDS and length SDS decreased between birth and term age and increased between term age and 3 months CA, and length SDS showed an additional increase between 3 and 6 months CA (Figure 3.1). Head circumference SDS increased between birth and term age and decreased between term age and 3 months CA (Figure 3.1). IGF-I levels and insulin levels increased between term age and 3 months CA and IGF-I levels showed a further increase between 3 and 6 months CA (Figure 3.2).

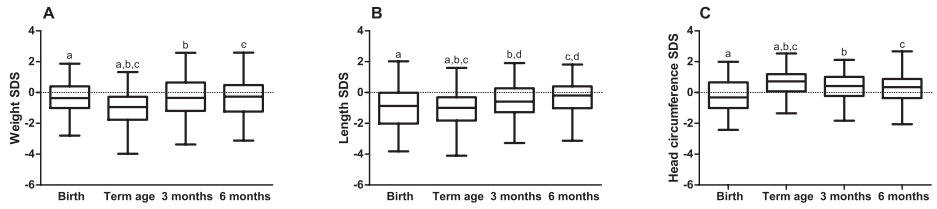

Figure 3.1. Weight SDS (A), length SDS (B), and head circumference SDS (C) between birth and 6 months CA

Tukey boxplots. Differences in weight SDS, length SDS, and head circumference SDS between time-points were evaluated by generalized estimating equations adjusted for gender and gestational age; similar letters indicate a significant difference between time-points, $P<0.01$.

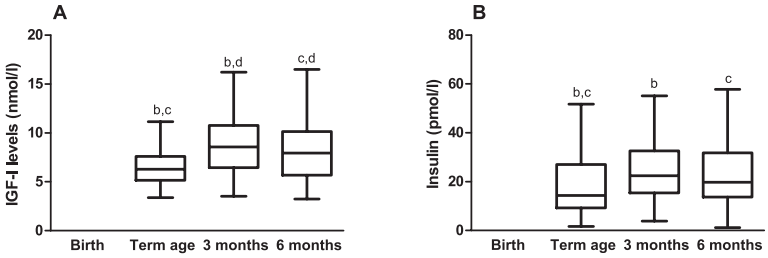

Figure 3.2. IGF-I (nmol/l) (A) and insulin levels (pmol/l) (B) between term age and 6 months CA
Tukey boxplots. Differences in IGF-I and insulin levels between time-points were evaluated by generalized estimating equations adjusted for gender and gestational age; similar letters indicate a significant difference between time-points, P<0.01.

Associations between IGF-I levels, insulin levels, and growth parameters at term age, 3 and 6 months CA

IGF-I levels and insulin levels were associated with weight SDS and length SDS at term age and at 3 and 6 months CA (Table 3.1 and Table 3.2). Insulin levels were associated with IGF-I levels at term age and at 3 months CA (β=0.12, 95% CI 0.06-0.18, p<0.001 and β=0.20, 95% CI 0.08-0.31, p=0.001, respectively), but not with IGF-I levels at 6 months CA. The associations between insulin and IGF-I levels at term age and 3 months CA remained significant after adjustment for severity of illness, type of diet, and nutritional intake (data not shown).

Table 3.1. IGF-I levels in relation to growth parameters at term age, 3 and 6 months CA

	IGF-I Term		IGF-I 3 m		IGF-I 6 m	
	β	95% CI	β	95% CI	β	95% CI
Weight SDS						
Term	1.99[a]	1.35-2.59				
3 m	1.57[a]	0.84-2.29	1.39[a]	0.84-1.94		
6 m	0.83[d]	0.13-1.54	1.18[a]	0.66-1.70	0.75[b]	0.25-1.26
Length SDS						
Term	1.77[a]	1.07-2.48				
3 m	1.40[a]	0.75-2.05	1.06[a]	0.55-1.58		
6 m	0.83[a]	0.16-1.51	1.08[a]	0.59-1.58	0.60[c]	0.12-1.07
Head circumference SDS						
Term	0.98[b]	0.43-1.53				
3 m	0.57	-0.01;1.15	0.60[b]	0.16-1.04		
6 m	0.31	-0.32;0.94	0.53[d]	0.05-1.0	-0.04	-0.50;0.42

Term: term age; m: months corrected age. Linear regression with log transformed IGF-I levels adjusted for gender and gestational age; [a] P<0.001, [b] P<0.01, [c] P<0.02, [d] P<0.05.

Table 3.2. Insulin levels in relation to growth parameters at term age, 3 and 6 months CA

	Insulin Term		Insulin 3 m		Insulin 6 m	
	β	95% CI	β	95% CI	β	95% CI
Weight SDS						
Term	0.30[c]	0.06-0.53				
3 m	0.27[d]	0.02-0.53	0.42[d]	0.01-0.83		
6 m	0.10	-0.14;0.35	0.38[d]	0.0-0.76	0.59[a]	0.31-0.87
Length SDS						
Term	0.37[b]	0.12-0.63				
3 m	0.31[c]	0.07-0.54	0.44[d]	0.06-0.81		
6 m	0.16	-0.06;0.39	0.41[d]	0.05-0.78	0.44[b]	0.18-0.71
Head circumference SDS						
Term	0.13	-0.07;0.33				
3 m	0.04	-0.16;0.24	0.05	-0.28;0.38		
6 m	-0.07	-0.28;0.15	0.12	-0.23;0.47	0.20	-0.06;0.46

Term: term age; m: months corrected age. Linear regression with log transformed insulin levels adjusted for gender, gestational age, and mean fasting duration; [a] P<0.001, [b] P<0.01, [c] P<0.02, [d] P<0.05.

Table 3.3. IGF-I and insulin levels in relation to preceding weight and length gain

	Term age			
	IGF-I		Insulin	
	β	95% CI	β	95% CI
Birth-term age				
ΔWeight SDS	0.20[a]	0.13;0.27		n.s.
ΔLength SDS	0.07[c]	0.02;0.13		n.s.
	3 months CA			
	β	95% CI	β	95% CI
Birth-3 months CA				
ΔWeight SDS	0.19[a]	0.12;0.25	0.12[d]	0.01;0.22
ΔLength SDS	0.11[b]	0.04;0.17	0.11[d]	0.004;0.22
	6 months CA			
	β	95% CI	β	95% CI
Birth-6 months CA				
ΔWeight SDS	0.13[a]	0.07;0.20	0.20[b]	0.08;0.32
ΔLength SDS	0.12[b]	0.05;0.18		n.s.

Δ: gain; CA: corrected age. Linear regression analysis with log transformed IGF-I and insulin levels adjusted for gender and gestational age (and mean fasting duration for insulin levels); [a] P<0.001, [b] P<0.01, [c] P<0.02, [d] P<0.05.

IGF-I levels and insulin levels in relation to preceding growth

IGF-I levels at term age, 3 and 6 months CA were associated with preceding weight SDS gain and length SDS gain between birth and term age, birth and 3 months CA, and birth

and 6 months CA, respectively (Table 3.3). Insulin levels at 3 months CA were associated with preceding weight SDS gain and length SDS gain between birth and 3 months CA (Table 3.3). Insulin levels at 6 months CA were associated with preceding weight SDS gain between birth and 6 months CA (Table 3.3). These associations were not influenced by severity of illness, the type of diet, or nutritional intakes. After adjustment for current weight or length, the associations between IGF-I levels at term age, 3 and 6 months CA and preceding weight SDS gain between birth and term age, birth and 3 months CA, and birth and 6 months CA, respectively remained significant, whereas those between IGF-I levels at term age, 3 and 6 months CA and preceding length SDS gain and the associations between insulin levels at 3 and 6 months and preceding weight SDS gain and length SDS gain disappeared.

IGF-I levels and insulin levels in relation to subsequent growth

IGF-I levels and insulin levels at term age, adjusted for growth between birth and term age, were negatively associated with weight SDS gain and length SDS gain between term age and 6 months CA (IGF-I: β=-1.03, 95% CI -1.65;-0.41, p=0.001 and β=-0.78, 95% CI -1.32;-0.23, p=0.005; insulin: β=-0.19, 95% CI -0.37;-0.01, p=0.044 and β=-0.18, 95% CI -0.35;-0.01, p=0.035). These associations were not influenced by severity of illness, the type of diet, or nutritional intakes. IGF-I levels and insulin levels at 3 months CA were not associated with weight SDS and length SDS gain between 3 and 6 months CA.

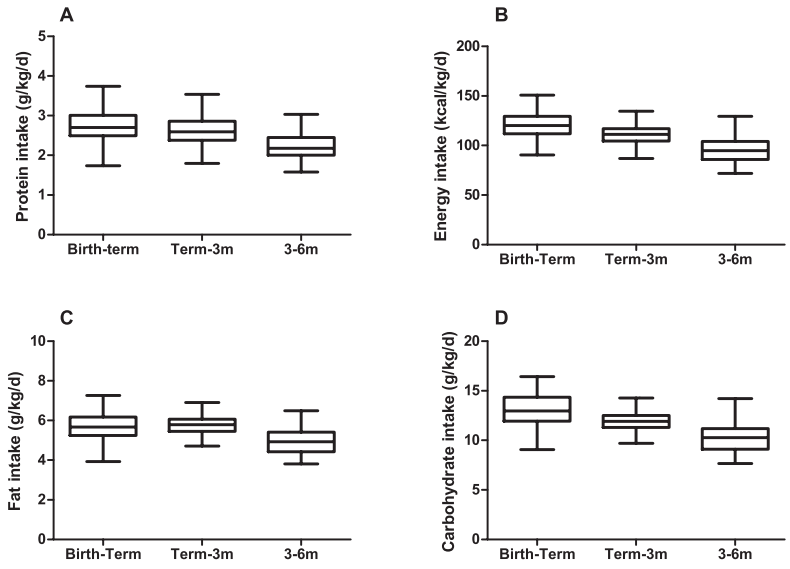

Figure 3.3. Protein (g/kg/d) (A), energy (kcal/kg/d) (B), fat (g/kg/d) (C), and carbohydrate (g/kg/d) (D) intake between birth and six months CA
Tukey boxplots.

IGF-I levels and insulin levels in relation to nutrient intake

Protein (g/kg/d), energy (kcal/kg/d), fat (g/kg/d), and carbohydrate (g/kg/d) intake decreased between birth and 6 months CA (Figure 3.3). Mean energy, protein, fat, and carbohydrate intake between birth and term age, between term age and 3 months CA, and between 3 and 6 months CA were neither associated with IGF-I levels nor with insulin levels at any point in time.

DISCUSSION

The present study suggests that IGF-I and insulin are important factors in growth regulation of preterm infants during the first 6 months post-term, as demonstrated by the relation between serum IGF-I and insulin levels, preceding growth, current growth parameters, and subsequent growth. The associations between IGF-I and insulin levels and current growth parameters are in accordance with previous studies of term infants at similar postmenstrual age [3-6] and with studies of preterm infants [9-11, 13-14]. Furthermore, the associations between IGF-I and insulin levels at term age and subsequent growth until 6 months CA, independent of growth before term age, are in line with the results of Chellakooty et al. and Madsen et al. in term infants at 3 months and 9 months of age, respectively [5, 8]. We hypothesize that the inverse association between IGF-I and insulin levels at term age and subsequent growth in the present study may reflect that most preterm infants experienced decreased growth between birth and term age, which resulted in lower IGF-I and insulin levels at term age, followed by accelerated growth after term age. If preterm infants experience the largest decrease in growth between birth and term age, they may demonstrate the most growth after term age. Thus, the findings of the present study may imply that IGF-I and insulin levels are regulators of post-term growth in preterm infants.

Previous studies have demonstrated that IGF-I levels are related to nutrient intake, in particular to protein intake, in term infants during late infancy [7] and in preterm infants before term age [12]. In contrast, other investigators did not find a direct association between nutrient intake and IGF-I levels in term infants at 9 months of age [8]. In the present study, IGF-I levels were not associated with nutrient intake in preterm infants after term age. We hypothesize that IGF-I levels in preterm infants might be independent of nutritional intake. In addition, IGF-I levels may be genetically determined.

On the other hand, the lack of association between IGF-I levels, insulin levels, and nutrient intake may be due to the insufficient data on the exact nutritional intake in the human milk fed infants. Between birth and term age, the composition of human milk was estimated based on average analytical information on the composition of human breast milk from the literature [20], while there is a large inter-individual and intra-individual

variation as well as a gradual change over time in the composition of human milk. In addition, between term age and 6 months CA, most human milk fed infants were breast-fed and, therefore, the exact amount and composition of their nutritional intake was unknown. Thus, we can only suggest that, with the post-term nutritional regimen used in the present study, nutrient intake does not play an essential role for IGF-I production in formula-fed infants.

During the third trimester of pregnancy, IGF-I is an important factor for fetal growth. During this phase, IGF-I is primarily controlled by the glucose/insulin axis that allows rapid responses to changes in nutrient intake [10]; insulin, in turn, may link nutrient intake to IGF-I. In the present study, insulin levels were positively related to IGF-I levels at term age and 3 months CA. This suggests that the post-term growth regulation in preterm infants shares similarities with the fetal growth regulation during the third trimester. Therefore, it is interesting to note that insulin levels were related to IGF-I levels at term age and at 3 months CA, but not at 6 months CA. In term infants, major changes in growth regulation occur with a shift from insulin/nutrition dependency at term age towards GH dependency at 6 months postnatal age [1]. The lack of association between IGF-I and insulin levels at 6 months CA in the present study may be due to this gradual maturation of the GH/IGF-I axis.

In addition, insulin is important for direct stimulation of postnatal growth, in particular body composition [21-23]. In our study, insulin levels were associated with preceding weight gain and subsequent growth but to a smaller extent than IGF-I. This might indicate that insulin mainly exerts a permissive role on the production of IGF-I instead of a direct enhancing role on postnatal growth and that insulin production is stimulated by gain in body weight.

This study had some limitations. A limitation of the present study is the small sample size. It should be considered that the number of subjects was too small to show significant associations between nutrient intake and IGF-I levels. In addition, this paper reports on post-hoc analyses, whereas the power analysis of the main randomized controlled trial was based on the primary objective of the complete study, notably differences in growth and body composition between preterm infants fed postdischarge formula, term formula, and human milk with the earlier mentioned limitations [15]. The lack of association between nutrient intake and IGF-I levels, therefore, needs to be interpreted with caution. Moreover, since this paper reports on post-hoc analyses, it can only suggest that IGF-I levels, insulin levels, and growth are associated. Within the design of the present study, causal relations between IGF-I levels, insulin levels, and growth cannot be established.

Furthermore, according to the study protocol of the randomized controlled trial, infants in the present study were fed different feeding regimes, e.g. postdischarge formula, term formula, or human milk. This type of feeding did not influence or explain any

of the associations found in the present study; however, the influence of the diet on any of the associations cannot be excluded completely.

In conclusion, this study suggests that IGF-I may play an important role in the regulation of growth in preterm infants during the first 6 months post-term, which shares similarities with the regulation of fetal growth and growth of term infants during infancy, and that insulin may exert a permissive role on the production of IGF-I. Similar to term infants, a shift from insulin dependent towards GH dependent growth regulation may occur in preterm infants at 6 months CA. In contrast to findings in term infants, our data could only permit to suggest that IGF-I levels in preterm infants until 6 months CA are not dependent on type of nutrition per se.

REFERENCES

1. Gluckman P, Pinal C. Regulation of fetal growth by the somatotrophic axis. J Nutr. 2003;133:1741S-1746S.
2. Oliver M, Harding J, Breier B, Evans P, Gluckman P. Glucose bot not a mixed amino acid infusion regulates insulin-like growth factor-I concentrations in fetal sheep. Pediatr Res. 1993;34:62-65.
3. Low L, Tam S, Kwan E, Tsang A, Karlberg J. Onset of significant GH dependence of serum IGF-1 and IGF-binding protein 3 concentrations in early life. Pediatr Res. 2001;50:737-742.
4. Ong K, Langkamp M, Ranke M, Whitehead K, Hughes I, Acerini C, Dunger D. Insulin-like growth factor 1 concentrations in infancy predict differential gains in body length and adiposity: the Cambridge Baby Growth Study. Am J Clin Nutr. 2009;90:156-161.
5. Chellakooty M, Juul A, Boisen K, Damgaard I, Kai C, Schmidt I, Petersen J, Skakkenaek N, Main K. A prospective study of serum insulin-like growth factor 1 (IGF-1) and IGF-binding protein-3 in 942 healthy infants: associations with birth weight, gender, growth velocity and breast feeding. J Clin Endocrinol Metab. 2006;91:820-826.
6. Skalkidou A, Petridou E, Papthoma E, Salvanos H, Trichopoulos D. Growth velocity during the first postnatal week of life is linked to a spurt of IGF-1 effect. Paediatr Perinatal Epidemiol. 2003;17:281-286.
7. Larnkjaer A, Hoppe C, Mølgaard A, Michaelsen K. The effect of whole milk and infant formula on growth and IGF-1 in late infancy. Eur J Clin Nutr. 2009;63:956-963.
8. Madsen A, Larnkjaer A, Mølgaard C, Michaelsen K. IGF-I and IGFBP-3 in healthy 9 month old infants from the SKOT cohort: breastfeeding, diet, and later obesity. Growth Hormone & IGF Research. 2011;21:199-204.
9. Hansen-Pupp I, Löfqvist C, Polberger S, Niklasson A, Fellman V, Hellström A, Ley D. Influence of insulin-like growth factor-I and nutrition during phases of postnatal growth in very preterm infants. Pediatr Res. 2011;69:448-453.
10. Kajantie E, Dunkel L, Rutanen E, Sappälä M, Koistinen R, Sarnesto A, Andersson S. IGF-1, IGF binding protein (IGFBP)-3, phosphoisoforms of IGFBP-1 and postnatal growth in very low birth weight infants. J Clin Endocrinol Metabol. 2002;87:2171-2179.
11. Löfqvist C, Engström E, Sigurdssin J, Hard A, Niklasson A, Ewald U, Holmström G, Smith L, Hellström A. Postnatal head growth deficit among premature infants parallels retinopathy of prematurity and insulin-like growth factor deficit. Pediatrics. 2006;117:1930-1938.
12. Engström E, Niklasson A, Albertsson-Wikland K, Ewald U, Hellström A. The role of maternal factors, postnatal nutrition, weight gain and gender in regulations of serum IGF-1 among preterm infants. Pediatr Res. 2005;57:605-610.
13. Ohkawa N, Shoji H, Kitamura T, Suganuma H, Yoshikawa N. IGF-1, leptin and active ghrelin levels in very low birth weight infants during the first 8 weeks of life. Acta Paediatr. 2010;99:37-41.
14. Patel L, Cavazzoni E, Whatmore A, Carney S, Wales J. The contributions of plasma IGF-1, IGFBP-3 and leptin to growth in extremely premature infants during the first two years. Pediatr Res. 2007;61:99-104.
15. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
16. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.

17. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
18. Gray J, Richardson D, McCormick M, Workman-Daniels K, Goldmann D. Neonatal therapeutic intervention scoring system: a therapy-based severity of illness index. Pediatrics. 1992;90:561-567.
19. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part I. J Clin Chem Clin Biochem. 1983;21:709-720.
20. Lafeber H, van Zoeren-Grobben D, Van Beek R, Chrisman V, editors. Enteral and parenteral nutrition in newborns [In Dutch: Werkboek enterale en parenterale voeding bij de pasgeborene]. Amsterdam: VU Uitgeverij; 2012.
21. Soto N, Bazaes R, Peña V, Salazar T, Avila A, Iñiguez G, Ong K, Dunger D, Mericq M. Insulin sensitivity and secretion are related to catch-up growth in small-for-gestational-age infants at age 1 year: results from a prospective cohort. J Clin Endocrinol Metab. 2003;88:3645-3650.
22. Rotteveel J, van Weissenbruch M, Twisk J, Delemarre-van de Waal H. Insulin sensitivity in prematurely born adults: relation to preterm growth restraint. Horm Res Pediatr. 2011;75:252-257.
23. Ibañez L, Ong K, Dunger D, de Zegher F. Early development of adiposity and insulin resistance after catch-up weight gain in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91:2153-2158.

Chapter 4

Lean mass and fat mass accretion between term age and six months post-term in growth restricted preterm infants

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Submitted

ABSTRACT

Background/Objectives

Early growth restriction followed by accelerated growth may result in metabolic disease in later life. This study compared growth, body composition, and nutritional intake between birth and six months post-term between appropriate-for-gestational-age preterm infants with and without growth restriction at term age (AGA GR+ and AGA GR-, respectively) and small-for-gestational-age (SGA) preterm infants.

Subjects/Methods

Eighty-three AGA GR-, 15 AGA GR+, and 33 SGA preterm infants were included. At term age and six months post-term, weight (g), length (cm), and head circumference (cm) were measured and expressed as standard deviation scores (SDS) and lean mass (LM; g) and fat mass (FM; g) were measured by whole-body dual-energy x-ray absorptiometry (Hologic QDR4500A). Between term age and six months post-term, gain (Δ) in weight, LM, and FM and intake of protein (g/kg/day) and energy (kcal/kg/day) were calculated.

Results

Higher protein and energy intake during the first six months post-term resulted in higher Δweight SDS, higher ΔLM, and lower ΔFM in AGA GR+ and SGA compared to AGA GR- preterm infants (Δweight SDS [expressed as mean ± SD]: 1.34 ± 0.93 and 0.97 ± 1.04 versus 0.45 ± 0.99 SDS; respectively, $P<0.05$; ΔLM [expressed as median (IQR)]: 2898 (587) and 3055 (581) versus 2552 (618) g, respectively, $P\leq0.05$; ΔFM: 1263 (834) and 1441 (772) versus 1722 (764) g, respectively, $P\leq0.07$).

Conclusions

During the first six months post-term, AGA GR+ and SGA preterm infants restore their LM without excessive FM despite higher energy and protein intake compared to AGA GR- preterm infants.

INTRODUCTION

Adequate prenatal and postnatal growth without excessive fat accumulation is important for metabolic health of preterm infants in later life. Body composition is affected by prenatal [1-2] as well as by early postnatal growth. As a result of their extrauterine third trimester, preterm infants are extremely susceptible for disruption of this early postnatal growth phase. Preterm infants born appropriate-for-gestational-age (AGA) are at risk of growth restriction at term age (AGA GR+; weight, length, or both at term age <-2 SDS), since postnatal nutrient supplies are directed towards survival at the expense of postnatal growth [3-4]. Similar to small-for-gestational-age (SGA) preterm infants, AGA GR+ preterm infants demonstrate catch-up growth during early infancy [3-4], which is associated with adiposity during infancy [5] and adolescence [6]. Adiposity, in turn, has been related to metabolic consequences in later life, including insulin resistance and cardiovascular disease.

Growth and body composition of preterm infants are influenced by nutritional intake. Some studies demonstrate that energy and protein intake enhance fat mass (FM) accumulation of preterm infants during infancy [7-8], whereas others do not [9-10]. Adequate growth without increased FM accumulation may be especially important for infants that are prone for accelerated growth during infancy, such as AGA GR+ and SGA preterm infants [3, 11]. Recently, Roggero et al. showed that growth and FM accumulation was similar in SGA preterm infants fed an energy- and protein-enriched formula compared to those fed a standard term formula during the first six months post-term and they suggest that SGA preterm infants have either a delay in FM restoration or a lower genetic potential [12]. However, another explanation for their findings may be that energy (kcal/kg/d) and protein intake (g/kg/d) were comparable between SGA and AGA preterm infants [12], as it has been suggested that SGA and AGA GR+ preterm infants may benefit from a higher nutrient intake [13-14]. Moreover, few studies have focused on body composition of AGA GR+ preterm infants in relation to their nutritional intake during early infancy. We hypothesized that, similar to SGA preterm infants, AGA GR+ preterm infants have catch-up growth, as a result of higher nutrient supply, that results in increased FM accumulation. Therefore, the present study compared growth, body composition, and nutritional intake until six months post-term between AGA preterm infants with and without growth restriction at term age (AGA GR+ and AGA GR-, respectively) and SGA preterm infants.

SUBJECTS AND METHODS

The present study was an observational analysis within a randomized controlled trial that evaluated the effects of an isocaloric, protein- and mineral-enriched postdischarge formula, term formula, and human milk on growth and body composition of preterm infants until six months post-term, as described in detail previously [13]. In short, 152 preterm infants with a gestational age of 32 weeks or less, a birth weight of 1,500 gram or less, or both were included shortly after birth. Preterm infants with congenital malformations or conditions known to affect growth or body composition were not included. One-hundred-and-thirty-nine preterm infants completed the randomized controlled trial at six months post-term (Figure 4.1). The present study included 131 preterm infants with available data on growth, body composition, and nutritional intake until six months post-term (Figure 4.1). These infants visited our outpatient clinic at term age (40.3 ±

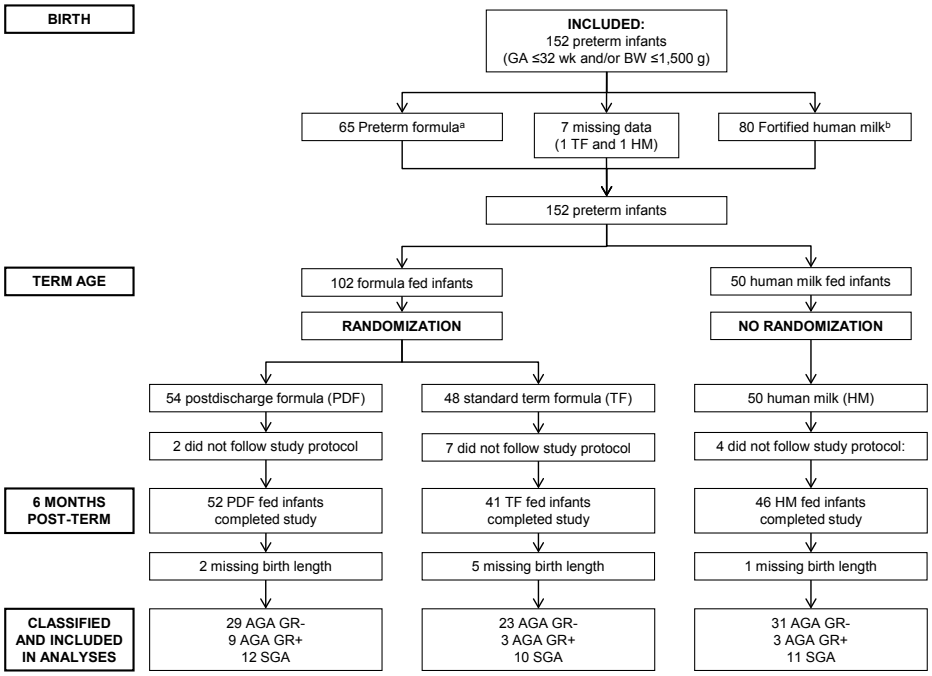


Figure 4.1. Flow chart of subject recruitment, inclusion and randomization of the original randomized controlled trial

AGA GR-: appropriate-for-gestational-age infants without growth restriction at term age; AGA GR+: appropriate-for-gestational-age infants with growth restriction at term age; BW: birth weight; GA: gestational age; HM: human milk; PDF: postdischarge formula; SGA: small-for-gestational-age infants; TF: term formula. [a] 35 AGA GR-, 8 AGA GR+, and 15 SGA preterm infants; 3 preterm infants did not follow study protocol, 4 preterm infants had missing birth length. [b] 48 AGA GR-, 7 AGA GR+, and 18 SGA preterm infants; 3 preterm infants did not follow study protocol, 4 preterm infants had missing birth length.

0.7 weeks postmenstrual age), three months post-term (53.0 ± 0.5 weeks postmenstrual age), and six months post-term (66.0 ± 0.5 weeks postmenstrual age). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the ethics committee of VU University Medical Center, Amsterdam, The Netherlands. Written informed consent was obtained from the parents of all participating children.

Between birth and term age, infants were fed preterm formula (per 100 ml: 80 kcal, 2.2-2.5 g protein, 4.3-4.4 g fat, 7.6-8.2 g carbohydrates) or fortified human milk (Figure 4.1). At term age, formula fed infants were randomized to isocaloric postdischarge formula (per 100 ml: 67 kcal, 1.7 g protein, 3.5 g fat, 7.0 g carbohydrates) or standard term formula (per 100 ml: 67 kcal, 1.47 g protein, 3.5 g fat, 7.2 g carbohydrates) (Figure 4.1), as described previously [13], and were fed this diet until six months post-term. Infants that were fed >80% human milk at term age were considered to be human milk fed. If human milk was insufficiently available, infants were fed standard term formula additionally. If complementary feeding was introduced between five and six months post-term, it was limited to fruit or vegetables once a day.

Details on enteral and parenteral intake, including volume intake (ml/d) and type of diet, were extracted from the infant's medical record between birth and discharge by a single person. Between discharge and six months post-term, parents recorded their infant's formula intake (ml/d), intake of supplements, and any changes in their infant's diet in a diary (per day between discharge and term age; once per week between term age and six months post-term). Human milk fed infants were predominantly fed human milk with fortifier by bottle before term age. In these infants, the volume of human milk intake was recorded and the composition of human milk was estimated (per 100 ml: 68 kcal, 1 g protein, 4 g fat, 7 g carbohydrates [15]). After term age, human milk fed infants were breast-fed and their exact intake was unknown. The available data on volume, type, and composition of intake and weight was used to calculated the mean intake of energy (kcal/d and kcal/kg/d), protein (g/d and g/kg/d), fat (g/d and g/kg/d), and carbohydrates (g/d and g/kg/d) between birth and term age in all infants and between term age and six months post-term only in formula fed infants. As previously demonstrated, between term age and six months post-term, growth was similar, gain in LM was higher and gain in FM was lower in preterm infants fed postdischarge formula compared to those fed term formula or human milk [13].

Weight (g) was measured with a digital scale to the nearest 1 gram, length (cm) was measured with a length board to the nearest 0.1 cm, and head circumference (cm) was measured with a non-stretchable measuring tape to the nearest 0.1 cm by a single research nurse at birth, term age, three months post-term, and six months post-term, as described previously [13]. Weight, length, and head circumference at birth and term age were expressed as standard deviations scores (SDS) based on Swedish references for

preterm infants [16]. Weight, length, and head circumference at three and six months post-term were expressed as SDS based on Dutch references [17]. Adapted from Roggero et al. and Finken et al. [3, 18], infants were classified as AGA GR- if weight and length at birth and term age were -2 SDS or above, as AGA GR+ if weight and length at birth were -2 SDS or above and weight, length, or both at term age were below -2 SDS, and as SGA if weight, length, or both at birth were below -2 SDS. Gain (Δ) in absolute and relative (SDS) weight, length, and head circumference between term age and six months post-term was calculated.

As length was included in the classification of preterm infants (AGA GR-, AGA GR+, or SGA), the influence of parental height was considered as a proxy of the genetic growth potential. Maternal height (cm) and paternal height (cm) were acquired through parental questionnaires. Target height was calculated as follows: target height boys (cm)=44.5+0.376*paternal height (cm)+0.411*maternal height (cm) and target height girls (cm)=47.1+0.334*paternal height (cm)+0.364*maternal height (cm) [19]. In addition, target height SDS was calculated as follows: target height SDS boys=(target height-183.8)/7.1 and target height SDS girls=(target height-170.7)/6.3 [19].

The severity of illness during hospital admission was evaluated by the Neonatal Therapeutic Intervention Scoring System, which has a maximum score of 130. It is a valid indicator of severity of illness in neonates, independent of birth weight [20].

Lean mass (LM; g) and fat mass (FM; g) were measured by whole-body dual-energy x-ray absorptiometry (DXA; Hologic QDR4500A, Hologic, Bedford, MA, USA) at term age and six months post-term and analyzed by Infant Whole Body Software version 12.3.3. [13]. FM was expressed as percentage FM (%FM) as follows: FM (g)/weight (g)*100%. One expert radiologist evaluated the DXA scan quality and was blinded for the study groups or the type of diet. Scans that were incomplete or that had severe movement artifacts were excluded. Good quality DXA scans were available from 110 preterm infants at term age (68 AGA GR-, 14 AGA GR+, and 28 SGA), from 102 preterm infants at six months post-term (66 AGA GR-, 12 AGA GR+, and 24 SGA), and from 84 preterm infants both at term age and six months post-term (52 AGA GR-, 11 AGA GR+, and 21 SGA).

Statistical analyses

The distribution of gender and type of diet were expressed as frequencies and were compared between AGA GR-, AGA GR+, and SGA infants by logistic regression. Gestational age, absolute growth parameters, LM and FM as well as energy and protein intakes between birth and term age were normally distributed after log transformation and were expressed as median (IQR). Relative growth (SDS), severity of illness, target height SDS, fat and carbohydrate intakes between birth and term age and nutritional intakes between term age and six months post-term were normally distributed and were expressed as mean ± SD. Gestational age, growth, target height SDS, nutritional

Table 4.1. Gestational age and absolute growth parameters in AGA GR-, AGA GR+, and SGA infants

Characteristics	AGA GR- (n=83)	AGA GR+ (n=15)	SGA (n=33)
Birth			
Gestational age (wk)	30.1 (2.0)†	30.7 (1.4)	31.1 (1.6)
Weight (g)	1465 (371)*,†	1182 (220)‡	1160 (468)
Length (cm)	39.0 (3.5)*,†	38.0 (2.0)‡	36.0 (3.5)
HC (cm)	28.0 (2.5)*,†	27.5 (4.0)‡	27.5 (2.6)
Term age			
Weight (g)	3230 (465)*,†	2641 (300)	2736 (574)
Length (cm)	49.5 (2.0)*,†	47.5 (3.0)‡	46.5 (3.5)
HC (cm)	36.0 (1.4)*,†	34.9 (2.2)	35.0 (2.4)
3 months post-term			
Weight (g)	5860 (980)*,†	4925 (420)	4990 (1240)
Length (cm)	60.0 (3.0)*,†	57.5 (3.0)‡	57.0 (3.5)
HC (cm)	40.8 (1.3)*,†	40.6 (2.1)	40.5 (2.9)
6 months post-term			
Weight (g)	7460 (1300)*,†	6840 (1503)	6625 (1560)
Length (cm)	67.0 (3.0)*,†	66.0 (3.5)‡	64.0 (4.0)
HC (cm)	44.0 (1.8)*,†	43.4 (1.2)	43.8 (2.4)

Values as median (IQR). AGA GR-: appropriate-for-gestational-age without growth restriction at term age; AGA GR+: appropriate-for-gestational-age with growth restriction at term age; SGA: small-for-gestational-age; HC: head circumference. Differences compared by regression analyses adjusted for gender and gestational age; * AGA GR- versus AGA GR+, P<0.02; † AGA GR- versus SGA, P<0.02; ‡ AGA GR+ versus SGA, P<0.05.

intakes, LM, and FM were compared between AGA GR-, AGA GR+, and SGA infants by regression analyses with the between-group comparisons as dummy variables adjusted for gender and gestational age (the comparison of gestational age was only adjusted for gender). In addition, all analyses were adjusted for type of diet and severity of illness score. A P value of 0.05 was considered significant and P values were two-sided. All statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

This study included 83 AGA GR-, 15 AGA GR+, and 33 SGA preterm infants (Figure 4.1). SGA infants were more often boys than AGA GR- and AGA GR+ infants (73% versus 43% and 40%, P<0.05) and had a higher gestational age than AGA GR- infants (Table 4.1). All SGA infants had a birth length below -2 SDS and 10 SGA infants also had a birth weight below -2 SDS (30.3%). Severity of illness score and target height SDS were not different

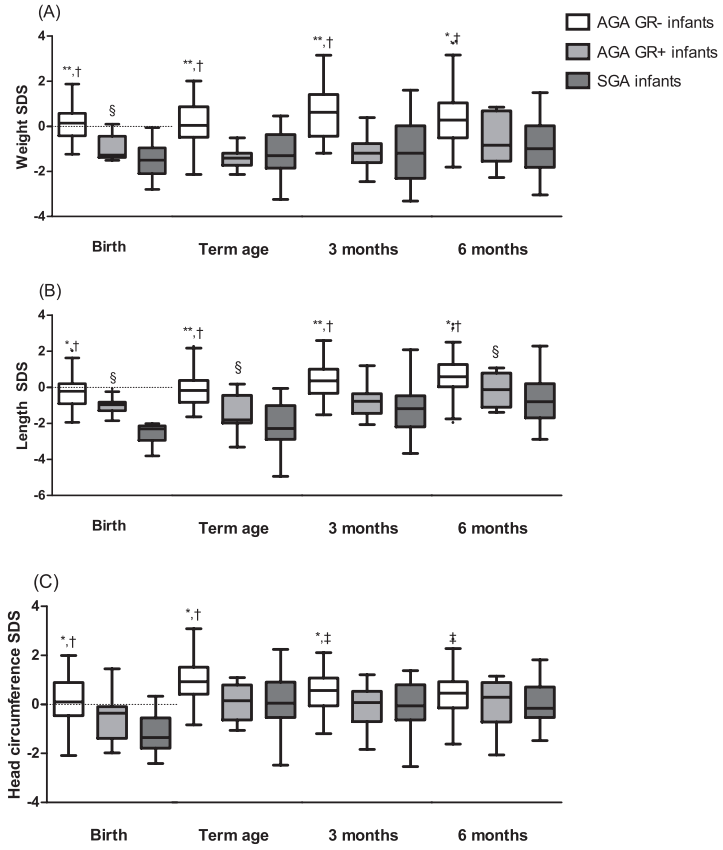


Figure 4.2. Weight SDS (A), length SDS (B), and head circumference SDS (C) between birth and six months post-term in AGA GR-, AGA GR+, and SGA infants

Tukey box plots. AGA GR-: appropriate-for-gestational-age infants without growth restriction at term age; AGA GR+: appropriate-for-gestational-age infants with growth restriction at term age; SDS: standard deviation scores; SGA: small-for-gestational-age infants. Differences between AGA GR-, AGA GR+, and SGA infants were compared by linear regression adjusted for gender and gestational age; ** AGA GR- versus AGA GR+, P<0.001; * AGA GR- versus AGA GR+, P<0.05; † AGA GR- versus SGA, P<0.001; ‡ AGA GR- versus SGA, P<0.05; § AGA GR+ versus SGA, P<0.05.

between AGA GR-, AGA GR+, and SGA infants (severity of illness score: 21.4 ± 8.3, 23.4 ± 8.2, and 21.3 ± 7.8; target height SDS: -0.26 ± 0.60, -0.34 ± 0.56, and -0.33 ± 0.84; respectively; all P≥0.05). The distribution of the type of diet was not different between the three groups (Figure 4.1; P≥0.05).

Anthropometric measurements were lower in AGA GR+ and SGA infants compared to AGA GR- infants at all time-points (Table 4.1 and Figure 4.2). Gain in weight SDS and gain in length SDS between term age and six months post-term were higher in AGA GR+ and SGA infants compared to AGA GR- infants (Table 4.2). Gain in head circumference

Table 4.2. Absolute and relative (SDS) growth and gain in lean mass and fat mass in AGA GR-, AGA GR+, and SGA infants between term age and six months post-term

Term age-6 months post-term		AGA GR- (n=83)	AGA GR+ (n=15)	SGA (n=33)
Growth				
ΔWeight	gram	4075 (1135)	4235 (1390)	3985 (1120)
	SDS	0.45 ± 0.99[*,†]	1.34 ± 0.93	0.97 ± 1.04
ΔLength	cm	18.0 (2.5)	18.0 (4.0)	18.0 (2.8)
	SDS	0.64 ± 0.80[*,†]	1.24 ± 1.02	1.28 ± 1.04
ΔHC	cm	8.0 (1.0)[†]	8.5 (2.0)	8.5 (1.2)
	SDS	-0.49 ± 0.73[†]	-0.10 ± 0.89	-0.23 ± 0.65
Body composition				
ΔLM	gram	2552 (618)[#,†]	2898 (587)	3055 (581)
ΔFM	gram	1722 (764)[§,†]	1263 (834)	1441 (772)

Values as mean ± SD or median (IQR). AGA GR-: appropriate-for-gestational-age without growth restriction at term age; AGA GR+: appropriate-for-gestational-age with growth restriction at term age; SGA: small-for-gestational-age; Δ: gain; SDS: standard deviation score; HC: head circumference; LM: lean mass; FM: fat mass. Differences compared by regression analyses adjusted for gender and gestational age; [*] AGA GR- versus AGA GR+, P<0.02; [#] AGA GR- versus AGA GR+, P=0.05; [§] AGA GR- versus AGA GR+, P=0.07; [†] AGA GR- versus SGA, P<0.05.

SDS between term age and six months post-term was higher in SGA infants compared to AGA GR- infants (Table 4.2).

With respect to nutrition, AGA GR+ and SGA infants had higher protein, energy, and fat intake per kilogram per day compared to AGA GR- infants between birth and term age as well as between term age and six months post-term (Table 4.3). However, absolute total protein, energy, and fat intake per day between birth and term age was lower in AGA GR+ and SGA infants compared to AGA GR- infants (Table 4.3).

With respect to body composition, AGA GR+ and SGA infants had lower LM, FM, and %FM at term age and lower FM and %FM at six months post-term compared to AGA GR- infants (Figure 4.3). Between term age and six months post-term, AGA GR+ and SGA infants had higher gain in LM and lower gain in FM compared to AGA GR- infants (Table 4.2).

The differences in growth, nutritional intakes, LM, and FM between the three groups remained unchanged after adjustment for severity of illness score and for type of diet (i.e. preterm formula or fortified human milk between birth and term age; postdischarge formula, term formula, or human milk between term age and six months post-term).

Table 4.3. Protein, energy, fat, and carbohydrate intake between birth and six months post-term in formula-fed AGA GR-, AGA GR+, and SGA infants

		AGA GR-	AGA GR+	SGA
Birth-term age				
Protein intake[a]	g/kg/d	2.63 (0.48)[†]	2.72 (0.98)	2.75 (1.55)
	g/d	8.85 (2.39)[*,†]	7.51 (0.90)	7.84 (2.01)
Energy intake[a]	kcal/kg/d	116.4 (89.5)[*,†]	125.4 (24.2)	128.4 (14.2)
	kcal/d	379.7 (68.7)[*,†]	342.8 (63.9)	357.4 (83.8)
Fat intake[a]	g/kg/d	5.48 ± 0.90[†]	5.90 ± 0.64	5.93 ± 0.82
	g/d	18.5 ± 3.5[*,†]	15.8 ± 2.4	16.3 ± 3.0
Carbohydrate intake[b]	g/kg/d	13.2 ± 2.0	12.9 ± 1.8	12.8 ± 1.4
	g/d	44.5 ± 8.0[*,†]	34.7 ± 6.0	34.8 ± 5.8
Term age-6 months post-term				
Protein intake[c]	g/kg/d	2.38 ± 0.25[*,†]	2.66 ± 0.27	2.58 ± 0.39
	g/d	18.2 ± 2.5	18.4 ± 2.6	18.1 ± 2.5
Energy intake[c]	kcal/kg/d	99.8 ± 7.5[*,†]	109.6 ± 10.0	106.9 ± 13.5
	kcal/d	760.9 ± 94.0	758.2 ± 101.1	753.1 ± 83.9
Fat intake[c]	g/kg/d	5.21 ± 0.38[*,†]	5.65 ± 0.54	5.56 ± 0.68
	g/d	39.7 ± 4.8	39.1 ± 5.0	39.2 ± 4.7
Carbohydrate intake[c]	g/kg/d	10.7 ± 0.9[*,†]	11.8 ± 1.2	11.5 ± 1.6
	g/d	81.4 ± 10.7	81.7 ± 11.8	80.7 ± 8.9

Values as mean ± SD or median (IQR). AGA GR-: appropriate-for-gestational-age without growth restriction at term age; AGA GR+: appropriate-for-gestational-age with growth restriction at term age; SGA: small-for-gestational-age [a] 80 AGA GR-, 15 AGA GR+, and 33 SGA infants included; [b] 78 AGA GR-, 15 AGA GR+, and 30 SGA infants included; [c] 52 AGA GR-, 12 AGA GR+, and 21 SGA infants included. Differences compared by regression analyses adjusted for gender and gestational age; [*] AGA GR- versus AGA GR+, P<0.05; [†] AGA GR- versus SGA, P<0.05.

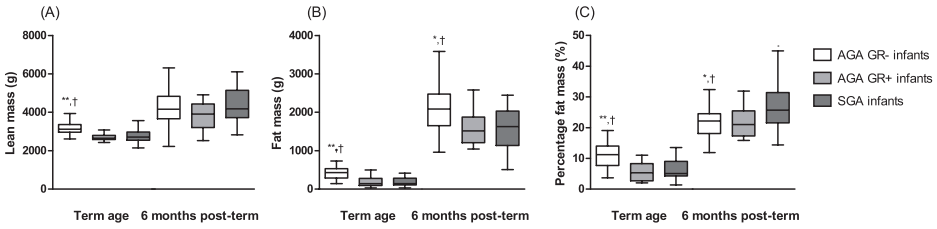


Figure 4.3. Lean mass (g) (A), fat mass (g) (B), and percentage fat mass (%) (C) between term age and six months post-term in AGA GR-, AGA GR+, and SGA infants

Tukey box plots. AGA GR-: appropriate-for-gestational-age infants without growth restriction at term age; AGA GR+: appropriate-for-gestational-age infants with growth restriction at term age; SGA: small-for-gestational-age infants. Differences between AGA GR-, AGA GR+, and SGA infants were compared by linear regression (with natural log transformed variables) adjusted for gender and gestational age; [**] AGA GR- versus AGA GR+, P<0.001; [*] AGA GR- versus AGA GR+, P<0.05; [†] AGA GR- versus SGA, P<0.001.

DISCUSSION

The present study shows that, compared to AGA GR- infants, higher weight gain in AGA GR+ and SGA infants consists of higher LM accretion and lower FM accretion during the first six months post-term. It has been demonstrated that FM accumulation of preterm infants is positively related to energy and protein intake after term age [7-8] as well as to weight gain during early infancy [5]. Therefore, it was remarkable in the present study that FM accretion was lower in AGA GR+ and SGA preterm infants while they had higher weight gain, energy intake (kcal/kg/d), and protein intake (g/kg/d) compared to AGA GR- infants between term age and six months post-term.

The lower FM deposition in AGA GR+ and SGA preterm infants may be a consequence of deficits in macronutrients that developed before term age and were not corrected thereafter, as may be suggested by the lower total nutritional intake between birth and term age in these infants. After term age, persisting deficits of fat and carbohydrates may delay fat mass restoration [21]. It has been suggested that AGA GR+ and SGA preterm infants may benefit from increased nutritional intakes to achieve adequate growth and body composition during infancy [13-14]. With respect to fat mass accretion, our findings might support the conceptual premise that AGA GR+ and SGA infants may benefit from even higher nutritional intakes than that were achieved with the current feeding regimen during infancy. On the other hand, not the absolute amount of fat but the higher rate of fat accumulation in AGA GR- infants may have adverse metabolic consequences in later life [22].

Another explanation for the lower FM accretion and adequate LM restoration in AGA GR+ and SGA preterm infants may be the fact that LM replenishment has lower energy requirements than FM accretion [23] and, therefore, precedes FM restoration during postnatal growth of preterm infants [24-25]. This may imply that the lack of FM restoration of AGA GR+ and SGA preterm infants in the present study is a consequence of the timing of body composition measurement and that complete FM restoration may occur later in life.

In addition to nutritional intake, body composition acquisition may depend on the genetic growth potential. A genetically smaller body size of AGA GR+ and SGA preterm infants may explain the lower FM accumulation during the first six months post-term [26]. However, in the present study, target height SDS of AGA GR+ and SGA preterm infants was not different than that of AGA GR- preterm infants.

The present study had several limitations. A limitation was that the sample size was relatively small, in particular after subgroup classification. The original randomized controlled trial investigated growth and body composition of preterm infants fed three different feeding regimens during the first six months post-term. The distribution of the type of diet was similar in the three groups and the differences between AGA GR-,

AGA GR+, and SGA infants persisted after adjustment for the type of diet. However, the influence of the nutritional regimen cannot be excluded completely. Another limitation was that the first body composition measurement occurred at term age and not a birth. This was a consequence of the design of the randomized controlled trial, which focused on the effects of a nutritional intervention between term age and six months post-term. Thus, the differences in body composition between AGA GR-, AGA GR+, and SGA infants could not be adjusted for differences in body composition at birth.

In addition, in the present study, SGA was used as a proxy for fetal growth restriction. Several studies in term infants suggest that customized fetal growth curves should be used to define SGA [27-29], as infants with fetal growth restriction are at increased risk of perinatal morbidity and chronic adult disease, such as insulin resistance, hypertension, and cardiovascular disease [27-28], whereas constitutionally small infants are not [28]. However, others suggest that SGA is justified as a proxy for fetal growth restriction after early preterm birth, since perinatal morbidity of SGA infants declines with advancing gestational age [26]. Furthermore, the composition of human milk that was used to calculate the nutritional intake between birth and term age was estimated [15]. However, there is a large inter-individual and intra-individual variation as well as a gradual change over time in the composition of human milk. Therefore, the comparison of nutritional intake between birth and term age needs to be interpreted with caution.

In conclusion, compared to AGA GR- preterm infants, SGA and AGA GR+ preterm infants restore their LM without excessive FM accumulation at six months post-term despite a higher nutritional intake per kilogram per day between birth and six months post-term. Nevertheless, it remains controversial if higher nutritional intakes that may potentially increase FM accretion are desirable in AGA GR+ and SGA infants, as the higher FM accumulation in AGA GR- preterm infants may even have adverse metabolic consequences. Therefore, it is recommended to carefully monitor early postnatal effects of specific nutritional intakes on body composition in preterm infants in order to prevent excessive FM accretion that is associated with a higher risk of adiposity and metabolic consequences in later life.

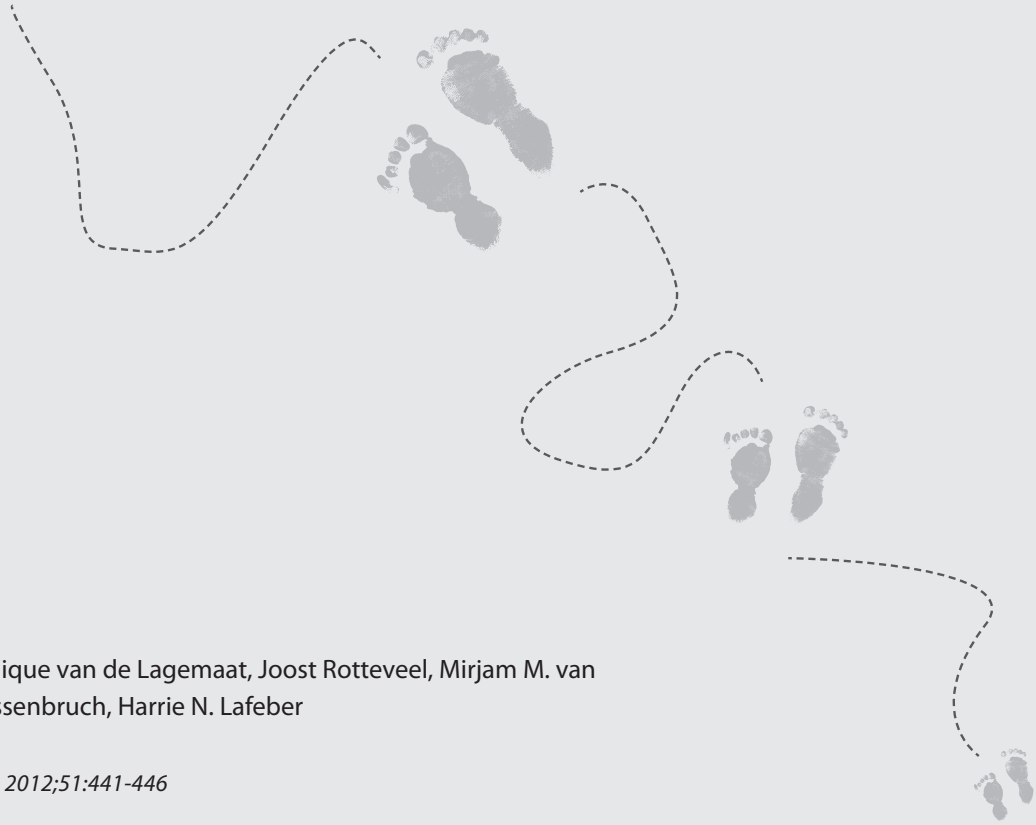
REFERENCES

1. Uthaya S, Thomas E, Hamilton G, Doré C, Bell J. Altered adiposity after extremely preterm birth. Pediatr Res. 2005;57:211-215.
2. Giannì M, Roggero P, Taroni F, Liotto N, Piemontese P, Mosca F. Adiposity in small for gestational age preterm infants assessed at term equivalent age. Arch Dis Child Fetal Neonatal Ed. 2009;94:F368-372.
3. Finken M, Dekker F, de Zegher F, Wit J. Long-term height gain of prematurely born children with neonatal growth restraint: parallellism with the growth pattern of short children born small for gestational age. Pediatrics. 2006;118:640-643.
4. Wit J, Finken M, Rijken M, de Zegher F. Preterm growth restraint: a paradigm that unifies intrauterine growth retardation and preterm extrauterine growth retardation and has implications for the small-for-gestational-age indication in growth hormone therapy. Pediatrics. 2006;117:e793-e795.
5. Griffin I, Cooke R. Development of whole body adiposity in preterm infants. Early Hum Dev. 2012;88:S19-S24.
6. Euser A, Finken M, Keijzer-Veen M, Hille E, Wit J, Dekker F. Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. Am J Clin Nutr. 2005;81:480-487.
7. Cooke R, Griffin I, McCormick K. Adiposity is not altered in preterm infants fed a nutrient-enriched formula after hospital discharge. Pediatr Res. 2010;67:660-664.
8. Cooke R, McCormick K, Griffin I, Embleton N, Faulkner K. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res. 1999;46:461-465.
9. Roggero P, Gianni M, Liotto N, Taroni F, Morniroli D, Mosca F. Small for gestational age preterm infants: nutritional strategies and quality of growth after discharge. J Matern Fetal Neonatal Med. 2011;24:144-146.
10. De Curtis M, Pieltain C, Rigo J. Body composition in preterm infants fed standard term or enriched formula after hospital discharge. Eur J Nutr. 2002;41:177-182.
11. Saenger P, Czernichow P, Hughes I, Reiter E. Small for gestational age: short stature and beyond. Endocr Rev. 2007;28:219-251.
12. Roggero P, Giannì M, Amato O, Liotto N, Morlacchi L, Orsi A, Piemontese P, Taroni F, Morniroli D, et al. Growth and fat-free mass gain in preterm infants after discharge: a randomized controlled trial. Pediatrics. 2012;130:e1215.
13. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
14. Aggett P, Agostoni C, Axelsson I, DeCurtis M, Goulet O. Feeding preterm infants after hospital discharge. J Pediatr Gastroenterol Nutr. 2006;42:596-603.
15. Lafeber H, van Zoeren-Grobben D, Van Beek R, Gerards L. Enteral and parenteral nutrition in newborns (In Dutch: Werkboek enterale en parenterale voeding bij pasgeborenen). 2nd ed. Amsterdam: VU; 2004.
16. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
17. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.

18. Roggero P, Gianni M, Liotto N, Taroni F, Orsi A, Amato O, Morlacchi L, Piemontese P, Agosti M, Mosca F. Rapid recovery of fat mass in small for gestational age preterm infants after term. PLoS ONE. 2011;6:e14489.
19. van Dommelen P, Schönbeck Y, van Buuren S. A simple calculation of target height. Arch Dis Child. 2012;97:182.
20. Gray J, Richardson D, McCormick M, Workman-Daniels K, Goldmann D. Neonatal therapeutic intervention scoring system: a therapy-based severity of illness index. Pediatrics. 1992;90:561-567.
21. van Goudoever J, Sulkers E, Lafeber H, Sauer P. Short-term growth and substrate use in very-low-birth-weight infants fed formulas with different energy contents. Am J Clin Nutr. 2000;71:816-821.
22. Howe L, Tilling K, Benfield L, Logue J, Sattar N, Ness A, Smith G, Lawlor D. Changes in ponderal index and body mass index across childhood and their associations with fat mass and cardiovascular risk factors at age 15. PLoS ONE. 2010;5:e15186.
23. Roberts S, Young V. Energy costs of fat and protein deposition in the human infant. Am J Clin Nutr. 1988;48:951-955.
24. Ahmad I, Nemet D, Eliakim A, Koeppel R, Grochow D, Coussens M, Gallitto S, Rich J, Pontello A, et al. Body composition and its components in preterm and term newborns: a cross-sectional, multimodel investigation. Am J Hum Biol. 2010;22:69-75.
25. Beltrand J, Nicolescu R, Kaguelidou F, Verkausiene R, Sibony O, Chevenne D, Claris O, Lévy-Marchal C. Catch-up growth following fetal growth restriction promotes rapid restoration of fat mass but without metabolic consequences at one year of age. PLoS ONE. 2009;4:e5343.
26. Ananth C, Vintzileos A. Distinguishing pathological from constitutional small for gestational age births in population-based studies. Early Hum Dev. 2009;85:653-658.
27. McCowan L, Stewart A. Term birth weight centiles for babies from New Zealand's main ethnic groups. Aust N Z J Obstet Gynaecol. 2004;44:432-435.
28. Milovanovic I, Njuieyon F, Deghmoun S, Chevenne D, Levy-Marchal C, Beltrand J. Innate small babies are metabolically healthy children. J Clin Endocrinol Metab. 2012;97:4407-4413.
29. Gardosi J. New definition of small for gestational age based on fetal growth potential. Horm Res. 2006;65:15-18.

Chapter 5

Small-for-gestational-age preterm-born infants already have lower bone mass during early infancy



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ABSTRACT

Background

In preterm-born infants, low birth weight and diminished bone accretion deteriorate peak bone mass. Whether low birth weight is already associated with decreased bone mass during infancy is unknown.

Objective

To study the effect of birth weight on bone accretion between term age (40 weeks postmenstrual age) and six months post-term in preterm-born infants.

Design

In 139 preterm-born infants (51% male, gestational age 30.3 ± 1.5 weeks, birth weight 1341 ± 288 g) weight and whole-body bone mineral content (BMC, g) were measured at term age and six months post-term. At birth, infants were small-for-gestational-age (SGA, n=33, weight and/or length <-2 SDS) or appropriate-for-gestational-age (AGA, n=98, weight and length ≥-2 SDS).

Results

At term age and six months post-term, BMC adjusted for gender and gestational age was lower in SGA than AGA infants (term age: 38.1 ± 9.5 versus 48.6 ± 10.1 g, β=-0.26, 95%CI -0.37;-0.16, p<0.001; six months: 130.1 ± 25.7 versus 145.4 ± 22.9 g, β=-0.16, 95%CI -0.25;-0.08, p<0.001). At six months post-term, BMC remained lower in SGA infants after adjustment for actual weight and length. Between term age and six months post-term, BMC gain adjusted for gender and gestational age was lower in SGA than AGA infants (91.7 ± 22.8 versus 98.2 ± 20.7 g; β=-0.12, 95%CI -0.24;-0.003, p=0.044). BMC gain remained lower in SGA infants after adjustment for weight and length gain.

Conclusion

The first six months post-term, SGA preterms have lower bone accretion, independent of body size, suggesting that prenatal conditions for bone accretion cannot be replicated postnatally.

INTRODUCTION

Preterm-born infants are prone to suboptimal bone mass. Preterm birth is frequently preceded by intra-uterine growth retardation, which is associated with inadequate intra-uterine bone formation and a smaller skeleton at birth. In addition, preterm-born infants are deprived of the intra-uterine third trimester, which is essential for 80% of the total bone mass of the newborn [1]. During the postnatal extra-uterine third trimester, mineral intake of preterm-born infants is insufficient for bone mass accretion, which aggravates the already present bone mineral deficit. During infancy and childhood, preterm-born children have lower bone mass accretion compared to their term-born peers [2-5]. This decreased bone mass tracks from infancy to adulthood resulting in lower peak bone mass in preterm-born adults [6-8], which is associated with a higher risk of osteoporosis [9].

During infancy, bone mass accretion can to a certain extent be modified by feeding and growth. The type of feeding provided during early infancy influences bone mass accretion, as emphasized by lower bone mineral content (BMC) in preterm-born infants fed human milk compared to formula [10-12] and by higher BMC in preterm-born infants fed nutrient-enriched formula compared to standard formula [13-15].

Bone mass accretion can also be modified by prenatal and postnatal growth trajectories. In term-born infants, fetal growth and low birth weight, as a proxy of intra-uterine growth, are associated with lower bone mass and decreased estimated bone strength at adult age [16-19]. In preterm-born infants, low birth weight is associated with decreased bone mass at term age and with lower bone mineral density (BMD) at 20 years of age [7, 20]. In particular, preterm-born infants that are small-for-gestational-age have decreased bone size and lower BMD at 20 years of age [7, 21]. In both term-born and preterm-born infants, growth during infancy and childhood determines bone mass in childhood and adulthood [17, 22-28].

Whether low birth weight in preterm-born infants is already associated with decreased bone mass during early infancy is unknown. Therefore, we studied the effect of birth weight on bone mass accretion between term age (40 weeks postmenstrual age) and six months post-term in preterm-born infants and investigated if this relation was modified by post-term growth and type of feeding.

SUBJECTS AND METHODS

Subjects

The present study was part of a randomized controlled trial that evaluated the effect of postdischarge formula, term formula, and human milk on growth and body composition

of preterm-born infants. The design of the randomized controlled trial has been described in detail elsewhere [29]. In short, 152 infants with a gestational age of 32 weeks or less and/or a birth weight of 1,500 gram or less were included shortly after birth. Infants with congenital malformations or conditions that affected growth and body composition were not included. The present study aimed to investigate size at birth in relation to prospectively collected anthropometry and bone mass between term age and six months post-term and included all infants with available data on anthropometry and bone mass between these time-points. Infants were evaluated at our outpatient clinic at term age (40.3 ± 0.7 weeks postmenstrual age), at three months post-term (53.0 ± 0.5 weeks postmenstrual age) and at six months post-term (66.0 ± 0.5 weeks postmenstrual age). At six months post-term, 139 preterm-born infants completed the randomized controlled trial (51% boys, gestational age 30.3 ± 1.5 weeks, birth weight 1341 ± 288 g) (Figure 5.1). Thirteen infants did not complete the randomized controlled trial for various reasons (Figure 5.1).

The study protocol was approved by the ethics committee of VU University Medical Center, Amsterdam, The Netherlands. All the parents of the participating children gave written informed consent.

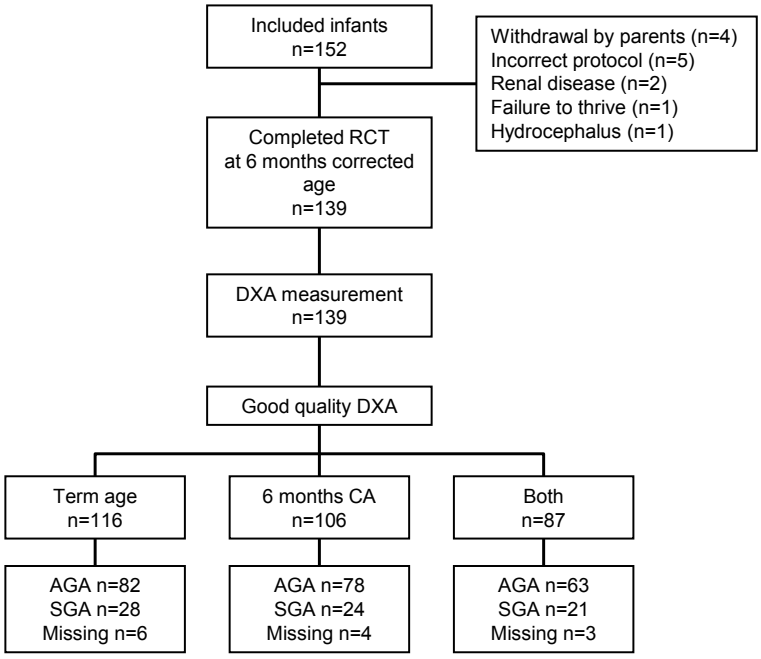

Figure 5.1. Flow diagram of study subject recruitment
N: number of infants; AGA: appropriate-for-gestational-age (weight and length ≥-2 SDS); SGA: small-for-gestational-age (weight and/or length <-2 SDS); missing: not classified as AGA or SGA due to unknown birth length.

Methods

Anthropometry, severity of illness, and type of diet

At birth, at term age, and at three and six months post-term, weight (gram) was measured with a digital scale, length (cm) was measured with a length board, and head circumference (cm) was measured with a non-stretchable measuring tape [29]. At birth, weight, length, and head circumference were expressed as standard deviation score (SDS) based on Swedish references for preterm-born infants, which adjusted for gender and gestational age [30]. Infants were classified as small-for-gestational-age (SGA) if birth weight and/or length were below -2 SDS and as appropriate-for-gestational-age (AGA) if birth weight and length were -2 SDS or above. The relative gain in weight and length between term age and six months post-term was calculated as follows: (gain between term age and six months post-term)/measurement at term age*100%.

The Neonatal Therapeutic Intervention Scoring System was used to evaluate severity of illness during hospital admission. The Neonatal Therapeutic Intervention Scoring System has a maximum score of 130 points and is a valid indicator of severity of illness in neonates, independent of birth weight [31]. In the present study, infants were fed different feeding regimens between term age and six months post-term according to the study protocol of the randomized controlled trial, namely postdischarge formula, term formula, or human milk [29].

Dual-energy x-ray absorptiometry (DXA)

At term age and at six months post-term, bone mass was measured with whole-body dual-energy x-ray absorptiometry (DXA; Hologic QDR4500A, Hologic Inc, Bedford, MA, USA), as described previously [29]. In short, naked infants were swaddled in a cotton sheet and placed in supine position on the scanning area. Infants were not sedated and were scanned after feeding when the infant was settled. A research nurse was in attendance during the scanning procedure to ensure an adequate position of the infant.

Scan analysis was performed with Infant Whole Body Software, version 12.3.3 and provided bone area (BA; cm^2), bone mineral content (BMC; g), and bone mineral density (BMD; $g.cm^{-2}$). Since body weight was a main determinant of bone mineral content in previous studies [32-33], the proportion BMC/weight (%) was calculated as follows: BMC (g)/weight (g)*100%. The relative gain in BA, BMC, and BMD between term age and six months post-term was calculated as follows: (gain between term age and six months post-term)/measurement at term age*100%.

DXA scan quality was evaluated by one expert radiologist who was blinded for the size at birth and the type of diet of the infants. Incomplete scans and scans with severe movement artifacts, as described by Koo et al. [34], were excluded from the analyses in the present study. Good quality DXA scans were available from 116 infants at term age,

from 106 infants at six months post-term, and from 87 infants at both term age and six months post-term (Figure 5.1).

Statistics

All parameters were normally distributed at all time-points. Values were expressed as mean ± standard deviation (SD), unless indicated otherwise. Cross tabulation and logistic regression analyses were used for between-group comparisons (AGA versus SGA) of gender and type of diet.

Multivariate regression analyses were used for between-group comparisons of gestational age, severity of illness, and growth with AGA versus SGA as the independent variable and gestational age, severity of illness, and growth as the dependent variables. Growth was adjusted for gender, gestational age, and postmenstrual age. Multivariate regression analyses were also used for between-group comparisons of bone mass with AGA versus SGA as the independent variable and bone mass parameters as the dependent variables. BA, BMC, BMC/weight, BMD, and covariates were natural log transformed to increase the power of the analyses [35]. BA, BMC, and BMD were adjusted for gender, gestational age, weight, length, and type of diet, and BMC/weight was adjusted for gender, gestational age, and type of diet. In addition, BMC was adjusted for gender, gestational age, weight, length, and bone area, as recommended by Prentice et al. [35]. A P value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Study population

Ninety-eight infants were classified as AGA and 33 infants as SGA. Eight infants could not be classified as AGA or SGA due to unknown birth length. Of the SGA infants, 10 infants had a birth weight as well as a birth length below -2 SDS and 23 infants had a birth length below -2 SDS and a birth weight of -2 SDS or higher (mean birth weight SDS -1.13 ± 0.45).

The SGA group comprised more boys than the AGA group (72.7% versus 42.9%, OR=3.6, 95%CI 1.5-8.4, p=0.004). Gestational age was lower in AGA than SGA infants (30.1 ± 1.5 versus 31.1 ± 1.1 weeks, p<0.001). Postmenstrual age at term age and at three months and six months post-term was not different between AGA and SGA infants (term age: 40.5 ± 0.7 versus 40.6 ± 0.9 weeks; three months: 52.9 ± 0.5 versus 52.9 ± 0.7 weeks; six months: 66.1 ± 0.8 versus 66.2 ± 0.8 weeks; all p≥0.05). Type of diet and severity of illness did not differ between AGA and SGA infants (severity of illness: 21.8 ± 8.3 versus 21.3 ± 7.8 points, p=0.78). Of the AGA infants, 38.8% was fed postdischarge formula, 26.5% term formula, and 34.7% human milk. Of the SGA infants, 36.4% was fed

postdischarge formula, 30.3% term formula, and 33.3% human milk. If only infants with a good quality DXA at term age and six months post-term were compared, similar results were found in AGA and SGA infants.

AGA and SGA infants: growth between term age and six months post-term

Weight, length, and head circumference were lower in SGA than AGA infants at birth, at term age, and at three and six months post-term, after adjustment for gender, gestational age, and postmenstrual age (Table 5.1). These differences were not explained by severity of illness or the type of diet (e.g. postdischarge formula, term formula, or human milk).

Gain in weight, length, and head circumference between term age and six months post-term was not different between AGA and SGA infants after adjustment for gender and gestational age (Table 5.1). If only infants with a good quality DXA at term age and six months post-term were compared, similar results were found in AGA and SGA infants.

Table 5.1. Growth between birth and six months post-term in AGA and SGA infants

	AGA (n=98)		SGA (n=33)		
	mean ± SD	range	mean ± SD	range	P
Birth					
Weight (g)	1394 ± 283	710-2065	1158 ± 254	760-1577	<0.001
Length (cm)	38.9 ± 2.6	32-45	35.5 ± 2.2	31-39	<0.001
Head circumference (cm)[1]	27.9 ± 1.9	22.2-32	27.0 ± 1.7	24-30.5	<0.001
Term age					
Weight (g)	3262 ± 439	2395-4260	2751 ± 447	2035-3540	<0.001
Length (cm)	49.2 ± 1.9	44.5-54	46.4 ± 2.3	42-50.5	<0.001
Head circumference (cm)	35.9 ± 1.3	33-39.6	35.2 ± 1.5	31.7-38.4	<0.001
3 months post-term					
Weight (g)	5755 ± 778	2395-4260	5102 ± 862	3680-7050	<0.001
Length (cm)	59.6 ± 1.9	44.5-54.0	57.0 ± 2.6	51.0-64.0	<0.001
Head circumference (cm)	40.9 ± 1.4	37.5-45.5	40.5 ± 1.5	37.6-42.7	0.002
6 months post-term					
Weight (g)	7509 ± 101	5520-11030	6872 ± 100	5070-8865	<0.001
Length (cm)	67.1 ± 2.6	61-75	64.8 ± 2.9	59.5-72.5	<0.001
Head circumference (cm)	44.0 ± 1.5	40-49.4	43.7 ± 1.5	40.7-46.8	0.023
Term age-6 months post-term					
ΔWeight (g)	4247 ± 828	2900-7165	4121 ± 808	2500-5680	n.s.
ΔLength (cm)	17.9 ± 2.0	13.7-24.0	18.4 ± 2.3	14.5-26.0	n.s.
ΔHead circumference	8.0 ± 1.0	4.7-10.4	8.5 ± 1.0	6.8-10.9	n.s.

AGA: appropriate-for-gestational-age (weight and length at birth ≥-2 SDS); SGA: small-for-gestational-age (weight and/or length at birth <-2 SDS); Δ: gain. Multivariate regression analyses for between-group differences adjusted for gender, gestational age, and postmenstrual age. [1] HC at birth was missing in two AGA infants.

Table 5.2. Bone mass in AGA and SGA infants between term age and six months post-term

	AGA		SGA		AGA versus SGA		
	n	mean ± SD	n	mean ± SD	β	95% CI	P
Term age							
BA (cm^2)	82	322.0 ± 39.1	28	274.0 ± 34.1	-0.17	-0.23;-0.12	<0.001
BMC (g)	82	48.6 ± 10.1	28	38.1 ± 9.5	-0.26	-0.37;-0.16	<0.001
BMD ($g.cm^{-2}$)	82	0.15 ± 0.02	28	0.14 ± 0.02	-0.09	-0.15;-0.03	0.003
BMC/weight (%)	82	1.37 ± 0.18	28	1.28 ± 0.17	-0.07	-0.13;-0.01	0.033
6 months post-term							
BA (cm^2)	78	627.3 ± 59.8	24	582.5 ± 50.2	-0.10	-0.15;-0.05	<0.001
BMC (g)	78	145.4 ± 22.9	24	130.1 ± 25.7	-0.16	-0.25;-0.08	<0.001
BMD ($g.cm^{-2}$)	78	0.23 ± 0.02	24	0.22 ± 0.03	-0.07	-0.12;-0.01	0.012
BMC/weight (%)	78	1.84 ± 0.15	24	1.73 ± 0.18	-0.07	-0.12;-0.02	0.003
Term age-6 months							
ΔBA (cm^2)	63	309.5 ± 55.2	21	312.7 ± 40.6			n.s.
ΔBMC (g)	63	98.2 ± 20.7	21	91.7 ± 22.8	-0.12	-0.24;-0.003	0.044
ΔBMD ($g.cm^{-2}$)	63	0.082 ± 0.026	21	0.083 ± 0.027			n.s.
ΔBMC/Δweight (%)	63	2.30 ± 0.30	21	2.07 ± 0.28	-0.11	-0.18;-0.03	0.007

AGA: appropriate-for-gestational-age (weight and length ≥-2 SDS); SGA: small-for-gestational-age (weight and/or length <-2 SDS); BA: bone area; BMC: bone mineral content; BMD: bone mineral density; Δ: gain. Multivariate regression analyses with AGA versus SGA as independent categorical variable (AGA as reference category) and natural log transformed bone mass parameters as dependent variables, adjusted for gender and gestational age. The negative beta coefficient (β) indicates lower bone mass in SGA than AGA infants.

AGA and SGA infants: bone mass at term age and six months post-term

At term age and at six months post-term, all bone mass parameters were lower in SGA than AGA infants after adjustment for gender and gestational age (Table 5.2 and Figure 5.2). BA, BMC, and BMD at term age were not lower in SGA than AGA infants after additional adjustment for actual weight and length. BMD at six months post-term was also not lower in SGA than AGA infants after additional adjustment for actual weight and length. However, BA and BMC at six months post-term remained lower in SGA than AGA infants after additional adjustment for actual weight and length (BA: β=-0.03, 95%CI -0.06;-0.01, p=0.013; and BMC: β=-0.06, 95%CI -0.12;-0.01, p=0.014).

BMC at term age and at six months post-term was not different between AGA and SGA infants after adjustment for gender, gestational age, weight, length, and bone area (data not shown). The differences in bone mass parameters at six months post-term between AGA and SGA infants were not explained by severity of illness or the type of diet. If only infants with a good quality DXA at term age and six months post-term were compared, similar results were found in AGA and SGA infants.

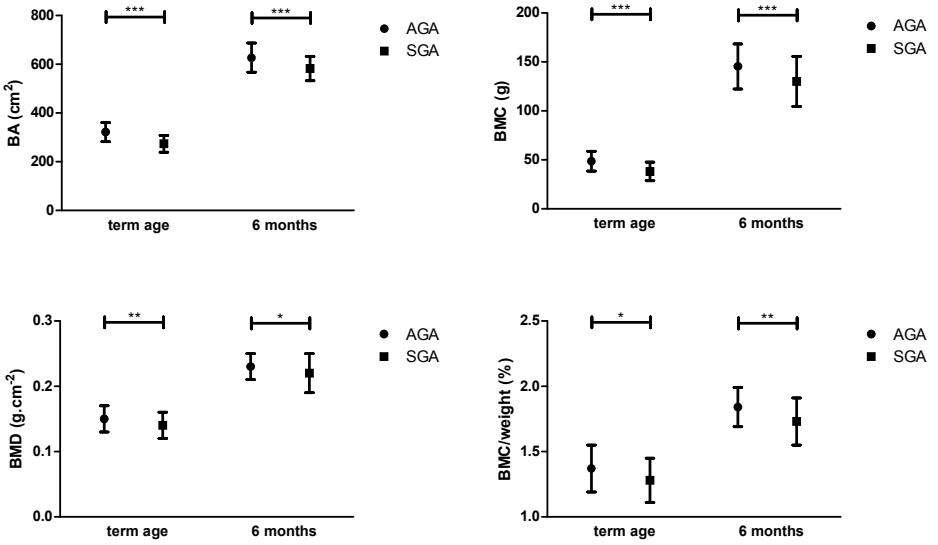

Figure 5.2. Bone mass accretion in AGA (●) and SGA (■) infants between term age and six months post-term
Values as mean ± SD; AGA: appropriate-for-gestational-age (weight and length ≥-2 SDS); SGA: small-for-gestational-age (weight and/or length <-2 SDS); BA: bone area; BMC: bone mineral content; BMD: bone mineral density. Multivariate regression analyses with natural log transformed parameters and covariates for between-group differences, adjusted for gender and gestational age; *** P<0.001, ** P<0.01, * P<0.05.

AGA and SGA infants: gain in bone mass between term age and six months post-term

All bone mass parameters increased in AGA and SGA infants between term age and six months post-term (Table 5.2 and Figure 5.2). Gain in BA and BMD was not different between AGA and SGA infants after adjustment for gender and gestational age (Table 5.2). Gain in BMC and BMC/weight was higher in AGA than SGA infants after adjustment for gender and gestational age (Table 5.2). Gain in BMC remained higher in AGA than SGA infants after additional adjustment for gain in weight and length (β=-0.11, 95%CI -0.18;-0.03, p=0.01) and after additional adjustment for gain in weight, length, and bone area (β=-0.10, 95%CI -0.18;-0.02, p=0.01). The differences in gain in bone mass between AGA and SGA infants were not explained by severity of illness or the type of diet.

AGA and SGA infants: relative gain in weight, length, and bone mass between term age and six months post-term

Relative gain in weight, length, and BA was higher in SGA than AGA infants adjusted for gender and gestational age (Table 5.3). After additional adjustment for relative gain in weight and length, relative gain in BA was not different between AGA and SGA infants. Relative gain in BMC and BMD was not different between AGA and SGA infants adjusted for gender and gestational age (Table 5.3).

Table 5.3. Relative gain in weight, length, and bone mass gain in AGA and SGA infants between term age and six months post-term

	AGA (n=63)	SGA (n=21)	AGA versus SGA		
	mean ± SD	mean ± SD	β	95% CI	P
% ΔWeight	134.4 ± 30.6	163.6 ± 25.5	0.17	0.06;0.29	0.004
% ΔLength	36.4 ± 4.5	41.2 ± 6.0	0.11	0.04;0.18	0.002
% ΔBA	98.6 ± 23.8	117.0 ± 21.0	0.16	0.04;0.29	0.012
% ΔBMC	213.4 ± 71.4	253.5 ± 71.2	0.15	-0.03;0.32	n.s.
% ΔBMD	56.9 ± 23.2	61.9 ± 22.4	0.08	-0.20;0.37	n.s.

AGA: appropriate-for-gestational-age (weight and length ≥-2 SDS); SGA: small-for-gestational-age (weight and/or length <-2 SDS); BA: bone area; BMC: bone mineral content; BMD: bone mineral density; Δ: gain. % ΔWeight: relative gain in weight=(Δweight between term age and six months post-term)/weight at term age*100%; other parameters were calculated similarly. Multivariate regression analyses with AGA versus SGA as independent categorical variable (AGA as reference category) and natural log transformed weight, length, and bone mass parameters as dependent variables, adjusted for gender and gestational age. The positive beta coefficient (β) indicates higher relative gain in SGA than AGA infants. Only infants with good quality DXA at both term age and six months post-term were included in the analyses.

DISCUSSION

This study is the first to demonstrate that incomplete intra-uterine bone mass accretion cannot be compensated in the extra-uterine environment in SGA preterm-born infants. In analogy with term-born SGA infants [36-38], preterm-born SGA infants have lower bone mass accretion during early infancy, independent of body size. Previous studies demonstrated lower bone mass in SGA than AGA infants directly after birth. Petersen et al. show that preterm-born infants with a birth weight below the 10th percentile have lower bone mineral mass at birth than preterm-born AGA infants [36]. The present study showed that lower bone mass in SGA preterm-born infants persists into early infancy due to lower gain in bone mass until six months post-term, independent of post-term growth.

Previous authors have suggested that, in preterm-born infants, the postnatal growth trajectory modifies bone mass accretion, since weight and length during infancy are positively associated with bone mineral content in childhood and adulthood [24, 26-27] and, indeed, this association is found in term-born infants [16, 22-24, 39]. However, in our study, the lower gain in bone mass in SGA preterm-born infants could not be attributed to smaller body size during infancy. Thus, it appears that in preterm-born infants bone mass during early infancy is mainly determined by the prenatal growth trajectory. This difference with term-born infants may be explained by the fact that preterm-born infants are exposed to the extra-uterine environment during the third trimester, when normally 80% of the total intra-uterine bone mass is attained [1]. The extra-uterine environment during the third trimester cannot meet the needs of preterm-born infants, and the bone mineral deficit is further ag-

gravated. Our results suggest that this deficit persists during early infancy. The lower bone mass accretion in SGA preterm-born infants could not be attributed to lower gain in skeletal size, as measured by bone area [40]. Since a low bone mineral content may reflect either a small skeleton (i.e. low bone area) or low bone density [40], this finding implies that SGA preterm-born infants in our study have lower bone mass accretion as a result of decreased bone density. Tracking of decreased bone mass from infancy to adulthood may explain the finding that preterm-born SGA adults have decreased bone mass [7, 21].

In the present study, bone mineral content was lower in SGA infants at term age and six months post-term (21.6% and 10.3%, respectively). However, the difference in relative gain in BMC was not significantly different. This suggests that SGA preterm-born infants in our study do not have a significantly higher bone mass accretion between term age and six months post-term to compensate for a lower bone mineral content at term age.

An important factor for bone mass accretion is type of feeding. During infancy, human milk results in lower bone mineral content than formula [10-12, 41], and term formula results in lower bone mineral content than nutrient-enriched formula in preterm-born infants [13-15]. Surprisingly, greater neonatal human milk exposure is associated with increased bone mineral content in preterm-born adults at 20 years of age, suggesting that the period before term age is sensitive for the effect of human milk on later bone mass [7]. Fewtrell et al. speculated that this enhancing effect of human milk on bone mass may be explained by the various growth factors and hormones present in human milk [7]. In the present study, the type of feeding after term age was not related to the lower gain in bone mass in SGA compared to AGA infants during the first six months post-term. It might be suggested that the sensitive period for the effect of human milk on bone mass exists before but not after term age.

Several characteristics of the study population may have influenced the results of the present study. First, SGA infants had a higher mean gestational age than AGA infants due to the inclusion criteria of the randomized controlled trial, which were a gestational age of 32 weeks or less and/or a birth weight of 1,500 gram or less. However, SGA infants had lower bone mass during infancy. Thus, our data suggest that, with regard to bone mineralization, SGA infants do not benefit from this longer exposure to the intra-uterine environment. This may indicate that the detrimental effect of intra-uterine growth restriction on bone mass accretion persists after term age.

Second, the SGA group comprised a higher percentage of boys. This may be a result of the inclusion criteria of the randomized trial because gender in combination with being SGA or AGA at birth was not taken into account at the moment of inclusion. In the present study, all analyses were adjusted for gestational age and gender to exclude any influence of these parameters on bone mass and growth.

Furthermore, SGA infants all had short stature at birth and 30% had low birth weight as well. This can be explained by the SGA classification, which was based on birth weight

as well as birth length. Post-term gain in weight and length was similar in SGA and AGA infants, and this resulted in a smaller body size in SGA infants at all time-points. Therefore, analyses with bone mass parameters were adjusted for body size to exclude a proportionally lower bone mass in SGA infants.

This study had several limitations. An important limitation was that the sample size was small, especially after subgroup classification. Another limitation was that size at birth was classified based on weight and length, since both are important indicators of body size and are related to bone mass. In contrast to several previous studies, a value below -2 SDS was used to define small body size and, thus, only the smallest infants were included in the SGA group.

In addition, statistical approaches for bone mass analyses vary considerably between studies. For statistical analyses, we adjusted for body size and bone area, as described by Prentice et al. and as used by Fewtrell et al. for analyses in a preterm-born adult cohort [7, 35]. Several studies did not adjust for differences in actual body size when comparing bone mass between AGA and SGA infants [36-38]. Therefore, lower bone mass in SGA infants in these studies may be explained by smaller body size and not by actual bone mass deficit. Nevertheless, our data showed that bone mass was lower in SGA infants, independent of body size.

Furthermore, it is difficult to compare the results of our study to those of previous studies due to differences in the methodology of bone mass measurement [33]. In previous studies, bone mass measurements were performed with different apparatus, e.g. single photon absorptiometry versus dual-energy x-ray absorptiometry, and with a different model and type of dual-energy x-ray absorptiometry, e.g. Lunar versus Hologic, and analyses of bone mass were performed with different software, e.g. Pediatric versus Infant Software. For these reasons, comparisons between our study and previous studies need to be interpreted with caution.

CONCLUSION

This study suggests that the lack of bone accretion of SGA preterm-born infants cannot be compensated during the first six months post-term. Their lower bone mass gain is not related either to gain in body or skeletal size or to the type of feeding. Thus, the prenatal growth trajectory, and not the postnatal growth trajectory, appears to be the main determinant of bone mass accretion in preterm-born infants during the first six months post-term. This novel idea that the extra-uterine environment cannot replicate the favorable intra-uterine conditions for bone mass accretion emphasizes the importance of the intra-uterine environment for sufficient bone mass accretion in preterm-born infants.

Furthermore, these findings question the efficacy of postnatal nutritional interventions for the improvement of bone mass accretion in SGA preterm-born infants.

REFERENCES

1. Ellis K, Shypailo R, Schanler R, Langston C. Body elemental composition of the neonate: new reference data. Am J Hum Biol. 1993;5:323-330.
2. Avila-Díaz M, Flores-Huerta S, Martínez-Muñiz I, Amato D. Increments in whole body bone mineral content associated with weight and length in pre-term and full-term infants during the first 6 months of life. Arch Med Res. 2001;32:288-292.
3. Kurl S, Heinonen K, Länsimies E. Effects of prematurity, intrauterine growth status, and early dexamethasone treatment on postnatal bone mineralisation. Arch Dis Child Fetal Neonatal Ed. 2000;83:F109-F111.
4. Bowden L, Jones C, Ryan S. Bone mineralisation in ex-preterm infants aged 8 years. Eur J Pediatr. 1999;158:658-661.
5. Fewtrell M, Prentice A, Jones S, Bishop N, Stirling D. Bone mineralization and turnover in preterm infants at 8-12 years of age: the effect of early diet. J Bone Miner Res. 1999;14:810-820.
6. Hovi P, Andersson S, Järvenpää A, Eriksson J, Strang-Karlsson S, Kajantie E, Mäkitie O. Decreased bone mineral density in adults born with very low birth weight: a cohort study. PLoS Med. 2009;6:e.1000135.
7. Fewtrell M, Williams J, Singhal A, Murgatroyd P, Fuller N. Early diet and peak bone mass: 20 year follow up of a randomized trial of early diet in infants born preterm. Bone. 2009;45:142-149.
8. Smith C, Wright N, Wales J, Mackenzie C, Primhak R, Eastell R, Walsh J. Very low birth weight survivors have reduced peak bone mass and reduced insulin sensitivity. Clin Endocrinol. 2011;75:443-449.
9. Hernandez C, Beaupré G, Carter D. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int. 2003;14:843-847.
10. Pieltain C, De Curtis M, Gérard P, Rigo J. Weight gain composition in preterm infants with dual energy x-ray absorptiometry. Pediatr Res. 2001;49:120-124.
11. Specker B, Beck A, Kalkwarf H, Ho M. Randomized trial of varying mineral intake on total body bone mineral accretion during the first year of life. Pediatrics. 1997;99:E12.
12. Wauben I, Atkinson S, Shah J, Paes B. Growth and body composition of preterm infants: influence of nutrient fortification of mother's milk in hospital and breastfeeding post-hospital discharge. Acta Paediatr. 1998;87:780-785.
13. Lapillonne A, Salle B, Glorieux F, Claris O. Bone mineralization and growth are enhanced in preterm infants fed an isocaloric, nutrient-enriched preterm formula through term. Am J Clin Nutr. 2004;80:1595-1603.
14. Bishop N, King F, Lucas A. Increased bone mineral content of preterm infants fed with nutrient enriched formula after discharge from hospital. Arch Dis Child. 1993;68:573-578.
15. Cooke R, McCormick K, Griffin I, Embleton N, Faulkner K. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res. 1999;46:461-465.
16. Ay L, Jaddoe V, Hofman A, Moll H, Raat H, Steegers E, Hokken-Koelega A. Foetal and postnatal growth and bone mass at 6 months: the Generation R study. Clin Endocrinol. 2011;74:181-190.
17. Dennison E, Syddall H, Sayer A, Gilbody H, Cooper C. Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire Cohort Study. Pediatr Res. 2005;57:582-586.
18. Cooper C, Fall C, Egger P. Growth in infancy and bone mass in later life. Ann Rheum Dis. 1997;56:17-21.

19. Oliver H, Jameson K, Sayer A, Cooper C, Dennison E. Growth in early life predicts bone strength in late adulthood: The Hertfordshire Cohort Study. Bone. 2007;41:400-405.
20. De Schepper J, Cools F, Vandenplas Y, Louis O. Whole body bone mineral content is similar at discharge from the hospital in premature infants receiving fortified breast milk or preterm formula. J Pediatr Gastroenterol Nutr. 2005;41:230-234.
21. Putzker A, Pozza R, Schwartz H, Schmidt H, Bechtold S. Endosteal bone storage in young adults born small for gestational age-a study using peripheral quantitative computed tomography. Clin Endocrinol. 2012;76:485-491.
22. Mølgaard C, Larnkjaer A, Budek Mark A, Michaelsen K. Are early growth and nutrition related to bone health in adolescence? The Copenhagen Cohort Study of infant nutrition and growth. Am J Clin Nutr. 2011;94:1865S-1869S.
23. Jensen R, Vielwerth S, Frystyk J, Veldhuis J, Larsen T, Mølgaard C, Greisen G, Juul A. Fetal growth velocity, size in early life and adolescence, and prediction of bone mass: association to the GH-IGF axis. J Bone Miner Res. 2008;23:439-446.
24. Weiler H, Yuen C, Seshia M. Growth and bone mineralization of young adults weighing less than 1500 g at birth. Early Hum Dev. 2002;67:101-112.
25. Saito T, Nakamura K, Okuda Y, Nashimoto M, Yamamoto N, Yamamoto M. Weight gain in childhood and bone mass in female college students. J Bone Miner Res. 2005;23:69-75.
26. Kurl S, Heinonen K, Länsimies E, Launiala K. Determinants of bone mineral density in prematurely born children aged 6-7 years. Acta Paediatr. 1998;87:650-653.
27. Fewtrell M, Prentice A, Cole T, Lucas A. Effects of growth during infancy and childhood on bone mineralization and turnover in preterm children aged 8-12 years. Acta Paediatr. 2000;89:148-153.
28. Leunissen R, Stijnen T, Boot A, Hokken-Koelega A. Influence of birth size and body composition on bone mineral density in early adulthood: the PROGRAM study. Clin Endocrinol. 2008;69:386-392.
29. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
30. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
31. Gray J, Richardson D, McCormick M, Workman-Daniels K, Goldmann D. Neonatal therapeutic intervention scoring system: a therapy-based severity of illness index. Pediatrics. 1992;90:561-567.
32. Bolt R, Van Weissenbruch M, Roos J, Delemarre-van de Waal H, Cranendonk A, Lafeber H. Body composition in infants with chronic lung disease after treatment with dexamethasone. Acta Paediatr. 2002;91:815-821.
33. Picaud J, Lapillonne A, Pieltain C, Reygrobellet B, Claris O, Salle B, Rigo J. Software and scan acquisition technique-related discrepancies in bone mineral assessment using dual-energy x-ray absorptiometry in neonates. Acta Paediatr. 2002;91:1189-1193.
34. Koo W, Walters J, Bush A. Technical considerations of dual energy x-ray absorptiometry based bone mineral measurements for pediatric studies. J Bone Miner Res. 1995;10:1998-2004.
35. Prentice A, Parsons T, Cole T. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. Am J Clin Nutr. 1994;60:837-842.
36. Petersen S, Gotfredsen A, Knudsen F. Total body bone mineral in light-for-gestational-age infants and appropriate-for-gestational-age infants. Acta Paediatr Scand. 1989;78:347-350.

37. Lapillonne A, Braillon P, Claris O, Chatelain P, Delmas P, Salle B. Body composition in appropriate and in small for gestational age infants. Acta Paediatr. 1997;86:196-200.
38. Beltrand J, Alison M, Nicolescu R, Verkausiene R, Deghmoun S, Sibony O, Sebag G, Lévy-Marchal C. Bone mineral content at birth is determined both by birth weight and fetal growth pattern. Pediatr Res. 2008;64:86-90.
39. Laitinen J, Kiukaanniemi K, Heikkinen J, Koiranen M, Nieminen P, Sovio U, Keinänen-Kiunaaniemi S, Järvelin M. Body size from birth to adulthood and bone mineral content and density at 31 years of age: results from the nothern Finland 1966 birth cohort study. Osteoporos Int. 2005;16:1417-1424.
40. Mølgaard C, Thomsen B, Prentice A. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child. 1997;76:9-15.
41. Abrams S, Schanler R, Garza C. Bone mineralization in former very low birth weight infants fed either human milk or commercial formula. J Pediatr. 1988;112:956-960.

Chapter 6

Procollagen type I N-terminal peptide (PINP) in preterm infants is associated with growth during the first six months post-term

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Submitted

ABSTRACT

Objective

To identify growth-related collagen and bone parameters in small-for-gestational-age (SGA) and appropriate-for-gestational-age (AGA) preterm infants during the first six months post-term. In SGA preterm infants, increased growth and decreased bone acquisition, which we demonstrated previously, may be reflected by these markers.

Design

Observational study within a randomized controlled trial.

Patients

Thirty-three SGA (weight, length, or both at birth <-2 SDS) and 98 AGA preterm infants (gestational age [median (IQR)]: 30.3 (2.0) versus 31.1 (1.6) weeks; 42.9% versus 72.7% boys).

Measurements

Weight (g), length (cm), procollagen type I N-terminal peptide (PINP; µg/l), urinary helical peptide (UHP; µg/mmol creatinine), and alkaline phosphatase (ALP; U/l) expressed as standard deviation scores (SDS) at term age, three and six months post-term.

Results

Weight and length gain during the first six months post-term and PINP SDS at term age, three months, and six months post-term were higher in SGA compared to AGA infants. UHP SDS and ALP SDS were similar in AGA and SGA infants. PINP SDS and UHP SDS at term age and PINP SDS at three months post-term were associated with subsequent weight and length gain until six months post-term.

Conclusions

Accelerated growth in SGA compared to AGA preterm infants is reflected by increased collagen type I synthesis during the first six months post-term, suggesting that PINP and UHP correspond with growth in preterm infants. An explanation for decreased bone acquisition of SGA preterm infants may be that increased collagen type I synthesis is not directly followed by increased bone mineralization.

INTRODUCTION

During growth, collagen turnover is abundant and leads to the production of specific products that may be used as markers of collagen synthesis and degradation [1]. For collagen type I, which is predominantly present in bone matrix, serum procollagen type I N-terminal peptide (PINP) is a synthesis marker [1-2] and urinary helical peptide (UHP) a degradation marker. Lower collagen type I synthesis markers in cord blood may reflect decreased intrauterine growth [3-4] that results in low weight, length, or both at birth. Furthermore, they may reflect decreased intrauterine bone formation that results in lower bone mineral content (BMC) of small-for-gestational-age (SGA) compared to appropriate-for-gestational-age (AGA) preterm infants at birth [5].

Postnatally, preterm infants and in particular those born SGA have increased growth in the presence of an adequate nutritional supply. In term infants, collagen type I synthesis markers are associated with growth during infancy [6], childhood, and adolescence [7-12]. In preterm infants, growth, which may also affect bone accretion [13], is reflected by increased formation and decreased degradation markers of collagen type I until term age (i.e. 40 weeks postmenstrual age) [1, 14-16]. No information on collagen type I turnover in relation to growth and bone accretion in preterm infants after term age has been published.

Moreover, collagen type I formation and degradation markers may allow a more dynamic assessment of bone formation and bone resorption [17]. Recently, we have demonstrated lower BMC gain in SGA compared to AGA preterm infants during the first six months post-term, independent of growth or gain in skeletal size [18]. The lower BMC gain may be attributed to decreased bone mineralization [18], in which case a lower serum alkaline phosphatase (ALP) is expected in SGA preterm infants. On the other hand, lower BMC gain may also reflect lower collagen type I deposition in the bone matrix.

The present study aimed to elucidate whether growth and bone accretion of AGA and SGA preterm infants are associated with collagen type I formation and degradation markers and investigated PINP, ALP, and UHP in relation to growth and BMC in AGA and SGA preterm infants between term age and six months post-term. We hypothesized that, during the first six months post-term, increased growth in SGA preterm infants was associated with higher PINP and lower UHP. Second, we hypothesized that lower gain in BMC was explained by lower bone mineralization reflected by lower ALP.

PATIENTS AND METHODS

Patients

The present study was part of a randomized controlled trial that evaluated the effects of postdischarge formula, term formula, and human milk on growth and body composition in preterm infants, as described previously [18-19]. In short, 152 infants born at a gestational age of 32 weeks or less, with a birth weight of 1,500 gram or less, or both were included and attended our outpatient clinic at term age (40.3 ± 0.7 weeks postmenstrual age), three months post-term (53.0 ± 0.5 weeks postmenstrual age), and six months post-term (66.0 ± 0.5 weeks postmenstrual age). One-hundred-and-thirty-nine infants completed the randomized controlled trial at six months post-term, as described previously [18-19]. The present study investigated size at birth, postnatal growth, and BMC in relation to serum collagen turnover and bone markers and included 98 AGA and 33 SGA infants with available data on collagen turnover and bone markers, growth, and BMC between term age and six months post-term. Eight preterm infants of the original randomized controlled trial were not included because they could not be classified as AGA or SGA due to missing data on birth length. This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving patients were approved by the Ethics Committee of VU University Medical Center, Amsterdam, The Netherlands. All the parents of the participating infants gave written informed consent.

Methods

Gestational age (weeks) at birth was extracted from the infants' medical records. Weight and length were measured at birth, term age, three and six months post-term, as described previously [18-19]. At birth and term age, weight and length were expressed as standard deviation scores (SDS) based on Swedish references for preterm infants [20]. At three and six months post-term, weight and length were expressed as SDS based on Dutch references [21]. Infants were classified as AGA if weight and length at birth were -2 SDS or above and as SGA if weight, length, or both at birth were below -2 SDS. Gain (Δ) in weight SDS and length SDS was calculated for the following intervals: birth-term age, birth-three months post-term, birth-six months post-term, term age-three months post-term, term age-six months post-term, and three-six months post-term.

PINP, ALP, urine creatinine, and UHP were measured at term age, three and six months post-term. PINP (µg/l) was measured at the Endocrine Laboratory of VU University Medical Center, Amsterdam, The Netherlands by a radioimmunoassay (Orion Diagnostica, Espoo, Finland) with an inter-assay variance of 9.0%. ALP (U/l) was measured at the Department of Clinical Chemistry of VU University Medical Center, Amsterdam, The Netherlands by IFCC method (Roche diagnostics, Mannheim, Germany) with an inter-assay

variance of 1.9%. Urine creatinine (mmol/l) was analyzed at the Department of Clinical Chemistry of VU University Medical Center, Amsterdam, The Netherlands by the Jaffé method (Roche diagnostics, Mannheim, Germany) with an inter-assay variance of 2.2%. UHP (µg/mmol creatinine) was measured at the University Medical Centre Groningen, Groningen, The Netherlands by ELISA (Quidel Corporation, San Diego, CA, USA) with an inter-assay variance of 8.8%. Change in PINP, ALP, and UHP was calculated between term age and three months post-term and between three and six months post-term. The ratio between PINP and UHP was calculated at term age, three and six months post-term, as a proxy for the ratio between collagen type I formation and degradation.

BMC (g) was measured at term age and six months post-term by whole-body dual-energy x-ray absorptiometry (DXA; Hologic QDR4500A, Hologic Inc., Bedford, MA, USA) and analyzed with Infant Whole Body Software, version 12.3.3, as described previously [18-19]. DXA scan quality was evaluated by one expert radiologist who was blinded for the size at birth and the type of feeding of the infants. Incomplete scans and scans with severe movement artefacts were excluded. Good quality DXA scans were available from 82 AGA and 28 SGA preterm infants at term age, 78 AGA and 24 SGA preterm infants at six months post-term, and 63 AGA and 21 SGA at both term age and six months post-term [18].

Statistics

The distribution of gender and type of feeding were expressed as frequency and compared between SGA and AGA infants by logistic regression, adjusted for gestational age and for gender and gestational age, respectively. Weight SDS and length SDS were normally distributed and expressed as mean ± standard deviation (SD). Gestational age at birth, PINP, ALP, UHP, PINP/UHP ratio, and BMC were normally distributed after natural log transformation and expressed as median (IQR).

Growth, PINP (µg/l), ALP (U/l), UHP (µg/mmol creatinine), and PINP/UHP ratio were compared between SGA and AGA infants by linear regression adjusted for gender and gestational age. Associations between PINP (µg/l), UHP (µg/mmol creatinine), PINP/UHP ratio, growth, and BMC (g) were evaluated by linear regression adjusted for gender and gestational age.

PINP, ALP, UHP, and BMC were expressed as internal SDS by gender with AGA infants as the reference category to improve the comparison between SGA and AGA infants, as SGA infants were more often boys and had a higher gestational age at birth. In addition, these internal SDS were used to exclude the influence of gender and being SGA in the associations. Unfortunately, SDS references were not available for this age group. Differences in PINP SDS, ALP SDS, and UHP SDS between SGA and AGA infants as well as associations between PINP SDS, UHP SDS, and growth and between PINP SDS, UHP SDS, and BMC SDS were analyzed by linear regression, all adjusted for gender and gestational age.

It was evaluated if type of feeding (postdischarge formula, term formula, or human milk) influenced the differences in growth, collagen turnover and bone markers between SGA and AGA infants. In addition, it was evaluated if associations between collagen turnover markers, growth, and BMC were modified by the type of feeding or by being SGA and if there was an interaction between gender, gestational age, and being SGA on these associations. Associations between growth intervals and collagen turnover markers were adjusted for gain in weight SDS or length SDS in the preceding interval. All statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). A P value of less than 0.05 was considered significant.

RESULTS

SGA and AGA infants: baseline characteristics and growth

Gestational age at birth was higher in SGA compared to AGA infants (31.1 (1.6) weeks versus 30.3 (2.0) weeks, P=0.001). SGA infants were more often boys compared to AGA infants (72.7% versus 42.9%, P=0.004). The type of feeding did not differ between SGA and AGA infants (SGA: 36.4% postdischarge formula; 30.3% term formula, 33.3% human milk; AGA: 38.8% postdischarge formula, 26.5% term formula, 34.7% human milk; P≥0.05).

Weight SDS and length SDS were lower in SGA compared to AGA infants at all time-points (Figure 6.1). Between birth and term age, gain in weight SDS and length SDS was similar in SGA and AGA infants (Table 6.1). Between term age and three months post-term and between term age and six months post-term, gain in length SDS was higher in SGA compared to AGA infants (Table 6.1). Between three and six months post-term, gain in weight SDS was higher in SGA compared to AGA infants (Table 6.1).The differences in growth between SGA and AGA infants were not related to the type of feeding (data not shown).

SGA and AGA infants: collagen turnover and bone markers

PINP (µg/l) and UHP (µg/mmol creatinine) at term age and PINP (µg/l) at three and six months post-term were higher in SGA compared to AGA infants; ALP (U/l) at term age, three and six months post-term was not different between SGA and AGA infants (Figure 6.2). Between term age and three months post-term, the decline in PINP (µg/l) and UHP (µg/mmol creatinine) and the increase in ALP (U/l) were similar in SGA and AGA infants (data not shown). Between three and six months post-term, SGA infants had a greater decline in UHP compared to AGA infants (-1016 (712) versus -718 (831) µg/mmol creatinine, P=0.036), whereas the decline in PINP (µg/l) and ALP (U/l) was not different (data not shown). As a result, SGA infants had higher PINP/UHP ratio at six months post-term compared to AGA infants (Figure 6.2).

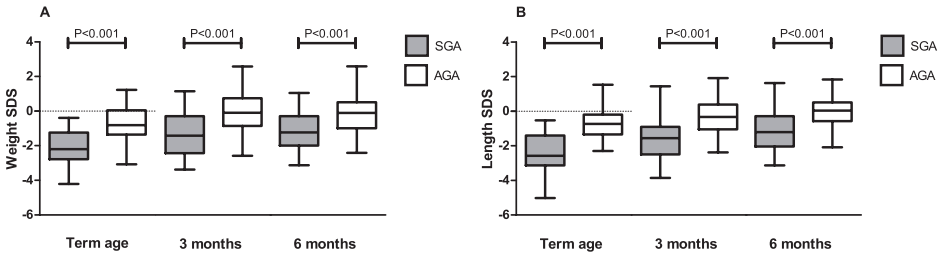

Figure 6.1. Weight SDS (A) and length SDS (B) in SGA and AGA preterm infants between term age and six months post-term

Tukey box plots. SGA: small-for-gestational-age; AGA: appropriate-for-gestational-age; months: months post-term. Weight SDS and length SDS were compared between SGA and AGA infants by linear regression, adjusted for gender and gestational age.

Table 6.1. Gain in weight SDS and length SDS in SGA and AGA preterm infants between birth and six months post-term

	SGA (n=33)	AGA (n=98)
Birth-term age		
ΔWeight SDS	-0.61 ± 0.74	-0.70 ± 0.68
ΔLength SDS	0.08 ± 1.04	-0.38 ± 1.07
Term age-3 months post-term		
ΔWeight SDS	0.82 ± 0.71	0.69 ± 0.77
ΔLength SDS	0.84 ± 0.78**	0.43 ± 0.69
3-6 months post-term		
ΔWeight SDS	0.15 ± 0.61*	-0.10 ± 0.60
ΔLength SDS	0.44 ± 0.70	0.30 ± 0.58
Term age-6 months post-term		
ΔWeight SDS	0.97 ± 1.04	0.59 ± 1.03
ΔLength SDS	1.28 ± 1.04**	0.73 ± 0.87

Values as mean ± SD. SGA: small-for-gestational-age; AGA: appropriate-for-gestational-age; Δ: gain; SDS: standard deviation score. Values compared between SGA and AGA preterm infants by linear regression adjusted for gender and gestational age; ** P<0.02; * P<0.05.

A challenge of working with collagen turnover and bone markers is that serum levels change with age and differ between boys and girls. Since SGA infants were more often boys compared to AGA infants, collagen turnover and bone markers were compared using SDS. PINP SDS at term age, three and six months post-term was higher in SGA compared to AGA infants, whereas UHP SDS, PINP/UHP ratio SDS, and ALP SDS were similar in SGA and AGA infants (Figure 6.3). The differences in collagen turnover and bone markers expressed as SDS between SGA and AGA infants were not influenced by the type of feeding (data not shown).

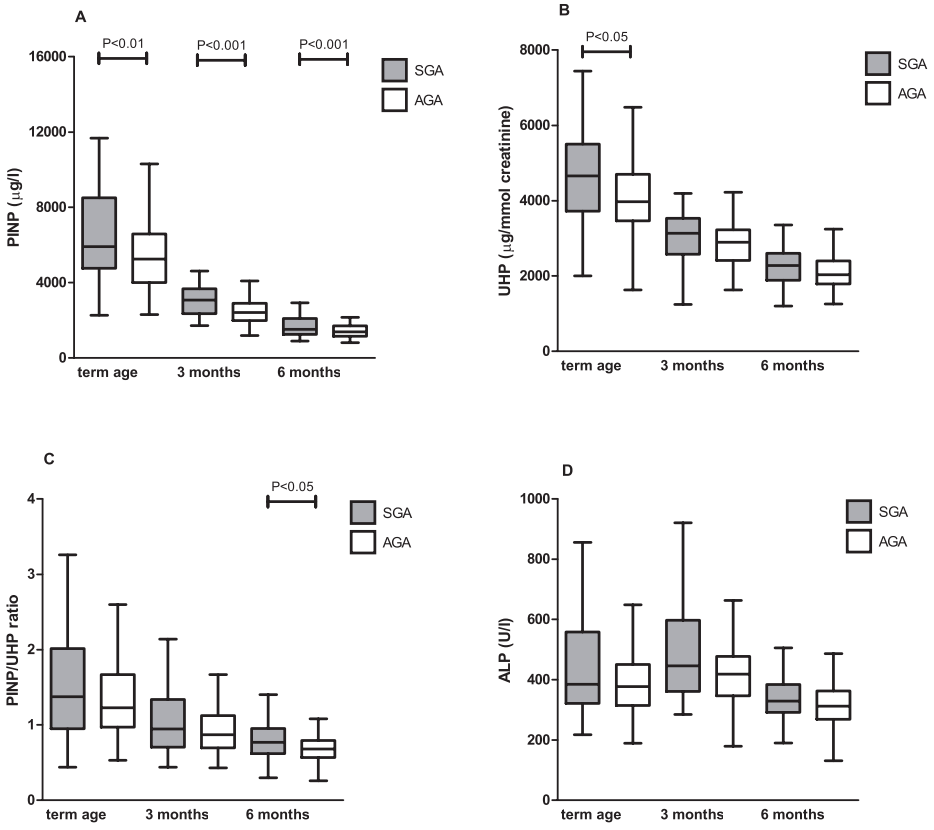

Figure 6.2. PINP (µg/l; A), UHP (µg/mmol creatinine; B), PINP/UHP ratio (C), and ALP (U/l; C) in SGA and AGA preterm infants between term age and six months post-term
Tukey boxplots. months: months post-term; SGA: small-for-gestational-age; AGA: appropriate-for-gestational-age; PINP: procollagen type I N-terminal peptide; UHP: urinary helical peptide; ALP: alkaline phosphatase. Markers were compared between SGA and AGA preterm infants by linear regression with natural log transformed parameters, adjusted for gender and gestational age.

Total group: collagen turnover markers in relation to growth

Gain in weight SDS between birth and term age was associated with PINP SDS and with PINP/UHP ratio SDS at term age (β=0.13, 95%CI 0.03;0.24, P=0.016; and β=0.16, 95%CI 0.05;0.27, P=0.004; respectively) but not with UHP SDS at term age. Gain in weight SDS and length SDS between birth and three months post-term as well as between birth and six months post-term was not associated with PINP SDS, UHP SDS, and the PINP/UHP ratio SDS at three and six months post-term, respectively (data not shown).

PINP SDS and UHP SDS at term age were associated with gain in weight SDS and length SDS between term age and six months post-term (Table 6.2). PINP/UHP ratio SDS at term age was not associated with gain in weight SDS and length SDS between term age and six months post-term (Table 6.2). These associations persisted after adjustment

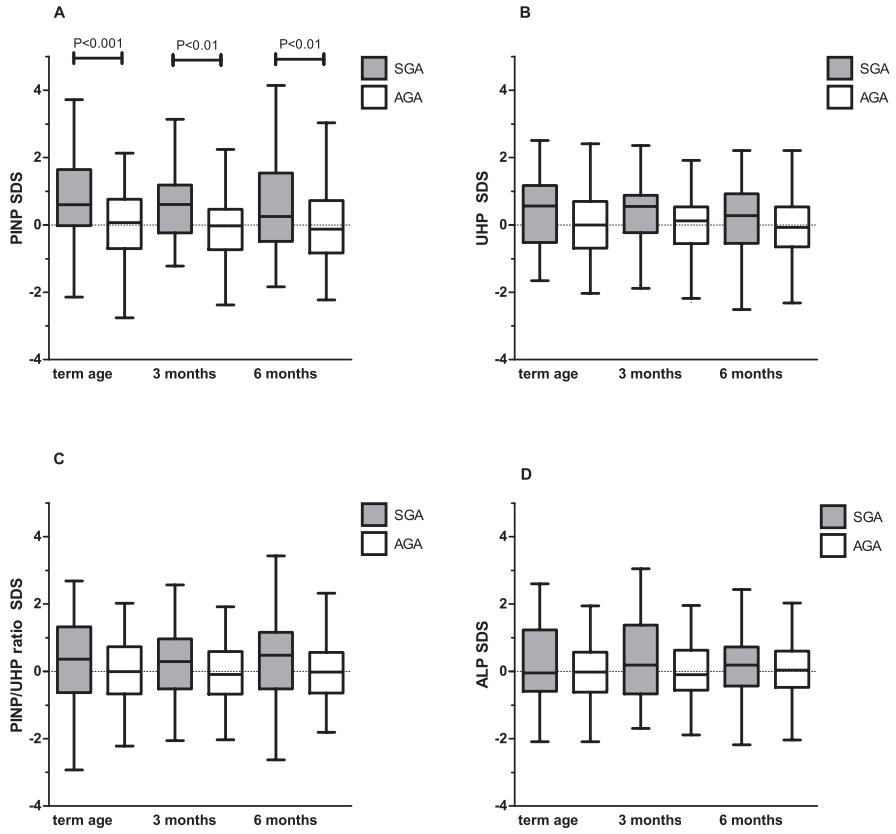

Figure 6.3. PINP SDS (A), UHP SDS (B), PINP/UHP ratio SDS (C), and ALP SDS (D) in SGA and AGA preterm infants between term age and six months post-term

Tukey boxplots. SGA: small-for-gestational-age; AGA: appropriate-for-gestational-age; months: months post-term; PINP: procollagen type I N-terminal peptide; UHP: urinary helical peptide; ALP: alkaline phosphatase. Markers were compared between SGA and AGA preterm infants by linear regression, adjusted for gender and gestational age.

for preceding gain in weight SDS or length SDS between birth and term age. In addition, adjusted for preceding gain in weight SDS between birth and term age, PINP SDS at term age was positively associated with gain in weight SDS between term age and six months post-term (β=0.17, 95%CI 0.01;0.33, P=0.033). PINP SDS at three months post-term was associated with gain in weight SDS and length SDS between three and six months post-term, whereas UHP SDS and PINP/UHP ratio SDS were not (Table 6.3). These associations persisted after adjustment for preceding gain in weight or length SDS between birth and three months post-term.

Similar associations were found between PINP (µg/l), UHP (µg/mmol creatinine), PINP/UHP ratio, and growth, adjusted for gender and gestational age (data not shown). The associations between collagen turnover markers, expressed as absolute values or SDS,

and growth were not modified by being SGA at birth or by the type of feeding (data not shown). There was no interaction between gender or gestational age and being SGA on the associations (data not shown).

Total group: collagen turnover markers in relation to BMC

PINP SDS and UHP SDS at term age were inversely associated with BMC SDS at term age (β=-0.30, 95%CI -0.48;-0.11, P=0.004; and β=-0.52, 95%CI -0.71;-0.34, P<0.001; respectively). UHP SDS at term age was inversely associated with BMC SDS at six months post-term (β=-0.36, 95%CI -0.57;-0.16, P=0.001), whereas PINP SDS at term age was not. PINP SDS at three and six months post-term was not associated with BMC SDS at six months post-term. UHP SDS at three and six months post-term was inversely associated with BMC SDS at six months post-term (β=-0.33, 95%CI -0.52;-0.13, P=0.001; and β=-0.34, 95%CI -0.55;-0.13, P=0.002; respectively).

Similar associations were found between PINP (µg/l), UHP (µg/mmol creatinine), and BMC (g), adjusted for gender and gestational age (data not shown). The associations between collagen turnover markers and BMC, expressed as absolute values or SDS, were not modified by being SGA at birth or by the type of feeding (data not shown). There was no interaction between gender or gestational age and being SGA on the associations (data not shown).

Table 6.2. PINP SDS, UHP SDS, and PINP/UHP ratio SDS at term age in relation to gain in weight SDS and length SDS in the total group (n=131) between term age and six months post-term

	Term age								
	PINP SDS			UHP SDS			PINP/UHP ratio SDS		
	R	β	95% CI	R	β	95% CI	R	β	95% CI
Term age-3 months post-term									
ΔWeight SDS	0.33	0.08	-0.03;0.20	0.32	0.07	-0.05;0.19	0.07	0.10	-0.16;0.36
ΔLength SDS	0.17	0.10	-0.01;0.22	0.21	0.13	0.01;0.25*	0.01	-0.02	-0.29;0.25
3-6 months post-term									
ΔWeight SDS	0.05	0.02	-0.07;0.12	0.22	0.12	0.02;0.22**	0.13	-0.24	-0.57;0.10
ΔLength SDS	0.18	0.06	-0.04;0.15	0.21	0.08	-0.03;0.18	0.01	0.01	-0.32;0.34
Term age-6 months post-term									
ΔWeight SDS	0.26	0.11	-0.05;0.27	0.30	0.19	0.02;0.35*	0.02	-0.02	-0.22;0.17
ΔLength SDS	0.21	0.16	0.01;0.30*	0.27	0.21	0.06;0.36**	0.01	-0.01	-0.22;0.21

PINP: procollagen type I N-terminal peptide (µg/l); UHP: urinary helical peptide (µg/mmol creatinine); R: model summary correlation coefficient; β: regression coefficient; 95%CI: 95% confidence interval of regression coefficient; Δ: gain in. Associations evaluated by linear regression, adjusted for gender and gestational age; ** P<0.02, * P<0.05.

Table 6.3. PINP SDS, UHP SDS, and PINP/UHP ratio SDS at three months post-term in relation to gain in weight SDS and length SDS in the total group (n=131) between three and six months post-term

	3 months post-term								
	PINP SDS			UHP SDS			PINP/UHP ratio SDS		
	R	β	95% CI	R	β	95% CI	R	β	95% CI
3-6 months post-term									
ΔWeight SDS	0.30	0.15	0.05;0.25**	0.03	0.02	-0.09;0.12	0.16	0.29	-0.04;0.62§
ΔLength SDS	0.26	0.15	0.05;0.25**	0.21	0.09	-0.02;0.20§	0.09	0.16	-0.17;0.49

PINP: procollagen type I N-terminal peptide (μg/l); UHP: urinary helical peptide (μg/mmol creatinine); R: model summary correlation coefficient; β: regression coefficient; 95%CI: 95% confidence interval of regression coefficient; Δ: gain in. Associations evaluated by linear regression, adjusted for gender and gestational age; ** P<0.01, § P=0.09.

DISCUSSION

The present study is the first to publish on collagen type I turnover in preterm infants after term age in relation to growth and bone accretion and demonstrates that SGA preterm infants have higher collagen type I synthesis, reflected by higher PINP levels, compared to AGA preterm infants during the first six months post-term. Collagen turnover markers as indicators of growth have only been reported in preterm infants until term age [1, 14-16]. We have now demonstrated that the collagen turnover markers PINP and UHP continue to be markers of growth of preterm infants during the first six months post-term.

Furthermore, our findings suggest that higher collagen type I synthesis in SGA compared to AGA preterm infants may reflect faster growth in SGA preterm infants during the first six months post-term. In contrast, others demonstrate equal PINP levels in SGA and AGA preterm infants until term age [14]. In the present study, the higher collagen type I synthesis markers may reflect accelerated growth of SGA preterm infants, similar to associations demonstrated in term subjects during infancy [6], childhood and adolescence [7-12]. In more detail, this study adds that PINP is associated with growth between three and six months post-term, which might imply that most of the collagen type I synthesis that contributes to growth takes place during this interval.

During the first year of life, collagen type I synthesis decreases, which is reflected by a decrease in PINP levels [22-23]. This decline may demonstrate a shift from rapid in utero and early postnatal growth towards slower growth during infancy [24]. In the present study, PINP levels decreased in both SGA and AGA preterm infants during the first six months post-term, which suggests a shift towards slower growth. The higher PINP levels at six months post-term in SGA compared to AGA infants may imply that preterm SGA infants approach this phase of growth deceleration later during infancy.

It may be hypothesized that collagen type I synthesis predominantly results in bone growth because collagen type I is mainly deposited in bone matrix [2]. Although, in the present study, SGA preterm infants had increased collagen type I synthesis, we demonstrated recently that the same group of infants has a decreased gain in BMC [18]. BMC is determined by skeletal size as well as by bone mineral density [25]. We hypothesize that higher collagen type I synthesis of SGA preterm infants may not result in higher gain in BMC because there is a lag time before bone mineralization starts, as suggested by a previous study in SGA term infants [26]. This hypothesis may be supported by the present study that demonstrated that increased collagen type I synthesis in SGA preterm infants was not accompanied by increased bone mineralization, as suggested by the finding that serum ALP was not increased in SGA preterm infants. ALP has inconsistently been associated with BMC [27]. This puts emphasis on adequate postnatal nutritional regimens that supply sufficient nutrients to SGA preterm infants, including sufficient amino acids for collagen type I synthesis [24] as well as adequate amounts of calcium, phosphorus, and vitamin D in order to enhance bone mineralization.

Several characteristics of the study population may be taken into account when the results of the present study are interpreted. Being SGA was classified based on birth weight and birth length, as both are important indicators of body size and are related to bone mass. In contrast to previous studies, only the smallest infants were included in the SGA group because weight and length below -2 SDS were used to define small body size. Furthermore, SGA preterm infants had a higher gestational age at birth and were more often boys. The latter may be due to the fact that gender in combination with being SGA was not taken into account during randomization. In order to control for the influence of this difference in gestational age and gender, all analyses were adjusted for these variables and standard deviation scores were used. For weight SDS and length SDS, large reference groups were available. For BMC, collagen turnover and bone markers, internal SDS were constructed. In addition, there was no interaction between gender, gestational age, and being SGA on the presented results.

The present study had several limitations. The sample size was small, especially after subgroup classification. Another limitation was that the original randomized controlled trial investigated growth and body composition of preterm infants fed three different nutritional regimens after discharge. The distribution of the type of feeding was comparable in SGA and AGA infants and the type of postdischarge nutrition neither influenced nor explained the associations between growth, BMC, and collagen turnover and bone markers or the differences between SGA and AGA infants. However, the influence of the nutritional regimen after discharge cannot be excluded completely.

In conclusion, increased collagen type I synthesis in SGA compared to AGA preterm infants is associated with accelerated growth during the first six months post-term, suggesting that PINP and UHP correspond with growth in preterm infants. Furthermore,

lower gain in BMC in SGA compared to AGA preterm infants is not a consequence of insufficient collagen type I synthesis or increased bone resorption in SGA preterm infants. This lower gain in BMC may be attributed to a delay in bone mineralization, as suggested by the finding that increased collagen type I synthesis in SGA preterm infants is not yet accompanied by increased ALP, which reflects bone mineralization. These findings emphasize the importance of an adequate nutritional supply to SGA preterm infants, including calcium, phosphorus, and vitamin D, in order to achieve adequate bone mineralization during the first half of infancy.

REFERENCES

1. Kajantie E, Dunkel L, Risteli J, Pohjavuori M, Andersson S. Markers of type I and III collagen turnover as indicators of growth velocity in very low birth weight infants. J Clin Endocrinol Metab. 2001;86:4299-4306.
2. Nakano K, Iwamatsu T, Wang C, Tarasima M, Nakayama T, Sasaki K, Tachikawa E, Noda N, Mizoguchi E, Osawa M. High bone turnover of type I collagen depends on fetal growth. Bone. 2006;38:249-256.
3. Harrast S, Kalkwarf H. Effects of gestational age, maternal diabetes, and intrauterine growth retardation on markers of fetal bone turnover in amniotic fluid. Calcif Tissue Int. 1998;62:205-208.
4. Saarela T, Risteli J, Kauppila A, Koivisto M. Type I collagen markers in cord serum of appropriate vs. small for gestational age infants born during the second half of pregnancy. Eur J Clin Invest. 2001;31:438-443.
5. Petersen S, Gotfredsen A, Knudsen F. Total body bone mineral in light-for-gestational-age infants and appropriate-for-gestational-age infants. Acta Paediatr Scand. 1989;78:347-350.
6. Doherty C, Crofton P, Sarkar M, Shakur M, Wade J, Kelnar C, Elmlinger M, Ranke M, Cutting W. Malnutrition, zinc supplementation and catch-up growth: changes in insulin-like growth factor I, its binding proteins, bone formation and collagen turnover. Clin Endocrinol. 2002;57:391-399.
7. Trivedi P, Risteli J, Hindmarsh P, Brook C, Mowat A. Serum concentrations of the type I and III procollagen peptides as biochemical markers of growth velocity in healthy infants and children and in children with growth disorders. Pediatr Res. 1991;30:276-280.
8. Saggese G, Bertelloni S, Baroncelli G, Di Nero G. Serum levels of carboxyterminal propeptide of type I procollagen in healthy children from 1st year of life to adulthood and in metabolic bone diseases. Eur J Pediatr. 1992;151:764-768.
9. Crofton P, Wade J, Taylor M, Holland C. Serum concentrations of carboxy-terminal propeptide of type I procollagen, amino-terminal propeptide of type III procollagen, cross-linked carboxy-terminal telopeptide of type I collagen, and their interrelationships in schoolchildren. Clin Chem. 1997;43:1577-1581.
10. Rotteveel J, Schoute E, Delemarre-van de Waal H. Serum procollagen I carboxyterminal propeptide (PICP) levels through puberty: relation to height velocity and serum hormone levels. Acta Paediatr. 1997;86:143-147.
11. van Coevorden S, Netelenbos J, de Ridder C, Roos J, Popp-Snijders C, Delemarre-van de Waal H. Bone metabolism markers and bone mass in healthy pubertal boys and girls. Clin Endocrinol. 2002;57:107-116.
12. Munday K, Ginty F, Fulford A, Bates C. Relationships between biochemical bone turnover markers, season, and inflammatory status indices in prepubertal Gambian boys. Calcif Tissue Int. 2006;79:15-21.
13. Kitazawa S, Itabashi K, Umeda Y, Inoue M, Nishioka T. Growth and bone mineralization in small for gestational age preterm infants. Pediatr Int. 2013;Aug 2013. doi 10.1111/ped.12189.
14. Crofton P, Shrivastava A, Wade J, Stephen R, Kelnar C, Lyon A, McIntosh N. Bone and collagen markers in preterm infants: relationship with growth and bone mineral content over the first 10 weeks of life. Pediatr Res. 1999;46:581-587.
15. Furmaga-Jablónska W, Kulik-Rechberger B, Kozlowska M. Association between leptin, markers of bone formation and physical growth of newborns. Ann Hum Biol. 2003;30:250-261.
16. Bhandari V, Fall P, Raisz L, Rowe J. Potential biochemical growth markers in premature infants. Am J Perinatol. 1999;16:339-349.

17. Ferretti M, Palumbo C, Contri M, Marotti G. Static and dynamic osteogenesis: two different types of bone formation. Anat Embryol. 2002;206:21-29.
18. van de Lagemaat M, Rotteveel J, van Weissenbruch M, Lafeber H. Small-for-gestational-age preterm-born infants already have lower bone mass during early infancy. Bone. 2012;51:441-446.
19. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
20. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
21. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
22. Crofton P, Evans N, Taylor M, Holland C. Procollagen type I amino-terminal propeptide: pediatric reference data and relationship with procollagen type I carboxyl-terminal propeptide. Clin Chem. 2004;50:2173-2176.
23. Jürimäe J. Interpretation and application of bone turnover markers in children and adolescents. Curr Opin Pediatr. 2010;22:494-500.
24. Shiff Y, Eliakim A, Shainkin-Kestenbaum R, Arnon S, Lis M, Dolfin T. Measurements of bone turnover markers in premature infants. J Pediatr Endocrinol Metab. 2001;14:389-395.
25. Mølgaard C, Thomsen B, Prentice A. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child. 1997;76:9-15.
26. Namgung R, Tsang R, Sierra R, Ho M. Normal serum indices of bone collagen biosynthesis and degradation in small for gestational age infants. J Pediatr Gastroenterol Nutr. 1996;23:224-228.
27. Faerk J, Peitersen B, Petersen S, Michaelsen K. Bone mineralisation in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. Arch Dis Child Fetal Neonatal Ed. 2002;87:F133-F136.

Part II

Postdischarge nutrition

Chapter 7

Increased gain in bone mineral content of preterm infants fed an isocaloric, protein- and mineral-enriched postdischarge formula

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ABSTRACT

Purpose

Preterm infants are at risk for suboptimal bone mineralization. Postnatal bone formation requires optimal nutritional composition. This study evaluated the effect of isocaloric, protein- and mineral-enriched postdischarge formula (PDF), standard term formula (TF), and human milk (HM) on gain in bone mineral content (BMC) of preterm infants between term age (40 weeks postmenstrual age) and 6 months corrected age (CA).

Methods

Between term age and 6 months CA, 93 preterm infants were randomized to be fed PDF (n=52) or TF (n=41) and 46 preterm infants were fed HM. Weight (g) and length (cm) were measured at birth, term age, and 6 months CA. BMC (g) was measured by whole-body dual-energy x-ray absorptiometry at term age and 6 months CA.

Results

Gain in BMC (expressed as median with interquartile range) between term age and 6 months CA was higher in PDF fed infants (102.3 (32.4) g) compared to TF and HM fed infants (91.6 (24.5) and 84.5 (33.3) g, respectively), adjusted for gender, gestational age, birth weight, and gain in weight and length.

Conclusion

Between term age and 6 months CA, isocaloric PDF enhances gain in BMC of preterm infants, independent of gain in weight and length. We speculate that higher gain in BMC during infancy may improve adult bone mass in preterm infants.

INTRODUCTION

Eighty percent of the total bone mass of a newborn is acquired during the intra-uterine third trimester. Since preterm infants are exposed to extra-uterine conditions during the third trimester, they are at risk for suboptimal bone mineralization. To achieve adequate bone accretion during the extra-uterine third trimester, preterm infants require an optimal composition of nutrient supply after birth [1].

Between birth and term age (40 weeks postmenstrual age), preterm infants fed an isocaloric, protein- and mineral-enriched formula reach a higher bone mineral content (BMC) compared to preterm infants fed a standard term formula [2]. After term age, the effect of a high-caloric, protein- and mineral-enriched formula on BMC has been investigated but remains controversial. Whereas some studies demonstrate that preterm infants fed a high-caloric, protein- and mineral-enriched formula reach a higher BMC than infants fed a standard term formula [3-5], other studies did not find a positive effect of a high-caloric, enriched formula on BMC after term age [6-7].

As the effect of an isocaloric, protein- and mineral-enriched formula on BMC has been demonstrated until term age [2], we hypothesized that protein and minerals added to a formula without additional energy enrichment improve bone accretion of preterm infants after term age. Therefore, the present study investigated the effects of isocaloric postdischarge formula (PDF), standard term formula (TF), and human milk (HM) on bone area (BA) and BMC of preterm infants between term age and 6 months corrected age (CA).

PATIENTS AND METHODS

The design of the present study and its participants have been described in detail previously [8]. In short, infants born at a gestational age of 32 weeks or less and/or with a birth weight of 1,500 g or less were eligible for the study. Exclusion criteria included congenital malformations or conditions known to affect growth or body composition. The study was approved by the local ethics committee. Parents of the participating children gave written informed consent.

At term age, formula-fed infants were randomized to PDF (n=52; Table 7.1) or TF (n=41) and were fed this diet until 6 months CA. Between birth and term age, preterm infants were fed preterm formula (n=62: 39 PDF and 23 TF fed infants) or fortified human milk (n=75: 13 PDF, 17 TF, and 45 HM fed infants) and the type of diet could not be classified in two infants due to missing data. Sixteen HM fed infants were supplemented with 200 IU/d vitamin D after human milk fortification was stopped at discharge. Between birth and term age, two PDF and two TF fed infants were supplemented with 200 IU/d vitamin

Table 7.1. Composition of study formulas[a]

	Postdischarge formula (PDF)[b]	Term formula (TF)[c]
Energy (kcal)	67	67
Protein (g)	1.7	1.47
Protein/energy ratio (g/100 kcal)	2.6	2.2
Carbohydrates (g)	7.0	7.2
Fat (g)	3.5	3.5
Calcium (mg)	65	50
Phosphorus (mg)	38	30
Vitamin D (IU)	56	48

[a] per 100 ml prepared formula; [b] Friso Prematuur 1®, FrieslandCampina, Leeuwarden, The Netherlands; [c] Friso 1 normaal®, FrieslandCampina, Leeuwarden, The Netherlands.

D during two or three weeks because they were fed unfortified human milk. Infants did not receive any mineral supplements between birth and term age. Two PDF and two TF fed infants were supplemented with vitamin D by their parents for unknown reasons during the first week after term age. At term age, infants were considered to be HM fed (n=46) if they received >80% HM and were supplemented with 200 IU/d of vitamin D. If HM was insufficiently available, TF was added to the diet. Between term age and 6 months CA, the total volume intake (ml/kg/day) was not different between PDF and TF fed infants [8]. In the present study, infants did not receive any mineral supplements between term age and 6 months CA.

Weight (g) and length (cm) were measured at birth, term age, and six months CA, as described previously [8]. Weight and length at birth and term age were expressed as standard deviation score (SDS) based on Swedish references of preterm infants [9] and weight and length at 6 months CA were expressed as SDS based on Dutch references [10]. BA (cm^2) and BMC (g) were measured at term age and 6 months CA by whole-body dual-energy x-ray absorptiometry (DXA; Hologic QDR4500A, Hologic Inc., Bedford, MA, USA) and were analyzed by Infant Whole Body Software version 12.3.3. One expert radiologist evaluated the DXA quality and was blinded for the type of diet. Scans with severe movement artifacts and incomplete scans were excluded [11]. Good quality scans were available from 116 infants at term age, 106 infants at 6 months CA, and 87 infants at both term age and 6 months CA. Gain in weight, length, BA, and BMC between term age and 6 months CA was calculated.

Statistics

The distribution of gender was expressed as a frequency and compared between PDF, TF, and HM fed infants by Fisher's exact test. Postmenstrual age, weight, length, BA, BMC, gain in BA, and gain in BMC were normally distributed after log transformation and were expressed as median with interquartile range (IQR). Postmenstrual age, adjusted for

gender, as well as weight and length, both adjusted for gender and gestational age, were compared between PDF, TF, and HM fed infants by linear regression analyses.

BA and BMC were compared between PDF, TF, and HM fed infants by linear regression analyses with gender, gestational age, birth weight, and current weight and length as covariates. Gain in BA and BMC was compared between PDF, TF, and HM fed infants by linear regression analyses with gender, gestational age, birth weight and gain in weight and length as covariates. In addition, BMC at term age, BMC at 6 months CA, and gain in BMC were adjusted for BA at term age, BA at 6 months CA, and gain in BA, respectively, as recommended by Prentice et al. and Mølgaard et al. [12-13]. All analyses were also performed with weight and length SDS and these results were similar to those with absolute weight and length. Therefore, only the analyses with absolute weight and length are described here. A P value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Postmenstrual age, weight, length, and the distribution of gender were not different between PDF, TF, and HM fed infants (Table 7.2). BMC at term age was lower in PDF and TF fed infants compared to HM fed infants (Table 7.3). BMC at 6 months CA was higher in PDF fed infants compared to TF and HM fed infants (Table 7.3). After adjustment for BA, BMC at term age and 6 months CA remained different between PDF and HM fed infants. Gain in BMC between term age and 6 months CA was higher in PDF fed infants compared to TF and HM fed infants (Table 7.3). However, after adjustment for gain in BA, gain in BMC was no longer different between PDF and TF fed infants but remained higher in PDF fed infants compared to HM fed infants. Gain in BMC was not different between TF and HM fed infants (Table 7.3).

All HM fed infants received fortified human milk, whereas PDF and TF fed infants received either preterm formula or fortified human milk between birth and term age. BMC at term age, BMC at 6 months CA, and gain in BMC was similar in PDF and TF fed infants that were fed preterm formula compared to those fed fortified human milk between birth and term age (data not shown). There was no interaction between the diet between birth and term age and the diet between term age and 6 months CA on bone mass parameters. In addition, there was no interaction between gender and diet on bone mass parameters.

Table 7.2. Growth parameters between birth and 6 months corrected age in PDF, TF, and HM fed infants

	PDF (n=52)	TF (n=41)	HM (n=46)
Boys[a] (%)	51.9	56.1	45.7
Birth			
PMA (weeks)	30.7 (1.5)	30.9 (2.3)	30.0 (1.9)
Weight (g)	1344 (483)	1394 (262)	1345 (605)
Length (cm)	38.0 (4.0)	38.3 (3.0)	38.0 (3.5)
Term age			
PMA (weeks)	40.6 (1.1)	40.3 (0.9)	40.6 (0.7)
Weight (g)	3173 (699)	3090 (603)	3158 (734)
Length (cm)	49 (3.0)	48.5 (3.0)	48.3 (2.6)
6 months CA			
PMA (weeks)	66.1 (1.0)	66.1 (1.0)	66.3 (1.0)
Weight (g)	7380 (1511)	7460 (1430)	7150 (1729)
Length (cm)	67.0 (2.5)	66.5 (4.8)	66.3 (4.0)

Values as median (IQR), unless specified otherwise. PDF: postdischarge formula; TF: term formula; HM: human milk; PMA: postmenstrual age; CA: corrected age. Linear regression analyses for comparisons between PDF, TF, and HM fed infants. No significant differences between PDF, TF, and HM fed infants. [a] Frequency (%).

Table 7.3. Bone area and bone mineral content in PDF, TF, and HM fed infants between term age and 6 months corrected age

	PDF		TF		HM	
	n	median (IQR)	n	median (IQR)	n	median (IQR)
Term age						
BA (cm^2)	43	309.7 (73.9)*	36	300.4 (67.2)$^{\#}$	37	314.8 (57.2)
BMC (g)	43	43.8 (20.4)‡	36	47.3 (12.7)$^{\#}$	37	48.5 (16.7)
6 months CA						
BA (cm^2)	42	632.3 (97.3)	30	597.8 (88.3)	34	601.4 (95.8)
BMC (g)	42	150.5 (42.5)$^{*,+}$	30	134.2 (35.1)	34	132.6 (33.4)
Term age-6 months CA						
ΔBA (cm^2)	36	306.2 (81.4)	25	300.6 (95.4)	26	317.1 (41.8)
ΔBMC (g)	36	102.3 (32.4)$^{*,+}$	25	91.6 (24.5)	26	84.5 (33.3)

PDF: postdischarge formula; TF: term formula; HM: human milk; BA: bone area; BMC: bone mineral content; CA: corrected age; Δ: gain. Linear regression analyses for differences between PDF, TF, and HM fed infants were adjusted for gender, gestational age, birth weight, and (gain in) weight and length; * PDF versus TF, $P<0.05$; $^{+}$ PDF versus HM, $P<0.01$; ‡ PDF versus HM, $P<0.05$; $^{\#}$ TF versus HM, $P<0.05$.

DISCUSSION

This study demonstrates that PDF fed infants have the highest gain in BMC between term age and 6 months CA, independent of gain in weight and length. Several studies demonstrate a beneficial effect of various high-caloric postdischarge formulae on BMC of preterm infants during infancy [3-4]. Nonetheless, the isocaloric PDF used in the present study also improves the BMC of preterm infants during early infancy.

In the present study, gain in BMC was higher in PDF fed infants compared to HM fed infants. In contrast to previous studies that found lower bone mineralization in infants fed human milk compared to infants fed standard term formula [14-18], gain in BMC was not different between HM and TF fed infants in the present study. This may be explained by a different composition and a different volume intake of the TF used in the present study compared to the standard term formula used in previous studies [14-18]. Another explanation may be that, in the present study, HM fed infants were fed TF if HM was insufficiently available, which occurred especially after 3 months CA. This may result in a comparable gain in BMC of HM and TF fed infants.

BMC is the product of BA and bone density. Therefore, low BMC may reflect either a small skeleton (i.e. low BA) or low bone density [13]. In the present study, the higher gain in BMC of PDF fed infants compared to TF fed infants was explained by a difference in gain in BA. This may imply that PDF fed infants have a higher gain in BMC as a result of a difference in skeletal size. A difference in skeletal size was not the explanation for higher gain in BMC in PDF fed infants compared to HM fed infants, since the gain in BMC remained higher in PDF fed infants after adjustment for gain in BA. In other words, this may imply that PDF fed infants have a higher gain in BMC compared to HM fed infants due to a higher gain in bone density. Both findings support our hypothesis that PDF enhances bone mineralization of preterm infants during early infancy.

Gain in BMC during early infancy may be very important for future bone health. Low BMC in infancy may persist into adulthood and result in lower adult bone mass [19-21], leading to a higher risk of osteoporosis in later life [22]. Therefore, it can be expected that increased gain in BMC in preterm infants as a result of nutritional intervention during early infancy is important for adult peak bone mass.

In conclusion, an isocaloric, protein- and mineral-enriched PDF between term age and 6 months CA enhances gain in BMC of preterm infants. We speculate that higher gain in BMC as a result of isocaloric PDF may be beneficial to attain higher adult peak bone mass in preterm infants.

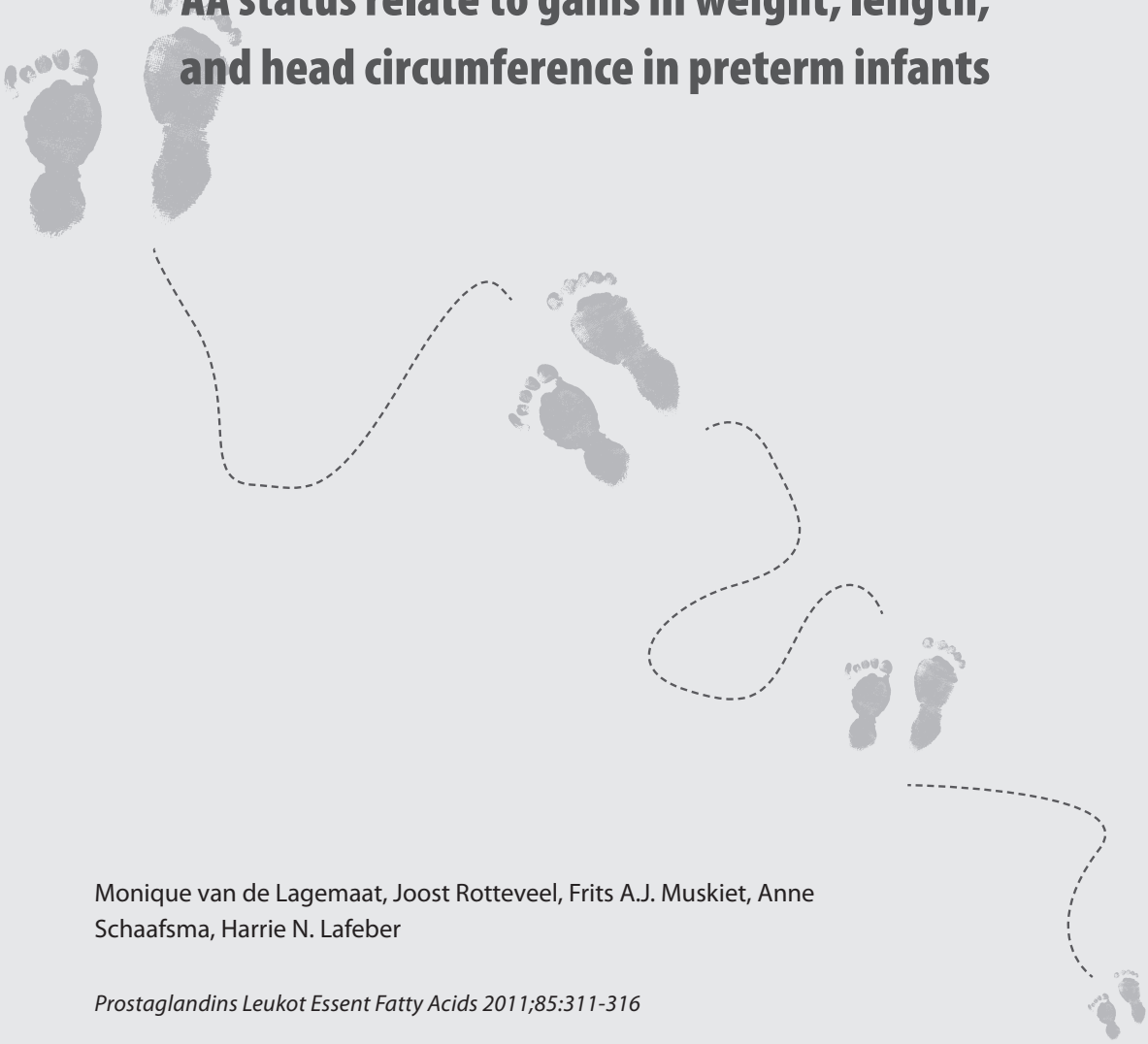
REFERENCES

1. Prentice A, Schoenmakers I, Laskey M, de Bono S, Ginty F, Goldberg G. Symposium on 'Nutrition and health in children and adolescents' Session 1: Nutrition in growth and development: Nutrition and bone growth and development. Proc Nutr Soc. 2006;65:348-360.
2. Lapillonne A, Salle B, Glorieux F, Claris O. Bone mineralization and growth are enhanced in preterm infants fed an isocaloric, nutrient-enriched preterm formula through term. Am J Clin Nutr. 2004;80:1595-1603.
3. Bishop N, King F, Lucas A. Increased bone mineral content of preterm infants fed with nutrient enriched formula after discharge from hospital. Arch Dis Child. 1993;68:573-578.
4. Picaud J, Decullier E, Plan O, Pidoux O, Bin-Dorel S, van Egroo L, Chapuis F, Claris O. Growth and bone mineralization in preterm infants fed preterm formula or standard term formula after discharge. J Pediatr. 2008;153:616-621.
5. Cooke R, McCormick K, Griffin I, Embleton N, Faulkner K. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res. 1999;46:461-465.
6. Chan G. Growth and bone mineral status of discharged very low birth weight infants fed different formulas or human milk. J Pediatr. 1993;123:439-443.
7. Pieltain C, De Curtis M, Gérard P, Rigo J. Weight gain composition in preterm infants with dual energy x-ray absorptiometry. Pediatr Res. 2001;49:120-124.
8. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
9. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
10. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
11. Koo W, Walters J, Bush A. Technical considerations of dual energy x-ray absorptiometry based bone mineral measurements for pediatric studies. J Bone Miner Res. 1995;10:1998-2004.
12. Prentice A, Parsons T, Cole T. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. Am J Clin Nutr. 1994;60:837-842.
13. Mølgaard C, Thomsen B, Prentice A. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child. 1997;76:9-15.
14. Abrams S, Schanler R, Garza C. Bone mineralization in former very low birth weight infants fed either human milk or commercial formula. J Pediatr. 1988;112:956-960.
15. Specker B, Beck A, Kalkwarf H, Ho M. Randomized trial of varying mineral intake on total body bone mineral accretion during the first year of life. Pediatrics. 1997;99:E12.
16. Schanler R, Burns P, Abrams S, Garza C. Bone mineralization outcomes in human milk fed preterm infants. Pediatr Res. 1992;31:583-586.
17. Wauben I, Atkinson S, Grad T, Shah J, Paes B. Moderate nutrient supplementation of mother's milk for preterm infants supports adequate bone mass and short-term growth: a randomized, controlled trial. Am J Clin Nutr. 1998;67:465-472.
18. Kurl S, Heinonen K, Länsimies E. Pre- and post-discharge feeding of very preterm infants: impact on growth and bone mineralization. Clin Physiol Funct Imaging. 2003;23:182-189.

19. Hovi P, Andersson S, Järvenpää A, Eriksson J, Strang-Karlsson S, Kajantie E, Mäkitie O. Decreased bone mineral density in adults born with very low birth weight: a cohort study. PLoS Med. 2009;6:e.1000135.
20. Fewtrell M, Williams J, Singhal A, Murgatroyd P, Fuller N. Early diet and peak bone mass: 20 year follow up of a randomized trial of early diet in infants born preterm. Bone. 2009;45:142-149.
21. Smith C, Wright N, Wales J, Mackenzie C, Primhak R, Eastell R, Walsh J. Very low birth weight survivors have reduced peak bone mass and reduced insulin sensitivity. Clin Endocrinol. 2011;75:443-449.
22. Hernandez C, Beaupré G, Carter D. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int. 2003;14:843-847.

Chapter 8

Post-term dietary-induced changes in DHA and AA status relate to gains in weight, length, and head circumference in preterm infants



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ABSTRACT

Preterms need supplementation with docosahexaenoic (DHA) and arachidonic (AA) acids to prevent steep postnatal declines. Associations between growth and erythrocyte (RBC) DHA and AA were studied in 139 preterms (51% male, gestational age 30.3 ± 1.5 weeks, birth weight 1341 ± 288 g) fed human milk with breast milk fortifier or preterm formula until term age followed by postdischarge formula (PDF; n=52, 0.4% DHA, 0.4% AA), term formula (TF; n=41, 0.2% DHA, 0.2% AA), or human milk (HM; n=46). At six months corrected age, PDF resulted in higher RBC-DHA than TF and HM, while RBC-AA was higher than TF but similar to HM. There were no between-group differences in growth between term age and six months corrected age. RBC-DHA related positively with gain in weight and length and negatively with gain in head circumference. RBC-AA related positively with gain in head circumference and negatively with gain in weight and length. In conclusion, PDF with higher DHA and AA than TF may promote postnatal growth of preterms.

INTRODUCTION

Long-chain polyunsaturated fatty acids (LC-PUFAs), notably docosahexaenoic (DHA) and arachidonic (AA) acids, are important for central nervous system development [1-3], growth, and body composition [4]. Especially in very low birth weight infants, biosynthesis of LC-PUFA from alpha-linolenic and linoleic acid is insufficient to prevent postnatal decline of DHA and AA, which might adversely affect growth and development [5-7]. Meta-analyses of randomized controlled trials have, however, not shown better growth by LC-PUFA supplementation, neither in term nor in preterm infants [8-10].

Preterm infants have lower LC-PUFA stores at birth compared to term infants [5, 11-13], since they lack part of the rapid LC-PUFA accretion of the last trimester of pregnancy [3, 14-15]. Insufficient dietary supply of LC-PUFA between birth and term age causes a steep decrease of LC-PUFA status in preterm infants. This LC-PUFA gap is not observed in term infants [5, 16]. The colostrum of preterm delivering mothers contains higher DHA and AA than milk from mothers delivering at term [17-18]. This apparent advantage vanishes rapidly with advancing lactation and is unable to fill the LC-PUFA gap [19]. For DHA this may in part be caused by the low DHA status of mothers living in Western countries [20], which adversely affects both the DHA status of preterm infants at birth and the ability to fill the imminent DHA gap after birth by feeding the mother's own milk.

Guidelines for LC-PUFA content of infant formula are based on the LC-PUFA content of milk from Western mothers [21-22]. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends 0.25-0.45 g% DHA and 0.38-0.64 g% AA for in-hospital preterm formula [23-25], but there are no specific recommendations for the LC-PUFA content of formula for preterm infants after discharge.

We aimed to prevent the imminent low LC-PUFA status of preterm infants by feeding a DHA and AA enriched postdischarge formula between term age and six months corrected age. Furthermore, we aimed to study the relation between red blood cell (RBC) DHA, AA, and eicosapentaenoic acid (EPA) concentrations and postnatal growth.

PATIENTS AND METHODS

Study design

The present study and its participants have been described previously [26]. In short, infants born at gestational ages of 32 weeks or less and/or with birth weights of 1,500 g or less were included in a randomized controlled trial, which evaluated the effect of postdischarge formula (PDF), term formula (TF), and human milk (HM) between term age and six months corrected age on growth and body composition. Until term age, infants received human milk with standard breast milk fortifier or preterm formula. The

estimated LC-PUFA content of the preterm formula was 0.4-0.5 g% for DHA and 0.5-0.6 g% for AA (Friso Prematuur, FrieslandCampina, Leeuwarden, The Netherlands; Nenatal start, Nutricia, Zoetermeer, The Netherlands). Infants were considered HM-fed if, at term age, they received predominantly human milk. If HM was insufficient, additional term formula was provided until six months corrected age. PDF contained the same quantity of energy, lower levels of carbohydrates, and higher levels of protein, DHA, AA, and some minerals and vitamins compared to TF (Table 8.1). Both TF and PDF were supplied by FrieslandCampina, Leeuwarden, The Netherlands. Of the 152 infants included in this study, 139 completed the study at six months corrected age. Informed consent from the parents and ethics committee approval were obtained.

Table 8.1. Composition of study formulae[a]

Formula content	Preterm formula (PTF)[b]	Postdischarge formula (PDF)	Term formula (TF)
Energy (kcal)	80	67	67
Protein (g)	2.2-2.5	1.7	1.4
Protein/energy ratio (g/100 kcal)	2.75-3.1	2.6	2.2
Carbohydrates (g)	7.6-8.2	7.0	7.2
Fat (g)	4.3-4.4	3.5	3.5
Linoleic acid (mg)	490-560	415	422
α-Linolenic acid (mg)	69-80	59	63
Docosahexaenoic acid (mg)	18-20 (0.4-0.5%)[c]	14 (0.4%)[c]	7 (0.2%)[c]
Arachidonic acid (mg)	20-26 (0.5-0.6%)[c]	14 (0.4%)[c]	7 (0.2%)[c]
Eicosapentaenoic acid (mg)	0-3.9 (0-0.09%)[c]	3.9 (0.09%)[c]	-
Calcium (mg)	100-120	65	50
Phosphorus (mg)	55-66	38	30
Vitamin D (μg)	2.4-3.0	1.4	1.2
Iron (mg)	0.78-1.4	1.00	0.78

[a] Data are for 100 ml prepared formula; [b] For PTF the ranges of composition is shown for Friso Prematuur (FrieslandCampina, Leeuwarden, The Netherlands) and Nenatal start (Nutritica, Zoetermeer, The Netherlands); [c] Percentage of total fat.

Methods

Weight, length, and head circumference were measured at birth and term age and at three and six months corrected age. At birth and term age, weight, length, and head circumference were expressed as standard deviation score (SDS) based on Swedish references for preterm infants [27]. At three and six months corrected age, weight, length, and head circumference were expressed as SDS based on Dutch references [28].

At term age and at three and six months corrected age, EDTA-anticoagulated venous blood samples for RBC-fatty acid analysis were collected at least 2.5 h after feeding. Blood samples were immediately cooled in melting ice. Within 2 h after collection,

plasma and buffy coat were removed. The RBC were washed three times with isotonic saline. The RBC were finally suspended to a hematocrit of about 50%. Two hundred µL of this suspension was stored at -20°C in a teflon-sealable tube containing 2 mL methanol-6 M HCl (5:1 v/v), 1 mg butylated hydroxytoluene (antioxidant) and internal standard (50 µg 17:0 in 100 µL methanol). Samples were transported in dry ice to the University Medical Center Groningen (The Netherlands) for RBC-fatty acid composition analysis. The ready-to-transmethylate mixture was heated to 90°C for 4 h. Fatty acid methyl esters were extracted into hexane and profiled by capillary gas chromatography and flame ionization detection, as described previously [29]. RBC-fatty acid composition was calculated from the peak areas and expressed as g% (fatty acid composition) or g/g (fatty acid ratios). The within-run and day-to-day precision for the RBC-fatty acid analysis has been described before [29]. Of the 139 infants that completed the study at six months corrected age, RBC-fatty acid analyses were available from 115 infants at term age, 128 infants at three months, and 114 infants at six months corrected age.

Between term age and six months corrected age, parents recorded their child's intake of the preceding week in a diary once a week. These diaries provided information about type and amount of enteral intake and any supplements and were used to calculate total milk volumes (ml/kg/day). Human milk fed infants were usually breastfed and therefore the exact total milk intake was unknown. In formula fed infants, protein intake (g/kg/day) and DHA, EPA, and AA intakes (mg/kg/day) between term age and six months corrected age were calculated based on total milk volumes and composition of the study formulae as described in Table 8.1. Nutrient intake, especially LC-PUFA intake, of the mother during pregnancy and lactation was unknown.

Statistics

All statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). RBC-fatty acid data were expressed as mean ± standard deviation (SD). After natural logarithmic transformation, RBC-EPA levels at term age and three months corrected age were normally distributed. All other parameters were normally distributed.

Regression analyses were used to evaluate between-group differences in gestational age at birth and between-group differences in weight, length, head circumference and RBC-fatty acids at term age and at three and six months corrected age. Between-group differences in RBC-fatty acids at three and six months corrected age were corrected for RBC-fatty acids at term age. Generalized estimating equations were used to evaluate within-group longitudinal changes in growth and RBC-fatty acids and the relation between growth and change in RBC-fatty acids between term age and six months corrected age. P values of less than 0.05 were considered significant.

RESULTS

Anthropometry and dietary intake

There were no between-group differences in gestational age or anthropometric characteristics (Table 8.2). Between term age and six months corrected age, total milk volume intakes (ml/kg/day) were not significantly different between PDF and TF-fed infants. Consequently, PDF-fed infants had significantly higher DHA, EPA, and AA intakes (mg/kg/day) compared to TF-fed infants (data not shown).

Between-group differences in RBC-fatty acids

Gestational age and gender did not influence RBC-DHA, EPA, and AA at term age and at three and six months corrected age (data not shown). At term age, HM-fed infants had lower RBC-DHA and DHA/AA ratio and similar RBC-AA and EPA compared to formula-fed infants (Table 8.3).

At three months corrected age, PDF-fed infants had higher RBC-DHA, EPA, and DHA/AA ratio compared to TF and HM-fed infants. Furthermore, PDF-fed infants had higher RBC-AA compared to TF-fed infants and lower AA compared to HM-fed infants. HM-fed infants had higher RBC-AA compared to TF-fed infants (Table 8.3). After correction for RBC-AA and EPA at term age, the differences in RBC-AA and EPA at three months corrected age between PDF and HM-fed infants were no longer significant.

At six months corrected age, PDF-fed infants had higher RBC-DHA, AA, EPA, and DHA/AA ratio compared to TF-fed infants and higher RBC-DHA, EPA, and DHA/AA ratio compared to HM-fed infants. At six months corrected age, RBC-AA was similar in PDF and

Table 8.2. Characteristics of the study population

Characteristics	PDF (n=52)	TF (n=41)	HM (n=46)
Boys[a]	27/52 = 51.9%	23/41 = 56.1%	21/46 = 45.7%
First born child[a]	33/52 = 63.5%	25/41 = 61.0%	36/46 = 78.3%
At birth[b]			
Gestational age (week)	30.5 ± 1.4	30.5 ± 1.4	30.0 ± 1.6
Weight (g)	1344 ± 304	1377 ± 210	1304 ± 330
Length (cm)	38.2 ± 3.0	38.2 ± 2.6	37.7 ± 3.1
Head circumference (cm)	27.8 ± 1.9	28.0 ± 1.7	27.4 ± 2.0
At term age[b]			
Postmenstrual age (week)	40.7 ± 0.7	40.4 ± 0.6	40.6 ± 0.9
Weight (g)	3137 ± 511	3193 ± 489	3138 ± 513
Length (cm)	48.7 ± 2.3	48.7 ± 2.1	48.2 ± 2.5
Head circumference (cm)	35.9 ± 1.2	35.8 ± 1.5	35.6 ± 1.5

PDF: postdischarge formula; TF: term formula; HM: human milk. [a] Frequency (%); [b] mean ± SD.

HM-fed infants (Table 8.3). These differences remained significant after correction for RBC-DHA, AA, and EPA at term age.

Within-group longitudinal changes in RBC-fatty acids

In PDF and HM-fed infants, RBC-DHA increased between term age and three months as well as between three and six months corrected age. In TF-fed infants, RBC-DHA increased only between three and six months corrected age. In PDF, TF, and HM-fed infants, RBC-AA decreased between term age and three months corrected age. HM-fed infants showed a further decrease of RBC-AA between three and six months corrected age. In PDF, TF, and HM-fed infants RBC-DHA/AA ratio increased between term age and three months as well as between three and six months corrected age (Figure 8.1).

Between-group differences in growth

At term age and at three and six months corrected age, there were no significant differences in weight, length, and head circumference SDS between PDF, TF, and HM-fed infants (Figure 8.2).

Within-group longitudinal changes in growth

In PDF, TF, and HM-fed infants, the main changes in weight, length and head circumference SDS occurred between term age and three months corrected age. In PDF, TF, and HM fed infants, weight and length SDS increased and head circumference SDS decreased between term age and three months corrected age (Figure 8.2).

Table 8.3. RBC-AA, DHA, and EPA (g%) and the DHA/AA ratio (g/g) at term age, three months and six months corrected age

Fatty acid (ratio)	Age	PDF	TF	HM	PDF vs TF[a]	PDF vs HM[a]	TF vs HM[a]
AA	Term age	13.74 ± 0.89	13.86 ± 0.93	14.06 ± 1.17	n.s.	n.s.	n.s.
	3 months	12.99 ± 1.20	12.53 ± 0.64	13.57 ± 1.06	0.039	0.008	<0.001
	6 months	13.12 ± 0.98	12.50 ± 0.63	13.15 ± 0.90	0.002	n.s.	0.001
DHA	Term age	4.71 ± 0.70	4.59 ± 0.76	4.08 ± 0.55	n.s.	<0.001	0.003
	3 months	5.73 ± 0.73	4.70 ± 0.49	4.43 ± 0.78	<0.001	<0.001	n.s.
	6 months	6.01 ± 0.67	4.96 ± 0.54	4.74 ± 0.67	<0.001	<0.001	n.s.
EPA	Term age	0.34 ± 0.05	0.32 ± 0.06	0.33 ± 0.13	n.s.	n.s.	n.s.
	3 months	0.37 ± 0.05	0.30 ± 0.04	0.32 ± 0.11	<0.001	<0.001	n.s.
	6 months	0.38 ± 0.04	0.30 ± 0.05	0.30 ± 0.07	<0.001	<0.001	n.s.
DHA/AA ratio	Term age	0.34 ± 0.05	0.33 ± 0.06	0.29 ± 0.04	n.s.	<0.001	<0.001
	3 months	0.44 ± 0.05	0.38 ± 0.04	0.33 ± 0.06	<0.001	<0.001	<0.001
	6 months	0.46 ± 0.05	0.40 ± 0.04	0.36 ± 0.06	<0.001	<0.001	0.005

Values as mean ± SD. n.s. : not significant; PDF: postdischarge formula; TF: term formula; HM: human milk; AA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid. [a] P-value, significance level P<0.05.

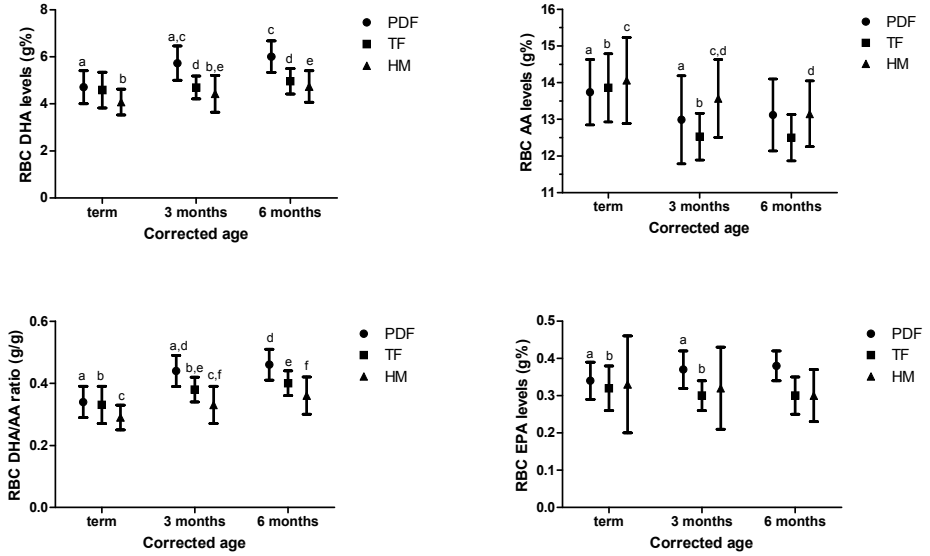

Figure 8.1. RBC-DHA, AA, and EPA (g%) and the DHA/AA ratio (g/g) at term age, three and six months corrected age for PDF, TF, and HM-fed preterm infants

RBC: red blood cell; DHA: docosahexaenoic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; PDF: postdischarge formula; TF: term formula; HM: human milk. Similar letters indicate a significant change in time, P<0.05.

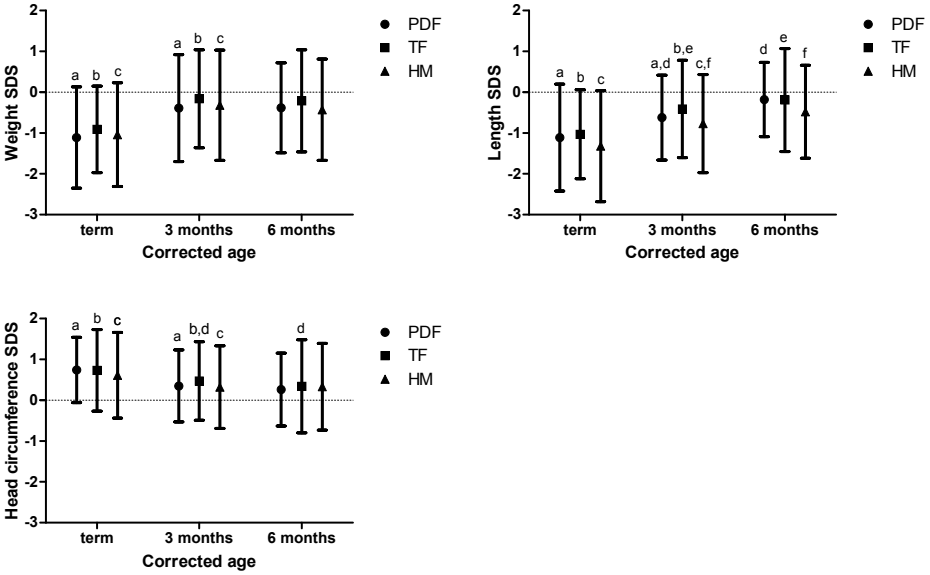

Figure 8.2. Weight, length, and head circumference SDS at term age, three and six months corrected age for PDF, TF, and HM-fed preterm infants

PDF: postdischarge formula; TF: term formula; HM: human milk. Similar letters indicate a significant change in time, P<0.05.

Relation between RBC-fatty acid changes and growth

For all infants, the changes in RBC-DHA were positively associated with gain in weight and length and negatively with gain in head circumference between term age and six months corrected age. Similarly, for all infants, the changes in RBC-AA were positively associated with gain in head circumference and negatively with gain in weight and length. Changes in the RBC-DHA/AA ratios were positively associated with gain in weight and length and negatively with gain in head circumference between term age and six months corrected age. Changes in RBC-EPA showed no associations with gain in weight, length, or head circumference between term age and six months corrected age (Table 8.4). All of the above significant associations remained significant after adjustment for protein intake in formula fed infants.

Table 8.4. Associations between the changes in RBC-DHA, AA, and EPA (g%) and the DHA/AA ratio (g/g) and postnatal growth between term age and six months corrected age

	Weight SDS 0-6 months		Length SDS 0-6 months		Head circumference SDS 0-6 months	
	β1	β2	β1	β2	β1	β2
DHA (g%) 0-6 months	0.27**	0.16*	0.42**	0.29**	-0.15**	-0.13*
AA (g%) 0-6 months	-0.28**	-0.17**	-0.23**	-0.11*	0.15**	0.15**
EPA (g%) 0-6 months	0.07	0.33	0.33	0.71	-0.83	-1.04
DHA/AA (g/g) ratio 0-6 months	4.53**	2.87**	5.91**	4.07**	-2.53**	-2.41**

DHA: docosahexaenoic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid. β1: unadjusted; β2: adjusted for protein intake; ** P< 0.001; * P< 0.05.

DISCUSSION

AA is an important regulator of fetal growth, but during the third trimester of pregnancy, DHA becomes increasingly more important [2, 6]. Preterm infants lack fetal DHA and AA accretion during the last trimester [3, 14-15], resulting in lower DHA and AA at birth and a lower nadir in DHA and AA compared to infants born at term. For that reason, adequate dietary LC-PUFA intake might be essential for preterm infants to prevent low postnatal LC-PUFA status and concomitant negative effects on growth and central nervous system development.

This randomized controlled trial with three post-term feeding regimens differing in DHA and AA intakes failed to show the influence of these LC-PUFAs on the growth of preterm infants. However, in contrast to several previous studies, we found associations between the LC-PUFAs in RBC and postnatal growth until six months post-term. Until now, AA and DHA supplementation of preterm formulae has been associated with increased growth up to two months post-term, but these associations disappear

thereafter [9, 30]. In our analyses, we have taken into account that protein intake is an important determinant of growth, but the current associations remained significant after adjustment for protein intake between term age and six months post-term.

Supplementation studies with DHA and EPA, without AA, have been shown to cause a decline of RBC-AA in preterm infants during the first half of infancy, especially between birth and term age. This might be caused by immature AA synthesis capacity postnatally or by the competition of dietary EPA with AA for incorporation into phospholipids [7]. Decreased RBC-AA has been associated with decreased gain in weight, length, and head circumference during the first year in preterm infants [31]. This might suggest that EPA supplementation has a negative effect on postnatal growth. However, in this study, we found no associations between changes in RBC-EPA and gain in weight, length, and head circumference between term age and six months post-term.

A postnatal AA decline might theoretically be prevented in several ways. The linoleic acid intake could be decreased to diminish the competition of the high linoleic acid content of milk with the available AA for incorporation into phospholipids [32]. Second, DHA intake could be decreased to prevent competition with AA, but, in preterm infants, the DHA status is already endangered. Finally, the AA intake could be increased, which seems the most practical. To achieve an AA status similar to term breast fed infants, preterm infants need to reach a RBC-AA of 15 g% at three months post-term [33]. In the present study, PDF-fed infants had an RBC-AA closer to this possible target than TF-fed infants (about 13 g%, Table 8.3). To meanwhile guarantee sufficient DHA status, preterm infants may also need to be supplemented with additional DHA to prevent unfavorable competition of a higher AA intake with the available DHA [34]. What target should be aimed at is currently unknown, but an adequate supply of DHA to e.g. brain becomes increasingly more important from the end of pregnancy and especially after delivery because of the rapid DHA accumulation [2, 6]. In contrast to AA, DHA status is highly dependent on DHA intake, notably from fish. Two reference points for postnatal infant RBC-DHA status have recently emerged from the study of mothers with lifetime stable dietary habits. Mothers with an RBC-DHA status of 6 g% at the end of pregnancy deliver infants with an RBC-DHA of 6 g% at term birth and the infant RBC-DHA will remain at 6 g% after three months exclusive breastfeeding. Second, infants of lactating mothers with an RBC-DHA of 8 g% at the end of pregnancy will increase their RBC-DHA from 7 g% at term birth to 8 g% after three months breastfeeding [33].

In our study, PDF-fed infants reached this 6 g% RBC-DHA reference point for term infant postnatal equilibrium [33] from about three months post-term, whereas TF and even HM-fed infants did not. The 8 g% RBC-DHA reference point, at which the lactating mother is in equilibrium during lactation [33], can only be reached if the PDF contains higher DHA or if additional DHA will be provided via the preterm formula. We conclude that the current feeding regimen almost reached term infant RBC-AA targets and also

reached the reference point of term infant RBC-DHA equilibrium. It is possible that feeding regimens with higher AA and DHA concentrations in notably preterm formula will cause an AA status closer to the term infant RBC-AA target of 15 g% and a higher DHA. We speculate that the resulting higher AA and DHA status may jointly improve growth of a lean body mass that is in need of LC-PUFA.

Early aggressive feeding protocols, notably those aiming at increased caloric intake, may lead to increased growth of very low birth weight infants that is nevertheless associated with increased risk of disease later in life, such as diabetes [35-36], cardiovascular disease [37-38], and obesity [39-40]. Without increased growth during infancy, preterm infants might have lower risk of the metabolic syndrome and associated cardiovascular disease in later life but may exhibit inappropriate brain growth and adverse neurodevelopmental outcome [41-42]. This study demonstrates that an increased AA intake with a balanced DHA/AA ratio was associated with enhanced gain in head circumference. Head growth and concomitant gain in brain volume is crucial for central nervous system development. We reported previously that the infants receiving the current feeding regimen with PDF had lower fat mass corrected for body size at six months corrected age compared to infants fed TF or HM [26]. Whether the current feeding regimen with PDF also promotes the growth of lean body mass in later life and causes a higher lean body mass/fat mass ratio with concomitant lower chance of the metabolic syndrome is currently unknown and should be the subject of further investigation.

The main limitation in the interpretation of the current results is in its emphasis on associations between RBC LC-PUFA and growth, which are not necessarily causal. No differences emerged from the randomized design. It must, however, be emphasized that most of the randomized studies with LC-PUFA are negative, while associations have consistently been found [43]. Several suggestions explaining this discrepancy have been made [43]. Second, the number of subjects was small. Third, HM-fed infants were fed (almost) exclusively with HM at randomization, but the number of exclusively HM-fed infants declined thereafter, in particular after three months. When HM was insufficient, TF was given. Therefore, comparisons between HM- and TF-fed infants should be interpreted with caution. Fourth, RBC-fatty acids were not measured at birth or at any time between birth and term age. Therefore, we cannot correct the RBC-fatty acid contents at term age for values at birth. Preterm infants born at lower gestational age are expected to have lower LC-PUFA stores, but we found no influence of gestational age at birth on RBC-fatty acids at term age. We have no explanation for this finding. Apparently, the dietary postnatal environment has a larger influence on LC-PUFA status at term age than gestational age and LC-PUFA status at birth.

In conclusion, at six months post-term, PDF-fed infants had RBC-AA levels that were higher than TF-fed infants but similar to HM-fed infants. At six months post-term, the RBC-DHA contents of PDF-fed infants exceeded those of TF and HM-fed infants to reach

the 6 g% reference point of RBC-DHA equilibrium for term HM-fed infants. There were no between-group differences in growth. Higher RBC-DHA was associated with increased gain in weight and length, and higher RBC-AA was associated with increased gain in head circumference between term age and six months post-term. Our data suggest that feeding preterm infants with a postdischarge formula with 0.4% DHA and 0.4% AA is superior for growth compared with a term formula with 0.2 % DHA and 0.2% AA.

REFERENCES

1. Lauritzen L, Hansen H, Jorgensen M, Michaelsen K. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res. 2001;40:1-94.
2. Martinez M, Mougan I. Fatty acid composition of human brain phospholipids during normal development. J Neurochem. 1998;71:2528-2533.
3. Fewtrell M, Morley R, Abbott R, Singhal A, Isaacs E, Stephenson T, MacFadyen U, Lucas A. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. Pediatrics. 2002;110:73-82.
4. Uauy R, Rojas C, Llanos A, Mena P. Dietary essential fatty acids in early postnatal life: long-term outcomes. Nestlé Nutr Workshop Ser Pediatr Program. 2005;55:101-133.
5. Uauy R, Mena P. Lipids and neurodevelopment. Nutr Rev. 2001;59:S34-S48.
6. Clandinin M, Chappell J, Leong S, Heim T, Swyer P, Chance G. Intrauterine fatty acid accretion in human brain: implications for fatty acid requirements. Early Hum Dev. 1980;4:121-129.
7. Carlson S, Cooke R, Rhodes P, Peeples J, Werkman S, Tolley E. Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: phospholipid fatty acids. Pediatr Res. 1991;30:404-412.
8. Makrides M, Gibson R, Udell T, Ried K, International LCPUFA Investigators. Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. Am J Clin Nutr. 2005;81:1094-1101.
9. Schulzke S, Patole S, Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev. 2011;Feb 16(2):CD000375.
10. Simmer K, Patole S, Rao S. Longchain polyunsaturated fatty acid supplementation in infants born at term. Cochrane Database of Syst Rev. 2011;Dec 7(12):CD000376.
11. Makrides M, Neumann M, Byard R. Fatty acid composition of the brain, retina and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr. 1994;60:180-194.
12. Farquharson J, Jamieson E, Abbasi K, Patrick W, Logan R, Cockburn F. Effect of diet on the fatty acid composition of the major phospholipids of infant cerebral cortex. Arch Dis Child. 1995;72:198-203.
13. Carlson S, Rhodes P, Rao V, Goldgar D. Effect of fish oil supplementation on the n-3 fatty acid content of red blood cell membranes in preterm infants. Pediatr Res. 1987;21:507-510.
14. Lapillonne A, Picaud J, Chirouze V, Goudable J, Reygrobellet B, Claris O, Salle B. The use of low-EPA fish oil for long-chain polyunsaturated fatty acid supplementation of preterm infants. Pediatr Res. 2000;48:835-841.
15. Klein C. Nutrient requirements for preterm infants formulas. J Nutr. 2002;123:1395S-1577S.
16. Innis S. Essential fatty acid transfer and fetal development. Placenta. 2005;26:S70-S75.
17. Brenna J, Varamini B, Jensen R, Diersen-Schade D, Boettcher J, Arterburn L. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007;85:1457-1464.
18. Bokor S, Koletzko B, Decsi T. Systematic review of fatty acid composition of human milk from mothers of preterm compared to full-term infants. Ann Nutr Metab. 2007;51:550-556.
19. van Goor S, Dijck-Brouwer D, Hadders-Algra M, Doornbos B, Erwich J, Schaafsma A, Muskiet F. Human milk arachidonic acid and docosahexaenoic acid contents increase following supplementation during pregnancy and lactation. Prostaglandins Leukot Essent Fatty Acids. 2009;80:65-69.

20. Muskiet F, van Goor S, Kuipers R, Velzing-Aarts F, Smit E, Bouwstra H, Dijck-Brouwer D, Boersma E, Hadders-Algra M. Long-chain polyunsaturated fatty acids in maternal and infant nutrition. Prostaglandins Leukot Essent Fatty Acids. 2006;75:135-144.
21. Koletzko B, Lien E, Agostoni C, Böhles H, Campoy C, Cetin I, Decsi T, Dudenhausen J, Dupont C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinatal Med. 2008;36:5-14.
22. van Goor S, Smit E, Schaafsma A, Dijck-Brouwer D, Muskiet F. Milk of women with lifetime consumption of the recommended daily intake of fish fatty acids should constitute the basis for the DHA contents of infant formula. J Perinatal Med. 2008;36:548-549.
23. Koletzko B, Agostoni C, Carlson S, Clandinin T, Hornstra G, Neuringer M, Uauy R, Yamashiro Y, Willats P. Long chain polyunsaturated fatty acids (LC-PUFA) and perinatal development. Acta Paediatr. 2001;90:460-464.
24. Koletzko B, Baker S, Cleghorn G, Neto U, Gopalan S, Hernell O, Hock Q, Jirapinyo P, Lönnderdal B, et al. Global standard for the composition of infant formula: recommendations of an ESPGHAN coordinated international expert group. J Pediatr Gastroenterol Nutr. 2005;41:584-599.
25. Agostoni C, Buonocore G, Carnielli V, De Curtis M, Darmaun D, Decsi T, Dömellof M, Embleton N, Fusch C, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50:85-91.
26. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
27. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
28. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
29. Muskiet F, van Doormaal J, Martini I, Wolthers B, van der Slik W. Capillary gas chromatographic profiling of total long-chain fatty acids and cholesterol in biological materials. J Chromatogr. 1983;278:231-244.
30. Woltil H, van Beusekom C, Schaafsma A, Muskiet F, Okken A. Long-chain polyunsaturated fatty acid status and early growth of low birth weight infants. Eur J Pediatr. 1998;157:146-152.
31. Carlson S, Werkman S, Peeples J, Cooke R, Tolley E. Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci USA. 1993;90:1073-1077.
32. Nelson G, Schmidt P, Bartolini G, Kelley D, Kyle D. The effect of dietary arachidonic acid on plasma lipoprotein distributions, apoproteins, blood lipid levels, and tissue fatty acid composition in humans. Lipids. 1997;32:427-433.
33. Kuipers R, Luxwolda M, Sango W, Kwesigabo G, Dijck-Brouwer D, Muskiet F. Maternal DHA equilibrium during pregnancy and lactation is reached at an erythrocyte DHA content of 8 g/100 g fatty acids. J Nutr. 2011;141:418-427.
34. Kuipers R, Luxwolda M, Dijck-Brouwer D, Muskiet F. Intrauterine, postpartum and adult relationships between arachidonc acid (AA) and docosahexaenoic acid (DHA). Prostaglandins Leukot Essent Fatty Acids. 2011;85:245-252.
35. Crowther N, Cameron N, Trusler J, Gray I. Association between poor glucose tolerance and rapid postnatal weight gain in seven-year-old children. Diabetologia. 1998;41:1163-1167.

36. Iñiguez G, Ong K, Bazaes R, Avila A, Salazar T, Dunger D, Mericq V. Longitudinal changes in insulin-like growth factor-1, insulin sensitivity and secretion from birth to age three years in small-for-gestational-age children. J Clin Endocrinol Metab. 2006;91:4645-4649.
37. Eriksson J. Commentary: Early 'catch-up' growth is good for later health. Int J Epidemiol. 2001;30:1330-1331.
38. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet. 2004;363:1642-1645.
39. Singhal A, Kennedy K, Lanigan J, Fewtrell M, Cole T, Stephenson T, Elias-Jones A, Weaver L, Ibhanesebhor S, et al. Nutrition in infancy and long-term risk of obesity: evidence from 2 randomized controlled trials. Am J Clin Nutr. 2010;92:1133-1144.
40. Monteiro P, Victora C. Rapid growth in infancy and childhood and obesity in later life-a systematic review. Obes Rev. 2005;6:143-154.
41. Latal-Hajnal B, von Siebenthal K, Kovari H, Bucher H, Largo R. Postnatal growth in very low birth weight infants: significant association with neurodevelopmental outcome. J Pediatr. 2003;143:163-170.
42. Brandt I, Sticker E, Lentze M. Catch up growth of head circumference of very low birth weight, small for gestational age preterm infants and mental development to adulthood. J Pediatr. 2003;142:463-468.
43. McCann J, Ames B. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am J Clin Nutr. 2005;85:281-295.

Chapter 9

Higher vitamin D intake in preterm infants fed an isocaloric, protein- and mineral-enriched postdischarge formula is associated with increased bone accretion

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ABSTRACT

During the first half of infancy, bone accretion of preterm infants fed an isocaloric, protein- and mineral-enriched postdischarge formula (PDF) is higher compared with those fed term formula (TF) or human milk (HM). This may be related to higher protein, calcium, phosphorus, and vitamin D intakes. This study investigated serum calcium, phosphate, and 25-hydroxyvitamin D [25(OH)D] in relation to bone mineral content (BMC) in PDF, TF, and HM fed preterm infants between term age (40 wk postmenstrual age) and 6 mo corrected age (CA). Between term age and 6 mo CA, 52 preterm infants were fed PDF (per 100 ml: 67 kcal, 1.7 g protein, 65 mg calcium, 38 mg phosphorus, 56 IU vitamin D), 41 were fed TF (per 100 ml: 67 kcal, 1.47 g protein, 50 mg calcium, 30 mg phosphorus, 48 IU vitamin D) and 46 were fed HM. Serum calcium, phosphorus, and 25(OH)D were measured at term age and at 3 and 6 mo CA. BMC (g) was measured by whole-body dual-energy x-ray absorptiometry at term age and 6 mo CA. Between term age and 6 mo CA, intakes of calcium, phosphorus, and vitamin D were significantly higher in PDF compared with TF fed infants, and PDF fed infants reached significantly higher serum 25(OH)D at 6 mo CA (103 ± 24.3 vs. 92.8 ± 15.5 nmol/L, P=0.003). Between term age and 6 mo CA, increases in serum 25(OH)D were associated with an increase in BMC (β=0.001, 95%CI 0.00-0.003, P=0.046). In conclusion, during the first 6 mo post-term, higher vitamin D intake and greater increase in serum 25(OH)D concentration in PDF fed preterm infants were associated with increased bone accretion.

INTRODUCTION

More than two thirds of the calcium, vitamin D, and bone accretion takes place during the third trimester of pregnancy [1-3]. Vitamin D accretion depends on the maternal vitamin D status [4]. Postnatally, in addition to the availability of dietary protein, calcium, and phosphate, the vitamin D status of the infant becomes very important for postnatal bone accretion [5]. The vitamin D status of the infant is related to maternal vitamin D status during pregnancy, dietary vitamin D intake, and 1,25-dihydroxyvitamin D synthesis [2, 4], which is already operative during intrauterine life and starts within 24 h after birth in infants born as early as 28 wk gestational age [6].

In term infants, vitamin D stores are depleted within the first 8 postnatal weeks [6] due to insufficient dietary vitamin D intake, especially in breast fed term infants. In preterm infants, vitamin D stores are lower at birth and, moreover, during early extrauterine life, dietary vitamin D supply is frequently lower than during the intrauterine third trimester [7]. As a consequence, vitamin D deficiency may develop, which results in secondary hyperparathyroidism with increased calcium resorption from the bone to maintain serum calcium concentrations [8].

In addition to bone-related effects, several studies demonstrate that a low maternal and concomitant low infant vitamin D status are associated with other conditions, such as type 1 diabetes in childhood [9], allergic diseases [10], and impaired neurocognitive development [11-12]. Furthermore, a low vitamin D status affects the immune system, such as the first line of defense in the intestine, which needs an adequate vitamin D status for the synthesis of the antimicrobial peptide cathelicidin [13]. This may contribute to increased (respiratory) infections in infants with a low vitamin D status during the first months of life [13-15].

Recently, we demonstrated that bone accretion during the first 6 mo post-term is higher in preterm infants fed an isocaloric, protein- and mineral-enriched postdischarge formula (PDF) compared with those fed a standard term formula (TF) or human milk (HM) [16]. PDF fed preterm infants may have higher availability of dietary protein, calcium, phosphorus, and vitamin D for bone accretion. However, controversy remains whether an improved vitamin D status is related to increased bone accretion of preterm infants. Some state that vitamin D status may only play a minor role in the presence of adequate calcium and phosphorus intake [17], whereas others demonstrate that vitamin D status is associated with bone accretion during infancy [18-19] and childhood [20]. The present study investigated serum calcium, phosphate, parathyroid hormone (PTH), and 25-hydroxyvitamin D [25(OH)D], a good marker of vitamin D status [4, 6, 8], in relation to bone accretion in preterm infants fed PDF, TF, and HM between term age (40 wk postmenstrual age) and 6 mo corrected age (CA).

PATIENTS AND METHODS

Details of this study have been described previously [16, 21]. In short, 139 infants born at a gestational age ≤32 wk and/or with a birth weight ≤1,500 g were included. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving patients were approved by the Ethics Committee of VU University Medical Center, Amsterdam, The Netherlands. Parents of the participating children gave written informed consent.

Between birth and term age, infants were fed fortified human milk (13 PDF, 17 TF, and 45 HM fed infants) or preterm formula (39 PDF and 23 TF fed infants) (Table 9.1). The type of feeding between birth and term age could not be determined in 2 infants due to missing data. Breast milk fortifier provided 64.9 mg calcium, 42.7 mg phosphorus, and 200 IU vitamin D/100 mL HM. According to the recommendations in The Netherlands between 2003 and 2006, preterm infants fed >50% HM were supplemented with breast milk fortifier or with 200 IU/d vitamin D when human milk fortification was discontinued at discharge. Preterm infants fed >50% formula, either preterm formula, PDF, or TF, were not supplemented with vitamin D. Of the infants fed human milk between birth and term age, 20 infants (2 PDF, 2 TF, and 16 HM fed infants) were supplemented with 200 IU/d vitamin D when HM fortification was discontinued after discharge. None of the infants fed preterm formula between birth and term age were supplemented with vitamin D.

At term age, formula-fed infants were randomized to PDF (n=52) or TF (n=41) and were fed this formula until 6 mo CA (Table 9.1). Infants were considered to be HM fed (n=46) if they received >80% HM at term age and these infants were supplemented with 200 IU/d vitamin D. If HM was insufficiently available, TF was added to the diet. Infants did not receive enteral calcium or phosphorus supplementation at any time. In contrast to the Dutch recommendations described above, 2 PDF and 2 TF fed infants

Table 9.1. Nutritional composition of study formulas

	Per 100 mL		
	Preterm formula[1]	Postdischarge formula (PDF)[2]	Term formula (TF)[3]
Energy (kcal)	80	67	67
Protein (g)	2.2-2.5	1.7	1.47
Protein/energy ratio (g/100 kcal)	2.75-3.1	2.6	2.2
Carbohydrates (g)	7.6-8.2	7.0	7.2
Fat (g)	4.3-4.4	3.5	3.5
Calcium (mg)	100-120	65	50
Phosphorus (mg)	55-66	38	30
Vitamin D (IU)	96-120	56	48

[1] Range of composition for Friso Prematuur (FrieslandCampina) and Nenatal Start (Nutricia); [2] Friso Prematuur 1 (FrieslandCampina); [3] Friso 1 Normaal (FrieslandCampina).

were supplemented with vitamin D by their parents for unknown reasons during the first week after term age [2 PDF fed infants received 62.4 IU/(kg•d) and 2 TF fed infants received 25.6 and 78.8 IU/(kg•d) vitamin D].

Between birth and discharge, data on parenteral and enteral intake (mL/d) and weight (g) were extracted from infants' medical records by a single person. Between discharge and 6 mo CA, parents recorded in a diary their infant's weight (g), formula intake (mL/d), vitamin D supplements, and any changes in their infant's diet (per day between discharge and term age and 1d/wk between term age and 6 mo CA). In formula (preterm formula, PDF, and TF) fed infants, intakes of calcium [mg/(kg•d)], phosphorus [mg/(kg•d)], and vitamin D [IU/(kg•d)], and vitamin D per day (IU/d) between birth and 6 mo CA were calculated for each week on the basis of the infant's weight, the volume intake (mL/d) of formula, and the nutritional composition of the formula. In HM fed infants, the vitamin D intake [IU/(kg•d)] and the vitamin D intake per day (IU/d) between birth and term age were calculated on the basis of the infant's weight, the volume intake (mL/d) of HM, the vitamin D content of breast milk fortifier, and the vitamin D intake from supplements. Between birth and term age, HM fed infants were fed expressed HM and, therefore, their volume intake was known. In contrast, between term age and 6 mo CA, HM-fed infants were breastfed and their exact intake was unknown. In addition, parents of HM fed infants did not record vitamin D supplementation consistently in the parental diaries, and some HM fed infants were additionally fed TF. As a consequence, the vitamin D intake of HM fed infants between term age and 6 mo CA could not be calculated.

Gestational age at birth was extracted from infants' medical records. At birth, term age, 3 and 6 mo CA, weight was measured to the nearest gram with a digital scale, length was measured to the nearest 0.1 cm with a length board, and head circumference was measured to the nearest 0.1 cm with a non-stretchable measuring tape, as described previously [21]. Weight, length, and head circumference were expressed as z-scores at birth and term age [22] and at 3 and 6 mo CA [23]. Ethnicity was based on paternal and maternal ethnicity and was classified as white if both parents were white and as nonwhite if one or both parents were of nonwhite race/ethnicity.

At term age, 3 and 6 mo CA, serum total calcium (mmol/L) and phosphate (mmol/L) were measured by colorimetric assay (Modular analytics, Roche diagnostics, Mannheim, Germany) with an inter-assay variance of 2.5 and 2.6%, respectively. At similar timepoints, PTH (pmol/L) was measured by luminescent immunometric assay (Nichols Institute Diagnostics, San Juan Capistrano, USA) with an inter-assay variance of 10% and 25(OH)D (nmol/L) was measured by a competitive binding protein assay (Diasorin, Stillwater, Minnesota, USA) with an inter-assay variance of 10%. Between term age and 6 mo CA, change in serum total calcium, phosphate, PTH, and 25(OH)D was calculated. Vitamin D deficiency was defined as serum 25(OH)D <30 nmol/L, vitamin D insufficiency as serum 25(OH)D <50 nmol/L, and high vitamin D concentrations as serum 25(OH)D

>150 nmol/L [3, 8, 24-25]. Because endogenous vitamin D synthesis in Amsterdam (latitude: 52.4° north) ceases between October and April [26], season of birth was defined as winter (November through April) or summer (May through October).

At term age and at 6 mo CA, bone area (cm^2) and bone mineral content (BMC; g) were measured by whole-body DXA (Hologic QDR4500A, Hologic, Inc.) and analyzed by Infant Whole Body Software version 12.3.3 (Hologic). The quality of the DXA was evaluated by 1 expert radiologist who was blinded for the type of feeding. Scans with severe movement artifacts and incomplete scans were excluded [27]. Good-quality DXA scans were available from 116 infants at term age (43 PDF, 36 TF, and 37 HM fed infants), 106 infants at 6 mo CA (42 PDF, 30 TF, and 34 HM fed infants), and 87 infants at both term age and 6 mo CA (36 PDF, 25 TF, and 26 HM fed infants), as described previously [16]. Gain in BMC between term age and 6 mo CA was calculated.

Statistical analyses were performed using SPSS 17.0 for Windows (SPSS Inc.). The distribution of sex, ethnicity, season of birth, vitamin D deficiency and insufficiency, and high vitamin D concentrations were expressed as frequencies and were compared between PDF, TF, and HM fed infants by χ^2 test. Gestational age at birth, vitamin D intake per day (IU/d), serum PTH, and BMC were normally distributed after ln transformation and were expressed as medians (IQR). Weight, length, and head circumference z-scores; intakes of calcium, phosphorus, and vitamin D; and concentrations of serum calcium, phosphate, and 25(OH)D were normally distributed and expressed as means ± SDs.

Gestational age at birth, growth, serum calcium, serum phosphate, serum PTH, and serum 25(OH)D were compared between PDF, TF, and HM fed infants by linear regression adjusted for sex and gestational age (analysis with gestational age was only adjusted for sex). Analyses with serum 25(OH)D were adjusted for season of birth and ethnicity. Bone area and BMC were compared between PDF, TF, and HM fed infants by linear regression adjusted for sex, gestational age, birth weight, and actual weight and length. Intakes of calcium [mg/(kg•d)], phosphorus [mg/(kg•d)], and vitamin D [IU/(kg•d)], and vitamin D per day (IU/d) between term age and 6 mo CA were compared between PDF and TF fed infants by linear regression adjusted for sex and gestational age. Associations between BMC and serum calcium, serum PTH, and serum 25(OH)D as well as between gain in BMC and change in serum PTH and change in serum 25(OH)D were evaluated by linear regression; we also evaluated whether type of feeding (PDF, TF, or HM), season of birth, ethnicity, or sex modified these associations. A P value <0.05 was considered significant.

RESULTS

PDF, TF, and HM fed infants

Infant characteristics at birth and growth variables between birth and 6 mo CA were not different among PDF, TF, and HM fed infants [Table 9.2 and Supplemental Table 9.1 (Appendix B)]. Between birth and term age, the mean intakes of calcium, phosphorus, and vitamin D were similar in PDF and TF fed infants who were fed preterm formula [calcium: 110 ± 24.9 vs. 108 ± 24.7 mg/(kg•d); phosphorus: 57.7 ± 11.5 vs. 56.9 ± 12.1 mg/(kg•d); vitamin D: 222 ± 42.4 vs. 221 ± 47.2 IU/(kg•d); all P≥0.05]. Between birth and term age, the mean vitamin D intake was similar in PDF, TF, and HM fed infants fed fortified HM [233 ± 43.7 vs. 247 ± 37.8 vs. 228 ± 50.7 IU/(kg•d), respectively; P≥0.05]. Between term age and 6 mo CA, the mean intakes of calcium, phosphorus, and vitamin D were significantly higher in PDF compared with TF fed infants [calcium: 100 ± 10.9 vs. 77.2 ± 6.9 mg/(kg•d); phosphorus 58.3 ± 6.3 vs. 46.1 ± 4.0 mg/(kg•d); vitamin D: 86.8 ± 9.6 vs. 74.4 ± 6.8 IU/(kg•d); all P<0.001]. Between term age and 6 mo CA, the vitamin D intake per day (IU/d) was significantly higher in PDF compared with TF fed infants [values indicate median (IQR); 445 (72.8) vs. 380 (60.8) IU/d, P<0.001].

Table 9.2. Infant characteristics at birth[1]

	PDF (n=52)		TF (n=41)		HM (n=46)	
	n	Value	n	Value	n	Value
Boys (%)	27	51.9	23	56.1	21	45.7
White (%)	34	65.4	32	78	34	73.9
Season of birth (%)						
winter	25	48.1	29	70.7	26	56.5
summer	27	51.9	12	29.3	20	43.5
Gestational age at birth (wk)	52	30.7 (1.5)	41	30.9 (2.3)	46	30.0 (1.9)

[1] Values as frequencies or medians (IQR). HM: human milk; PDF: postdischarge formula; TF: term formula.

25(OH)D and PTH in PDF, TF, and HM fed infants

The frequency of vitamin D deficiency [serum 25(OH)D <30 nmol/L] at term age and of vitamin D insufficiency [serum 25(OH)D <50 nmol/L] at term age and 3 mo CA were similar in PDF, TF, and HM fed infants (vitamin D deficiency at term age: 7.7, 0, and 2.3%, respectively; vitamin D insufficiency at term age: 13.5, 10, and 9.1%, respectively; and vitamin D insufficiency at 3 mo: 2, 2.4, and 0%, respectively; all P≥ 0.05). None of the infants was vitamin D deficient or insufficient at 6 mo CA. The frequency of high serum 25(OH)D (>150 nmol/L) was significantly higher in TF compared with PDF and HM fed infants at term age and similar at 3 and 6 mo CA (term age: 10% vs. 1.9% and 0%; respectively; P<0.05; 3 mo: 0% and 0% vs. 0%, P≥0.05; 6 mo: 0% vs. 3.9% and 0%, P≥0.05). PDF

fed infants had slightly lower serum 25(OH)D at term age and significantly higher serum 25(OH)D at 6 mo CA compared with TF and HM fed infants (Table 9.3). Between term age and 6 mo CA, PDF fed infants had a significantly greater increase in serum 25(OH)D compared with TF and HM fed infants [change in 25(OH)D: 18.4 ± 33.1 vs. -4.2 ± 35.7 and 4.5 ± 33.6 nmol/L, respectively; P<0.05].

Although the frequency of vitamin D deficiency was similar in PDF, TF, and HM infants at term age, PDF and TF fed infants had significantly higher serum PTH compared with HM fed infants at term age, but this difference disappeared thereafter (Table 9.3). Between term age and 6 mo CA, PDF and TF fed infants had a significantly greater decrease in serum PTH compared with HM fed infants [change in PTH: -3.17 (4.99) and -2.44 (3.55) vs. 0.01 (3.55) pmol/L, P<0.01].

Table 9.3. Serum total calcium, phosphate, PTH, 25(OH)D, BA, and BMC between term age and 6 mo CA in preterm infants fed PDF, TF, and HM[1]

	PDF (n=52)		TF (n=41)		HM (n=46)	
	n	Value	n	Value	n	Value
Term age						
Calcium (mmol/L)	52	2.56 ± 0.10	41	2.54 ± 0.09	46	2.55 ± 0.10
Phosphate (mmol/L)	52	2.17 ± 0.17[a]	41	2.20 ± 0.16[a]	46	2.05 ± 0.29[b]
PTH (pmol/L)	49	5.40 (5.55)[a]	38	4.86 (3.93)[a]	36	2.41 (3.66)[b]
25(OH)D (nmol/L)	52	85.2 ± 31.9	40	97.2 ± 37.9	44	90.5 ± 28.1
BA (cm^2)	43	310 (73.9)[a]	36	300 (67.2)[b]	37	315 (57.2)[a]
BMC (g)	42	43.8 (20.4)	30	47.3 (12.7)	34	48.5 (16.7)
3 mo CA						
Calcium (mmol/L)	51	2.66 ± 0.09[a]	41	2.61 ± 0.11[b]	46	2.67 ± 0.09[a]
Phosphate (mmol/L)	51	2.22 ± 0.14[a]	41	2.18 ± 0.15[a]	46	2.06 ± 0.22[b]
PTH (pmol/L)	47	2.96 (1.89)	41	2.87 (2.01)	45	2.50 (2.13)
25(OH)D[2] (nmol/L)	50	95.0 ± 21.9	41	89.3 ± 20.9	46	87.1 ± 17.8
6 mo CA						
Calcium (mmol/L)	52	2.67 ± 0.09	41	2.66 ± 0.09	46	2.66 ± 0.07
Phosphate (mmol/L)	52	2.06 ± 0.14	41	2.06 ± 0.12	46	2.06 ± 0.17
PTH (pmol/L)	47	2.23 (1.33)	41	2.08 (1.30)	43	2.65 (1.70)
25(OH)D[2] (nmol/L)	51	103 ± 24.3[a]	41	92.8 ± 15.5[b]	45	95.6 ± 18.9[a]
BA (cm^2)	43	632 (97.3)[a]	36	598 (88.3)[a]	37	601 (95.8)[b]
BMC (g)	42	151 (42.5)[a]	30	134 (35.1)[b]	34	133 (33.4)[b]

[1] Values are means ± SD or median (IQR) and show comparison between PDF, TF, and HM fed infants by linear regression adjusted for sex and gestational age [and 25(OH)D for season of birth and ethnicity]. Labeled means or medians in a row without a common letter differ, P<0.05.

BA: bone area; BMC: bone mineral content; CA: corrected age; HM: human milk; PDF: postdischarge formula; PTH: parathyroid hormone; TF: term formula; 25(OH)D: 25-hydroxyvitamin D.

[2] PDF versus HM, P=0.06.

Serum calcium, PTH, and 25(OH)D in relation to bone mass

At term age, serum total calcium and 25(OH)D were positively associated and serum PTH was negatively associated with BMC (Table 9.4). At 3 and 6 mo CA, serum 25(OH)D was positively associated and serum PTH was negatively associated with BMC at 6 mo CA (Table 9.4).

Table 9.4. Serum total calcium, serum PTH, and serum 25(OH)D in relation to bone mineral content (BMC) in preterm infants between term age and 6 mo CA[1]

	BMC[2] (g)			
	Term age		6 mo CA	
	n	β (95% CI)	n	β (95% CI)
Term age				
Calcium (mmol/L)	116	0.88** (0.43;1.32)	106	0.27 (-0.08;0.61)
PTH[2] (pmol/L)	103	-0.15** (-0.21;-0.09)	92	0.01 (-0.04;0.06)
25OHD (nmol/L)	114	0.003** (0.002;0.005)	103	0.00 (0.00;0.001)
3 mo CA				
Calcium (mmol/L)		N/A	105	0.17 (-0.16;0.51)
PTH[2] (pmol/L)		N/A	101	-0.06* (-0.12;0.00)
25OHD (nmol/L)		N/A	104	0.002* (0.00;0.003)
6 mo CA				
Calcium (mmol/L)		N/A	106	0.36 (-0.01;0.73)
PTH[2] (pmol/L)		N/A	101	-0.13** (-0.20;-0.07)
25OHD (nmol/L)		N/A	105	0.001† (0.00;0.003)

[1] Associations by linear regression; * P<0.05; ** P<0.001; †P=0.083. BMC: bone mineral content; CA: corrected age; N/A: not applicable; PTH: parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D.
[2] ln transformed

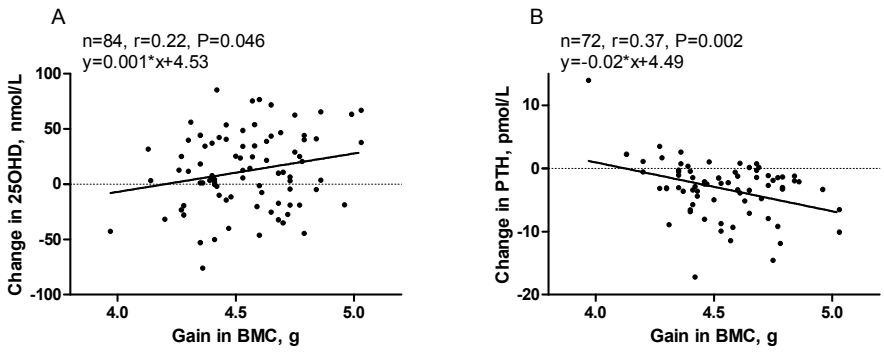

Figure 9.1. Change in serum 25(OH)D (A) and change in serum PTH (B) in relation to gain in BMC in preterm infants between term age and 6 mo corrected age
BMC: bone mineral content; PTH: parathyroid hormone; 25(OH)D: 25-hydroxyvitamin D.

Between term age and 6 mo CA, change in serum 25(OH)D was positively associated and change in serum PTH was negatively associated with gain in BMC (Figure 9.1). There was no effect modification of type of feeding, season of birth (winter/summer), ethnicity (white/nonwhite), or sex on the associations between serum total calcium, serum PTH, serum 25(OH)D, and BMC.

DISCUSSION

The present study demonstrates that higher vitamin D intake with an isocaloric, protein- and mineral-enriched PDF leads to a greater increase in serum 25(OH)D in preterm infants during the first 6 mo post-term. The greater increase in serum 25(OH)D in PDF fed preterm infants that results in higher serum 25(OH)D at 6 mo CA may be compensatory for the slightly lower 25(OH)D at term age. Change in serum 25(OH)D was positively associated with gain in BMC during the first 6 mo post-term. This may reflect that a greater increase in 25(OH)D enhances calcium absorption and availability for bone mineralization and may suggest that, in addition to dietary calcium, phosphorus, and protein availability, a higher vitamin D intake and greater increase in serum 25(OH)D contribute to higher bone accretion of PDF fed infants [16]. In addition, we hypothesize that the negative association between change in serum PTH and gain in BMC may imply that calcium resorption from bone is decreased in PDF fed infants with higher bone accretion. However, the higher dietary calcium, phosphorus, and protein intakes from PDF may also have an important role for the enhancement of bone accretion of preterm infants. It has been hypothesized that a higher bone mass during infancy may track to adulthood [16] and that higher adult bone mass, in turn, is associated with a lower risk of osteoporosis in later life [28].

PDF fed infants reached the recommended intake of 400 IU/d vitamin D during the first 6 mo post-term, whereas TF fed infants had a vitamin D intake slightly below 400 IU/d. This vitamin D intake is in accordance with the current guidelines on vitamin D supplementation for term infants, as recommended by the Institute of Medicine, the American Association of Pediatrics, the Pediatric Endocrine Society, and the Dutch Health Council [3, 8, 29-30] as well as in accordance with the current guidelines on vitamin D requirements for preterm infants after discharge [31], but is far below the 800-1000 IU/d vitamin D for preterm infants as recommended by the European Society of Paediatric Gastroenterology Hepatology and Nutrition [32]. However, the vitamin D intake from the formula used in the present study is sufficient to prevent vitamin D deficiency in preterm infants at 6 mo CA. Furthermore, PDF fed infants may already benefit from their higher vitamin D intake, as suggested by higher bone accretion [16]. This might suggest that if the current Dutch and American advice on vitamin D supplementation [3, 8, 29-

30] is combined with the higher vitamin D intake from PDF, preterm infants may reach even higher bone accretion during the first 6 mo post-term. On the other hand, these preterm infants may be at risk of serum 25(OH)D concentrations >150 nmol/L (60 ng/L), which have been associated with acute toxicity mostly related to hypercalcemia and growth retardation [25]. In the present study, the higher vitamin D intake from PDF did not lead to serum 25(OH)D concentrations >150 nmol/L (60 ng/L) at 6 mo CA.

On the other hand, during the first year of life, the vitamin D status of preterm infants is influenced by rapid growth and it has been suggested that vitamin D dosage should be adjusted for the actual body weight of the infant [33-34]. A recent study in healthy term infants demonstrates that a vitamin D intake close to 100 IU per kg body weight per day is sufficient to prevent vitamin D deficiency [33]. In the present study, the vitamin D intake per kilogram body weight per day was closer to the advised 100 IU/(kg•d) in PDF fed infants than in TF fed preterm infants and PDF fed infants had a low frequency of vitamin D deficiency at 3 and 6 mo CA. This may suggest that PDF approximates an adequate amount of vitamin D intake in preterm infants during the first 6 mo post-term.

The question remains whether the observed bone accretion in preterm infants in our study depends on vitamin D status. It has been suggested that vitamin D plays a minor role in bone accretion when adequate dietary calcium and phosphorus are available [17] because these nutrients are mandatory to achieve sufficient bone accretion. Nevertheless, in line with previous studies during infancy [18-19] and childhood [20], serum 25(OH)D was clearly associated with bone accretion, whereas calcium and phosphorus were not. Furthermore, in the present study, the frequency of vitamin D deficiency was low and serum calcium, phosphate, and PTH were within normal range (normal range of serum calcium: 2.10-2.60 mmol/L; phosphate: 1.10-2.10 mmol/L; and PTH: <8 pmol/L; respectively [35]). Because serum 25(OH)D was associated with bone accretion and there was a low frequency of vitamin D deficiency, it seems likely that vitamin D may play an important role in bone accretion in preterm infants.

A limitation of the present study was that the randomized controlled trial was designed and powered to evaluate the effect of postdischarge nutrition on growth and body composition between term age and 6 mo CA [21]. The study was not designed to test causal relations, thus, only associations can be demonstrated. Therefore, the results of the present study need to be interpreted with caution.

Another limitation may be that calcium, phosphorus, and vitamin D absorption from infant formula may vary considerably. The absorption of calcium from formula varies between 35 and 60% [1], while the absorption of phosphate is >90%. However, calcium salts within the infant formula may precipitate [1], which decreases intestinal calcium absorption. The absorption of vitamin D from infant formula may be complicated, since vitamin D is a fat-soluble vitamin and may adhere to the surface of feeding bottles [36]. Furthermore, in the present study, it was difficult to determine the vitamin D intake of

HM fed infants because the exact volume intake of HM fed infants was unknown and information on maternal vitamin D status or supplementation was not available. In addition, parents of HM fed infants did not consistently record the amount of vitamin D supplements administered in the diaries, and in a number of cases it can only be assumed that these infants received 200 IU/d vitamin D as recommended at the time.

Moreover, the vitamin D status of infants is not only determined by their dietary vitamin D intake. Maternal vitamin D status during pregnancy has a major influence on infant vitamin D status in early life [2, 4, 37-38] as well as on bone accretion during infancy [39-41] and childhood [42]. In addition, sunlight exposure [2, 8, 20], which is related to season of birth [18, 38, 43], skin pigmentation, and geographic location [6], may influence the vitamin D status of infants. However, the effect of vitamin D intake from formula on vitamin D status in infants may be dominant over the effect of sunlight exposure and, in turn, of geographic location [17]. Unfortunately, in the present study, no data were available on maternal vitamin D status or on infant vitamin D status before term age.

In addition, serum total calcium and not ionized calcium was measured at term age and at 3 and 6 mo CA. Serum total calcium may have a wide range due to variations in serum albumin and the state of hydration and can change without affecting the ionized calcium concentration. On the other hand, ionized calcium concentration can change without affecting the serum total calcium concentration.

Furthermore, the current guidelines vary with regard to the target 25(OH)D recommended for infants. The American Association of Pediatrics recommends a target 25(OH)D >50 nmol/L for term and preterm infants [29, 31], whereas the Endocrine Society recommends a target 25(OH)D >75 nmol/L for the overall population [8]. In line with the guidelines of the American Association of Pediatrics, vitamin D sufficiency was defined as serum 25(OH)D >50 nmol/L in the present study.

In conclusion, with the nutritional regimen of the present study, the additional vitamin D intake from an isocaloric, protein- and mineral-enriched PDF, which is in accordance with current recommendations of 400 IU/d vitamin D [3, 8, 29-30] and which approximates the advised 100 IU/(kg•d) vitamin D for term infants [33], results in a greater increase in serum 25(OH)D. This increase in serum 25(OH)D may, in addition to dietary protein, calcium, and phosphorus availability [44], contribute to increased bone accretion compared with standard TF or HM [16]. Furthermore, in addition to an isocaloric, protein- and mineral-enriched PDF, higher vitamin D supplementation, as advised with the current guidelines [3, 8, 29-30, 32], may improve bone accretion even further.

REFERENCES

1. Rigo J, De Curtis M, Pieltain C, Picaud J, Salle B, Senterre J. Bone mineral metabolism in the micropremie. Clin Perinatol. 2000;27:147-165.
2. Prentice A. Micronutrients and the bone mineral content of the mother, fetus and newborn. J Nutr. 2003;133:1693S-1699S.
3. Abrams S. What are the risks and benefits to increasing dietary bone minerals and vitamin D intakes in infants and small children. Ann Rev Nutr. 2011;31:285-297.
4. Mølgaard C, Michaelsen K. Vitamin D and bone health in early life. Proc Nutr Soc. 2003;62:823-828.
5. Kovacs C. Vitamin D in pregnancy and lactation: maternal, fetal, and neonatal outcomes from human and animal studies. Am J Clin Nutr. 2008;88:520S-528S.
6. Salle B, Delvin E, Lapillonne A, Bishop N, Glorieux F. Perinatal metabolism of vitamin D. Am J Clin Nutr. 2000;71:1317S-1324S.
7. Henriksen C, Helland I, Rønnestad A, Grønn M, Iversen P, Drevon C. Fat-soluble vitamins in breast-fed preterm and term infants. Eur J Clin Nutr. 2006;60:756-762.
8. Holick M, Binkley N, Bischoff-Ferrari H, Gordon C, Hanley D, Heaney R, Murad M, Weaver C. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011;96:1911-1930.
9. Zipitis C, Akobeng A. Vitamin D supplementation in early childhood and risk of type 1 diabetes: a systematic review and meta-analysis. Arch Dis Child. 2008;93:512-517.
10. Reinholz M, Ruzicka T, Schauber J. Vitamin D and its role in allergic disease. Clin Exp Allergy. 2012;42:817-826.
11. Morales E, Guxens M, Llop S, Rodríguez-Bernal C, Tardón A, Riaño I, Ibarluzea J, Lertxundi N, Espada M, et al. Circulating 25-hydroxyvitamin D3 in pregnancy and infant neuropsychological development. Pediatrics. 2012;130:e913-e920.
12. Whitehouse A, Holt B, Serralha M, Holt P, Kusel M, Hart P. Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics. 2012;129:485-493.
13. Walker V, Zhang X, Rastegar I, Liu P, Hollis B, Adams J, Modlin R. Cord blood vitamin D status impacts innate immune responses. J Clin Endocrinol Metab. 2011;96:1835-1843.
14. Belderbos M, Houben M, Wilbrink B, Lentjes E, Bloemen E, Kimpen J, Rovers M, Bont L. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics. 2011;127:e1513-e1520.
15. Camargo C, Ingham T, Wickens K, Thadhani R, Silvers K, Epton M. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and astma. Pediatrics. 2011;127:e180-e187.
16. van de Lagemaat M, Rotteveel J, van Weissenbruch M, Lafeber H. Increased gain in bone mineral content of preterm infants fed an isocaloric, protein-, and mineral-enriched postdischarge formula. Eur J Nutr. 2013;52:1781-1785.
17. Koo W, Krug-Wispe S, Neylan M, Succop P, Oestrich A, Tsang R. Effect of three levels of vitamin D intake in preterm infants receiving high mineral-containing milk. J Pediatr Gastroenterol Nutr. 1995;21:182-189.
18. Namgung R, Tsang R. Bone in the pregnant mother and newborn at birth. Clin Chem Acta. 2003;333:1-11.
19. Holmlund-Suila E, Viljakainen H, Hytinantti T, Lamberg-Allardt C, Andersson S, Mäkitie O. High-dose vitamin D intervention in infants-effects on vitamin D status, calcium homeostasis, and bone strength. J Clin Endocrinol Metab. 2012;97:4139-4147.

20. Sayers A, Fraser W, Lawlor D, Tobias J. 25-Hydroxyvitamin-D_3 levels are positively related to subsequent cortical bone development in childhood: findings from a large prospective cohort study. Osteoporos Int. 2012;23:2117-2128.
21. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
22. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
23. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
24. Abrams S. Vitamin D requirements of children: "all my life's a circle". Nutr Rev. 2012;70:201-206.
25. Institute of Medicine. Dietary Reference intake for vitamin D and calcium. Washington, DC: The National Academies Press; 2011.
26. Webb A, Kline L, Holick M. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988;67:373-378.
27. Koo W, Walters J, Bush A. Technical considerations of dual energy x-ray absorptiometry based bone mineral measurements for pediatric studies. J Bone Miner Res. 1995;10:1998-2004.
28. Hernandez C, Beaupré G, Carter D. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int. 2003;14:843-847.
29. Wagner C, Greer F. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. Pediatrics. 2008;112:1142-1152.
30. Health Council of the Netherlands. Evaluation of dietary reference values for vitamin D: The Hague: Health Council of the Netherlands; publication no. 2012/15; 2012.
31. Abrams S, Committee on Nutrition. Calcium and vitamin D requirements of enterally fed preterm infants. Pediatrics. 2013;131:e1676-e1683.
32. Agostoni C, Buonocore G, Carnielli V, De Curtis M, Darmaun D, Decsi T, Dömellof M, Embleton N, Fusch C, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition. J Pediatr Gastroenterol Nutr. 2010;50:85-91.
33. Pludowski P, Socha P, Karczmarewicz E, Zagorecka E, Lukaszkiewicz J, Stolarczyk A, Piotrowska-Jastrzebska J, Kryskiewicz E, Lorenc R, Socha J. Vitamin D supplementation and status in infants: a prospective cohort observational study. J Pediatr Gastroenterol Nutr. 2011;53:93-99.
34. [No authors listed]. Vitamin D supplementation: Recommendations for Canadian mothers and infants. Paediatr Child Health. 2007;12:583-598.
35. Derksen-Lubsen G, Mol H, Oudesluys-Murphy H, Sprij A. Compendium Pediatrics. Houten, The Netherlands: Bohn Stafleu van Loghum; 2011.
36. Backström M, Mäki R, Kuusela A, Koivisto A, Ikonen R, Kouri T, Mäli M. Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants. Arch Dis Child Fetal Neonatal Ed. 1999;80:F161-F166.
37. Pettifor J, Prentice A. The role of vitamin D in paediatric bone health. Best Pract Res Clin Endocrinol Metab. 2011;25:573-584.

38. Merewood A, Mehta S, Grossman X, Chen T, Mathieu J, Holick M, Bauchner H. Widespread vitamin D deficiency in urban Massachusetts newborns and their mothers. Pediatrics. 2010;125:640-647.
39. Viljakainen H, Saarnio E, Hytinantti T, Miettinen M, Surcel H, Mäkitie O, Andersson S, Laitinen K, Lamberg-Allardt C. Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab. 2010;95:1749-1757.
40. Marwaha R, Tandon N, Chopra S, Agarwal N, Garg M, Sharma B, Kanwar R, Bhadra K, Singh S, et al. Vitamin D status in pregnant Indian women across trimesters and different seasons and it correlation with neonatal serum 25-hydroxyvitamin D levels. Br J Nutr. 2011;106:1383-1389.
41. Shaw N, Mughal M. Vitamin D and child health part 1 (skeletal aspects). Arch Dis Child. 2013;98:363-367.
42. Javaid M, Crozier S, Harvey N, Gale C, Dennison E, Boucher B, Arden N, Godfrey K, Cooper C. Maternal vitamin D status during pregnancy and childhood bone mass at 9 years: a longitudinal study. Lancet. 2006;367:36-43.
43. Namgung R, Tsang R, Specker B, Sierra R, Ho M. Low bone mineral content and high serum osteocalcin and 1,25-dihydroxyvitamin D in summer- versus winter-born newborn infants: an early fetal effect? J Pediatr Gastroenterol Nutr. 1994;19:220-227.
44. Shiff Y, Eliakim A, Shainkin-Kestenbaum R, Arnon S, Lis M, Dolfin T. Measurements of bone turnover markers in premature infants. J Pediatr Endocrinol Metab. 2001;14:389-395.

Chapter 10

Iron deficiency and anemia in iron-fortified formula and human milk fed preterm infants until six months post-term

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ABSTRACT

Purpose

An iron intake of >2 mg/kg/d is recommended for preterm infants. We hypothesized that human milk (HM) fed preterm infants require iron supplementation after discharge, whereas iron-fortified formulae (IFF; 0.8-1.0 mg iron/100 ml) may provide sufficient dietary iron until six months post-term.

Methods

At term age, three and six months post-term, ferritin (µg/l) was measured in 92 IFF-fed infants (gestational age [median (interquartile range)] 30.7 (1.4) weeks, birth weight 1,375 (338) gram) and 46 HM-fed infants (gestational age 30.0 (1.7) weeks, birth weight 1,400 (571) gram). Iron intake (mg/kg/d) between term age and six months post-term was calculated.

Results

Iron was supplemented to 71.7% of the HM-fed infants and 83.7% of IFF-fed infants between term age and three months post-term and to 13% of HM-fed infants and 0% of IFF-fed infants between three and six months post-term. IFF-fed infants had an iron intake from supplements and formula of 2.66 (1.22) mg/kg/d between term age and three months post-term and 1.19 (0.32) mg/kg/d between three and six months post-term. At three and six months post-term, the incidence of ferritin <12 µg/l was higher in HM- compared to IFF-fed infants (23.8% versus 7.8% and 26.3% versus 9.5%, $P<0.02$).

Conclusion

This observational study demonstrates that ferritin <12 µg/l is more prevalent in HM-fed infants until six months post-term. This may be due to early cessation of additional iron supplementation. We speculate that additional iron supplementation is not necessary in preterm infants fed IFF (0.8-1.0 mg iron/100 ml), as they achieve ferritin ≥12 µg/l without additional iron supplements between three and six months post-term.

INTRODUCTION

Preterm infants lack the rapid fetal iron accretion during the third trimester of pregnancy [1-2]. In addition, frequent blood sampling and rapid postnatal growth exacerbate the depletion of body iron stores after birth [1, 3]. As a result, iron deficiency, defined as decreased ferritin (<12 µg/l), develops in 25-85% of preterm infants, which is mostly due to inadequate dietary iron intake during the first year [4-6]. Other markers of iron deficiency are decreased mean corpuscular volume (MCV <90 fl) and increased red cell distribution width (RDW >14.5%). Iron deficiency is associated with poor growth and reduced brain development [2, 7-8]. However, there is inconclusive but plausible evidence that enteral iron supplementation of preterm and low birth weight infants is beneficial for growth and neurodevelopment [9-12]. Nevertheless, an elemental iron intake of 2-4 mg/kg/d is recommended for preterm infants between 6 weeks and 12 months postnatal age [11, 13-14].

Several studies show that enteral iron supplementation results in a slight improvement of hemoglobin and ferritin after eight weeks postnatal age [4-5, 9]. It has been suggested that iron-fortified formulae (IFF) are sufficient to prevent iron deficiency. However, in current practice, preterm infants fed these formulae frequently receive additional enteral iron supplementation, which may have adverse effects due to iron overload [15]. On the other hand, enteral iron supplementation is important for human milk (HM) fed preterm infants because HM has a low iron content (0.5 mg/L) [9].

We hypothesized that HM-fed preterm infants require enteral iron supplementation to achieve an iron intake >2 mg/kg/d, whereas preterm infants fed IFF that provide an iron intake >2 mg/kg/d do not require additional enteral iron supplementation to prevent iron deficiency. With the IFF used in this study a volume intake of 200 ml/kg/d results in an iron intake of approximately 2 mg/kg/d. The present study evaluated the iron intake of IFF- and HM-fed preterm infants and its effect on the incidence of iron deficiency between term age (40 weeks postmenstrual age) and six months post-term.

SUBJECTS AND METHODS

Subjects

This study was part of a randomized controlled trial that evaluated the effects of an isocaloric, protein- and mineral-enriched postdischarge formula compared to a standard term formula and human milk as a control group on growth and body composition of preterm infants between term age and six months post-term, as described previously [16]. Growth and body composition during the first six months post-term were the primary outcomes of the randomized controlled trial. Signs of anemia and iron deficiency

were secondary outcomes of the randomized controlled trial. In short, 152 infants with a gestational age of 32 weeks or less and/or a birth weight of 1,500 gram or less were included shortly after birth (Figure 10.1). Between birth and term age, 80 infants were fed human milk with breast milk fortifier, which did not contain iron, and 75 infants were fed preterm formula (Friso Prematuur®, FrieslandCampina, Amersfoort, The Netherlands; 0.8 mg iron/100 ml or Nenatal start®, Nutricia, Zoetermeer, The Netherlands; 0.9 mg iron/100 ml). The type of feeding between birth and term age could not be determined in two postdischarge formula, four standard term formula, and one human milk fed infant due to missing data. Between term age and six months post-term, 102 formula-fed infants were randomized to postdischarge formula (n=54; Friso Prematuur 1®, FrieslandCampina, Amersfoort, The Netherlands; 1.0 mg iron/100 ml) or standard term formula (n=48; Friso Normaal 1®, FrieslandCampina, Amersfoort, The Netherlands; 0.8 mg iron/100 ml). Without randomization, 50 preterm infants that received predominantly (>80%) HM at term age were considered to be HM-fed. If HM was insufficiently available in this control group at some point between term age and six months post-term, standard term formula was added to the diet after parents consulted the coordinating investigator (E.M.A). At

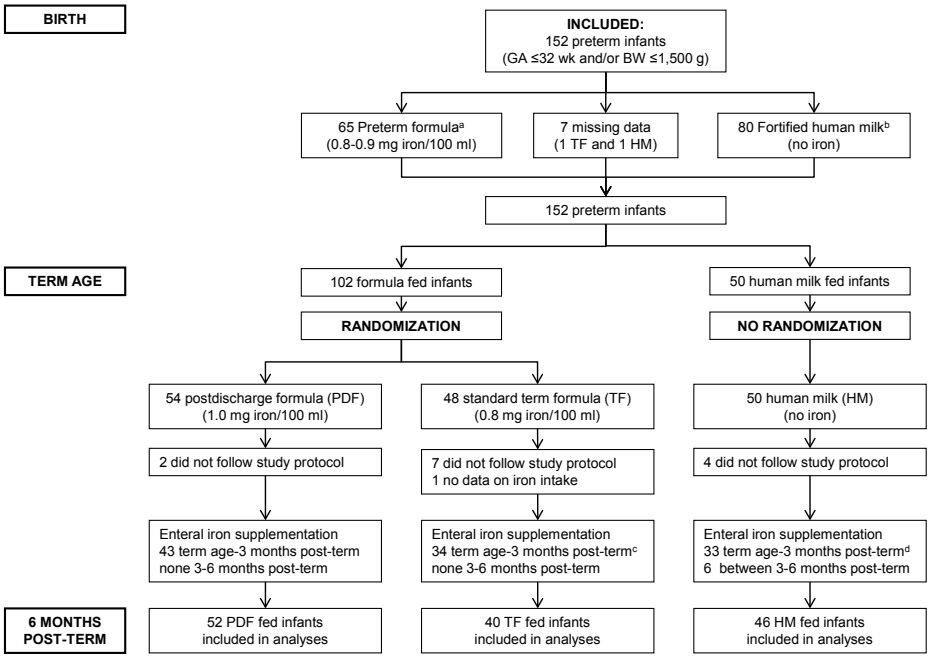

Figure 10.1. Flowchart of subject inclusion and randomization of the randomized controlled trial
BW: birth weight; GA: gestational age; HM: human milk; PDF: postdischarge formula; TF: term formula. [a] 39 PDF- and 26 TF-fed infants; [b] 13 PDF-, 18 TF-, and 49 HM-fed infants; [c] data on iron supplementation were missing in 1 TF-fed infant; [d] data on iron supplementation were missing in 3 HM-fed infants.

six months post-term, 138 preterm infants completed the study (Figure 10.1). Between term age and three months post-term, 28, 3, and 15 HM-fed infants were fed 75-100%, 50-75%, and <50% HM, respectively. Between three and six months post-term, 13, 7, and 9 HM-fed infants were fed 75-100%, 50-75%, and <50% HM, respectively. The remainder of the diet consisted of standard term formula (0.8 mg iron/100 ml).

The present observational study combined data on iron intake and the incidence of iron deficiency and anemia of 52 postdischarge formula, 40 standard term formula, and 46 HM-fed preterm infants during the first six months post-term. Infants attended the outpatient clinic of VU University Medical Center, Amsterdam, The Netherlands at term age (40.3 ± 0.7 weeks postmenstrual age), three months post-term (53.0 ± 0.5 weeks postmenstrual age), and six months post-term (66.0 ± 0.5 weeks postmenstrual age) for anthropometry and blood samples. The study protocol was approved by the local ethics committee and the study was conducted according to the guidelines laid down in the Declaration of Helsinki. All parents of the included infants gave written informed consent.

Methods

Gestational age at birth and the number and volume (ml) of erythrocyte transfusions during the study period were extracted from medical records. Weight (g), length (cm), and head circumference (cm) were measured at birth, term age, three months post-term, and six months post-term, as described previously [16]. To adjust for gender and postmenstrual age, weight, length, and head circumference were expressed as standard deviation score (SDS) based on Swedish references for preterm infants at birth and term age and based on Dutch references at three and six months post-term [17-18].

Between birth and discharge, details on enteral and parenteral intake were collected from the infant's medical record by one single person. Between discharge and six months post-term, parents recorded their child's intake in a diary (daily until term age and weekly after term age). The medical records and parental diaries provided information on type and amount of intake (ml) and on dosage and frequency of iron supplements. In general, the local pediatricians were discouraged to prescribe iron supplementation to IFF-fed infants, but they were free to follow their local routine. Unfortunately, no nationwide guidelines regarding iron supplementation in very low birth weight infants existed at that time, and in many cases no justification or rationale for the enteral iron supplementation could be given other than the finding of a rather low hemoglobin. In all infants, the intake of elemental iron (mg/kg/d) from iron supplements was calculated for each postnatal week. In the IFF-fed infants, the total intake of elemental iron (mg/kg/d) for each postnatal week was calculated based on the total volume intake (ml/kg/d) and the iron content of the formulae and on the intake of any iron supplements. The median iron intake from supplements (all infants) and the median total iron intake (IFF-

fed infants) were calculated for the following intervals: birth-term age, term age-three months post-term, three-six months post-term.

It was routine clinical practice to measure hemoglobin on the first day of life. At term age, three and six months post-term, venous blood samples were collected for the analysis of hemoglobin, hematocrit, mean corpuscular volume (MCV), red cell distribution width (RDW), serum ferritin, and C-reactive protein (CRP). Hemoglobin (g/l) was measured by colorimetry (Cell-Dyn Sapphire, Abbott Diagnostics, IL, USA) with an inter-assay variance of 1.6%. Hematocrit (l/l) and MCV (fl) were measured by impedance and optical flow cell measurement (Cell-Dyn Sapphire, Abbott Diagnostics, IL, USA) with an inter-assay variance of 2.6% and 1.6%, respectively. RDW (%) was measured by SE-9000 (Sysmex, Kobe, Japan). Ferritin was measured by an electrochemiluminescence immunoassay (Modular E, Roche diagnostics, Mannheim, Germany) with and inter-assay variance of 4.2%. CRP (mg/l) was measured by an immunoturbidimetric assay (CRPLX, Roche Diagnostics, Mannheim, Germany) with an inter-assay variance of 2.7%. All measurements were performed at the Department of Clinical Chemistry of VU University Medical Center, Amsterdam, The Netherlands.

Criteria that were considered indicative of iron deficiency were: serum-ferritin <12 µg/l [19], MCV <80 fl, and/or RDW >14.5%. Low ferritin was considered the most reliable indicator of iron deficiency, as it is inversely associated with iron absorption in infants and it reflects decreased iron stores that result in iron deficiency [11, 20]. Since ferritin is an acute phase reactant, ferritin was excluded from the analyses if CRP was ≥5 mg/l. Anemia was defined as hemoglobin <94.9 g/l and/or hematocrit <0.32 l/l.

Statistics

Distribution of gender, number of transfusions, number of infants receiving iron supplementation, and number of infants with signs of iron deficiency and anemia were expressed as frequencies and compared between IFF- and HM-fed infants by chi-square test. Hemoglobin at birth, total transfusion volume before term age, and weight, length, and head circumference SDS at birth, term age, three and six months post-term were normally distributed. Gestational age and weight (g), length (cm), and head circumference (cm) at birth, term age, three and six months post-term were normally distributed after natural log transformation. For consistency, hemoglobin at birth, total transfusion volume, gestational age, and growth parameters were expressed as median with interquartile range (IQR) and compared between IFF- and HM-fed infants by linear regression adjusted for gender and gestational age.

The dosage of oral iron supplements and parameters of iron deficiency and anemia were not normally distributed. These parameters were expressed as median with IQR and were compared between IFF- and HM-fed infants by non-parametric Kruskall-Wallis and post-hoc Mann-Whitney test with Bonferroni correction ($P<0.017$ was considered

significant). The change in parameters of iron deficiency and anemia between time-points was evaluated by Wilcoxon signed rank test. The correlation between ferritin at different time-points was evaluated by the non-parametric Spearman's rho (r_s). A P value of less than 0.05 was considered significant. All statistical analyses were performed in SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA).

Table 10.1. Iron-fortified formula (IFF) and human milk (HM) fed infants

	Iron-fortified formula (n=92)					
	PDF (n=52)		TF (n=40)		HM (n=46)	
	n		n		n	
Birth						
Male[a] (%)	27	51.9	23	57.5	21	45.7
Gestational age (weeks)	52	30.7 (1.5)	40	30.8 (2.4)	46	30.0 (1.93)
Weight (g)	52	1344 (483)	40	1390 (261)	46	1345 (605)
Length (cm)	50	38.0 (4.0)	35	38 (3.0)	45	38.0 (3.5)
Head circumference(cm)	50	27.9 (2.5)	37	28.0 (2.0)	46	28.0 (2.8)
Hemoglobin (g/l)	52	169.9 (33.8)	38	165.8 (48.3)	46	165.8 (27.4)
Birth-term age						
Erythrocyte transfusion[a] (%)	28	53.8	19	47.5	31	67.4
Erythrocyte transfusion (ml)	28	72.0 (52.0)	19	50.0 (65.0)	31	51.0 (56.0)
Term age						
Weight (g)	52	3173 (699)	40	3090 (584)	46	3158 (734)
Length (cm)	52	49.0 (3.0)	40	48.5 (3.0)	46	48.3 (2.6)
Head circumference (cm)	52	35.9 (1.4)	40	35.8 (1.6)	46	35.8 (2.3)
3 months post-term						
Weight (g)	52	5573 (1410)	40	5495 (1240)	46	5508 (1210)
Length (cm)	52	59.0 (3.8)	40	59.0 (4.4)	46	58.8 (3.5)
Head circumference (cm)	52	40.7 (2.1)	40	40.8 (1.0)	46	40.8 (2.2)
6 months post-term						
Weight (g)	52	7380 (1511)	40	7428 (1442)	46	7150 (1729)
Length (cm)	51	67.0 (2.5)	40	66.5 (4.4)	46	66.3 (4.0)
Head circumference (cm)	52	43.8 (1.6)	40	43.7 (1.8)	46	44.1 (2.0)

Values as median (IQR) and between-group comparisons by linear regression adjusted for gender and gestational age, unless specified otherwise. [a] Frequency (%) and between-group comparisons by chi-square test. There were no significant between-group differences. HM: human milk; IFF: iron-fortified formula; PDF: postdischarge formula; TF: term formula.

RESULTS

Erythrocyte transfusions and growth

Hemoglobin levels at birth and the frequency and volume of erythrocyte transfusions given before discharge were not different between IFF- and HM-fed infants (Table 10.1). Growth between birth and six months post-term was comparable between IFF- and HM-fed infants (Table 10.1 and Figure 10.2).

Iron intake

The total iron intake (i.e. from formula and supplements) was higher in preterm infants fed postdischarge formula (1.0 mg iron/100 ml) compared to those fed standard term formula (0.8 mg iron/100 ml) between term age and six months post-term [2.07 (0.87) versus 1.75 (0.52) mg/kg/d; P<0.05]. Even though the total iron intake was higher, parameters of anemia and iron deficiency were not different between infants fed post-discharge formula and standard term formula (Table 10.2). Therefore, only comparisons between IFF-fed infants (fed postdischarge formula or term formula between term age and six months post-term) and HM-fed infants are further described here.

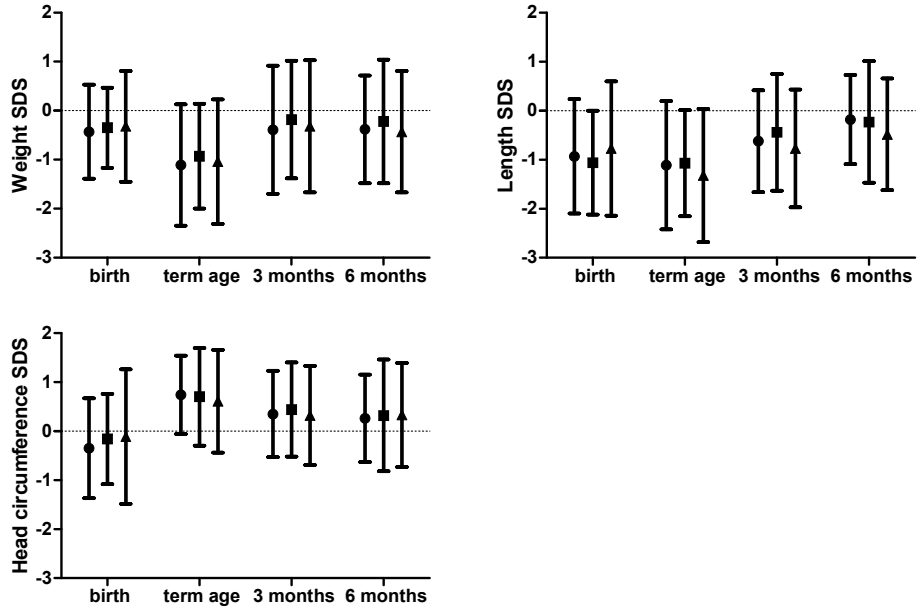

Figure 10.2. Weight SDS, length SDS, and head circumference SDS in IFF- and HM-fed preterm infants between birth and six months post-term

Values as median with interquartile range (bars) in postdischarge formula (●), standard term formula (■), and HM-fed infants (▲). Weight, length, and head circumference SDS were compared between groups by linear regression adjusted for gender and gestational age and there were no significant differences.

In this study, 79.7% of the infants received iron supplements between term age and three months post-term (71.7% of the HM-fed infants and 83.7% of the IFF-fed infants). Most infants received iron supplements within one month after term age. In iron supplemented infants, the dosage of iron supplementation was higher in HM- compared to IFF-fed infants between birth and term age as well as between term age and three months post-term [birth-term age: 2.78 (0.94) versus 2.16 (1.43) mg/kg/d; and term age-three months post-term: 2.51 (0.85) versus 2.26 (0.81) mg/kg/d; respectively; all P<0.05] (Figure 10.3). Between three and six months post-term, none of the IFF-fed infants and six HM-fed infants received iron supplements [HM-fed infants: 1.91 (1.42) mg/kg/d].

Table 10.2. Parameters of iron deficiency and anemia in iron-fortified formula (IFF) and human milk (HM) fed infants between term age and six months post-term

	Iron-fortified formulae (n=92)						
	PDF (n=52)		TF (n=40)		HM (n=46)		P[a]
	n		n		n		
Hemoglobin(g/l)							
Term age	52	96.6 (9.7)[*]	40	96.6 (19.3)[*]	46	94.9 (14.5)[*]	n.s.
3 months post-term	52	115.9 (8.1)[#]	40	115.9 (12.9)[‡]	46	114.3 (16.1)[‡]	n.s.
6 months post-term	52	120.8 (12.9)	40	119.1 (11.3)	46	117.5 (9.6)	n.s.
Hematocrit (l/l)							
Term age	52	0.29 (0.03)[*]	40	0.28 (0.05)[*]	45	0.29 (0.05)[*]	n.s.
3 months post-term	52	0.34 (0.03)[#]	40	0.35 (0.04)[#]	46	0.34 (0.05)[‡]	n.s.
6 months post-term	52	0.36 (0.04)	40	0.36 (0.03)	46	0.35 (0.03)	n.s.
MCV (fl)							
Term age	52	87 (5)[*]	40	88 (5)[*]	45	86 (6)[*]	n.s.
3 months post-term	52	79 (5)[‡]	40	79 (4)	46	77 (5)[‡]	<0.017
6 months post-term	52	78 (5)	40	78 (4)	46	77 (6)	<0.017
RDW (%)							
Term age	51	14.4 (2.3)[*]	40	14.9 (2.1)[*]	44	14.5 (1.5)[*]	n.s.
3 months post-term	52	12.9 (1.5)	40	13.1 (1.6)[‡]	45	13.1 (2.5)	n.s.
6 months post-term	52	12.5 (1.9)	39	12.3 (1.4)	46	13.5 (2.6)	<0.01
Ferritinb (µg/l)							
Term age	46	44.3 (63.0)[*]	32	38.6 (79.9)[$]	44	52.6 (61.1)[*]	n.s.
3 months post-term	45	21.1 (20.1)	32	18.9 (15.0)	42	21.1 (23.0)[‡]	n.s.
6 months post-term	40	21.5 (19.2)	34	18.4 (8.9)	38	15.0 (9.2)	<0.01

Values as median (IQR). [a] Between group comparisons by Kruskal-Wallis test and post-hoc Mann-Whitney test; Bonferroni correction, P<0.017 considered significant. [b] If CRP < 5 mg/L. Between time-points, values were compared by Wilcoxon signed rank test; [*] P<0.001 between term age and 3 months post-term; [$] P<0.05 between term age and 3 months post-term; [#] P<0.001 between 3 and 6 months post-term; [‡] P<0.05 between 3 and 6 months post-term. HM: human milk; IFF: iron-fortified formula; PDF: postdischarge formula; TF: standard term formula; n.s.: not significant.

IFF-fed infants had a total iron intake (i.e. iron intake from formula and supplements) above 2 mg/kg/d between term age and three months post-term (Table 10.3). HM-fed infants that were fed additional IFF had a total iron intake below 2 mg/kg/d between birth and six months post-term (Table 10.3).

Parameters of iron deficiency and anemia

Between term age and three months post-term, hemoglobin and hematocrit increased and MCV, RDW, and ferritin decreased in HM and IFF-fed infants (Table 10.2). Between three and six months post-term, hemoglobin and hematocrit increased in HM- and IFF-fed infants, while ferritin decreased in HM-fed infants and RDW decreased in IFF-fed infants (Table 10.2). Ferritin at term age correlated positively with ferritin at three months post-term (r_s=0.27, P<0.01); ferritin at three months post-term correlated positively with ferritin at six months post-term (r_s=0.33, P<0.01).

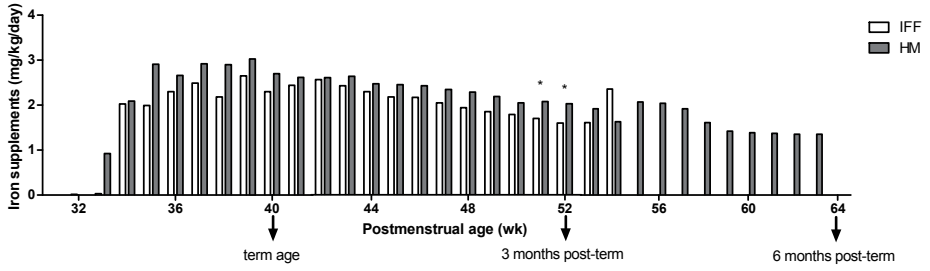

Figure 10.3. Median dosage (mg/kg/d) of oral iron supplements in iron-fortified formula (IFF) and human milk (HM) fed infants between birth and six months post-term
IFF: iron-fortified formula; HM: human milk. 77 IFF and 33 HM fed infants included between term age and three months post-term; no IFF and 6 HM fed infants included between three and six months post-term. Dosage of iron supplements (mg/kg/d) per week was compared between IFF- and HM-fed infants by Mann-Whitney test; * P<0.05.

Table 10.3. Total iron intake in iron-fortified formula (IFF) and human milk (HM) fed infants that received additional iron-fortified formula between birth and six months post-term

Total daily iron intake[a] (mg/kg/d)	IFF[b]		HM[c]		IFF versus HM[d]
	n	median (IQR)	n	median (IQR)	P
Birth-term age	88	1.19 (0.92)	43	0.82 (1.1)	<0.001
Term age-3 months post-term	91	2.66 (1.22)	19	1.42 (0.36)	<0.001
3-6 months post-term	90	1.19 (0.32)	33	1.04 (0.19)	0.001

[a] Based on iron intake from IFF and from enteral iron supplements; [b] preterm formula between birth and term age; postdischarge formula and standard term formula between term age and six months post-term; [c] only HM-fed infants that were fed additional iron-fortified formula were included; [d] between group values were compared by Mann-Whitney test. IFF: iron-fortified formula; HM: human milk.

At three months post-term, the incidence of ferritin <12 µg/l and RDW >14.5% was higher in HM- compared to IFF-fed infants (Table 10.4). At six months post-term, the incidence of ferritin <12 µg/l and RDW >14.5% was higher in HM- compared to IFF-fed infants (Table 10.4). At six months post-term, the incidence of hemoglobin <94.9 g/l and hematocrit <0.32 l/l was not different between HM- and IFF fed infants (Table 10.4).

If only infants with ferritin <12 µg/l were considered, the incidence of hemoglobin <94.9 g/l, hematocrit <0.32 l/l, or both was similar in HM- and IFF-fed infants at term age and higher in HM-fed infants at three months post-term compared to IFF-fed infants (term age: 80% versus 88.9%; P≥0.05; and three months post-term: 60% versus 0%; P<0.02; respectively). At six months post-term, none of the infants with ferritin <12 µg/l, fed either IFF or HM, had hemoglobin <94.9 g/l, hematocrit <0.32 l/l, or both.

Table 10.4. Anemia and iron deficiency in iron-fortified formula (IFF) and human milk (HM) fed infants between term age and six months post-term

		IFF (n=92)	HM (n=46)	IFF versus HM[a]		
		%	%	OR	95%CI	P
Hemoglobin <94.9 g/l	Term age	43.5	50			n.s.
	3 months post-term	1.1	4.3			n.s.
	6 months post-term	0.0	2.2			n.s.
Hematocrit <0.32 l/l	Term age[b]	82.6	82.2			n.s.
	3 months post-term	6.5	26.1	0.15	0.05-0.46	0.001
	6 months post-term	2.2	2.2			n.s.
MCV <80 fl	Term age[b]	4.3	4.4			n.s.
	3 months post-term	59.8	78.3	0.39	0.17-0.92	0.031
	6 months post-term	67.4	80.4			n.s.
RDW >14.5%	Term age[c]	52.7	48.9			n.s.
	3 months post-term[b]	9.8	26.7	0.25	0.09-0.68	0.006
	6 months post-term[d]	9.9	32.6	0.19	0.07-0.50	0.001
Ferritin <12 µg/l	Term age[e]	11.5	11.4			n.s.
	3 months post-term[f]	7.8	23.8	0.22	0.07-0.68	0.009
	6 months post-term[g]	9.5	26.3	0.27	0.09-0.79	0.017

Values as frequencies (%); [a] compared between IFF- and HM-fed infants by logistic regression adjusted for gender and gestational age; [b] 45 HM-fed infants; [c] 91 IFF-fed infants and 45 HM-fed infants; [d] 91 IFF-fed infants; [e] 78 IFF-fed infants and 44 HM-fed infants; [f] 77 IFF-fed infants and 42 HM-fed infants; [g] 74 IFF-fed infants and 38 HM-fed infants. HM: human milk; IFF: iron-fortified formula; n.s.: not significant.

DISCUSSION

In the present study, the incidence of decreased ferritin (<12 µg/l) or increased RDW (>14.5%), suggestive of iron deficiency, at three and six months post-term was higher in HM- compared to IFF-fed preterm infants. In addition, we observed that the number of HM-fed infants receiving additional iron supplements decreased during the first six months post-term. Some HM-fed infants were fed additional TF (0.8 mg iron/100 ml) because HM was insufficiently available. However, the additional intake of this iron-fortified formula did not increase their iron intake above 2 mg/kg/d (Table 10.3). If iron supplements were prescribed to HM-fed infants, the dosage was in accordance with the current guidelines that recommend 2-4 mg/kg/d elemental iron supplementation in preterm infants during the first 12 postnatal months [11, 13-14]. The decreased frequency of iron supplementation and the resultant higher incidence of iron deficiency in HM-fed preterm infants probably reflects the standard pediatric practice in The Netherlands between 2003 and 2006, when a rather low hemoglobin was often used as the only indicator for the initiation of iron supplementation. In addition, the higher incidence of iron deficiency in HM-fed infants in the present study was not explained by lower birth weight or faster postnatal growth, which are both associated with a higher risk of iron deficiency [21-23].

Controversy exists about the exact amount of iron required in infant formulae for preterm infants, partly because iron absorption from infant formulae is problematic and iron fortification of formulae may be associated with gastrointestinal symptoms [9]. Formulae with 0.5-0.9 mg iron/100 ml lead to serum ferritin <10µg/L in 2.5% of preterm infants before two months post-term and in 14.3% of preterm infants between two and six months post-term [24]. In contrast, we demonstrated a lower incidence of ferritin <12 µg/l in preterm infants fed formulae with 0.8-1.0 mg iron/100 ml between three and six months post-term, when only a few IFF-fed infants received iron supplements. With the IFF (0.8-1.0 mg iron/100 ml) used in this study, preterm infants need a volume intake of at least 200 ml/kg/d of IFF to approximate the recommended minimal iron intake of 2 mg/kg/d [11, 13-14]. Remarkably, the preterm infants in our study had a total iron intake below the recommended minimal intake of 2 mg/kg/d [11, 13-14]. Therefore, we speculate that infant formulae with an iron concentration of 0.8-1.0 mg/100 ml may prevent iron deficiency between three and six months post-term and that routine iron supplementation in preterm infants fed such iron-fortified formulae appears to be unnecessary. The participating pediatricians were not restricting the prescription of iron supplements, even though they were requested to restrict iron supplementation. For that reason, it is not possible to make a firm statement on the necessary iron concentration in postdischarge formulae during the first three months post-term. However, since no differences were found in parameters of iron deficiency and anemia between the

formulae with 0.8 and 1.0 mg iron/100 ml, the lower concentration seems to be sufficient during the first three months post-term under the circumstances of the present study.

This study demonstrates that a serum ferritin <12 µg/l is found in IFF- and HM-fed infants without signs of anemia (defined as hemoglobin <94.9 g/l, hematocrit <0.32 l/l, or both). At three months post-term, 60% of the HM-fed infants with ferritin <12 µg/l were anemic, whereas at six months post-term, none of the infants with ferritin <12 µg/l, fed either IFF or HM, were anemic. This might suggest that a biochemical marker, such as ferritin [20], is preferred for the diagnosis of iron deficiency and the initiation of iron supplementation in preterm infants. However, it should be taken into account that ferritin is an acute-phase reactant and an infection marker [for instance C-reactive protein (CRP)] should be measured to exclude a false-negative diagnosis of iron sufficiency. In addition, ferritin can be disturbed by several other conditions. For example, ferritin may be elevated as a result of liver or kidney disease and it may be decreased due to malabsorption syndromes [25]. If blood samples are grossly hemolytic, serum ferritin can be increased by 60% in these samples due to the release of intracellular ferritin [25]. Furthermore, ferritin may track between term age and six months post-term, as suggested by the positive correlations between time-points in the present study. This might imply that ferritin can be used for the early identification of preterm infants that are at risk of iron deficiency at three or six months post-term and may be amendable to early iron supplementation.

In conclusion, this observational study shows that ferritin <12 µg/l is more prevalent in HM- than IFF-fed infants until six months post-term. We might speculate that this may be due to (too) early cessation of iron supplementation in HM-fed preterm infants, which may reflect the standard pediatric practice in The Netherlands in 2003-2006. In addition, we speculate that additional iron supplements may not be necessary in infants fed IFF with 0.8-1.0 mg iron/100 ml, as these infants achieve ferritin levels above 12 µg/l without additional iron supplementation between three and six months post-term, which is defined as the cut off value for iron deficiency [19]. Future research may investigate if initiation and continuation of enteral iron supplements during the first six months post-term prevent ferritin <12 µg/l in HM-fed infants. Furthermore, it may clarify if IFF with 0.8-1.0 mg iron/100 ml is sufficient to prevent iron deficiency during the first six months post-term if no additional iron supplementation is initiated.

REFERENCES

1. Dallman P, Siimes M, Stekel A. Iron deficiency in infancy and childhood. Am J Clin Nutr. 1980;33:86-118.
2. Rao R, Georgieff M. Iron therapy for preterm infants. Clin Perinatol. 2009;36:27-42.
3. Aher S, Malwatkar K, Kadam S. Neonatal anemia. Semin Fetal Neonatal Med. 2008;13:239-247.
4. Hall R, Wheeler R, Benson J, Harris G, Rippetoe L. Feeding iron-fortified premature formula during initial hospitalization to infants less than 1800 grams birth weight. Pediatrics. 1993;92:409-414.
5. Lundström U, Siimes M, Dallman P. At what age does iron supplementation become necessary in low-birth-weight infants? J Pediatr. 1977;91:878-883.
6. Franz A, Mihatsch W, Sander S, Kron M, Pohlandt F. Prospective randomized trial of early versus late enteral iron supplementation in infants with a birth weight of less than 1301 grams. Pediatrics. 2000;106:700-706.
7. Lozoff B, Georgieff M. Iron deficiency and brain development. Semin Pediatr Neurol. 2006;13:158-165.
8. Hay W. Strategies for feeding the preterm infant. Neonatology. 2008;94:245-254.
9. Mills R, Davies M. Enteral iron supplementation in preterm and low birth weight infants (Review). Cochrane Database of Syst Rev. 2012;14:CD005095.
10. Long H, Yi J, Hu P, Li Z, Qiu W, Wang F, Zhu S. Benefits of iron supplementation for low birth weight infants: a systematic review. BMC Pediatr. 2012;12:99.
11. Baker R, Greer F, The Committee on Nutrition. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0-3 years of age). Pediatrics. 2010;126:1040-1050.
12. Lozoff B, Beard J, Connor J, Felt B, Georgieff M, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev. 2006;64:S34-S43.
13. American Academy of Pediatrics. Iron fortification of infant formulas. Pediatrics. 1999;104:119-123.
14. Siimes M, Järvenpää A. Prevention of anemia and iron deficiency in very-low-birth-weigh infants. J Pediatr. 1982;101:277-280.
15. Domellöf M. Benefits and harms of iron supplementation in iron-deficient and iron-sufficient children. Nestlé Nutr Instr Workshop Ser Pediatr Program. 2010;65:153-165.
16. Amesz E, Schaafsma A, Cranendonk A, Lafeber H. Optimal growth and lower fat mass in preterm infants fed a protein-enriched postdischarge formula. J Pediatr Gastroenterol Nutr. 2010;50:200-207.
17. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
18. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
19. WHO. Iron Deficiency Anaemia. Assessment, Prevention and Control. A Guide for Programme Managers. Geneva: WHO; 2001.
20. Domellöf M. Iron requirements, absorption and metabolism in infancy and childhood. Curr Opin Clin Nutr Metab Care. 2007;10:329-335.
21. Thorsdottir I, Gunnarsson B, Atladottir H, Michaelsen K, Palsson G. Iron status at 12 months of age-effects of body size, growth and diet in a population with high birth weight. Eur J Clin Nutr. 2003;57:505-513.

22. Wharf S, Fox T, Fairweather-Tait S, Cook J. Factors affecting iron stores in infants 4-8 months of age. Eur J Clin Nutr. 1997;51:504-509.
23. Michaelsen K, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects early iron status, growth velocity and dietary factors. Acta Paediatr. 1995;84:1035-1044.
24. Griffin I, Cooke R, Reid M, McCormick K, Smith J. Iron nutritional status in preterm infants fed formulas fortified with iron. Arch Dis Child Fetal Neonatal Ed. 1999;81:F45-F49.
25. Wang W, Knovich M, Coffman L, Torti F, Torti S. Serum ferritin: past, present and future. Biochem Biophys Acta. 2010;1800:760-769.

Chapter 11

General discussion

The last three decades, the incidence and survival of preterm infants increased worldwide [1-3]. In The Netherlands, the incidence of very preterm birth (<32 weeks gestational age) increased from 0.63% in 1983 [4-5] to 1.5% in 2008 [3], while mortality rates decreased from 30% in 1983 [4-6] to 16% in 2008 [3]. Increased survival of preterm infants can be attributed to regionalization of perinatal care [5, 7], to improved obstetric care [6-8], and to improved neonatal care [8-9]. This improved survival results in an increased incidence of long-term morbidity [7]. Consequently, the focus of research in preterm infants shifted towards improving growth and long-term morbidity. In subsequent investigations, the quantity and quality of prenatal and postnatal growth, which might be influenced by postnatal nutritional interventions, have been related to several long-term outcomes, such as neurodevelopment, metabolic problems, and cardiovascular disease [10]. In this thesis, studies that aimed to investigate the effects of prenatal growth, early postnatal growth, and postdischarge nutrition on growth, body composition, and bone accretion of preterm infants during the first six months post-term were described. In this chapter, the results obtained by these studies are discussed and these may contribute to necessary recommendations for preterm infants with regard to nutrition and growth for the coming future.

PART I. PRENATAL AND POSTNATAL GROWTH OF PRETERM INFANTS

Postnatal growth regulation of preterm infants

There is increasing evidence that postnatal growth of preterm infants is, among other factors, regulated by IGF-I, insulin, and nutrition, similar to the regulation of fetal growth during the second half of pregnancy [11]. Several studies that focused on the role of IGF-I, insulin, and nutrition in postnatal growth of term infants demonstrate that growth depends on nutrition during the first half of infancy and on growth hormone (GH) thereafter [12]. This thesis demonstrated that IGF-I and insulin are important for growth regulation of preterm infants during the first half of infancy [Chapter 3]. The hepatic production of IGF-I is increased by the nutrient supply and its resultant insulin secretion [13]. This indicates that growth is enhanced through higher IGF-I and insulin secretion that is achieved by nutrition. In Chapter 3, the associations between serum IGF-I and insulin might suggest that growth regulation of preterm infants is nutrition dependent at least until three months post-term, since it has been stated that these hormones are regulated by the nutrient supply [13]. Between three and six months post-term, a shift towards GH dependency occurs that, in contrast to the first three months post-term, may not be strongly affected by nutritional intake [11]. This finding may support previous studies suggesting that the critical window for nutritional interventions to affect

long-term growth and body composition of preterm infants may be early in life, in particular during the first three months of life [14-18].

As opposed to the aforementioned relation between IGF-I and nutrient supply, controversy exists on the associations between nutrient intake, in particular protein intake, and IGF-I during infancy. Some studies show that IGF-I is related to nutrient intake in preterm infants before term age [19] and in term infants during late infancy [20], whereas others did not find any association in term infants [21]. In this thesis, nutrient intake was not directly associated with insulin or IGF-I concentrations during the first half of infancy [Chapter 3]. This contradicts the previous suggestion that growth regulation of preterm infants is nutrition dependent during the first six months post-term. It may be hypothesized that the postnatal growth pattern and its regulating hormones are determined prenatally. Another explanation may be that, in the present study, preterm infants had such cumulative nutritional deficits that a positive effect on growth could not be reached. These cumulative nutritional deficits frequently develop early in life as a result of a mismatch between increased nutritional demands due to postnatal morbidity and limited feeding possibilities in preterm infants. It may be hypothesized that these nutritional deficits need to be corrected before nutrient intake can affect IGF-I and insulin secretion and function. Therefore, we can only suggest that with the post-term nutritional regimen used in the randomized controlled trial nutrient intake does not influence IGF-I or insulin during the first half of infancy [Chapter 3].

Intrauterine and extrauterine growth restriction (IUGR and EUGR): growth, body composition and bone accretion

Preterm infants that are small-for-gestational-age (SGA) at birth may have either experienced intrauterine growth restriction (IUGR) due to fetal, maternal, or placental morbidity or may be genetically small [22]. SGA preterm infants with IUGR can demonstrate accelerated growth during infancy, defined as upward centile crossing (>0.67 SDS) on a growth reference chart, to achieve their genetic growth potential, whereas SGA preterm infants that are genetically small do not. In addition, extrauterine growth restriction (EUGR) before term age (early EUGR), as a result of a mismatch between nutritional demand and supply, is also associated with accelerated growth during infancy. Accelerated growth during infancy is associated with an increased risk of metabolic and cardiovascular disease in later life. Furthermore, decreased intrauterine growth in preterm infants with IUGR and decreased early postnatal growth in preterm infants with early EUGR do not only result in a lower weight and length but also in lower bone mass, which needs to be compensated during the phase of increased growth. This indicates that the prenatal and early postnatal growth trajectories are important for growth, body composition, and bone accretion that are achieved during the first half of infancy and may, among other factors, be determined by the fetal and postnatal nutrient supply.

Similar to Roggero et al. [23], this thesis showed that SGA preterm infants and preterm infants with early EUGR remained smaller during the first six months post-term, despite increased relative growth and higher energy and macronutrient intake [Chapter 4]. With respect to body composition, this thesis demonstrated that the higher relative weight gain of SGA preterm infants and preterm infants with early EUGR consisted of higher gain in lean mass and lower gain in fat mass, resulting in a lower percent body fat during the first six months post-term. The increased relative growth of SGA preterm infants and preterm infants with early EUGR suggests that they did not achieve their full genetic growth potential during the intrauterine and early extrauterine period. Surprisingly, as mentioned before, this accelerated growth was not accompanied by increased adiposity in these infants, which may favor their metabolic health in the coming future [24]. For now, it is only speculative whether these infants are also at lower risk of cardiovascular and metabolic disease in later life.

The SGA classification used in this thesis is based on birth weight and birth length, which are indirect markers of the prenatal growth trajectory [25], and relates the size of the infant to the general population. It does not reflect the actual intrauterine growth pattern and cannot differentiate between SGA preterm infants with IUGR and SGA preterm infants that are genetically small. Serial ultrasonographic evaluation of intrauterine growth is the only method than can be used to differentiate SGA preterm infants with IUGR from SGA preterm infants that are genetically small.

An explanation for the lack of adiposity in SGA preterm infants and preterm infants with early EUGR may be that the restoration of fat mass takes longer than the study period of the randomized controlled trial and might require a higher energy intake compared to lean mass restoration [26]. Moreover, it is possible that fat mass of these infants further increases after the study period due to nutritional intervention. Other studies show that fat accumulation of preterm infants during the first two years of life [27-28] may track to increased adiposity at adult age [29-30] and that this is associated with risk of cardiovascular disease in later life [31]. Therefore, the optimal nutritional requirements of SGA preterm infants and preterm infants with early EUGR during the first half of infancy to achieve an adequate body composition without excessive fat mass have to be elucidated.

Increased relative growth of SGA preterm infants was accompanied by increased collagen type I synthesis during the first six months post-term [Chapter 6], which may indicate an increase in skeletal size. In SGA preterm infants, gain in bone area (BA) was indeed slightly but not significantly higher [Chapter 5], which may suggest that these infants have increased bone matrix formation that leads to increased skeletal size [32-33]. However, increased collagen type I synthesis was not accompanied by higher gain in bone mineral content (BMC) in SGA preterm infants during the first six months post-term [Chapter 5 and 6]. This indicates that, although bone matrix formation is

increased, deposition of mineral in the bone matrix is diminished and/or delayed. It may be hypothesized that SGA preterm infants benefit from increased protein, calcium, phosphorus, and vitamin D intake to improve bone accretion and mineralization during the first six months post-term. As lower bone mass of SGA preterm infants at birth [34] and at term age [35] is a risk factor for osteoporosis in later life [36-39], it is important to stimulate the postnatal bone accretion with nutritional interventions, as supported by previous studies in preterm subjects [40-42].

The smaller body size, lower fat deposition, and lower bone accretion during the first half of infancy in SGA preterm infants and preterm infants with early EUGR may imply that the prenatal and early postnatal trajectory are dominant over any postnatal nutritional intervention during the first half of infancy. On the other hand, it emphasizes the need for an adequate nutrient supply. Although it has been stated that enriched formulae and human milk fortification are effective strategies to address nutritional deficits and poor growth of preterm infants early after discharge [43], the exact nutritional intake that is required is unknown and the current nutritional guidelines are not adapted to the growth pattern until term age [44]. Furthermore, special interest should be addressed to the nutritional intake before term age to prevent that cumulative nutritional deficits develop in early life. To date, however, these early nutritional deficits cannot be fully prevented [43], which emphasizes the need for specialized enriched nutrition after term age.

PART II. POSTDISCHARGE NUTRITION

Nutrition is important for postnatal growth, body composition, and bone accretion. Preterm infants remain smaller [45-48] and have a suboptimal bone mass [49-52] throughout infancy, childhood, and adolescence compared to term infants. This may be a consequence of inadequate growth and bone accretion due to cumulative nutritional deficits before term age. These nutritional deficits underline the importance of an adequate nutritional regimen after term age, which may be achieved with postdischarge formula. Since the first publication on postdischarge formula in 1992 by Lucas et al. [53], several studies report that postdischarge formulae enriched with energy, protein, and minerals enhance growth [53-59] and bone accretion [58, 60-61] of preterm infants during the first year of life. Therefore, specialized postdischarge formula is now recommended for all preterm infants, if human milk is not available [44, 62].

The effect of postdischarge formula on growth and body composition

The debate continues on the effect of postdischarge formulae on growth and body composition of preterm infants. Some studies on postdischarge formulae with a high energy (>72 kcal/100 ml) and a high protein content (>1.7 g/100 ml) show no clear

benefit on growth and body composition [23, 63-65], whereas others report increased fat accretion during the first year of life [58, 66], which may be directly related to the higher energy intake [67-68]. The present study used a postdischarge formula (PDF) that was isocaloric but high in protein, minerals, vitamin D, and LC-PUFAs [Chapter 2], in accordance with ESPGHAN guidelines for postdischarge formulae [44]. This PDF resulted in similar growth, lower fat deposition, and higher bone accretion during the first six months post-term compared to standard term formula [Chapter 2, 7, 8]. In more detail, the higher protein availability from this isocaloric PDF may explain the similar growth with lower fat deposition [Chapter 2]. It may be hypothesized that this reduced infant fat accumulation tracks to adulthood [31, 69] and so reduces the risk of cardiovascular and metabolic diseases.

Growth of preterm infants fed the isocaloric PDF used in the present study may be further promoted by long-chain polyunsaturated fatty acids (LC-PUFAs). Preterm infants have a lower docosahexaenoic acid (DHA) and arachidonic acid (AA) status at birth and require an adequate dietary LC-PUFA intake to prevent postnatal deficits, which have been associated with adverse effects on growth and central nervous system development. Previous studies in preterm infants report that DHA and AA supplementation of preterm formula enhances growth up to two months postnatal age [70] but not thereafter [71-72]. We found that higher RBC-DHA was associated with increased gain in weight and length and decreased gain in head circumference and that higher RBC-AA was associated with increased gain in head circumference and decreased gain in weight and length [Chapter 8]. Increased head growth within 2 standard deviations of the mean may prevent an adverse neurodevelopmental outcome [73-74]. Moreover, the results described in this thesis suggests that the dietary supply of DHA and AA from PDF, resulting in a DHA and AA status within the range of the targets of human milk fed term infants, is associated with balanced growth of preterm infants [Chapter 8].

The effect of postdischarge formula on bone accretion

The combined higher protein, calcium, phosphorus, and vitamin D intake from PDF may attribute to higher collagen type I synthesis [75-76] and bone accretion [77] due to higher bone matrix formation combined with higher bone mineralization. In this thesis, the higher gain in bone mineral content of PDF compared to TF fed preterm infants was explained by the slightly higher increase in skeletal size in PDF fed preterm infants, whereas the difference with HM fed preterm infants was explained by higher bone mineral deposition in PDF fed preterm infants [Chapter 7]. Furthermore, higher vitamin D intake from PDF resulted in a higher increase in serum 25-hydroxyvitamin D concentrations, which was related to a higher increase in bone mineral content [Chapter 9]. It may be speculated that since a higher infant bone mineral content may track to adulthood [36-37], PDF fed preterm infants may have a lower risk of osteoporosis in later life [39].

Nevertheless, although the results of the randomized controlled trial suggest that an isocaloric, high-protein, mineral-enriched postdischarge formula improves quality of growth as well as bone accretion of preterm infants during the first six months post-term, there is an urgent need to investigate these long-term effects in the coming future.

Postdischarge nutrition and iron

Another micronutrient that is important for preterm infants is iron. Iron deficiency has been related to anemia, growth faltering, adverse neurodevelopmental outcome, and an impaired immune status [78-80]. As iron deficiency is frequently not accompanied by anemia, a biochemical marker of iron deficiency, such as ferritin [81], should be preferred over hemoglobin and hematocrit as an indicator of iron deficiency [Chapter 10]. In this thesis, we demonstrated that, at six months post-term, the incidence of iron deficiency (ferritin <12 µg/l) was higher in preterm infants fed human milk compared to those fed iron-fortified formulae [PDF (1.0 mg iron/100 ml) and TF (0.8 mg iron/100 ml)] [Chapter 10]. This may be explained by the finding that the number of human milk fed infants that were iron supplemented decreased rapidly during the first six months post-term, especially after three months post-term. The decision on iron supplementation may reflect the pediatric practice in The Netherlands during the study period, which was not in line with the current Dutch recommendations of 2-3 mg/kg/d iron supplementation between two and six to twelve months of age in preterm infants fed human milk, if their birth weight is below 2,000 gram [82-83]. Furthermore, it was stated that preterm infants fed iron-fortified formulae do not need additional enteral iron supplementation [82-83]. The finding that human milk fed preterm infants were poorly supplemented with iron during the first six months of life may underline that the importance of iron supplementation in these infants needs to be brought to the attention of pediatricians in The Netherlands.

Preterm infants fed iron-fortified formulae with 0.8-1.0 mg iron/100 ml may not need additional iron supplementation, as also suggested by the Dutch guidelines on iron supplementation [82-83]. In the present study, these infants achieve ferritin concentrations above 12 µg/l between three and six months post-term without additional iron supplementation, which implies that the iron intake from the iron-fortified formulae prevents iron deficiency between three and six months post-term [Chapter 10]. However, many iron-fortified formula fed preterm infants in our study were iron supplemented between term age and three months post-term and, therefore, we cannot state that iron-fortified formulae also prevent iron deficiency between term age and three months post-term.

METHODOLOGICAL CONSIDERATIONS

This thesis was based on a randomized controlled trial that was designed to evaluate the effects of postdischarge nutrition on growth, body composition, and nutritional status of preterm infants during the first six months post-term. Although all analyses presented in this thesis were adjusted for the type of diet (i.e. PDF, TF, or HM), influence of the type of diet on the demonstrated associations cannot be excluded. In addition, only associations and no causal relations can be established. Unfortunately, body composition, laboratory parameters, and bone parameters were not measured at birth.

This thesis presents differences in growth, body composition, and bone accretion between SGA preterm infants, AGA preterm infants, and preterm infants with early EUGR. These analyses were based on post-hoc analyses, as they were not part of the original randomized controlled trial. This should be taken into account when the results of these analyses are interpreted.

Controversy exists on which growth references should be used to define 'normal' or 'adequate' growth of preterm infants. Growth references are based on intrauterine growth reflected by size at birth [84], on postnatal growth of term infants [85], or on postnatal growth of preterm infants [86]. Growth references that are based on cross-sectional birth data reflect the intrauterine condition of a reference population that cannot be compared to preterm infants [22, 86]. Growth references that are based on postnatal growth of term infants require adjustment for prematurity, although postnatal growth of term infants cannot be compared to that of preterm infants [86]. Furthermore, growth references that are based on postnatal growth of preterm infants [86] imminently reflect postnatal growth restriction of preterm infants [22]. Although individualized growth references based on the in utero growth pattern are preferred to evaluate postnatal growth of preterm infants [22], these references require ultrasonographic measurements of fetal growth. The present study used growth references based on cross-sectional birth data of preterm infants until term age [84] and growth references based on growth of Dutch term infants thereafter, adjusted for postmenstrual age [85].

The present study used dual-energy x-ray absorptiometry (DXA) to determine body composition and bone accretion, which is sensitive to movement artifacts. Therefore, an experienced research nurse ensured the adequate position of the infants. It is difficult to compare DXA measurements of the present study to that measured in previous studies. Previous studies used different methods (e.g. air displacement plethysmography (PEAPOD), bioelectrical impedance analysis, single-photon absorptiometry, or DXA), or a different DXA model (e.g. Hologic or Lunar), type, or software [87]. As a consequence, comparisons of body composition and bone accretion between the present study and previous studies need to be interpreted with caution.

GENERAL CONCLUSIONS

This thesis shows that the prenatal and early postnatal trajectory are important for growth, body composition, and bone accretion of preterm infants, with an important growth regulating role for the endocrine factors IGF-I and insulin during the first half of infancy. Preterm infants born SGA or those with early EUGR remain smaller with lower fat mass and bone mass, despite higher nutritional intake during the first six months post-term, and these infants may benefit from even higher nutritional intakes to attain a fat mass and bone mass similar to AGA preterm infants. On the other hand, with respect to fat mass, it might be hypothesized that the lower fat accumulation in SGA preterm infants and preterm infants with early EUGR may be appropriate for their body size.

In addition, this thesis demonstrates that preterm infants benefit from an isocaloric, protein- and mineral-enriched postdischarge formula with regard to their quantity and quality of growth, bone accretion, and vitamin D status during the first six months post-term. In particular, preterm infants may benefit from this postdischarge formula because it is not energy-enriched and results in lower fat accumulation, which may be associated with a lower risk of adiposity and concomitant metabolic and cardiovascular consequences in later life.

Finally, iron deficiency is more common in preterm infants fed human milk compared to those fed iron-fortified formulae during the first half of infancy. This may be related to the lower frequency of iron supplementation in human milk fed preterm infants. Dutch guidelines on iron supplementation for preterm infants, similar to those described by Lafeber et al. [83], are required for the daily pediatric practice. In addition, the findings of this thesis may indicate that iron-fortified formulae prevent iron deficiency between three and six months post-term.

FUTURE RESEARCH

In the present study, preterm infants frequently experienced nutritional deficits before term age. Future studies should aim to improve the nutritional intake between birth and term age in order to reduce nutritional deficits and growth faltering before term age. This may be achieved by high protein administration early after birth [88-90]. We hypothesize that with an optimal nutritional regimen before term age, preterm infants may benefit even more from postdischarge formula because the higher nutrient supply from postdischarge formula is no longer needed to compensate for the nutritional deficits that developed before term age and can be fully utilized to improve growth, body composition, and bone accretion after term age. However, the effect of higher nutritional intake before term age as well as the additional effect of postdischarge

formula after term age on the quality and quantity of growth need to be investigated. These increased nutritional intakes may result in accelerated growth, in particular in SGA preterm infants and preterm infants with some degree of early EUGR, followed by metabolic and cardiovascular consequences in later life.

Overall, although postdischarge formula has short-term benefits during infancy, the long-term outcomes with respect to growth, body composition, bone accretion and cardio-metabolic consequences in preterm infants fed this formula need to be further investigated. A follow-up study of the randomized controlled trial described in this thesis (STEP 2) is currently in progress and evaluates growth, body composition, bone accretion, and metabolic health during childhood of preterm infants fed postdischarge formula, standard term formula, or human milk. Based on the benefits of the isocaloric, protein- and mineral-enriched postdischarge formula for body composition and bone accretion during the first half of infancy, it was hypothesized that preterm infants fed this formula have less fat mass, higher bone mineralization, lower blood pressure, and better metabolic health at 7 years of age compared to preterm infants fed standard term formula. Based on this hypothesis, growth, body composition and bone mass (both measured by DXA), blood pressure, and several biochemical parameters (e.g. insulin, glucose, IGF-I, cholesterol, and 25-hydroxyvitamin D) are evaluated in participants of the STEP at 7 years.

Moreover, it was hypothesized that less fat mass during childhood may lead to less adiposity during adulthood, which may decrease the risk of metabolic and cardiovascular consequences, such as diabetes, hypertension, and cardiovascular disease. To address this hypothesis, another follow-up study of the STEP cohort needs to be conducted at adult age. This study is expected to yield important results with respect to the long-term metabolic and cardiovascular effects of the early intervention with postdischarge formula and these may contribute to the establishment of optimal nutritional management of preterm infants in early life.

REFERENCES

1. Martin J, Hamilton B, Sutton P, Ventura S, Menacker F, Kirmeyer S. Births: final data for 2004. Natl Vital Stat Rep. 2006;55:1-101.
2. Joseph K, Kramer M, Allen A, Cyr M, Fair M, Ohlsson A, Wen S. Gestational age- and birthweight-specific declines in infant mortality in Canada, 1985-94. Fetal and Infant Health Study Group of the Canadian Perinatal Surveillance System. Paediatr Perinatal Epidemiol. 2000;14:332-339.
3. Stichting Perinatale Registratie Nederland. Perinatal Care in the Netherlands 2008 [In Dutch: Perinatal Zorg in Nederland 2008]. 2011.
4. Verloove-Vanhorick S, Verwey R, Brand R, Bennebroek Gravenhorst J, Keirse M, Ruys J. Neonatal mortality risk in relation to gestational age and birth weight. Result of a national survey of preterm and very-low-birthweight infants in The Netherlands. Lancet. 1986;333:55-57.
5. Walther F, den Ouden A, Verloove-Vanhorick S. Looking back in time: outcome of a national cohort of very preterm infants born in The Netherlands in 1983. Early Hum Dev. 2000;59:175-191.
6. Stoelhorst G, Rijken M, Martens S, Brand R, den Ouden A. Changes in neonatology: comparison of two cohorts of very preterm infants (gestational age <32 weeks): the Project on Preterm and Small for Gestational Age Infants 1983 and the Leiden Follow-up Project on Prematurity 1996-1997. Pediatrics. 2005;115:396-405.
7. Goldenberg R, Rouse D. Prevention of preterm birth. N Eng J Med. 1998;339:313-320.
8. Wen S, Smith G, Yang Q, Walker M. Epidemiology of preterm birth and neonatal outcome. Semin Fetal Neonatal Med. 2004;9:429-435.
9. Sullivan M, Hawes K. A decade comparison of preterm motor performance at age 4. Res Nurs Health. 2007;30:641-654.
10. Lucas A. Programming by early nutrition in man. Ciba Found Symp. 1991:38-55.
11. Fowden A. The insulin-like growth factors and feto-placental growth. Placenta. 2003;24:803-812.
12. Gluckman P, Pinal C. Regulation of fetal growth by the somatotrophic axis. J Nutr. 2003;133:1741S-1746S.
13. Gluckman P. Endocrine and nutritional regulation of prenatal growth. Acta Paediatr Suppl. 1997;423:153-157.
14. Singhal A, Cole T, Fewtrell M, Deanfield J, Lucas A. Is slower early growth beneficial for long-term cardiovascular health. Circulation. 2004:1108-1113.
15. Euser A, Finken M, Keijzer-Veen M, Hille E, Wit J, Dekker F. Associations between prenatal and infancy weight gain and BMI, fat mass, and fat distribution in young adulthood: a prospective cohort study in males and females born very preterm. Am J Clin Nutr. 2005;81:480-487.
16. Kerkhof G, Leunissen R, Hokken-Koelega A. Early origins of metabolic syndrome: role of small size at birth, early postnatal weight gain, and adult IGF-I. J Clin Endocrinol Metab. 2012;97:2637-2643.
17. Bazaes R, Alegria A, Pittaluga E, Avila A, Iñiguez G. Determinants of insulin sensitivity and secretion in very-low-birth-weight children. J Clin Endocrinol Metab. 2004;89:1267-1272.
18. Finken M, Keijzer-Veen M, Dekker F, Fröhlich M, Hilke E. Preterm birth and later insulin resistance: effects of birth weight and postnatal growth in a population based longitudinal study from birth into adult life. Diabetologia. 2006;49:478-485.
19. Engström E, Niklasson A, Albertsson-Wikland K, Ewald U, Hellström A. The role of maternal factors, postnatal nutrition, weight gain and gender in regulations of serum IGF-1 among preterm infants. Pediatr Res. 2005;57:605-610.
20. Larnkjaer A, Hoppe C, Mølgaard A, Michaelsen K. The effect of whole milk and infant formula on growth and IGF-1 in late infancy. Eur J Clin Nutr. 2009;63:956-963.

21. Madsen A, Larnkjaer A, Mølgaard C, Michaelsen K. IGF-I and IGFBP-3 in healthy 9 month old infants from the SKOT cohort: breastfeeding, diet, and later obesity. Growth Hormone & IGF Research. 2011;21:199-204.
22. Gardosi J. New definition of small for gestational age based on fetal growth potential. Horm Res. 2006;65:15-18.
23. Roggero P, Giannì M, Amato O, Liotto N, Morlacchi L, Orsi A, Piemontese P, Taroni F, Morniroli D, et al. Growth and fat-free mass gain in preterm infants after discharge: a randomized controlled trial. Pediatrics. 2012;130:e1215.
24. Milovanovic I, Njuieyon F, Deghmoun S, Chevenne D, Levy-Marchal C, Beltrand J. Innate small babies are metabolically healthy children. J Clin Endocrinol Metab. 2012;97:4407-4413.
25. Gluckman P, Hanson M. The consequences of being born small-An adaptive perspective. Horm Res. 2006;65:5-14.
26. Roberts S, Young V. Energy costs of fat and protein deposition in the human infant. Am J Clin Nutr. 1988;48:951-955.
27. Roggero P, Giannì M, Liotto N, Taroni F, Orsi A, Amato O, Morlacchi L, Piemontese P, Agosti M, Mosca F. Rapid recovery of fat mass in small for gestational age preterm infants after term. PLoS ONE. 2011;6:e14489.
28. Hernandez M, Rossel K, Peña V, Cavada G, Avila A, Iñiguez G, Mericq V. Leptin and IGF-I/II during the first weeks of life determine body composition at 2 years in infants born with very low birth weight. J Pediatr Endocrinol Metab. 2012;25:951-955.
29. Meas T, Deghmoun S, Alberti C, Carreira E, Armoogum P, Chevenne D, Lévy-Marchal C. Independent effects of weight gain and fetal programming on metabolic complications in adults born small for gestational age. Diabetologia. 2010;53:907-913.
30. Meas T, Deghmoun S, Armoogum P, Alberti C, Lévy-Marchal C. Consequences of being born small for gestational age on body composition: an 8-year follow-up study. J Clin Endocrinol Metab. 2008;93:3804-3809.
31. Howe L, Tilling K, Benfield L, Logue J, Sattar N, Ness A, Smith G, Lawlor D. Changes in ponderal index and body mass index across childhood and their associations with fat mass and cardiovascular risk factors at age 15. PLoS ONE. 2010;5:e15186.
32. Kajantie E, Dunkel L, Risteli J, Pohjavuori M, Andersson S. Markers of type I and III collagen turnover as indicators of growth velocity in very low birth weight infants. J Clin Endocrinol Metab. 2001;86:4299-4306.
33. Nakano K, Iwamatsu T, Wang C, Tarasima M, Nakayama T, Sasaki K, Tachikawa E, Noda N, Mizoguchi E, Osawa M. High bone turnover of type I collagen depends on fetal growth. Bone. 2006;38:249-256.
34. Petersen S, Gotfredsen A, Knudsen F. Total body bone mineral in light-for-gestational-age infants and appropriate-for-gestational-age infants. Acta Paediatr Scand. 1989;78:347-350.
35. De Schepper J, Cools F, Vandenplas Y, Louis O. Whole body bone mineral content is similar at discharge from the hospital in premature infants receiving fortified breast milk or preterm formula. J Pediatr Gastroenterol Nutr. 2005;41:230-234.
36. Fewtrell M, Williams J, Singhal A, Murgatroyd P, Fuller N. Early diet and peak bone mass: 20 year follow up of a randomized trial of early diet in infants born preterm. Bone. 2009;45:142-149.
37. Smith C, Wright N, Wales J, Mackenzie C, Primhak R, Eastell R, Walsh J. Very low birth weight survivors have reduced peak bone mass and reduced insulin sensitivity. Clin Endocrinol. 2011;75:443-449.

38. Dennison E, Syddall H, Sayer A, Gilbody H, Cooper C. Birth weight and weight at 1 year are independent determinants of bone mass in the seventh decade: the Hertfordshire Cohort Study. Pediatr Res. 2005;57:582-586.
39. Hernandez C, Beaupré G, Carter D. A theoretical analysis of the relative influences of peak BMD, age-related bone loss and menopause on the development of osteoporosis. Osteoporos Int. 2003;14:843-847.
40. Fewtrell M, Prentice A, Jones S, Bishop N, Stirling D. Bone mineralization and turnover in preterm infants at 8-12 years of age: the effect of early diet. J Bone Miner Res. 1999;14:810-820.
41. Weiler H, Yuen C, Seshia M. Growth and bone mineralization of young adults weighing less than 1500 g at birth. Early Hum Dev. 2002;67:101-112.
42. Kurl S, Heinonen K, Länsimies E, Launiala K. Determinants of bone mineral density in prematurely born children aged 6-7 years. Acta Paediatr. 1998;87:650-653.
43. Lapillonne A, O'Connor D, Wang D, Rigo J. Nutritional recommendations for the late-preterm infant and the preterm infant after hospital discharge. J Pediatr. 2013;162:S90-S100.
44. Aggett P, Agostoni C, Axelsson I, DeCurtis M, Goulet O. Feeding preterm infants after hospital discharge. J Pediatr Gastroenterol Nutr. 2006;42:596-603.
45. Hack M, Schluchter M, Cartar L, Rahman M, Cuttler L. Growth of very low birth weight infants to age 20 years. Pediatrics. 2003;112:e30-e38.
46. Ford G, Doyle L, Davis N, Callanan C. Very low birth weight and growth into adolescence. Arch Pediatr Adolesc Med. 2000;154:778-784.
47. Carver J. Nutrition for preterm infants after hospital discharge. Adv Pediatr. 2005;52:23-47.
48. Hovi P, Andersson S, Eriksson J, Järvenpää A, Strang-Karlsson S. Glucose regulation in young adults with very low birth weight. N Eng J Med. 2007;356:2053-2063.
49. Avila-Díaz M, Flores-Huerta S, Martínez-Muñiz I, Amato D. Increments in whole body bone mineral content associated with weight and length in pre-term and full-term infants during the first 6 months of life. Arch Med Res. 2001;32:288-292.
50. Kurl S, Heinonen K, Länsimies E. Effects of prematurity, intrauterine growth status, and early dexamethasone treatment on postnatal bone mineralisation. Arch Dis Child Fetal Neonatal Ed. 2000;83:F109-F111.
51. Bowden L, Jones C, Ryan S. Bone mineralisation in ex-preterm infants aged 8 years. Eur J Pediatr. 1999;158:658-661.
52. Fewtrell M, Prentice A, Cole T, Lucas A. Effects of growth during infancy and childhood on bone mineralization and turnover in preterm children aged 8-12 years. Acta Paediatr. 2000;89:148-153.
53. Lucas A, Bishop N, King F, Cole T. Randomised trial of nutrition for preterm infants after discharge. Arch Dis Child. 1992;67:324-327.
54. Lucas A, Fewtrell M, Morley R, Singhal A, Abbott R. Randomized trial of nutrient-enriched formula versus standard formula for postdischarge preterm infants. Pediatrics. 2001;108:703-711.
55. Cooke R, Embleton N, Griffin I, Wells J, McCormick K. Feeding preterm infants after hospital discharge: growth and development at 18 months of age. Pediatr Res. 2001;49:719-722.
56. Agosti M, Vegni C, Calciolari G, Marini A. Post-discharge nutrition of the very low-birth weight infant: interim results of the multicentric GAMMA study. Acta Paediatr Suppl. 2003;441:39-43.
57. Cooke R, Griffin I, McCormick K, Wells J, Smith J, Robinson S, Leighton M. Feeding preterm infants after hospital discharge: effect of dietary manipulation on nutrient intake and growth. Pediatr Res. 1998;43:355-360.

58. Picaud J, Decullier E, Plan O, Pidoux O, Bin-Dorel S, van Egroo L, Chapuis F, Claris O. Growth and bone mineralization in preterm infants fed preterm formula or standard term formula after discharge. J Pediatr. 2008;153:616-621.
59. Jeon G, Jung Y, Koh S, Lee Y, Kim K, Shin S, Kim S, Shim J, Chang Y, Park W. Preterm infants fed nutrient-enriched formula until 6 months show improved growth and development. Pediatr Int. 2011;53:683-688.
60. Bishop N, King F, Lucas A. Increased bone mineral content of preterm infants fed with nutrient enriched formula after discharge from hospital. Arch Dis Child. 1993;68:573-578.
61. Cooke R, McCormick K, Griffin I, Embleton N, Faulkner K. Feeding preterm infants after hospital discharge: effect of diet on body composition. Pediatr Res. 1999;46:461-465.
62. Tudehope D, Page D, Gilroy M. Infant formulas for preterm infants: in-hospital and post-discharge. J Paediatr Child Health. 2012;48:768-776.
63. De Curtis M, Pieltain C, Rigo J. Body composition in preterm infants fed standard term or enriched formula after hospital discharge. Eur J Nutr. 2002;41:177-182.
64. Taroni E, Liotto N, Orsi A, Piemontese P, Amato O, Morlacchi L, Mosca F. [Quality of post-discharge growth in small for gestational age preterm infants: an explorative study]. Pediatr Med Chir. 2009;31:121-125.
65. Young L, Morgan J, McCormick F, McGuire W. Nutrient-enriched formula versus standard term formula for preterm infants following hospital discharge. Cochrane Database of Syst Rev. 2012;14:CD004696.
66. Cooke R, Griffin I, McCormick K. Adiposity is not altered in preterm infants fed a nutrient-enriched formula after hospital discharge. Pediatr Res. 2010;67:660-664.
67. Kashyap S, Schulze K, Forsyth M, Zucker C, Dell R, Ramakrishnan R, Heird W. Growth, nutrient retention, and metabolic response in low birth weight infants fed varying intakes of protein and energy. J Pediatr. 1988;113:713-721.
68. Romera G, Figueras J, Rodríguez-Miquélez J, Ortega J, Jiménez R. Energy intake, metabolic balance and growth in preterm infants fed formulas with different nonprotein energy supplements. J Pediatr Gastroenterol Nutr. 2004;38:407-413.
69. Cheng S, Völgyi E, Tylavsky F, Lyytikäinen A, Törmäkangas T, Xu L, Cheng S, Kröger H, Alèn M, Kujala U. Trait-specific tracking and determinants of body composition: a 7-year follow-up study of pubertal growth in girls. BMC Med. 2009;7:5.
70. Woltil H, van Beusekom C, Schaafsma A, Muskiet F, Okken A. Long-chain polyunsaturated fatty acid status and early growth of low birth weight infants. Eur J Pediatr. 1998;157:146-152.
71. Makrides M, Gibson R, Udell T, Ried K, International LCPUFA Investigators. Supplementation of infant formula with long-chain polyunsaturated fatty acids does not influence the growth of term infants. Am J Clin Nutr. 2005;81:1094-1101.
72. Schulzke S, Patole S, Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. Cochrane Database Syst Rev. 2011;Feb 16(2):CD000375.
73. Latal-Hajnal B, Von Siebenthal K, Kovari H, Bucher HU, Largo RH. Postnatal growth in very low birth weight infants: significant association with neurodevelopmental outcome. J Pediatr. 2003;143:163-170.
74. Brandt I, Sticker E, Lentze M. Catch up growth of head circumference of very low birth weight, small for gestational age preterm infants and mental development to adulthood. J Pediatr. 2003;142:463-468.

75. Shiff Y, Eliakim A, Shainkin-Kestenbaum R, Arnon S, Lis M, Dolfin T. Measurements of bone turnover markers in premature infants. J Pediatr Endocrinol Metab. 2001;14:389-395.
76. Mølgaard C, Thomsen B, Prentice A. Whole body bone mineral content in healthy children and adolescents. Arch Dis Child. 1997;76:9-15.
77. Rigo J, De Curtis M, Pieltain C, Picaud J, Salle B, Senterre J. Bone mineral metabolism in the micropremie. Clin Perinatol. 2000;27:147-165.
78. Long H, Yi J, Hu P, Li Z, Qiu W, Wang F, Zhu S. Benefits of iron supplementation for low birth weight infants: a systematic review. BMC Pediatr. 2012;12:99.
79. Yadav D, Chandra J. Iron deficiency: beyond anemia. Indian J Pediatr. 2011;78:65-72.
80. Aggett P, Agostoni C, Axelsson I, Bresson J, Goulet O. Iron metabolism and requirements in early childhood: do we know enough?: a commentary by the ESPGHAN Committee on Nutrition. J Pediatr. 2002;34:337-345.
81. Domellöf M. Iron requirements, absorption and metabolism in infancy and childhood. Curr Opin Clin Nutr Metab Care. 2007;10:329-335.
82. Lafeber H, van Zoeren-Grobben D, Van Beek R, Gerards L. Enteral and parenteral nutrition in newborns (In Dutch: Werkboek enterale en parenterale voeding bij pasgeborenen). 2nd ed. Amsterdam: VU; 2004.
83. Lafeber H, van Zoeren-Grobben D, Van Beek R, Chrisman V. Enteral and parenteral nutrition in newborns [In Dutch: Werkboek enterale en parenterale voeding bij de pasgeborene]. Amsterdam: VU Uitgeverij; 2012.
84. Niklasson A, Albertsson-Wikland K. Continous growth reference from 24th weeks of gestation to 24 months by gender. BMC Pediatrics. 2008;8:8.
85. Fredriks A, van Buuren S, Burgmeijer R, Meulmeester J, Beuker R, Brugman E, Roede M, Verloove-Vanhorick S, Wit J. Continuing positive secular growth change in the Netherlands 1955-1997. Pediatr Res. 2000;47:316-323.
86. Bocca-Tjeertes I, Van Buuren S, Bos A, Kerstjens J, Ten Vergert E, Reijneveld S. Growth of preterm and full-term children aged 0-4 years: integrating median growth and variability in growth charts. J Pediatr. 2012;161:460-465.
87. Picaud J, Lapillonne A, Pieltain C, Reygrobellet B, Claris O, Salle B, Rigo J. Software and scan acquisition technique-related discrepancies in bone mineral assessment using dual-energy x-ray absorptiometry in neonates. Acta Paediatr. 2002;91:1189-1193.
88. Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and improvement of growth in extremely preterm infants. Acta Paediatr. 2012;101:64-70.
89. Senterre T, Rigo J. Optimizing early nutritional support based on recent recommendations in VLBW infants and postnatal growth restriction. J Pediatr Gastroenterol Nutr. 2011;53:536-542.
90. Embleton N, Pang N, Cooke R. Postnatal malnutrition and growth retardation: an inevitable consequence of current recommendations in preterm infants? Pediatrics. 2001;107:270-273.

Chapter 12

Summary
Nederlandse samenvatting

SUMMARY

During the extrauterine third trimester, preterm infants develop nutritional deficits as postnatal morbidity increases nutritional demands, which are not reached because of limited feeding possibilities. Consequently, many preterm infants experience extrauterine growth restriction (EUGR) followed by accelerated growth. Nutritional deficits may also lead to decreased bone accretion, micronutrient deficiencies, and neurodevelopmental impairment. The quantity and quality of prenatal and postnatal growth of preterm infants are important with respect to metabolic and cardiovascular consequences in later life. In-hospital feeding regimens aim to prevent cumulative postnatal nutritional deficits in preterm infants. To date, however, in-hospital regimens cannot fully prevent cumulative nutritional deficits or EUGR in preterm infants. Therefore, specialized postdischarge nutrition has a key role in the nutritional management of preterm infants after discharge to improve growth and bone accretion and to limit fat accumulation, which is associated with long-term cardiovascular and metabolic consequences. The background of this thesis is further addressed in **Chapter 1**.

This thesis is based on a single-blinded randomized controlled trial in 152 preterm infants that compared the effects of an isocaloric, protein- and mineral enriched postdischarge formula (PDF), a standard term formula (TF), and human milk (HM) on growth, body composition, bone accretion, and micronutrient status between term age and six months corrected age (CA). **Chapter 2** describes the study design and the main results of the randomized controlled trial. In short, preterm infants fed PDF had higher protein intake and similar growth with less fat accumulation compared to those fed TF during the first half of infancy. This thesis evaluates the effects of prenatal growth, early postnatal growth, and postdischarge nutrition on growth, body composition, and bone accretion of preterm infants during the first half of infancy, as stated in **Chapter 2**.

The first part of this thesis focuses on postnatal growth regulation and on the effects of prenatal growth and early postnatal growth on body composition and bone accretion of preterm infants. **Chapter 3** demonstrates that insulin-like growth factor type I (IGF-I) and insulin are important for growth regulation of preterm infants during the first six months post-term, independent of nutritional intake. In addition, the lack of association between IGF-I and insulin at six months CA suggests that growth regulation shifts towards growth hormone (GH) dependency at six months CA due to a gradual maturation of the GH/IGF-I axis.

Prenatal growth and early postnatal growth are important for body composition and bone accretion. **Chapter 4** reports that small-for-gestational-age (SGA) preterm infants and preterm infants with EUGR before term age (early EUGR) remained smaller and had

a lower percentage of body fat during the first six months post-term, despite increased relative growth and higher energy and macronutrient intakes. It might be hypothesized that accelerated infant growth without increased adiposity favors later metabolic health. However, the optimal nutritional requirements of preterm infants needed to achieve accelerated growth without excessive fat deposition have to be elucidated.

Chapter 5 describes that SGA preterm infants had lower gain in bone mineral content (BMC) during the first six months post-term, independent of gain in body size. In addition, **Chapter 6** shows that SGA preterm infants had higher collagen type I synthesis during the first half of infancy, which may reflect increased relative growth. Although collagen type I is predominantly found in bone matrix, higher collagen type I synthesis did not lead to increased gain in BMC in SGA preterm infants. This may be explained by insufficient bone mineralization. Since the lower bone mass of SGA preterm infants is a risk factor for osteoporosis, it is important to focus on nutritional interventions, for example with calcium, phosphorus, and vitamin D, that may stimulate bone accretion in these infants.

The second part of this thesis focuses on the effects of postdischarge nutrition on bone accretion, growth, and micronutrient status. **Chapter 7** describes that PDF enhanced gain in BMC of preterm infants during the first six months post-term, independent of gain in weight and length. Higher BMC of preterm infants during infancy, as a result of PDF, may track to higher adult bone mass, which decreases the risk of osteoporosis in later life.

Chapter 8 relates growth of preterm infants to long-chain polyunsaturated fatty acid (LC-PUFA) levels, namely docosahexaenoic acid (DHA) and arachidonic acid (AA). During the first six months post-term, PDF fed preterm infants had higher red blood cell (RBC) DHA and AA concentrations. RBC-DHA was positively associated with gain in weight and length and negatively with gain in head circumference. RBC-AA was positively associated with gain in head circumference and negatively with gain in weight and length. These findings suggest that the dietary supply of DHA and AA by PDF, which was within the targets of term infants fed human milk, may be related to balanced growth of preterm infants.

Chapter 9 shows that, in preterm infants, higher vitamin D intake with an isocaloric, protein- and mineral-enriched PDF resulted in a greater increase in serum 25-hydroxyvitamin D during the first six months post-term. Increase in serum 25-hydroxyvitamin D was positively associated with gain in BMC. This may suggest that, in addition to the availability of dietary protein, calcium, and phosphorus, higher vitamin D intake contributes to increased bone accretion in PDF fed preterm infants.

Chapter 10 demonstrates that iron deficiency was more frequent in preterm infants fed HM compared to those fed iron-fortified formula at three and six months CA. This

may be due to early cessation of additional iron supplementation in HM fed preterm infants. This might suggest that the importance of iron supplementation for HM fed preterm infants during the first half of infancy needs to be brought to the attention of Dutch pediatricians. In accordance with Dutch guidelines, iron-fortified formula appears to suffice to prevent iron deficiency between three and six months CA.

Chapter 11 discusses the growth regulating role of the endocrine factors IGF-I and insulin as well as prenatal growth and early postnatal growth in relation to growth, body composition, and bone accretion of preterm infants during the first half of infancy. In addition, this chapter discusses the benefits of an isocaloric, protein- and mineral-enriched PDF with regard to quantity and quality of growth, bone accretion, LC-PUFA status, and vitamin D status during the first six months post-term. Finally, the implications of the incidence of iron deficiency of preterm infants fed HM and iron-fortified formulae with regard to iron supplementation are discussed. Several suggestions for future research are described with a special interest in the nutritional intake between birth and term age and the long-term outcomes of preterm infants fed the isocaloric, protein- and mineral-enriched PDF described in this thesis.

NEDERLANDSE SAMENVATTING

In vergelijking met op tijd geboren kinderen groeien te vroeg geboren kinderen (premature) na de geboorte minder goed. Dit wordt deels verklaard door de beperkte mogelijkheden om direct na de geboorte voldoende voedingsstoffen toe te dienen. Bovendien is de vraag naar voedingsstoffen dan juist verhoogd als gevolg van problemen die met de vroeggeboorte samenhangen, zoals infecties en ademhalingsproblemen. Hierdoor ontwikkelen veel premature direct na de geboorte een groeivertraging (extra-uteriene groeirestrictie), die vaak gevolgd wordt door een fase van versnelde groei na de uitgerekende (a terme) datum. Tot de gevolgen van het tekort aan voedingsstoffen behoren ook een vermindering van de botaanmaak, een tekort aan micronutriënten (vitamines en mineralen) en stoornissen in de ontwikkeling. De gevolgen van het groeipatroon in de prenatale en postnatale fase (d.w.z. de fase voor en na de geboorte) kunnen zich uitstrekken tot op volwassen leeftijd. Groeivertraging en een ongunstige lichaamssamenstelling met veel vetmassa zijn bij prematuur geboren mensen geassocieerd met het ontwikkelen van het metabool syndroom en cardiovasculaire aandoeningen. Het is daarom van belang om al heel vroeg, tijdens de postnatale zorg in het ziekenhuis, een tekort aan essentiële voedingsstoffen te voorkomen. Echter, tot op heden kunnen deze tekorten evenals de genoemde groeivertraging na de geboorte tijdens de ziekenhuisopname niet worden voorkomen. Daarom heeft aangepaste voeding na ontslag ('postdischarge' voeding) een speciale plaats in het voedingsbeleid voor premature. Deze voeding wordt gegeven om de groei, de lichaamssamenstelling en de botaanmaak zodanig te verbeteren dat de kans op cardiovasculaire en metabole gevolgen op de lange termijn zoveel mogelijk wordt beperkt. De achtergrond van dit proefschrift wordt verder besproken in **Hoofdstuk 1**.

In dit proefschrift worden in **Deel I** de gevolgen van prenatale groei (d.w.z. groei voor de geboorte), vroeg postnatale groei (d.w.z. groei tussen de geboorte en de uitgerekende datum) beschreven. In **Deel 2** worden de effecten van 'postdischarge' voeding op de groei, de lichaamssamenstelling en de botaanmaak van premature gedurende het eerste half jaar bestudeerd.

Dit proefschrift is gebaseerd op een gerandomiseerd voedingsonderzoek uitgevoerd bij 152 premature. Deze premature werden na een zwangerschapsduur van 32 weken of minder geboren en/of hadden een geboortegewicht van 1500 gram of minder. Tussen de a terme leeftijd (d.w.z. de verwachte geboortedatum) en de gecorrigeerde leeftijd (d.w.z. de leeftijd gerekend vanaf de verwachte geboortedatum) van zes maanden werden deze premature met een 'postdischarge' voeding, met een standaard voeding voor op tijd geboren zuigelingen of met moedermelk gevoed. De 'postdischarge' voeding

bevatte evenveel calorieën maar meer eiwitten en mineralen dan de standaard voeding voor op tijd geboren zuigelingen. Bij dit onderzoek werden de groei, de lichaamssamenstelling en de botaanmaak van deze prematuren tijdens het eerste half jaar vergeleken.

Hoofdstuk 2 beschrijft de studieopzet en de belangrijkste resultaten van het gerandomiseerde onderzoek. Het belangrijkste feit uit de analyse was de bevestiging dat prematuren die 'postdischarge' voeding kregen tijdens het eerste half jaar inderdaad een hogere eiwitinname hadden. Deze prematuren lieten een vergelijkbare groei zien maar met minder vettoename dan prematuren die standaard voeding kregen.

Het eerste deel van dit proefschrift richt zich op de gevolgen van prenatale en postnatale groei van prematuren.

In **Hoofdstuk 3** wordt het belang van insulin-like growth factor type I (IGF-I) en insuline voor de groeiregulatie van prematuren tot zes maanden gecorrigeerde leeftijd beschreven. Deze regulatie was niet gerelateerd aan het type voeding. Er was op de a terme leeftijd en op de gecorrigeerde leeftijd van drie maanden een verband tussen IGF-I en insuline onderling. Later, op de gecorrigeerde leeftijd van zes maanden, werd dit verband tussen IGF-I en insuline niet meer gevonden. Mogelijk duidt dit op een afnemend belang van insuline en een toenemend belang van groeihormoon (GH) voor de groeiregulatie middels IGF-I.

In **Hoofdstuk 4** wordt aangetoond dat prematuren die small-for-gestational-age geboren zijn (SGA; d.w.z. te klein voor de zwangerschapsduur) en prematuren met vroege extra-uteriene groei restrictie (vroege EUGR; d.w.z. met een groeivertraging tussen de geboorte en de a terme leeftijd) een relatief hogere groeisnelheid hebben. Deze SGA prematuren en prematuren met vroege EUGR hadden een hogere inname van energie, eiwitten, koolhydraten en vetten maar bleven desondanks kleiner dan de andere kinderen. Tevens hadden zij een lager vetpercentage gedurende het eerste half jaar. Voor de latere gezondheid is een snelle groei, die niet gepaard gaat met aanwas van vetweefsel, wellicht gunstig. Echter, de optimale samenstelling van voeding voor prematuren, die resulteert in een snelle groei zonder vetaanwas, is op dit moment helaas nog niet bekend.

In **Hoofdstuk 5** wordt beschreven dat SGA prematuren tussen de a terme leeftijd en de gecorrigeerde leeftijd van zes maanden een lagere botaanmaak [uitgedrukt als toename van bone mineral content (BMC)] hadden. Deze lagere botaanmaak werd niet verklaard doordat SGA prematuren kleiner waren.

Aansluitend toont **Hoofdstuk 6** dat de collageen type I aanmaak van SGA prematuren hoger was dan die van de andere kinderen gedurende het eerste half jaar. Deze verhoogde aanmaak is mogelijk een teken van snelle groei. Echter, de verhoogde aanmaak van collageen type I, dat vooral voorkomt in de botmatrix, was bij SGA prematuren niet geassocieerd met een verhoogde aanmaak van botweefsel. Deze discrepantie zou verklaard kunnen worden door onvoldoende botmineralisatie. Aangezien onvoldoende aanmaak van botweefsel een risicofactor is voor het ontwikkelen van osteoporose op volwassen leeftijd, hebben juist deze kinderen mogelijk baat bij voedingsinterventies die gericht zijn op de stimulatie van botaanmaak. Hierbij kan worden gedacht aan suppletie van extra calcium, fosfaat en vitamine D.

Het tweede deel van dit proefschrift richt zich op de effecten van 'postdischarge' voeding op de botaanmaak, de groei en voldoende aanwezigheid van micronutriënten.

In **Hoofdstuk 7** wordt beschreven dat de 'postdischarge' voeding de botaanmaak (uitgedrukt als de toename van BMC) van prematuren tussen de a terme leeftijd en zes maanden gecorrigeerde leeftijd verbeterde. Deze verbetering was onafhankelijk van de toename in gewicht en lengte. Deze toename van BMC bij prematuren heeft mogelijk een gunstig effect op de botmassa op volwassen leeftijd. Dit laatste zou kunnen leiden tot een verminderd risico op osteoporose op latere leeftijd.

In **Hoofdstuk 8** wordt de relatie tussen de groei van prematuren en de inname van de lange keten meervoudig onverzadigde vetzuren gelegd. De 'postdischarge' voeding bevatte het omega-3 vetzuur docosahexeenzuur (DHA) en het omega-6 vetzuur arachidonzuur (AA). Bij prematuren die 'postdischarge' voeding kregen, werden gedurende het eerste half jaar hogere waarden van DHA en AA gevonden in de rode bloedcellen (RBC waarde). Er werd een positief verband gevonden tussen de RBC waarde van DHA en de toename in gewicht en lengte. Eenzelfde verband werd gevonden tussen de RBC waarde van AA en de toename in schedelomtrek. Voorts bleek er een negatief verband te bestaan tussen de RBC waarde van DHA en de toename in schedelomtrek evenals tussen de RBC waarde van AA en de toename in gewicht en lengte. Het aanbod van DHA en AA uit 'postdischarge' voeding leidde tot bloedwaarden van DHA en AA die goed vergelijkbaar waren met de bloedwaarden van op tijd geboren kinderen die moedermelk krijgen. Deze bloedwaarden van prematuren zijn mogelijk van belang voor voldoende groei.

In **Hoofdstuk 9** wordt aangetoond dat kinderen in de 'postdischarge' voeding groep tijdens het eerste half jaar een hogere vitamine D inname en een grotere toename van serum 25-hydroxyvitamine D (een maat voor de vitamine D status) hadden dan kinderen

uit de andere voedingsgroepen. Er was een positieve associatie tussen de toename van serum 25-hydroxyvitamine D en de toename van BMC. Het is waarschijnlijk dat vitamine D, naast eiwit, calcium en fosfaat, bijdraagt aan de hogere botaanmaak van prematuren die 'postdischarge' voeding krijgen.

In **Hoofdstuk 10** wordt beschreven dat ijzertekort op de gecorrigeerde leeftijd van drie en zes maanden vaker voorkwam bij prematuren die moedermelk kregen dan bij prematuren die ijzer verrijkte kunstvoeding, zoals 'postdischarge' voeding of standaard voeding, kregen. Dit wordt mogelijk verklaard doordat de ijzersuppletie bij prematuren die moedermelk kregen ruim voor de gecorrigeerde leeftijd van drie maanden werd gestaakt. Dit terwijl ijzersuppletie voor deze kinderen de enige bron van ijzervoorziening is. Overeenkomstig de Nederlandse richtlijnen lijkt ijzer verrijkte kunstvoeding voldoende om bij prematuren een ijzertekort tussen de gecorrigeerde leeftijd van drie en zes maanden te voorkomen.

Hoofdstuk 11 geeft een overzicht van de onderwerpen en conclusies van dit proefschrift. Prenatale groei en vroeg postnatale groei zijn belangrijk voor de groei, de lichaamssamenstelling en de botaanmaak van prematuren gedurende het eerste half jaar. In deze periode is IGF-I van belang voor de regulatie van groei.

De 'postdischarge' voeding, die gebruikt werd in dit onderzoek, leidt tot een hogere inname van eiwitten, lange keten vetzuren (DHA en AA), vitamines (vitamine D), en mineralen. Al deze factoren zijn van invloed op de groeimogelijkheden, op de ontwikkeling van de lichaamsamenstelling en op de botaanmaak van prematuren gedurende de eerste zes maanden na de uitgerekende datum. Ten slotte worden de implicaties van ijzersuppletie voor de ijzervoorraad van prematuren, die moedermelk en ijzer verrijkte kunstvoeding krijgen, aan de orde gesteld. De resultaten suggereren dat prematuren die moedermelk krijgen in ieder geval gedurende het eerste half jaar ijzersuppletie nodig hebben, terwijl prematuren die ijzer verrijkte kunstvoeding krijgen dit niet nodig hebben.

Dit hoofdstuk sluit af met aanbevelingen voor toekomstig onderzoek. Nieuw onderzoek zal zich moeten richten op een optimaal voedingsbeleid voor prematuren tussen de geboorte en de a terme leeftijd. Daarnaast is het van belang dat onderzoek plaatsvindt naar de lange termijn effecten van 'postdischarge' voeding, zoals die gebruikt is in het gerandomiseerde onderzoek waarop dit proefschrift is gebaseerd. Er wordt kort inzage gegeven in de methodieken waarmee thans dit lange termijn onderzoek plaatsvindt op 7-jarige leeftijd.

Appendix

APPENDIX A

Composition of the study formulae

	Postdischarge formula (PDF)	Term formula (TF)
Volume (ml/kg/d)	175	175
Protein (g)	1.7	1.47
Arginine (mg)	70	-
Taurine (mg)	4.6	4.6
Fat (g)	3.5	3.55
C8 – C10 (mg)	324	-
LA (mg)	415	422
ALA (mg)	59	63
GLA (mg)	11.5	-
DHA (mg)	14.0	6.7
AA (mg)	14.0	6.7
Carbohydrates	7.0	7.23
Lactose (g)	6.6	7.0
Saccharose (g)	-	-
GOS (g)	0.14	0.24
Maltodextrin (g)	0.16	0.26
Energy (kcal)	67	67
Na (mg)	25	20
K (mg)	68	67
Cl (mg)	43	40
Ca (mg)	65	50
Mg (mg)	6.14	6.0
P (mg)	38	30
Fe (mg)	1.0	0.8
Cu (µg)	65	50
Mn (µg)	34	33.5
Zn (mg)	0.64	0.6
I (µg)	17	7.4
Vitamin A (µg)	65	58
Beta-carotene (µg)	52	41
Vitamin D (µg)	1.4	1.2
Vitamin E (µg)	2.6	1.3
Vitamin K (µg)	6.35	5.1
Vitamin B1 (µg)	51	44
Vitamin B2 (µg)	115	90
Niacin (µg)	1920	777

	Postdischarge formula (PDF)	Term formula (TF)
Vitamin B6 (µg)	77	39.5
Vitamin B12 (µg)	0.29	0.16
Folic acid (µg)	30	11
Pantothenic acid (µg)	500	224
Biotin (µg)	2.05	1.14
Vitamin C (µg)	17	9.4
Choline (mg)	14	14
Carnitine (mg)	2.3	1.9
Osmolality (mOsmol/L)	270	280

All values are per 100 ml of prepared formula, except for volume (ml/kg/d).
AA: arachidonic acid; ALA: alpha linolenic acid; DHA: docosahexaenoic acid; GLA: gamma linolenic acid; GOS: galactooligosaccharides; LA: linoleic acid.

APPENDIX B

Supplemental Table 9.1. Weight, length, and head circumference z-scores between birth and six months corrected age (CA) in preterm infants fed postdischarge formula (PDF), term formula (TF), and human milk (HM)[1]

	Birth		Term age		3 months CA		6 months CA	
	n	Value	n	Value	n	Value	n	Value
Weight (z-score)								
PDF	52	-0.43 ± 0.96	52	-1.11 ± 1.24	52	-0.39 ± 1.31	52	-0.38 ± 1.10
TF	41	-0.32 ± 0.82	41	-0.91 ± 1.06	41	-0.16 ± 1.20	41	-0.21 ± 1.24
HM	46	-0.32 ± 1.13	46	-1.04 ± 1.27	46	-0.32 ± 1.35	46	-0.43 ± 1.24
Length (z-score)								
PDF	50	-0.93 ± 1.17	52	-1.11 ± 1.31	52	-0.62 ± 1.04	51	-0.18 ± 0.91
TF	41	-1.03 ± 1.06	41	-1.03 ± 1.09	41	-0.41 ± 1.19	41	-0.19 ± 1.26
HM	46	-0.77 ± 1.37	46	-1.32 ± 1.36	46	-0.77 ± 1.20	46	-0.48 ± 1.14
HC (z-score)								
PDF	50	-0.35 ± 1.02	52	0.74 ± 0.80	52	0.35 ± 0.88	52	0.26 ± 0.89
TF	41	-0.14 ± 0.92	41	0.73 ± 1.0	41	0.47 ± 0.96	41	0.34 ± 1.14
HM	46	-0.11 ± 1.37	46	0.61 ± 1.05	46	0.32 ± 1.01	46	0.33 ± 1.06

[1] Values as mean ± SD. HC: head circumference; HM: human milk; PDF: postdischarge formula; TF: term formula. Compared between PDF, TF, and HM fed infants by linear regression adjusted for gender and gestational age; no significant differences between groups.

Abbreviations

ABBREVIATIONS

AA	arachidonic acid
AGA	appropriate-for-gestational-age
AGA GR-	appropriate-for-gestational-age without growth restriction at term age
AGA GR+	appropriate-for-gestational-age with growth restriction at term age
ALA	alpha-linolenic acid
ALP	alkaline phosphatase
BA	bone area
BMC	bone mineral content
BMD	bone mineral density
CA	corrected age
CRP	C-reactive protein
DoHaD	developmental origins of adult health and disease
DXA	dual-energy x-ray absorptiometry
DHA	docosahexaenoic acid
Early EUGR	extrauterine growth restriction before term age
EPA	eicosapentaenoic acid
ESPGHAN	European Society of Paediatric Gastroenterology Hepatology and Nutrition
EUGR	extrauterine growth restriction
FM	fat mass
GH	growth hormone
GLA	gamma-linolenic acid
GOS	galacto-oligosaccharides
HM	human milk
IFF	iron-fortified formula
IGF	insulin-like growth factor
IGF-I	insulin-like growth factor type I
IGF-II	insulin-like growth factor type II
IQR	interquartile range
IUGR	intrauterine growth restriction
LC-PUFA	long-chain polyunsaturated fatty acid
LA	linoleic acid
LM	lean mass
MCV	mean corpuscular volume
Mo	month(s)
PDF	postdischarge formula
PEAPOD	air displacement plethysmography
PINP	procollagen type I N-terminal peptide

PMA	postmenstrual age
PTF	preterm formula
PTH	parathyroid hormone
RBC	red blood cell
RDW	red cell distribution width
SD	standard deviation
SDS	standard deviation score
SGA	small-for-gestational-age
STEP	Study Towards the Effects of Postdischarge nutrition
TF	standard term formula
UHP	urinary helical peptide
VLBW	very low birth weight (<1500 g)
Wk	week(s)
25(OH)D	25-hydroxyvitamin D
%FM	percentage fat mass

Publications

PUBLICATIONS

This thesis

van de Lagemaat M, Rotteveel J, Lafeber HN, van Weissenbruch MM. Lean mass and fat mass accretion between term age and six months post-term in growth restricted preterm infants. *Submitted.*

van de Lagemaat M, van der Veer E, van Weissenbruch MM, Lafeber HN, Rotteveel J. Procollagen type I N-terminal peptide (PINP) in preterm infants is associated with growth during the first six months post-term. *Submitted*.

van de Lagemaat M, Amesz EM, Schaafsma A, Lafeber HN. Iron deficiency and anemia in iron-fortified formula and human milk fed preterm infants until six months post-term. *Eur J Nutr 2013; doi 10.1007/s000394-013-0629-0.*

van de Lagemaat M, Rotteveel J, Schaafsma A, van Weissenbruch MM, Lafeber HN. Higher vitamin D intake in preterm infants fed an isocaloric, protein- and mineral-enriched postdischarge formula is associated with increased bone accretion. *J Nutr 2013;143:1439-1444.*

Lafeber HN, **van de Lagemaat M**, Rotteveel J, van Weissenbruch MM. Timing of nutritional interventions in very low birth weight infants: optimal neurodevelopment compared with the onset of the metabolic syndrome. *Am J Clin Nutr 2013;98:556S-560S.*

van de Lagemaat M, Rotteveel J, Heijboer AC, Lafeber HN, van Weissenbruch M. Growth in preterm infants until six months post-term: the role of insulin and IGF-I. *Horm Res Paediatr 2013;80:92-99.*

van de Lagemaat M, Rotteveel J, van Weissenbruch MM, Lafeber HN. Increased gain in bone mineral content of preterm infants fed an isocaloric, protein- and mineral-enriched postdischarge formula. *Eur J Nutr 2013;52:1781-1785.*

van de Lagemaat M, Rotteveel J, van Weissenbruch MM, Lafeber HN. Small-for-gestational-age preterm-born infants already have lower bone mass during early infancy. *Bone 2012;51:441-446.*

van de Lagemaat M, Rotteveel J, Muskiet FAJ, Schaafsma A, Lafeber HN. Post-term dietary-induced changes in DHA and AA status relate to gains in weight, length, and head circumference in preterm infants. *Prostaglandins Leukot Essent Fatty Acids 2011;85:311-316.*

Other

van de Lagemaat M, Lafeber HN. Dutch Youth Health Care guidelines: Follow-up of preterm or small-for-gestational-age infants. Section on postdischarge nutrition in preterm infants (In Dutch: JGZ richtlijn Nazorg vroeg/SGA-geboorte. Voeding na ontslag bij te vroeg geboren kinderen). 2012 (revised in 2013).

van de Lagemaat M, Nuver CJA. Local guidelines on mild traumatic brain injury (In Dutch: Protocol Licht traumatisch hoofd/hersenletsel). 2009, Sint Franciscus Gasthuis, Rotterdam, The Netherlands.

Curriculum vitae

CURRICULUM VITAE

Monique van de Lagemaat was born May 5th, 1983, in Everdingen, The Netherlands. In 2001, she graduated from secondary school (Gymnasium) at Alkwin College, Uithoorn, The Netherlands and started Medical School at VU University, Amsterdam, The Netherlands. As a medical student, she assisted at the outpatient clinic of the "Study Towards the Effects of Postdischarge nutrition on growth and body composition of infants born ≤32 weeks gestational age and/or ≤1,500 gram birth weight (STEP)" (department of Neonatology at VU University Medical Center, Amsterdam, The Netherlands) under supervision of drs. E.M. Amesz and prof. dr. H.N. Lafeber. As part of this research project, she focused on the iron status of preterm infants. In 2007, she did an internship in Neonatology at Karolinska University Hospital, Stockholm, Sweden. In 2007, she graduated medical school.

Between 2007 and 2010, Monique worked as resident (ANIOS) at the department of Pediatrics at Rijnland ziekenhuis, Leiderdorp, The Netherlands and at the department of Pediatrics at Sint Franciscus Gasthuis, Rotterdam, The Netherlands. During her second year in Rotterdam, she performed additional analyses on the iron status of preterm infants that participated in the STEP and prepared the study protocol for the STEP 2 study, a follow-up study of the STEP cohort at 7 year of age. In October 2010, she started her thesis under supervision of prof. dr. H.N. Lafeber, dr. M.M. van Weissenbruch, and dr. J. Rotteveel at VU University Medical Center, Amsterdam, The Netherlands. In 2012, she was awarded the Dutch Neonatal Chiesi Fellow Award for her presentation at the Dutch Neonatal Fellow Meeting in Utrecht, The Netherlands. Between 2012 and 2013, Monique was part of the Committee for Young Investigators in Pediatrics (Jonge Onderzoekers Kindergeneeskunde; JOK) of VU University Medical Center, Amsterdam, The Netherlands and Academic Medical Center, Amsterdam, The Netherlands. She works as a resident (ANIOS) at VU University Medical Center, Amsterdam, The Netherlands, where she starts her residency training in Pediatrics in 2014.

Dankwoord

DANKWOORD

"Go confidently in the direction of your dreams. Life the life you have imagined."
Henry David Thoureau

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