POSTNATAL GROWTH ANALYSIS OF PRETERM INFANTS

DEVELOPING PREDICTIVE MODELS FOR BODY WEIGHT CHANGES OF PRETERM INFANTS DURING AND AFTER UNIMPAIRED POSTNATAL ADAPTATION

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Abstract

Background: Postnatal growth of preterm infants does not match recommended intrauterine growth, due to the initial weight loss that accompanies healthy body composition rearrangements after birth. Thus, optimal postnatal growth for preterm infants is currently unknown.

Objectives: (1) Collect longitudinal postnatal growth data of 30–36 week GA preterm infants with unimpaired postnatal adaptation; (2) Develop regressions that predict the growth trajectory such an infant will adjust to by days of life 7/14/21; (3) Extrapolate and validate the regressions downwards to 25 weeks.

Methods: Infants of 30–36 week GA, born/admitted to 1/5 participating centres between 2008–2012, who met pre-specified criteria for unimpaired postnatal adaptation and who had at minimum 14 days of data were included. Day-specific anthropometric data from birth to discharge were abstracted retrospectively. Z-score regressions for days 7/14/21 were developed. Regressions were then extrapolated to 25 weeks and validated using an independent study population.

Results: Of 6203 infants, 665 met the screening criteria. By day 14, infants adjusted to stable growth trajectories that were 84±13% of the recommended weight-for-age. Using the following predictors: GA, z-score at birth and hospital-centre, regressions accurately predicted z-scores at days 7, 14 (n=665; R^2=0.939, 0.889) and 21 (n=333; R^2=0.841). Validation using 25-29 week GA infants (n=173) suggested models were also accurate within this age-range.

Conclusion: These results provide robust estimates of a hypothesis of healthy postnatal growth for preterm infants. Future steps include assessing long-term outcomes in a randomized control trial and assessing the quality of growth using body composition analyses.

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Abbreviations

AC:	abdominal circumference
AGA:	appropriate for gestational age
BMI:	body mass index
BPD:	biparietal diameter
BW:	birth weight
BW%ile:	birth weight percentile
CPAP:	continuous positive airway pressure
DOHaD:	developmental origins of health and disease
ELBW:	extremely low birth weight (<1000 g)
EFW:	estimated fetal weight
EPT:	extremely preterm
FL:	femur length
GA:	gestational age
GUH:	Greifswald University Hospital
HUH:	Heidelberg University Hospital
IVH:	intraventricular haemorrhage
LBW:	low birth weight
LGA:	large for gestational age
MUMC:	McMaster University Medical Centre
NEC:	necrotizing enterocolitis
PGR:	postnatal growth restriction
PVL:	periventricular leukomalacia
SDS:	standard deviation score
SGA:	small for gestational age
SJH:	St. Joseph’s Hamilton Healthcare
SMH:	St. Michael’s Hospital
VPT:	very preterm
VLBW:	very low birth weight (<1500 g)

Declaration of academic achievement

My supervisor Dr. Christoph Fusch, mentor Dr. Niels Rochow and myself developed the study design. I presented the project to the Neonatal Research Committee and Research Ethics Board for ethics approval and completed amendment requests. Wendy Seidlitz, Kathy John and Dr. Douglas Campbell identified patients who were eligible for the study at McMaster University, St. Joseph's Healthcare Hamilton and St. Michael's Hospital, respectively.

I completed data abstraction from McMaster University Medical Centre, St. Joseph's Healthcare Hamilton (Charlton and West 5th sites) and St. Michael's Hospital Toronto. Andrea Olbrich collected all data from Greifswald University Hospital, and Susanne Goettler completed all data abstraction from Heidelberg University Hospital in Germany. All submitted abstracts, presentations, analyses, figures and tables were constructed by myself.

Introduction

Over the last few decades there have been many advancements in the field of neonatal care, including the introduction of antenatal steroids, artificial ventilation and artificial surfactant therapy. This has resulted in increased survival rates of preterm infants, in that infants born as early as 24 weeks of gestation are able to survive. The improvement of survival rates has shifted the focus of current research to improve the long-term outcomes of these surviving preterm infants.

There is vast epidemiological evidence that intrauterine growth is associated with the later-life health of the preterm infant. A commonly used marker of poor intrauterine growth is low BW, because it is often a result of nutritional restriction during fetal life. Low BW is associated with an increased risk of developing many of the risk factors of cardiovascular disease in later life, including high blood pressure and insulin resistance (Barker *et al.* 1993). This suggests that the time period between birth and term corrected age is a critical period for epigenetic modifications that predispose infants to later-life morbidities. Thus, in order to optimize long-term outcomes of preterm infants, identifying and promoting healthy early postnatal growth for infants born prematurely is a topic of great concern for neonatologists.

Background

Early postnatal growth and long-term outcomes: the dilemma

Low growth rates during early postnatal life may result in postnatal growth restriction (PGR), which is commonly defined as a body weight below the 10th birth weight percentile (BW%ile) by the time the infant is discharged (Velaphali *et al.* 2011). In preterm and very low birth weight (VLBW, birth weight <1500 g) infants, PGR has been associated with later-life development of chronic lung disease, infection (Ho *et al.* 2003) and poor neurodevelopmental outcomes (Latal-Hajnal *et al.* 2003). Thus, in order to prevent such adverse long-term outcomes, rapid growth is promoted to erase the growth deficit by adjusting the given nutrition. As a result, the neonate attains a body weight that is closer to the recommended weight for their GA.

However, recent studies have also associated rapid growth during early postnatal life in VLBW infants with an increased risk for later-life adiposity, insulin resistance and cardiovascular disease (Jain *et al.* 2012). In LBW infants, postnatal catch-up growth has similarly been associated with increased systolic blood pressure at 3 years of age (Min *et al.* 2007). There is great epidemiological evidence in literature that there is an association between catch-up growth in preterm infants during early postnatal life and the development of cardiovascular disease risk factors in later-life.

In fact, studies on a cohort of preterm infants (mean GA: 31 weeks) highlighted that although rapid early postnatal growth was associated with reduced neurodevelopmental deficits (Lucas *et al.* 1998), it was also associated with an increased risk for insulin resistance in the same cohort during adolescence (Singhal *et al.* 2003). Similar associations have been established elsewhere, emphasizing the importance of achieving early postnatal growth rates that are neither too slow nor too fast. Thus, a delicately balanced postnatal growth rate during this period is important for promoting optimal long-term health outcomes for preterm infants.

DOHaD Hypothesis

The epidemiological evidence summarized above that LBW and rapid catch-up growth during early postnatal life are associated with an increased risk of developing CVD (Barker *et al.* 1986) and/or type 2 diabetes mellitus (Hales *et al.* 1991) in later life, led to the development of the Developmental Origins of Health and Disease (DOHaD) hypothesis. DOHaD states that prenatal and early postnatal periods are “critical windows” during which environmental factors can impact the aetiology of adult-onset diseases. The hypothesis is based on the theory that the fetus that is exposed to a substrate-limited intrauterine environment, marked by LBW, undergoes a predictive adaptive response (Gluckman *et al*. 2007). The predictive adaptive response entails irreversible adaptations such as tissue differentiation, ‘programming’ the neonate to increase its likeliness of survival at the cost of sub-optimal health outcomes at later-life.

Further, these costs to an increased likeliness of survival may be amplified when the fetus is abruptly exposed to a substrate-rich environment following birth.

Impact of nutrition on early postnatal growth

In accordance with the DOHaD hypothesis, an important environmental factor during prenatal and postnatal life for the growth and health outcomes of neonates is nutrition. Fetal and early postnatal nutrition play important roles in determining the somatic status and postnatal growth pattern of the preterm infant, which in turn programs the later-life health of the infant. Many epidemiological studies and randomized trials have associated fetal under-nutrition with adverse later-life outcomes. For instance, Barker *et al.* (1993) associated LBW, a marker of fetal under-nutrition, with an increased risk for developing cardiovascular disease risk factors as discussed above. Infants born during the Dutch Hunger Famine who were exposed to severe maternal under-nutrition during gestation have an increased risk for developing metabolic and cardiovascular disease in later life. Similar to fetal nutrition, postnatal nutrition prior to term age has also been associated with short-term and long-term health outcomes (Singhal *et al.* 2003).

The rate of postnatal growth of preterm infants can be adjusted upon modification of the given nutrition. Studies that have investigated the effects of various nutritional management strategies have successfully altered the preterm infant’s short-term growth and predisposition to later-life morbidities. For instance, early advancement of feeds has been positively associated with higher growth

velocities during days of life 7 - 28 in 23 - 27 week GA infants (Martin *et al.* 2009). Similarly, early administration of parenteral amino acids in extremely low birth weight infants (ELBW, birth weight <1000 g) was associated with significantly higher body weights, lengths and head circumferences at 36 weeks postmenstrual age and a lower prevalence of suboptimal head growth at 18 months corrected age (Poindexter *et al.* 2006). Prospective observational studies that implemented nutrition with high protein to energy ratios confirmed that it is clinically feasible to significantly minimize or erase the growth deficit in very low birth weight (VLBW, birth weight <1500 g) (Senterre *et al. J Pediatr Gastroenterol Nutr.,* 2011), and extremely preterm (EPT, <28 weeks GA) and very preterm (VPT, 28-30 weeks GA) infants (Senterre *et al. Acta Paediatrica,* 2011).

More recently, it was shown that with the use of an electronic system that provides up-to-date individual growth trajectories, the nutrition administered could be modified to guide postnatal growth of preterm infants in a manner that allowed the desired postnatal growth trajectories to be attained (Rochow *et al.* 2012). The described study defined the desired postnatal growth trajectory as one standard deviation score (SDS) below the BW%ile on which the infant was born. A lower incidence of late-onset sepsis and mortality was observed in preterm infants whose postnatal growth matched the desired trajectory. Although long-term studies are needed to confirm that this represents optimal growth, the described study proves that it is clinically feasible to adjust the postnatal growth of a preterm infant to attain desired rates by adjusting the given nutrition.

Current postnatal growth recommendations for preterm infants

The optimal postnatal growth of a preterm infant is currently unknown. However the current recommendation is that the postnatal growth of the preterm infant match the growth of an age-matched healthy fetus, in terms of both quantity and quality of growth (*American Academy of Pediatrics Committee on Nutrition*, 1985), (*Canadian Paediatric Society,* 1995). To date, this has been the most logical approach and has been thought to promote conditions that allow for optimal subsequent growth. During 23 – 27 weeks GA, the growth rate of the fetus is approximately 21 g/kg/day, and by 35 – 37 weeks GA, the growth rate of the fetus falls to 12 g/kg/day (Kramer *et al.* 2001). Thus, the nutrition administered to preterm infants is often adjusted with the goal of attaining age-appropriate growth rates which approximate the fetal growth rates.

Preterm infants of 23 – 27 weeks GA have been shown to consistently grow below the current recommendations despite achieving growth rates that are above the recommended intrauterine growth rate (15 g/kg/d) (Martin *et al.* 2009). Similar cases of PGR in preterm infant and VLBW infant populations are abundant (Ehrenkranz *et al.* 1999; Ziegler *et al.* 2002). However, due to major improvements in neonatal care, it is becoming increasingly feasible to optimize nutrition to attain postnatal growth trajectories that match intrauterine curves (Senterre *et al.* 2011). Despite these major achievements, the long-term metabolic outcomes of infants with such rapid catch up growth must be evaluated

in the future to determine whether such rates are in fact optimal for the preterm infant to attain.

Strategies for monitoring postnatal growth of preterm infants

Intrauterine growth charts

In order to abide by the American Academy of Pediatrics' recommendation of postnatal growth for preterm infants, clinicians utilize growth charts which provide an approximation of fetal growth to monitor postnatal growth. There are two types of charts that are commonly used: fetal growth charts and neonatal anthropometric charts such as BW%ile charts.

Estimated fetal weight charts

Fetal growth charts are based on estimations of *in utero* fetal weights that are used to calculate percentiles, which are plotted by the GA of the fetus at the time of measurement. Fetal weight may be estimated by (1) applying biometric measurements of the fetus that are obtained using either ultrasonography (more commonly) or magnetic resonance imaging (Baker *et al.* 1994) to non-linear regression models; (2) using volumetric methods that use either 2-dimensional biometric measurements or 3-dimensional ultrasonography to obtain volumes of body parts that are then applied to ratios that allow estimation of the whole-body volume of the fetus; or (3) using customized regressions that include maternal factors shown to impact fetal growth (Figueras *et al.* 2007; Gardosi *et al.* 2004; Clausson *et al.* 2001).

If multiple sonographic biometric measurements are obtained of an individual fetus at different stages of the pregnancy, the resulting fetal growth data represents longitudinal changes of the fetus while it is *in utero.* The longitudinal nature of such EFW curves provides an advantage over other cross-sectional intrauterine growth charts, such as BW%ile charts. Further, the approximation of fetal growth can be based on fetuses from healthy pregnancies that remain *in utero* for the entire length of a full term pregnancy, thereby representing healthy unimpaired fetal growth standards.

A comprehensive review (Melamed *et al.* 2009) which evaluated the accuracy of 26 different EFW models found that within the body weight range of 1000 – 4500 g, models which were based on 3 or 4 biometric indices produced the most accurate estimations of fetal weight. Specifically, the most accurate models were those published by Hadlock *et al.* (1985) and Woo *et al.* (1985):

Hadlock *et al.*:

Log_{10} EFW = 1.326 – 0.00326(AC)(FL) + 0.0107(HC) + 0.0438(AC) + 0.158(FL)

Woo *et al.*:

Log_{10} EFW = 1.54 + 0.15(BPD) + 0.00111$(AC)^2$ – 0.0000764 (BPD)$(AC)^2$

AC: abdominal circumference; FL: femur length; BPD: biparietal diameter

Gender-specific reference EFW-for-age values using the Hadlock formula have been published in a large French study (n = 9 577 fetuses), (Salomon *et al.* 2007) (**Figure 1**):

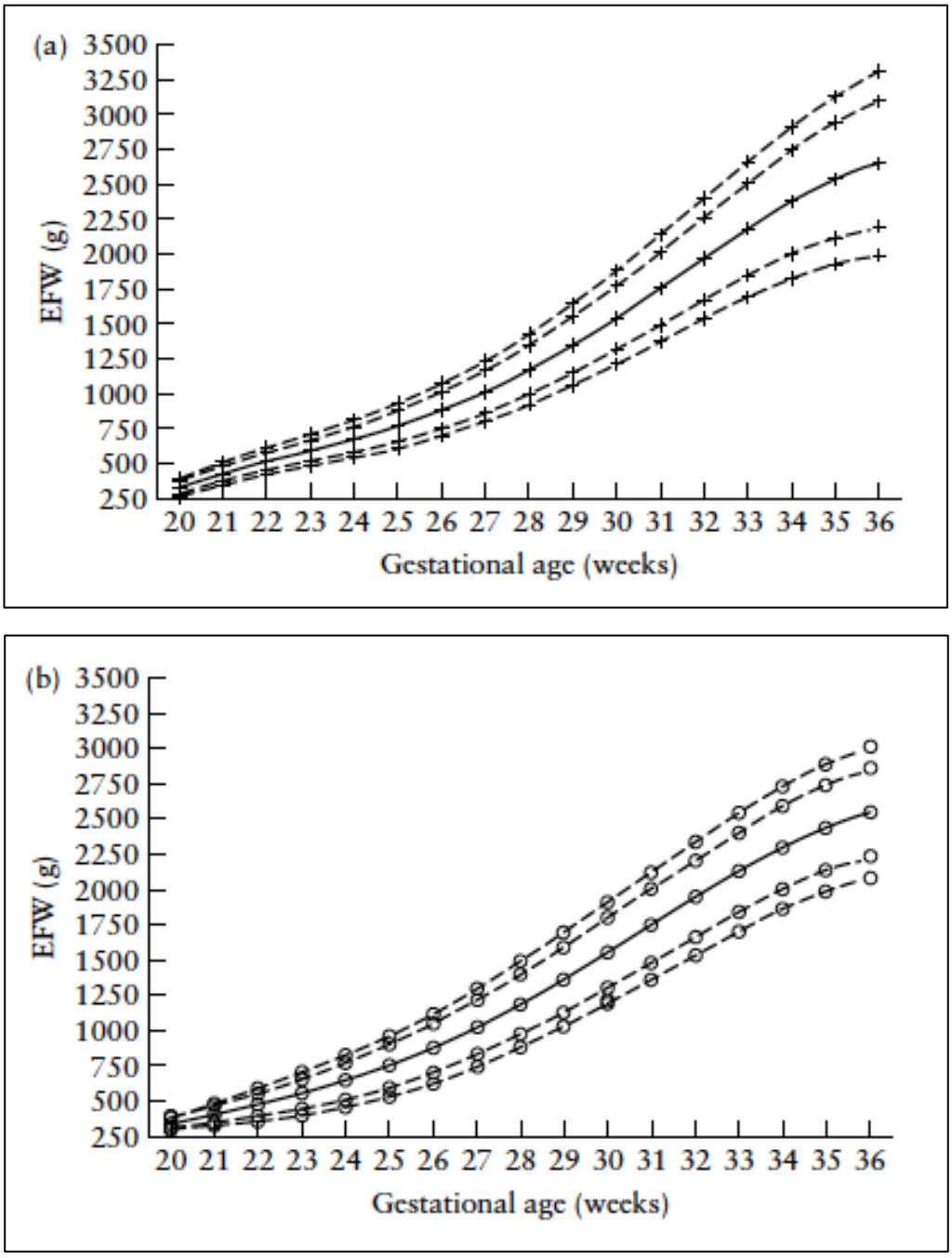


Figure 1: Gender-specific EFW charts (Salomon *et al.* 2007) of 3rd, 10th, 50th, 90th, and 97th EFW percentiles for (a) males (n = 4 869), and (b) females (4 708).

Birth weight percentile (BW%ile) charts

The most commonly used growth chart to monitor postnatal growth of preterm infants is the BW%ile chart. Such charts are constructed using cross-sectional BW measurements of infants that are born at varying GAs. Percentiles are then calculated from these BW values and are plotted by GA at birth. BWs are used to estimate intrauterine growth because they provide an accurate value that approximates the weight of the unborn fetus prior to delivery. In addition to providing accurate measurements of weight, other advantages associated with this method of monitoring postnatal growth are that BWs are easily measured, allowing for reliable values and the inclusion of very large study populations.

A commonly used growth chart that is used in clinical practice is one by Fenton *et al.* (2003). This chart was based on a systematic review and meta-analysis on intrauterine and post-term growth during 1980-2002. The preterm range of the growth chart used birth weight data from a large Canadian study by Kramer *et al.* (2001). The Canadian references were based on a population size of n = 676 605 infants born between 22 to 43 weeks of gestation during 1994 to 1996. BW%iles were presented as gender-specific values **(Table 1**).

Table 1: Published gender-specific BW%ile values, means and standard deviations for 22 to 43 completed weeks GA, for (a) males, and (b) females. (Kramer *et al.* 2001).

A.

Gestational Age	*n**	3rd Percentile	5th Percentile	10th Percentile	50th Percentile	90th Percentile	95th Percentile	97th Percentile	Mean	SD
22	82	338	368	401	490	587	627	659	501	111
23	114	406	434	475	589	714	762	797	598	114
24	156	468	498	547	690	844	902	940	697	125
25	202	521	557	617	795	981	1048	1092	800	147
26	234	571	614	686	908	1125	1200	1251	909	178
27	254	627	677	763	1033	1278	1358	1416	1026	209
28	330	694	752	853	1173	1445	1532	1598	1159	241
29	392	780	845	964	1332	1629	1729	1809	1312	273
30	467	885	959	1099	1507	1837	1955	2053	1487	306
31	584	1012	1098	1259	1698	2069	2209	2327	1682	339
32	997	1164	1266	1444	1906	2319	2478	2614	1896	369
33	1368	1344	1460	1648	2127	2580	2750	2897	2123	391
34	2553	1552	1677	1866	2360	2851	3029	3184	2361	410
35	4314	1783	1907	2091	2600	3132	3318	3475	2607	428
36	9648	2024	2144	2321	2845	3411	3604	3759	2855	443
37	19 965	2270	2384	2552	3080	3665	3857	4003	3091	449
38	51 947	2498	2605	2766	3290	3877	4065	4202	3306	448
39	77 623	2684	2786	2942	3465	4049	4232	4361	3489	445
40	112 737	2829	2927	3079	3613	4200	4382	4501	3638	447
41	54 139	2926	3025	3179	3733	4328	4512	4631	3745	459
42	8791	2960	3070	3233	3815	4433	4631	4773	3800	485
43	276	2954	3081	3249	3864	4528	4747	4941	3793	527

* Sample size at each gestational age after exclusions.

B.

Gestational Age	*n**	3rd Percentile	5th Percentile	10th Percentile	50th Percentile	90th Percentile	95th Percentile	97th Percentile	Mean	SD
22	80	332	347	385	466	552	576	576	472	72
23	106	379	403	450	557	669	706	726	564	95
24	148	424	456	513	651	790	839	887	656	121
25	184	469	508	578	751	918	982	1060	754	152
26	191	516	562	645	858	1060	1139	1247	860	186
27	188	569	624	717	976	1218	1313	1446	976	222
28	287	634	697	802	1109	1390	1499	1657	1107	254
29	299	716	787	903	1259	1578	1701	1885	1256	286
30	390	814	894	1022	1427	1783	1918	2121	1422	319
31	461	938	1026	1168	1613	2004	2150	2347	1604	345
32	795	1089	1184	1346	1817	2242	2399	2578	1808	368
33	1055	1264	1369	1548	2035	2494	2664	2825	2029	389
34	2018	1467	1581	1768	2266	2761	2948	3097	2266	409
35	3391	1695	1813	1998	2506	3037	3242	3384	2512	426
36	8203	1935	2052	2227	2744	3307	3523	3660	2754	439
37	17 308	2177	2286	2452	2968	3543	3752	3886	2981	443
38	47 516	2406	2502	2658	3169	3738	3931	4061	3181	439
39	75 068	2589	2680	2825	3334	3895	4076	4202	3350	434
40	110 738	2722	2814	2955	3470	4034	4212	4331	3486	434
41	52 063	2809	2906	3051	3576	4154	4330	4444	3588	439
42	7970	2849	2954	3114	3655	4251	4423	4554	3656	448
43	277	2862	2975	3159	3717	4333	4495	4685	3693	459

* Sample size at each gestational age after exclusions.

Longitudinal postnatal growth charts

To date, there have been four major studies that have used serial body weight measurements over the first few weeks of life to develop longitudinal postnatal growth curves. Studies led by Wright *et al.* (1993) and Ehrenkranz *et al.* (1999) longitudinally analyzed the postnatal growth of VLBW infants (n = 205, n = 1660, respectively). In both studies, despite achieving postnatal growth rates that were similar to intrauterine rates (14.4 – 16.1 g/kg/d), the infants did not attain a position on the 50th BW%ile by discharge. However, a limitation to both studies was that subjects were stratified by BW rather than GA, leading to heterogeneous groups of infants with varying growth patterns (Moutquin *et al.* 2003). As a result of selecting infants based on low birth weights, there was likely an over-representation of SGA infants and IUGR infants in study populations of such studies (Volpe *et al.* 2008).

In an attempt to overcome the limitation of analyzing heterogeneous groups of preterm infants, studies have stratified preterm infants by GA. Niklasson *et al.* (2003) analyzed the postnatal growth of very preterm (< 29 completed weeks of gestation) infants who were stratified by GA (n = 52) and more recently, Horemuzova *et al.* (2011) analyzed the postnatal growth of extremely preterm infants (< 26 weeks of gestation; n=162). It is important to note however, that both of these studies had low population sizes and required the infants to only meet the screening criteria of survival until term age. Thus, neither of the published longitudinal postnatal growth curves for preterm infants

represents a prescriptive standard to be met of postnatal growth for preterm infants, but instead both provide a characterization of the growth pattern of very and extremely preterm infants during early postnatal life.

Drawbacks of current strategies for monitoring postnatal growth of preterm infants

The limitation of using EFW charts to monitor postnatal growth of preterm infants, is that the ultrasound biometric measurements used to estimate fetal weight are often inaccurate. The inaccuracy has been attributed to random errors in volumetric methods and large intra- and interobserver variablitiy (Dudley *et al.* 2005). At low weights, ultrasonographic methods often over-estimate fetal weight, while at higher weights that exceed 4000 g, the fetal weight is often under-estimated (Melamed *et al.* 2009; Goetzinger *et al.* 2013). This lack of accuracy at the extremes of weights has been suggested to be a result of the varying porportions of the fetal head to abdomen in comparison to normal-weight fetuses (Dudley *et al.* 2005). Additionally, due to cost barriers, studies that use such methods use low study population sizes.

Although BW%ile charts are based on accurate measurements of body weight, they are associated with their own unique drawbacks when used to monitor postnatal growth of preterm infants. Firstly, the approximations of fetal weight do not represent healthy intrauterine growth in the range of 22 weeks of gestation to term-age. Preterm delivery is associated with fetal growth restriction, and thereby, BW measurements between the 22 to 37 week GA range of preterm

infants are more reflective of pathological growth (Cooke *et al.* 2007). In fact, when BW%iles of a French population were compared to EFW curves of another French population, it was evident that the BW%ile curves were significantly lower than the EFW curves in preterm gestations (Salomon *et al.* 2007). Similar comparisons and conclusions have also been reported elsewhere (Reeves *et al.* 2011). Secondly, growth is a longitudinal parameter, but the BWs are cross-sectional data. As a result, such charts may be appropriate for assessing the size of the infant at birth, but may be inappropriate for monitoring growth, which is a longitudinal measure (Villar *et al.* 2010). Another limitation of both types of growth references that estimate intrauterine growth, is that neither type takes into consideration the physiological weight loss that occurs during postnatal adaptation (Villar *et al.* 2010).

Lastly, two of the four longitudinal postnatal growth charts that are currently available select infants based on low birth weight instead of GA, leading to a selection bias of IUGR and SGA infants. Additionally, none of the published longitudinal postnatal growth references that have correctly-stratified by GA have evaluated maternal characteristics that may impair neonatal growth, have low population sizes, are based on single-centre studies and/or do not select infants that are free of congenital malformations and major clinical conditions that are associated with impaired postnatal growth or later-life adverse health outcomes. Thus, such growth charts provide only descriptive postnatal growth values of preterm infants.

Postnatal adaptation

Following delivery, the preterm infant must adjust to its new extrauterine environment. During the first few days of life, there is a signficant reduction in total body water, as the extracellular fluid compartment of the preterm infant irreversibly contracts (Bauer *et al.* 1991). Appropriate for gestational age (AGA) infants experienced a maximal postnatal weight loss on day 6 of life, and regained their BW by day 14 of life (Bauer *et al.* 1993). More immature infants experienced greater weight losses due to the higher porportion of total body water (Modi *et al.* 2004). In fact, total body water content decreases from 90% at 24 weeks of gestation to approximately 75% by term age (Velaphi *et al.* 2011). Small for gestational age (SGA) infants and infants with respiratory distress syndrome have also been shown to take longer to regain their BW (Bauer *et al.* 1993; Modi *et al.* 1990). The period of postnatal adaptation from maximal weight loss to BW regain (growth phase) was associated with increases in body solids from day 6 to day 23 of life (Bauer *et al.* 1993), and was followed by a stable growth phase.

Rationale

As neonatal medicine and nutritional strategies advance, the need for standardized postnatal growth standards for preterm infants is increasing. The first step toward developing a hypothesis of optimal postnatal growth for preterm infants is to characterize the postnatal growth trajectories of the healthiest subset

of preterm infants. Ideally postnatal growth references should be based on a multi-centre approach, accurate GA estimates and serial growth measurements of healthy preterm infants who require little to no postnatal support (Gardosi *et al.* 2004). Included infants should be free of congenital malformations and major clinical conditions that are associated with impaired postnatal growth, as well as not have undergone major surgery as it has been postulated that during acute postnatal stress, infants divert protein and energy from growth to instead be used for tissue repair (Vilar *et al.* 2010). Additionally, infants should be included based on preterm delivery instead of using low birth weight as a proxy (Moutquin *et al.* 2003, Bertino *et al.* 2011) and the screening period should span the last decade to reflect current neonatal care (Villar *et al.* 2010). Such postnatal growth references could then be used to develop predictive regression models that require clinical information available at birth to predict the postnatal growth measure a healthy preterm infant would adjust to during and following unimpaired postnatal adaptation on an individual-basis. Such longitudinal postnatal growth references will improve upon the limitations of existing growth charts, and provide a hypothesis of optimal postnatal growth for preterm infants that can be further investigated in future studies.

Overall research objectives

1. Describe body weight changes during and after postnatal adaptation of preterm infants born between 30 – 36 completed weeks of gestation who require minimal postnatal intervention, and who are admitted to either McMaster University Medical Centre (2008-2012), St. Joseph's Healthcare Hamilton (2008-2011), Greifswald University Hospital (2008-2012), Heidelberg University Hospital (2009-2011), or St. Michael's Hospital (2009-2010).

2. Use the postnatal growth data obtained in Aim #1 to develop multiple-linear regressions that predict the body weight that a preterm infant with little/no postnatal support adjusts to by days 7, 14 and 21 of postnatal life. Independent variables assessed will include clinical information available at birth, such as: GA, BW, hospital centre, mode of delivery, gestation, head circumference, and body length.

3. Validate the predictive models developed in Aim #2 by **(A)** apparent validation, **(B)** internal validation (split-sample technique)

4. Extrapolate the predictive models with the greatest accuracy into earlier weeks of gestation (24-29 weeks) and validate using an independent study population of preterm infants with little/no postnatal support who were born between 24 – 29 completed weeks of gestation.

Overall hypotheses

The null hypothesis for Aim #1 is that there will be no difference between postnatal growth trajectories and intrauterine growth trajectories during the 30 – 36 weeks GA range. However, preterm infants initially lose weight after birth, due to the contraction of the extracellular fluid compartment during postnatal adaptation. Thus, it is hypothesized that postnatal growth trajectories will be lower than intrauterine trajectories. This hypothesis is also supported by clinical observation and literature.

The null hypothesis for Aim #2 is that GA at birth, BW, hospital centre, mode of delivery, single vs. multiple gestation, head circumference at birth and body length at birth will not make statistically significant contributions to the predictive models. However, literature supports that GA, BW, feeding practices (which may differ between hospital centres), mode of delivery and gestation will significantly impact the day 7, 14 and 21 predictive growth models. For instance, it has been shown that from 30 weeks of gestation onwards, postnatal growth trajectories of singleton vs. multiple gestation preterm infants diverged, where singleton infants had lower body weights (Sankilampi *et al.* 2013).

The null hypotheses for Aim #3 are that **(A)** applying the developed predictive models on the same population used to construct it (30 – 36 week GA preterm infants) will result in no statistically significant differences between actual and predicted growth measures, and residual values that are clinically irrelevant; **(B)** the same independent variables incorporated in the predictive models that are based on all included 30 – 36 week GA preterm infants will remain significant in the split-sample groups that will be used for internal validation and the coefficient of determination will remain within 5% of those of the regressions being validated.

The null hypothesis for Aim #4 is that preterm infants born at GAs between 24 and 29 weeks will adjust to the same trajectory that infants born between 30 – 36 weeks adjust to after postnatal adaptation is complete. Therefore, there will be no statistically significant difference in predicted and observed postnatal growth at days 7, 14 and 21 in infants born between 24 – 29 completed weeks, when values are predicted based on regressions developed using the growth data of 30 – 36 week GA infants.

Specific Aims

1.1 Describe postnatal growth of preterm infants (24 – 36 weeks GA) who require minimal postnatal support, during postnatal adaptation period and after stable growth is achieved.

1.2 Evaluate how postnatal growth trajectories compare to recommended intrauterine growth trajectories using raw body weight values, z-scores, and percentage of recommended weight-for-age values.

1.3 Evaluate how postnatal growth rates compare to recommended intrauterine growth rates.

1.4 Explore how nutrition, ethnicity and gender affect early postnatal growth

1.5 Identify the differences in study population characteristics (ie. neonatal classification, GA, BW) and nutritional practices between hospital centres

2.1 Using a stepwise-forward selection technique, determine which independent variables are significant predictors of growth outcomes on days 7, 14 and 21.

2.2 Evaluate the accuracy of each model using residual analyses.

3.1 Indicate the optimism of the apparent performance of the most accurate predictive models, by determining whether there is a statistically significant

difference between predicted and actual values using a paired-samples t-test.

3.2 Internally validate the most accurate predictive models using a split-sample technique (compare relationship between dependent and independent variables, R^2 values and residuals).

3.4 Explore limitations of the obtained predictive models, upon residual analyses of various factors: GA at birth, gender and ethnicity.

4.1 Collect longitudinal growth data for 24 – 29 completed week GA preterm infants who require minimal/ no medical intervention.

4.2 Validate most accurate predictive models in the extrapolated region (24 to 29 weeks) by assessing the statistical and clinical significance of the residuals obtained when the regressions being validated are applied to an independent study population of preterm infants of 24 – 29 weeks GA.

Methods

Study Design

This study follows a multi-centre, longitudinal, descriptive design. Two independent retrospective cohorts were used (Study population A and B), which both comprised of low-risk preterm infants who required minimal to no medical intervention during the postnatal adaptation period. Subjects were admitted to one of the five participating centres: McMaster University Medical Centre (MUMC; Hamilton ON, Canada), St. Joseph's Healthcare Hamilton (SJH; Hamilton ON, Canada), Greifswald University Hospital (GUH; Greifswald, Germany), Heidelberg University Hospital (HUH; Heidelberg, Germany), and St. Michael's Hospital (SMH; Toronto ON, Canada). All infants were fed according to the nutritional guidelines at the corresponding centre. The study was approved by each of the centres' Research Ethics Boards.

SECTION A: Development of normative postnatal growth values for "low-risk" preterm infants

Study population: Group A

The inclusion criteria for the Group A study population was that the infants were admitted to one of the five participating hospital centres within the first 24 hours of life during the following screening periods: MUMC (2008-2012), SJH (2008-2011), GUH (2008-2012), HUH (2009-2011), SMH (2009-2010), and that

they had a GA at birth between 30 and 36 6/7 weeks. The infants were then subjected to strict screening criteria to select the healthiest subset of infants from the population.

Infants were excluded if there was antenatal alcohol/ drug/ cigarette use, maternal diabetes mellitus, histological chorioamnionitis, if the infants had any major pathology, had undergone major intracavitary surgery (except hernia repair), there was a requirement for mechanical ventilation or continuous positive airway pressure (CPAP) therapy for greater than 3 days of life, $FiO_2 \geq 0.3$ between 6-72 hours of life, nosocomial sepsis confirmed by positive blood culture results, confirmed necrotizing enterocolitis (NEC), ileus, neuronal intestinal dysplasia, esophageal atresia, pyloric hypertrophic stenosis, intestinal invagination, intraventricular haemorrhage (IVH; level III, IV), periventricular leukomalacia (PVL), hydrops fetalis and/or if the infant had not attained full enteral feeding (defined as 120 mL/kg/d) within the first ten days of life. Exclusion criteria were determined based on identifying morbidities that increased energy expenditure, and/ or affected body composition, nutrition or growth of the infant. For example, histological chorioamnionitis was used as an exclusion criterion because it has been shown to result in poor postnatal growth for preterm infants (<32 weeks GA) (Trevisanuto *et al.* 2013). Additionally, infants with uncertain GAs (discrepancy between obstetric and pediatric estimates, or GA estimates based on last-menstrual-period) and/or infants with <14 days of available health

records were also excluded, to maintain the integrity of the longitudinal-nature of the results.

Study population: Group B

The inclusion criteria for the Group B study population was that the infants were admitted to MUMC or GUH during 2008-2012 within the first 24 hours of life, and that they had a GA at birth between 24 and 29 6/7 weeks. The exclusion criteria were modified to reflect the greater prematurity of this population. As such, infants were excluded if there was antenatal alcohol/ drug/ cigarette use, maternal diabetes mellitus, histological chorioamnionitis, if the infant had any major pathology, required major intracavitary surgery (except for hernia repair), required mechanical ventilation for greater than 3 days of life, $FiO_2 \geq 0.3$ within the first 21 days of life, nosocomial sepsis confirmed by positive blood culture results during the first 21 days of life, confirmed NEC, ileus, neuronal intestinal dysplasia, esophageal atresia, pyloric hypertrophic stenosis, intestinal invagination, IVH (level III, IV), PVL, and hydrops fetalis. Additionally, infants were also excluded if the GA was uncertain.

Data abstraction

Paper and electronic health records were retrospectively scrutinized on-site on an individual-basis for the screening criteria outlined above. For study population A, GA, year of admission, day-specific anthropometric measurements until discharge (body weight [g], body length [cm], head circumference [cm]), nutritional information (day of life enteral feeds were begun, day of life full enteral

feeds was attained), mode of delivery (vaginal vs. C-section), ethnicity of parents, and single vs. multiple gestation information was collected. For study population B, GA, year of admission, day-specific body weight data for the first 21 days of life, nutritional information, mode of delivery, ethnicity of parents, and single vs. multiple gestation information was collected. All patients were randomly allocated a study number to maintain anonymity.

For the anthropometric measurements, body weight had been routinely measured daily using a weight scale, and head circumference and body length (crown-heel length) were both routinely measured using a measuring tape, once a week at German centres and once during hospital stay at Canadian centres. Trained healthcare professionals obtained all measurements. Illegible or improbable values were omitted from data analysis, where growth measurements were considered as improbable when they differed from the previous day's measurement by greater than 10%.

Characterizing postnatal growth trajectories

Infants were stratified by GA at birth into the following groups: 30, 31, 32, 33, 34 and 35 completed weeks. Day-specific body weight values were approximately normally distributed and were plotted as a mean ± standard deviation for each infant group. The day of life that maximum weight loss occurred and that BW was regained were determined based on the mean day-specific body weight values of each infant group.

SECTION B: Identifying trends in early postnatal growth

Comparison of postnatal and intrauterine growth

Infants were stratified by GA at birth into the following groups: 30, 31, 32, 33, 34 and 35 completed weeks. Day-specific body weight values were plotted as a mean ± standard deviation for each infant group. The values were plotted by corrected GA at the middle of the respective completed week, as previously recommended (Rochow *et al.* 2012). The resulting postnatal growth trajectories were then superimposed on reference growth curves that approximate intrauterine growth (gender-specific BW%ile charts and EFW charts). The reference BW%ile values were re-plotted to correct for the systematic plotting error described by Rochow *et al.* (2012) and to avoid the previously reported artificial leftward shift of the growth curve.

The BW%ile values used to represent intrauterine growth in the present study were obtained from gender-specific Canadian BW%ile charts (Kramer *et al.* 2001), which were used to develop the commonly-used Fenton growth charts (Fenton *et al.* 2003) between the GA period: 22 and 43 completed weeks. Intrauterine growth was also represented in the present study with EFW values obtained from Salomon *et al.* (2007), because of its high population size and use of accurate models to estimate fetal weight (Hadlock formula).

For quantitative comparison of postnatal growth trajectories and the recommended weight-for-age values (BW%ile or EFW values), the comparisons were expressed as z-scores, z-score differences from birth, and as percentages

of the recommended weight-for-age. Gender-specific recommended weight-for-age values were interpolated using an exponential model according to the corrected age of the infant at the time of measurement, on a day-to-day basis from the 50th BW%ile values presented by Kramer *et al.* (2001) and EFW values presented by Salomon *et al.* (2007). Z-scores, z-score differences and percentages of the recommended weight-for-age were calculated using the formulas described below. All values were calculated on an individual and day-to-day basis.

Z-score = [observed body weight – recommended weight-for-age]/SD

Z-score difference = Z-score at day *n* of life - Z-score at birth

Percentage of recommended weight-for-age
= Observed body weight of infant / recommended weight-for-age

Of note, since EFW curves only exist for the GA range of 20 to 36 weeks, postnatal growth measurements that corresponded to corrected ages beyond 36 weeks could not be compared to EFW references.

Postnatal growth rates were calculated using an exponential growth model described by Patel *et al.* (2005) (see below), and compared to recommended intrauterine growth rates calculated for each completed week from: BW%ile charts (Kramer *et al.* 2001) and EFW references (Salomon *et al.* 2007). Growth

rates were calculated for weeks two and three of postnatal life, and expressed as mean ± standard deviation. Thus, the presented postnatal growth values were representative of a seven-day period [completed week] during periods of stable postnatal growth.

$$\mathbf{PNG = [1000 * \ln (W_n / W_1)] / (D_n - D_1)}$$

PNG: postnatal growth rate [g/kg/d]
W: body weight [g]
D: day of measurement

Analysis of the differences between participating centers

The distributions of Group A BW, GA and z-scores at birth and day of life 14 were compared across hospital centres using a Kruskal-Wallis Test. In order to further evaluate the differences between specific centres, Mann-Whitney U Tests were used. Further, nutritional practices between centres were compared by evaluating the difference in medians of the days of life that full enteral feeds were administered to Group A infants between hospital centres. Full enteral feeds was defined as 120 mL/kg/d. Differences in medians were identified using the Median Test. All statistical analyses were performed using SPSS version 21.0 and differences were considered statistically significant at the 0.05 level.

Analysis of how factors affect postnatal growth

The effect of nutrition on postnatal growth was evaluated upon correlation analysis of the z-score difference infants adjusted to by day 14 of life and the day

of life full enteral feeds was administered. The effect of ethnicity and gender was also evaluated using a one-way ANOVA. The following ethnic categories were assessed: unknown, Asian, African, Caucasian, Caribbean, Mixed, Mediterranean, Native and South American.

SECTION C: Development and selection of models to predict early postnatal growth in "low-risk" preterm infants

Development of predictive models

A step-wise forward selection procedure was used to construct multiple linear regression models to predict the body weight of Group A preterm infants on days of life 7, 14 and 21. Body weight was expressed as a raw value, z-score, or percentage of the recommended weight-for-age. The independent variables (predictor variables) analyzed were: GA at birth, body weight at birth (expressed as a raw BW value, z-score, or percentage of recommended weight-for-age), hospital centre at which the infant was admitted and treated, gestation (singleton vs. multiple gestation), mode of delivery, head circumference at birth and body length at birth. The hospital centre was dichotomized into two groups based on patterns in the advancement of enteral feeds, where the first group included MUMC and SJH, which administered full enteral feeds (120 ml/kg/d) at a statistically significant earlier date than the second category of hospital centres (GUH, HUH, SMH).

Predictive models were also developed using the described forward step-wise selection procedure, where the categorical variable of hospital centre was recoded as four separate variables, using MUMC as the reference variable ("dummy" coding). Each coefficient was associated with one of the four other hospital centres (SJH, GUH, HUH, SMH, respectively). The pertinent coefficient was set to one for each of the corresponding centres, while the coefficients that corresponded to the other centres where the infant was not born/ admitted, were set to zero (ex: SJH: $x_{SJH}=1$, $x_{GUH}=0$, $x_{HUH}=0$, $x_{SMH}=0$ vs. GUH: $x_{SJH}=0$ $x_{GUH}=1$, $x_{HUH}=0$, $x_{SMH}=0$). This method categorized the centres based on all confounding differences between centres.

GA was included as a predictor variable in all models tested, because of the physiological understanding that the degree of prematurity affects the growing capacity of the infant. The remaining predictor variables were included in the multiple linear regression equation if they increased the R^2 value of the regression by at least 0.01 and if the *p*-value associated with the variable was less than 0.001. Therefore, terms that were non-significant or did not improve the R^2 value by at least 0.01 were removed from the equation.

Evaluation of the accuracy of predictive models

Predictive models for days 7, 14 and 21 using each of the dependant variables (raw body weight, z-score and percentage of the recommended weight-for-age value) were compared based on R^2 values and range of residual values.

Residual values were calculated as the difference between the predicted and actual growth measure.

SECTION D: Validation of predictive models

Apparent validation

In order to identify if there was a statistically significant difference between predicted and actual values, actual and predicted values of Group A infants were analyzed using a paired-samples t-test at the 0.05 significance level.

Internal validation

Internal validation of the predictive models was completed using a split-sample validation technique (Ma *et al.* 2013), in order to avoid a selection bias in the study population used to develop the model (Mark *et al.* 2001). The data of Group A subjects was randomly split into a training set and a validation set. Step-wise forward selection was used again to develop predictive models for days 7, 14 and 21 that were based on the training and validation sets separately. The relationship between the dependant variables and the independent variable was evaluated for its significance in all models. Pearson correlation coefficients for each of the three predictive models that were based on either the training set or validation set were also determined. The model was accepted as validated if (1) the relationship between dependent and independent variables was the same as in the overall predictive models, and if (2) the Pearson correlation coefficients for

each of the training and validation set models were within 5% of the coefficient of the overall predictive models. Additionally, the predicted values of Group A infants computed using the training and validation set models were also compared for statistically significant differences using a paired-samples t-test.

Analysis of the effect of factors on the accuracy of predictive models

The effects of gender, GA and ethnicity on the accuracy of predictive models were evaluated for day 7, 14 and 21 models. The difference between the actual body weight value and predicted value (residual) was plotted for each infant by the BW at birth. Additionally, residuals were also plotted by actual values, where infants were categorized by gender and ethnicity. Trends of inaccurate predictions were qualitatively assessed for each factor.

SECTION E: Extrapolation of predictive models downwards to 24 weeks

The validated predictive equations that were developed using Group A subjects' data were used to predict growth parameters of Group B subjects (GA: 24 – 29 weeks) on days of life 7, 14 and 21. The predicted growth measures were then compared to the actual values for each Group B infant using paired-sample t-tests. Residual plots were constructed for day 7, 14 and 21 predictive models, where the residual range, mean and confidence intervals at the 95% confidence level were plotted.

SECTION F: Combining Groups A and B to develop overall regressions

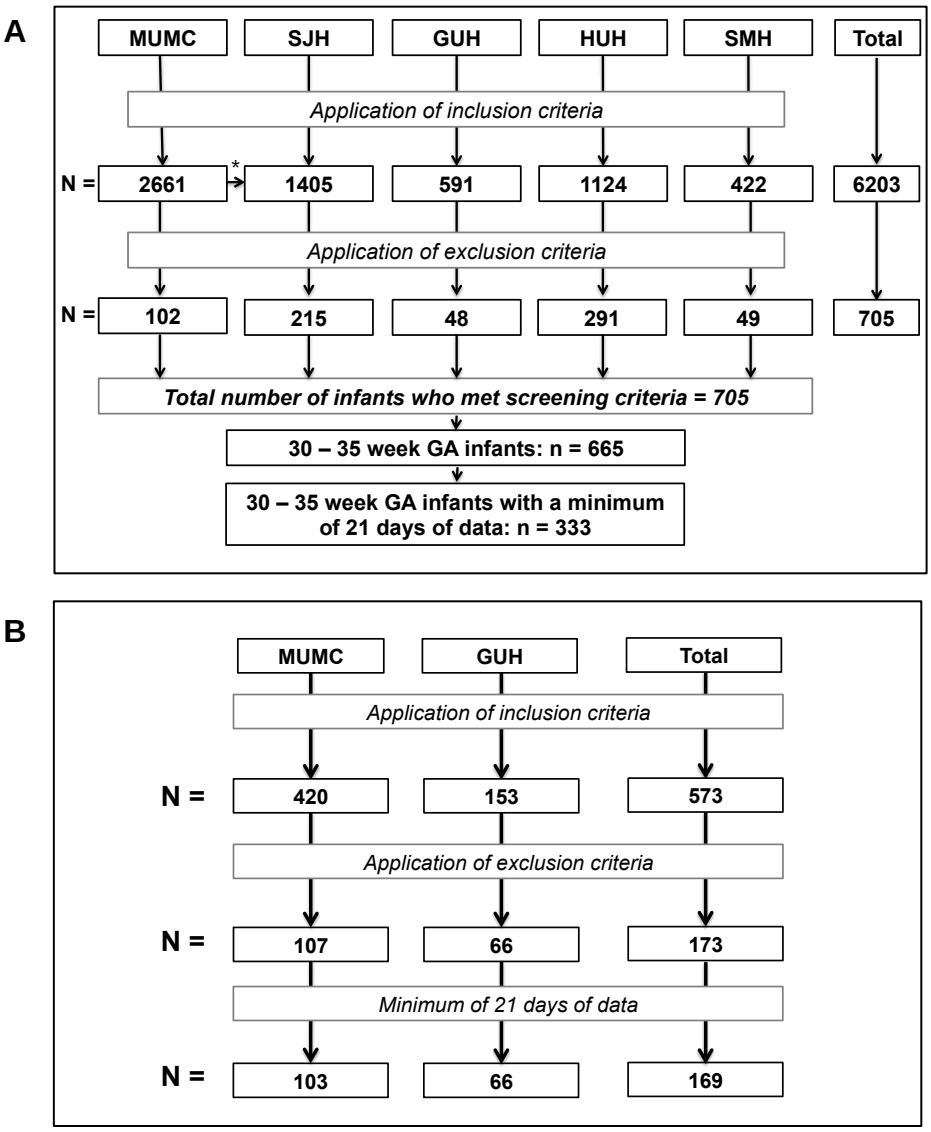
Multiple linear regression models were also developed using the same dependent variables and selection procedure that are described above, using the entire study population (Group A and Group B combined). Apparent validation was completed by using a paired-samples t-test to compare actual and predicted growth measures for days 7, 14 and 21 of life. Additionally, residual plot analyses were conducted and confidence intervals were plotted at the 95% confidence level.

Results

SECTION A: Development of normative postnatal growth values for "low-risk" preterm infants

Selection of Group A and Group B subjects

Sample size was a direct reflection of the number of charts received and the number of infants that satisfied our screening criteria. For Group A, a total of 6 203 preterm infants of GAs between 30 and 36 completed weeks were screened, from which 705 infants met the screening criteria **(Figure 2A).** 665 infants were born at gestational ages (GA) between 30 and 35 completed weeks, and of these, health records and growth data were available for a minimum of 21 days after birth for 333 preterm infants. Infants born between 36 – 36 6/7 (n = 40) had BWs that were significantly lower than the 50th BW%ile, in comparison to other GA groups of infants; average BW for 36 – 36 6/7 GA infants as a percentage of the 50th BW%ile was 79.5% vs. 96.9% of 30 – 35 6/7 GA infants. Thus, due to the selection bias of infants with lower BWs, infants in the 36 – 36 6/7 GA group were excluded from subsequent analyses. For Group B, a total of 573 preterm infants with GAs between 24 and 29 completed weeks were screened, of which 173 infants met the screening criteria **(Figure 2B)**. Health records and growth data were available for a minimum of 21 days after birth for 169 of these preterm infants.



* 117 infants were transferred from MUMC to SJH before 14 days of life
MUMC: McMaster University Medical Centre
SJH: St. Joseph's Healthcare Hamilton
GUH: Greifswald University Hospital
HUH: Heidelberg University Hospital
SMH: St. Michael's Hospital

Figure 2: Screening process for all infants admitted to participating hospital centres for selection of (A) Group A and (B) Group B study populations.

Study population characteristics

Table 2 presents demographic information of Group A (30 – 35 6/7 GA) and Group B (24 – 29 6/7 GA) study populations. An approximately even gender distribution was observed for both study groups. Of note, there were no 24 – 24 6/7 week GA infants who met the strict screening criteria for selecting preterm infants born between 24 – 29 completed weeks who required minimal medical intervention during the first 21 days of hospital stay. Additionally, there were a greater number of C-section deliveries (77.5% vs. 55.9%) in the more premature population of Group B, and the majority of births in this group were of singleton gestation (91.3%).

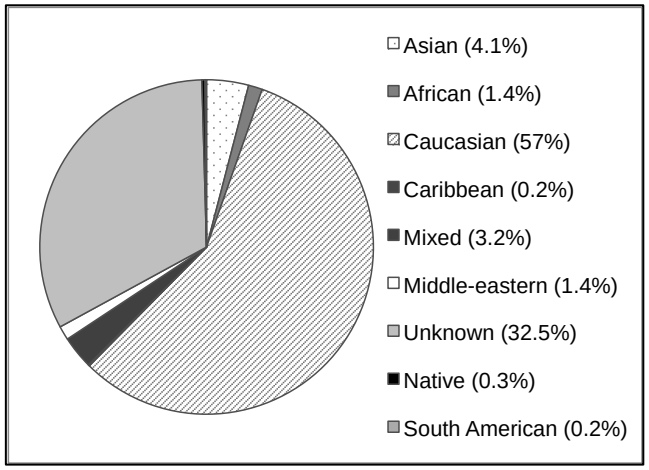
TABLE 2: Population characteristics of study populations Group A (n = 665) and Group B (n = 173). The frequency and percentage within Group A and Group B infants were presented for the following categories: hospital centre the infant was born/ admitted to, gender, GA (stratified by completed weeks), mode of delivery, gestation and neonatal classification based on the 10th and 90th BW%ile cut offs for SGA, AGA and LGA classification.

		Number, (%)	
		Group A	**Group B**
Hospital centre	MUMC	94 (14.1)	107 (61.8)
	SJH	207 (31.1)	-
	GUH	45 (6.8)	66 (38.2)
	HUH	271 (40.8)	-
	SMH	48 (7.2)	-
Gender	Male	363 (54.6)	93 (53.8)
	Female	302 (45.4)	80 (46.2)
Gestational age [completed weeks]	24	-	0 (0.0)
	25	-	6 (3.5)
	26	-	29 (16.8)
	27	-	34 (19.7)
	28	-	52 (30.1)
	29	-	52 (30.1)
	30	25 (3.8)	-
	31	56 (8.4)	-
	32	113 (17.0)	-
	33	138 (20.8)	-
	34	197 (29.6)	-
	35	136 (20.5)	-
Mode of delivery	Vaginal	194 (29.0)	38 (22.0)
	C-section	372 (55.9)	134 (77.5)
Gestation	Single	284 (42.7)	158 (91.3)
	Multiple	262 (39.4)	63 (36.4)
Neonatal classification	SGA	75 (11.3)	18 (10.4)
	AGA	562 (84.5)	151 (87.3)
	LGA	28 (4.2)	4 (2.3)

MUMC: McMaster University
SJH: St. Joseph's Healthcare Hamilton
GUH: Greifswald University Hospital
HUH: Heidelberg University Hospital
SMH: St. Michael's Hospital
SGA: small for gestational age
AGA: appropriate for gestational age
LGA: large for gestational age

The ethnic distribution of included infants in Group A (30 – 35 6/7 week GA) and Group B (24 – 29 6/7 week GA) are presented in **Figure 3 A** and **B,** respectively. In Group A, 57.0% of included infants were Caucasian, while the ethnicity of 32.5% infants was not indicated or illegible in health records. In Group B, 53.2% of included infants were Caucasian, and the ethnicity of 38.7% of infants was unknown.

A



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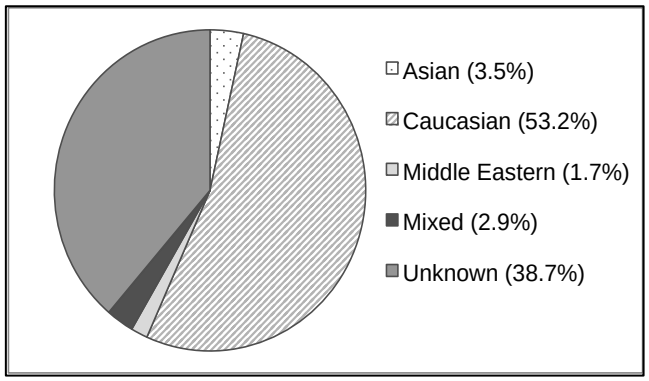


Figure 3: Ethnic distribution of included preterm infants of (A) Group A: 30 – 35 weeks GA, and (B) Group B: 24 – 29 weeks GA infants.

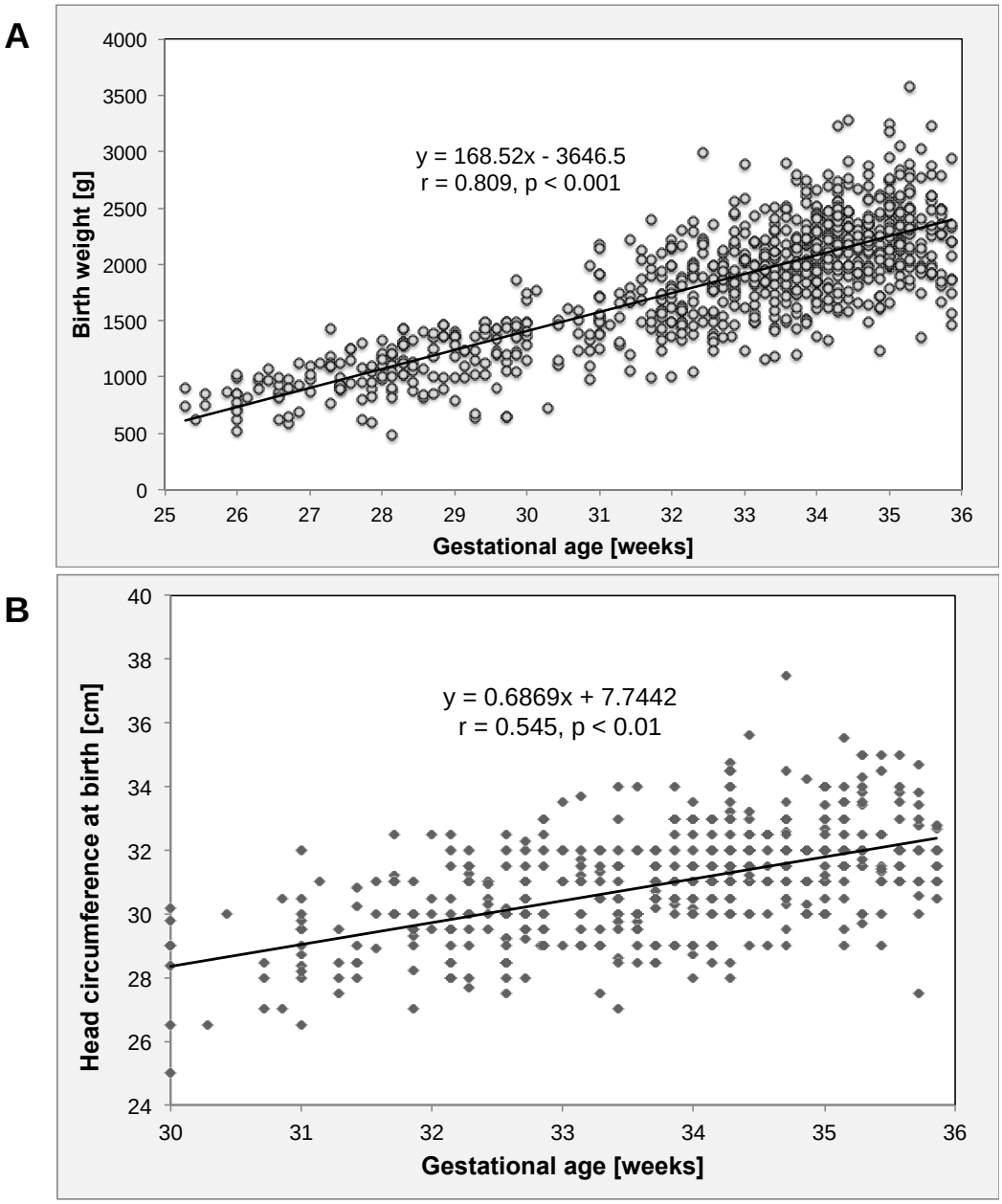
Group A and Group B BWs were approximately normally distributed. Means and standard deviations of body weights of all included infants were stratified by GA and presented for the gestational period between 25 and 35 completed weeks **(Table 3)**. It was observed that BWs for girls were lower than BWs for boys for all GAs except 29 completed weeks.

TABLE 3: Overall and gender-specific birth weights over 25 – 35 completed weeks of gestation, from combined study populations (Groups A and B). Mean values and standard deviations of body weights reported were stratified by GA [completed weeks] for all infants and for each gender separately.

	Birth weights (g)								
	All (n = 836)			Girls (n = 381)			Boys (n = 455)		
GA (completed weeks)	n	Mean	SD	n	Mean	SD	n	Mean	SD
25	6	789	100	3	743	110	3	835	80
26	28	845	157	12	824	168	16	861	152
27	34	179	179	17	909	150	17	1098	157
28	52	1136	206	20	1122	160	32	1145	232
29	51	1229	251	27	1240	229	24	1218	278
30	25	1383	273	10	1268	269	15	1460	256
31	56	1650	298	27	1621	319	29	1677	280
32	113	1817	329	47	1785	322	66	1840	334
33	138	2013	352	55	1935	386	83	2064	320
34	197	2167	333	90	2151	341	107	2181	327
35	136	2299	379	73	2213	372	63	2399	366

GA: gestational age at birth [completed weeks]
SD: standard deviation

Figure 4 presents anthropometric data of infants at birth. There was a positive correlation between BW and GA (r = 0.650, n = 838, p < 0.001). Amongst Group A infants (30 – 35 weeks GA), positive correlations were also observed between GA and head circumference (r = 0.545, n = 489, p < 0.001) and body length (r = 0.419, n = 303, p < 0.001).



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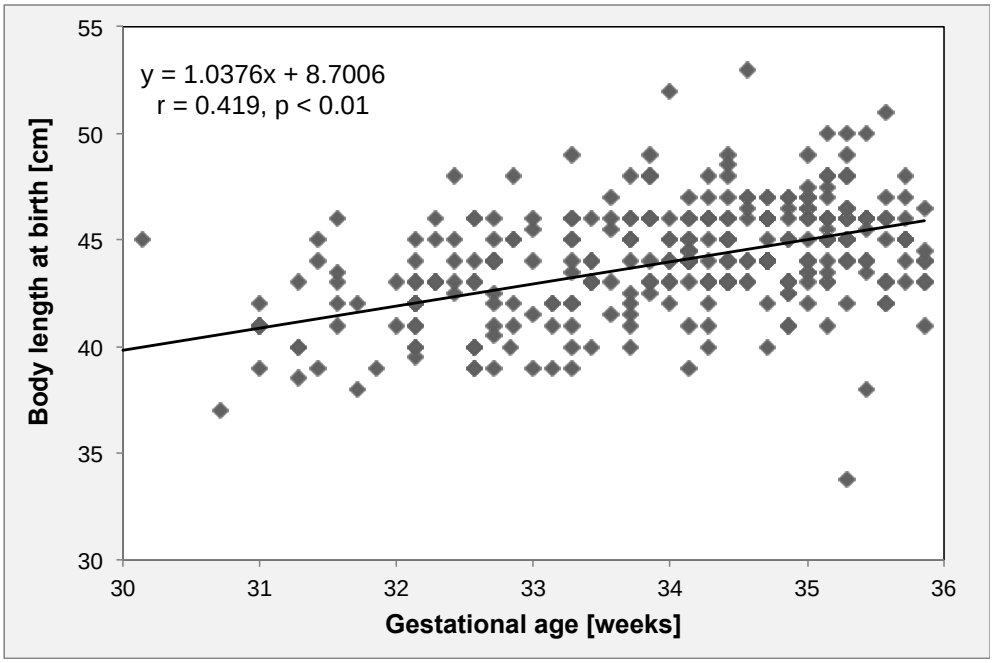


Figure 4: Anthropometric measurements of Group A and B subjects on day of birth. Group A subjects' **(A)** Body weight (n = 838), **(B)** Head circumference (n = 489), and **(C)** Body length (n = 303) were plotted by GA at birth.

Longitudinal postnatal growth analysis

On average, preterm infants of GAs at birth between 30 and 35 6/7 weeks (Group A) experienced maximal weight loss on day five of life. This maximal weight loss corresponded to 5.7% of the BW. BW was subsequently regained on average, by day 11 of life. Similarly, in the Group B study population (GA between 25 0/7 and 29 6/7), on average, maximal weight loss occurred on day five of life and BW was regained by day 12 of life. In contrast, the maximum weight loss seen by day five of life in Group B corresponded to 9.4% of the BW. Upon combining Group A and Group B infants, a negative correlation between percent of BW lost and GA at birth was observed ($r = 0.46$, $n = 838$, $p < 0.001$). Additionally, the mean postnatal body weights of each group of infants, when stratified by GA at birth, were significantly different from each other on days 1, 5, 11 and 12 at the 0.05 significance level.

The postnatal growth rates of Group A infants calculated as an average over a seven day period, over weeks two and three of postnatal life, ranged between a minimum of 13.5 g/kg/d to a maximum of 16.9 g/kg/d over the 31 to 37 week gestation period **(Figure 5A)**. The postnatal growth rates of Group B infants over weeks two and three of postnatal life, ranged from a minimum of 10.3 g/kg/d at 26 weeks corrected age to a maximum of 20.0 g/kg/d at 31 weeks corrected age **(Figure 5B)**.

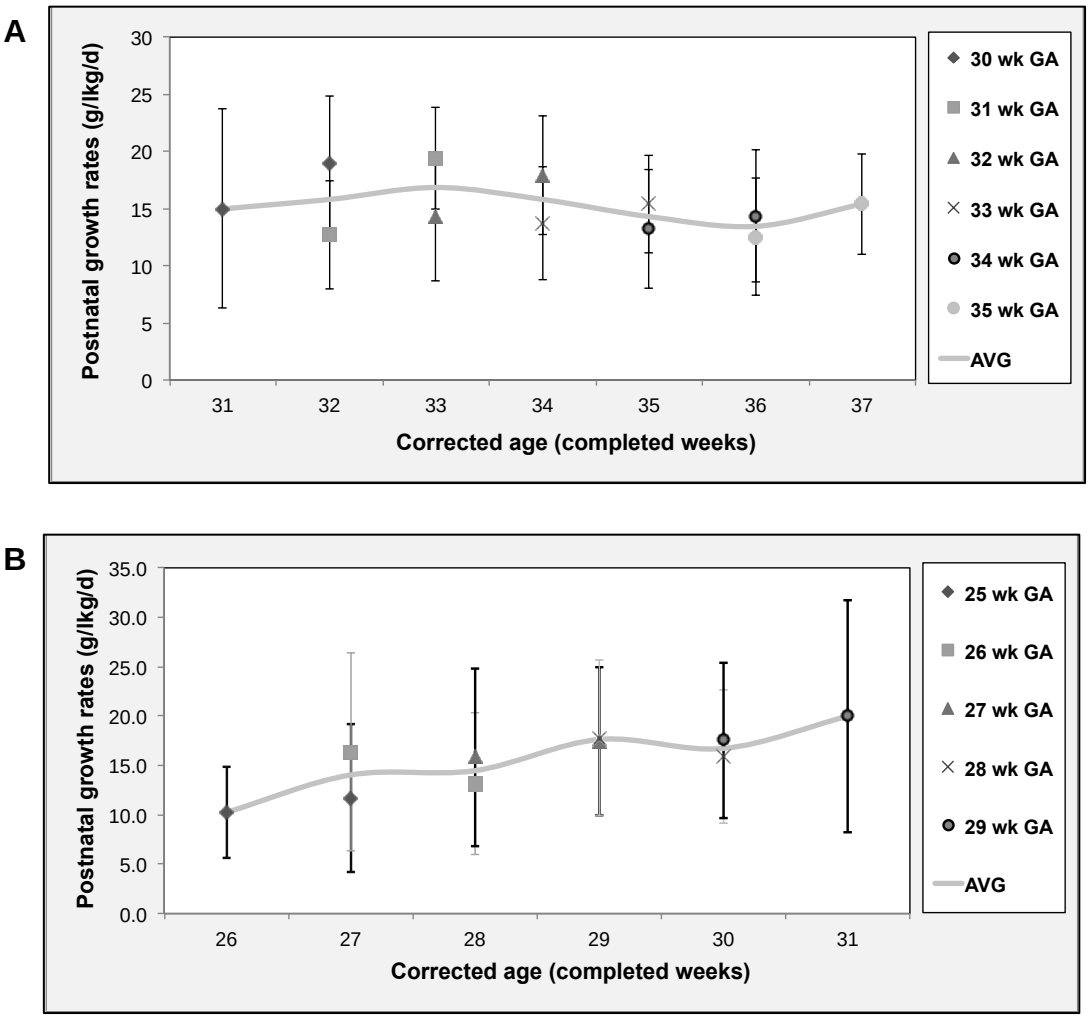


Figure 5: Postnatal growth rates of (A) Group A infants (30 - 35 weeks GA) and (B) Group B infants (25 - 29 weeks GA). Growth rates are calculated using an exponential growth model (Patel *et al.*, 2011). Values are presented as a mean ± standard deviation, and are representative of a seven-day period during the second and third week of postnatal life. Infants were stratified by GA at birth in completed weeks, and the average postnatal growth rates were plotted by corrected age (AVG).

SECTION A: Summary of results

Upon application of the strict screening criteria to select for preterm infants who required minimal medical intervention and who were not exposed to pre-defined factors known to impair postnatal growth, only 11.4% of 30 – 36 week GA infants and 30.2% of 24 – 29 week GA infants were eligible for the study.

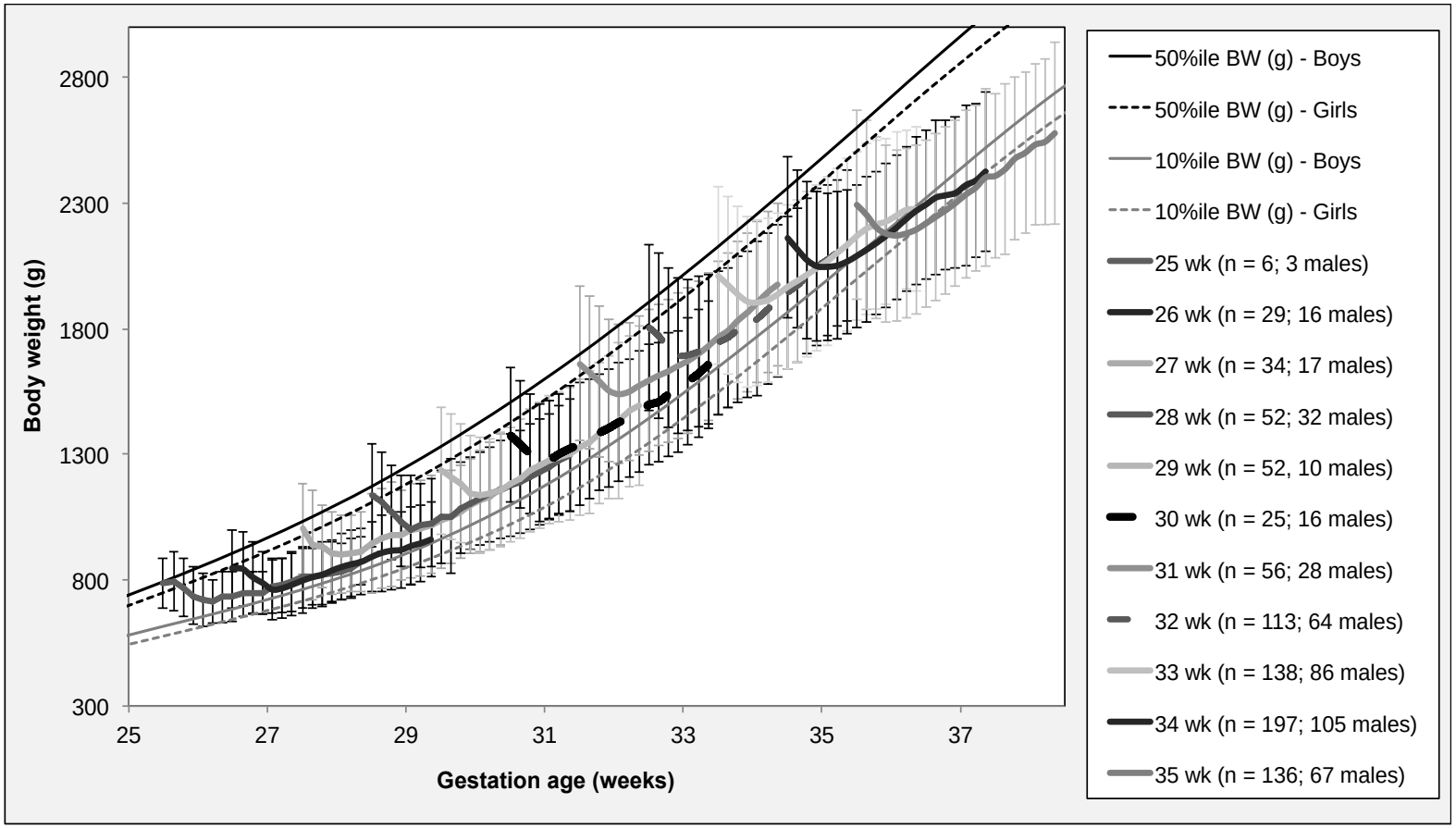
On average, Group A and B infants experienced maximal weight loss on day 5, however Group A infants regained BW by day 11, while Group B infants regained BW by day 12. However the percentage of BW lost on the day of body weight nadir was negatively correlated with GA at birth of the infant. Postnatal growth rates of Group A infants during the second and third week of life approximated 15 g/kg/d over the corrected age range of 31 to 37 weeks, while postnatal growth rates for Group B infants ranged from 10.3 – 20.0 g/kg/d over the 26 to 31 corrected age period.

SECTION B: Identifying trends in early postnatal growth

Comparison of postnatal growth to intrauterine growth references

Postnatal growth trajectories of Group A and Group B infants did not approximate recommended intrauterine growth references, using either BW%ile or EFW values as references **(Figure 6)**. By week two of life, infants in both groups had adjusted to stable growth trajectories that were below both the percentile on which the infant was born and below the reference curves.

A


Body weight (g)
2800
2300
1800
1300
800
300
25
27
29
31
33
35
37
Gestation age (weeks)
50%ile BW (g) - Boys
50%ile BW (g) - Girls
10%ile BW (g) - Boys
10%ile BW (g) - Girls
25 wk (n = 6; 3 males)
26 wk (n = 29; 16 males)
27 wk (n = 34; 17 males)
28 wk (n = 52; 32 males)
29 wk (n = 52, 10 males)
30 wk (n = 25; 16 males)
31 wk (n = 56; 28 males)
32 wk (n = 113; 64 males)
33 wk (n = 138; 86 males)
34 wk (n = 197; 105 males)
35 wk (n = 136; 67 males)

B

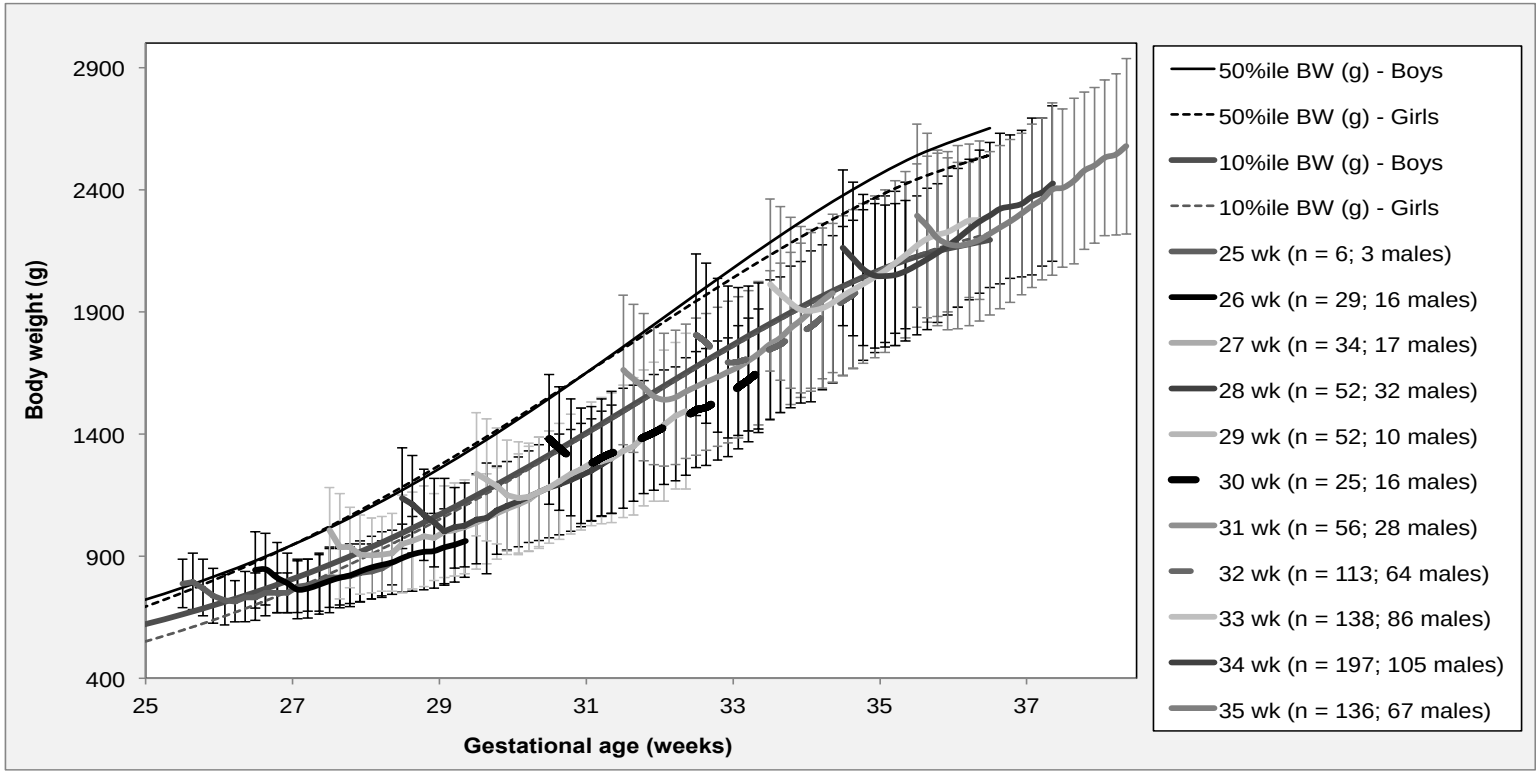


Figure 6: Postnatal growth trajectories of Group A (30 - 35 6/7 weeks GA) and Group B (25 – 29 6/7 weeks GA) infants (n_{total} = 838) superimposed on gender-specific (A) BW%iles (Kramer *et al.* 2001)**, and (B) EFW curves** (Salomon *et al.* 2007)**.** Day-specific mean body weight values ± standard deviation were plotted by corrected GA for each group of infants that were stratified by GA at birth. BW%ile values and mean body weight values were plotted at the middle of the completed week in order to correct for and prevent systematic plotting error, as recommended by Rochow *et al.* 2012.

On average, the BW of Group A (30 – 35 6/7 weeks GA) infants was 0.22 ± 0.885 SDS (n = 665) below the 50th BW%ile **(Figure 7A)** and 0.91 ± 1.811 SDS (n = 665) below the EFW value **(Figure 7B).** By day 14 and day 21, infants had adjusted to 0.97 ± 0.753 SDS (n = 665) and 0.98 ± 0.742 SDS (n = 333) below the 50th BW%ile **(Figure 7A)**, and to 1.54 ± 1.466 (n = 476) and 1.48 ± 1.407 (n = 236) below the EFW values **(Figure 7B)**.

Similarly, using the 50th BW%ile as the intrauterine growth reference, the average BW of Group A infants was 96 ± 16.4% (n = 665) of the reference, and 84 ± 12.5% on day 14 (n = 665) and 84 ± 11.9% on day 21 (n = 333) of postnatal life **(Figure 8A)**. Using the EFW curves as the intrauterine growth reference, the average BW of Group A infants was 95 ± 15.9% (n = 665) of the reference, and 86 ± 13.3% on day 14 (n = 476) and 87 ± 12.7% on day 21 (n = 236) of postnatal life **(Figure 8B)**.

Lastly, in order to take into consideration the initial body weight of the infant at birth when examining the percentile that the infant adjusted to following postnatal adaptation, z-score differences were calculated. Using the 50th BW%ile as a reference for the recommended weight-for-age, infants from Group A (30 – 35 completed weeks GA) adjusted on average to a trajectory that was associated with a z-score difference of -0.75 ± 0.310 on day 14 (n = 665) and -0.67 ± 0.375 on day 21 (n = 333) **(Figure 9A)**. Using the EFW curve as a reference for the recommended weight-for-age, infants from Group A (30 – 35 completed weeks) adjusted on average to a trajectory that was associated with a z-score difference

of -0.52 ± 0.734 on day 14 (n = 476) and -0.038 ± 1.019 on day 21 (n = 236) **(Figure 9B)**. Since z-scores could not be calculated for corrected ages beyond 36 weeks using EFW curves as the recommended weight-for-age, the number of infants for which z-scores and z-score differences were calculated was reduced to n = 236 by day 21.

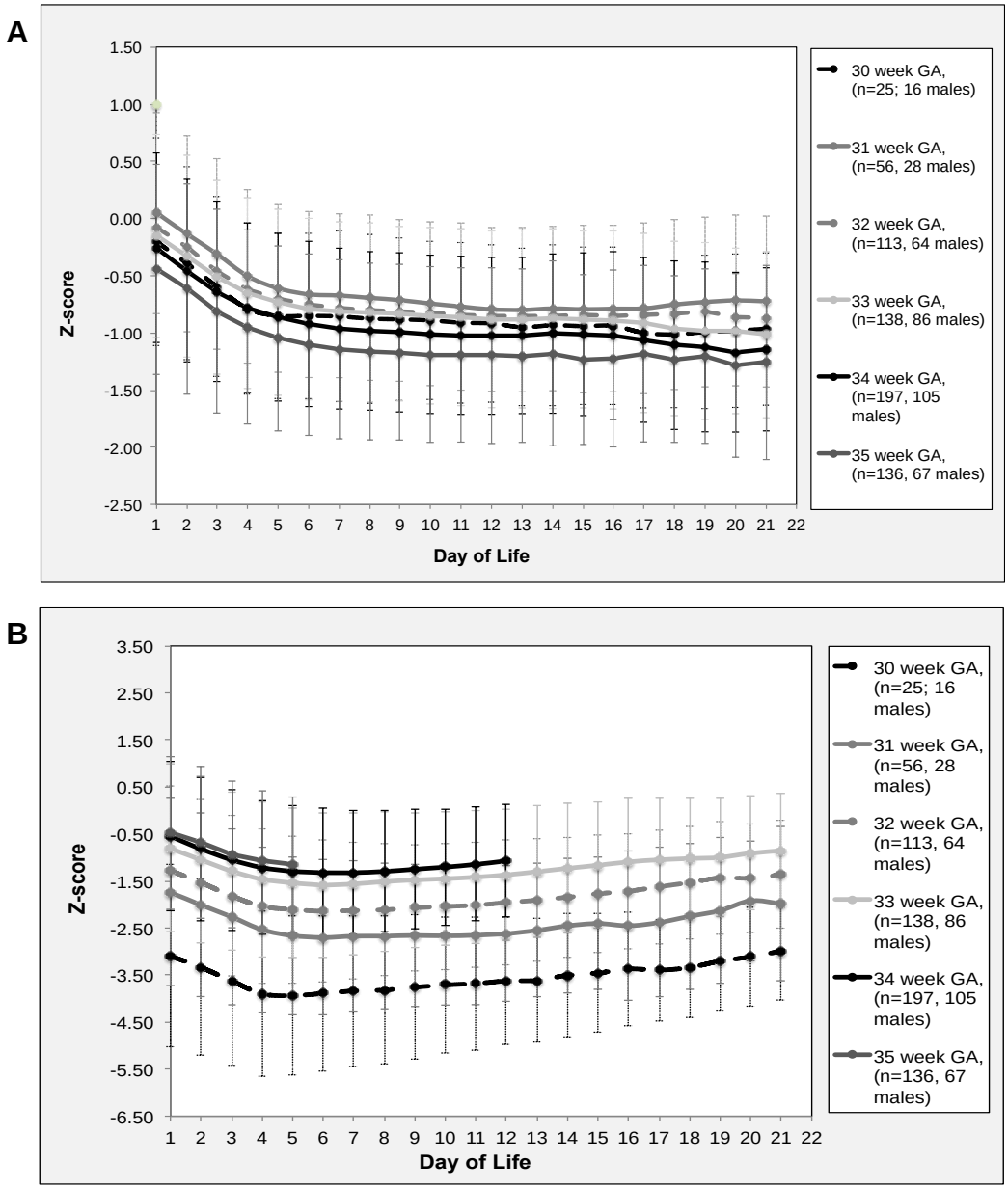


Figure 7: Z-scores of Group A infants (30 – 35 6/7 weeks GA) over first three weeks of postnatal life, using intrauterine growth references as recommended weight-for-age values. Z-scores were calculated on an individual-basis using day- and gender-specific reference values obtained from (A) 50th BW%iles (Kramer *et al.* 2001), and (B) mean EFW values (Salomon *et al.* 2007). Z-scores were presented as mean ± standard deviation for each group of infants stratified by GA.

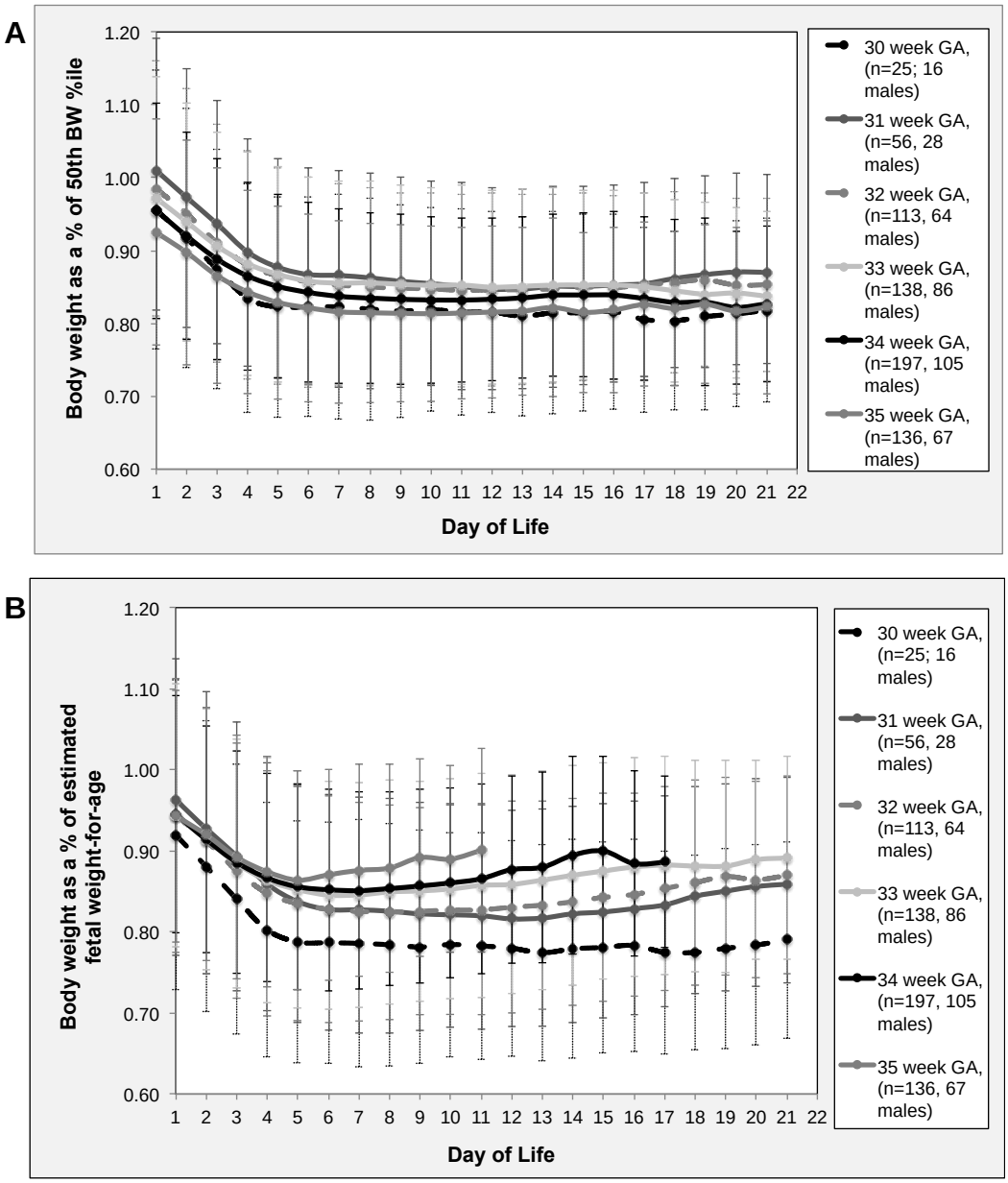


Figure 8: Day-specific body weights of Group A infants (30 – 35 6/7 weeks GA) over first three weeks of postnatal life expressed as percentages of recommended weight-for-age values. Percentages were calculated for each GA group and presented as mean ± standard deviation. Recommended weight-for-age values were represented using the gender-specific (A) 50th BW%iles from Kramer *et al.* 2001, and (B) mean EFW values from Salomon *et al.* 2007.

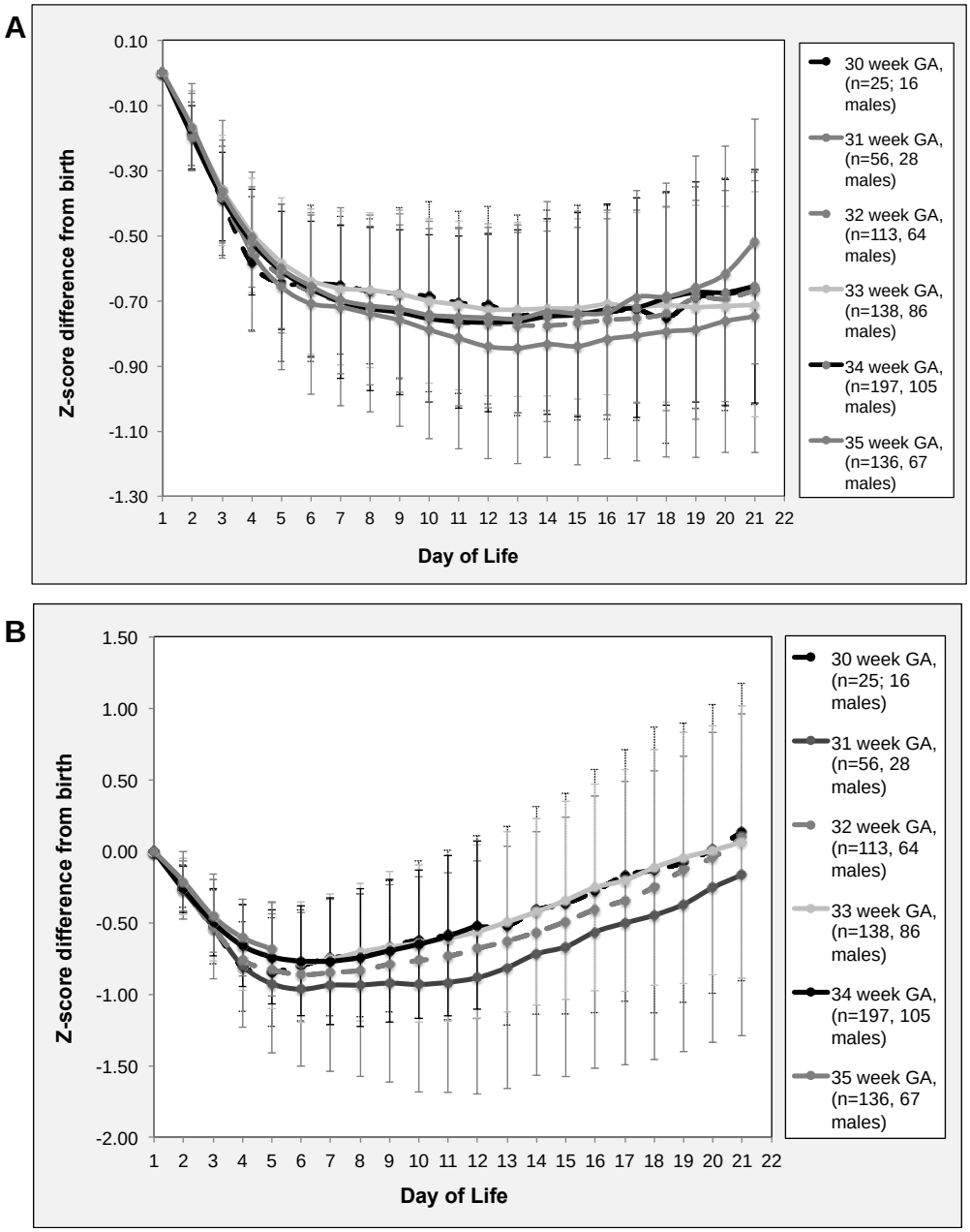


Figure 9: Z-score differences since birth of Group A infants (30 – 35 6/7 weeks GA) over first three weeks of postnatal life. Z-score differences were calculated by subtracting the mean z-score at each day from the z-score at birth, and were presented as mean ± standard deviation. Recommended weight-for-age values were represented using gender-specific (A) 50th BW%iles from Kramer *et al.* 2001, and (B) mean EFW values from Salomon *et al.* 2007.

On average, the BW of Group B (25 – 29 6/7 weeks GA) infants was 0.01 ± 0.869 SDS (n = 173) below the 50th BW%ile **(Figure 10A)** and 0.96 ± 1.314 SDS (n = 173) below the EFW value **(Figure 10B).** By day 14 and day 21, infants had adjusted to 0.88 ± 0.672 SDS (n = 173) and 0.92 ± 0.718 SDS (n = 169) below the 50th BW%ile **(Figure 10A)**, and to 2.45 ± 1.014 (n = 173) and 2.50 ± 1.079 (n = 169) below the EFW values **(Figure 10B)**.

Using the 50th BW%ile as the intrauterine growth reference, Group B infants were born with an average BW that was 100 ± 18.3% (n = 173) of the reference, and adjusted to body weights that were 81 ± 14.2% and 79 ± 19.1% of the 50th BW%ile by days 14 (n = 173) and 21 (n = 169) **(Figure 11A)**. Using the EFW curves as the intrauterine growth reference, the average BW of Group B infants was 91 ± 17.0% (n = 173) of the reference, and 73 ± 13.0% on day 14 (n = 476) and 71 ± 17.2% on day 21 (n = 169) of postnatal life **(Figure 11B)**.

Lastly, using the 50th BW%ile as the reference for the recommended weight-for age, Group B infants adjusted on average to a trajectory that was associated with a z-score difference of -0.87 ± 0.378 on day 14 (n = 173) and -0.89 ± 0.454 on day 21 (n = 169) **(Figure 12A)**. Using the EFW curve as a reference for the recommended weight-for-age, infants from Group B adjusted on average to a trajectory that was associated with a z-score difference of -1.49 ± 0.555 on day 14 (n = 173) and -1.55 ± 0.702 on day 21 (n = 169) **(Figure 12B)**.

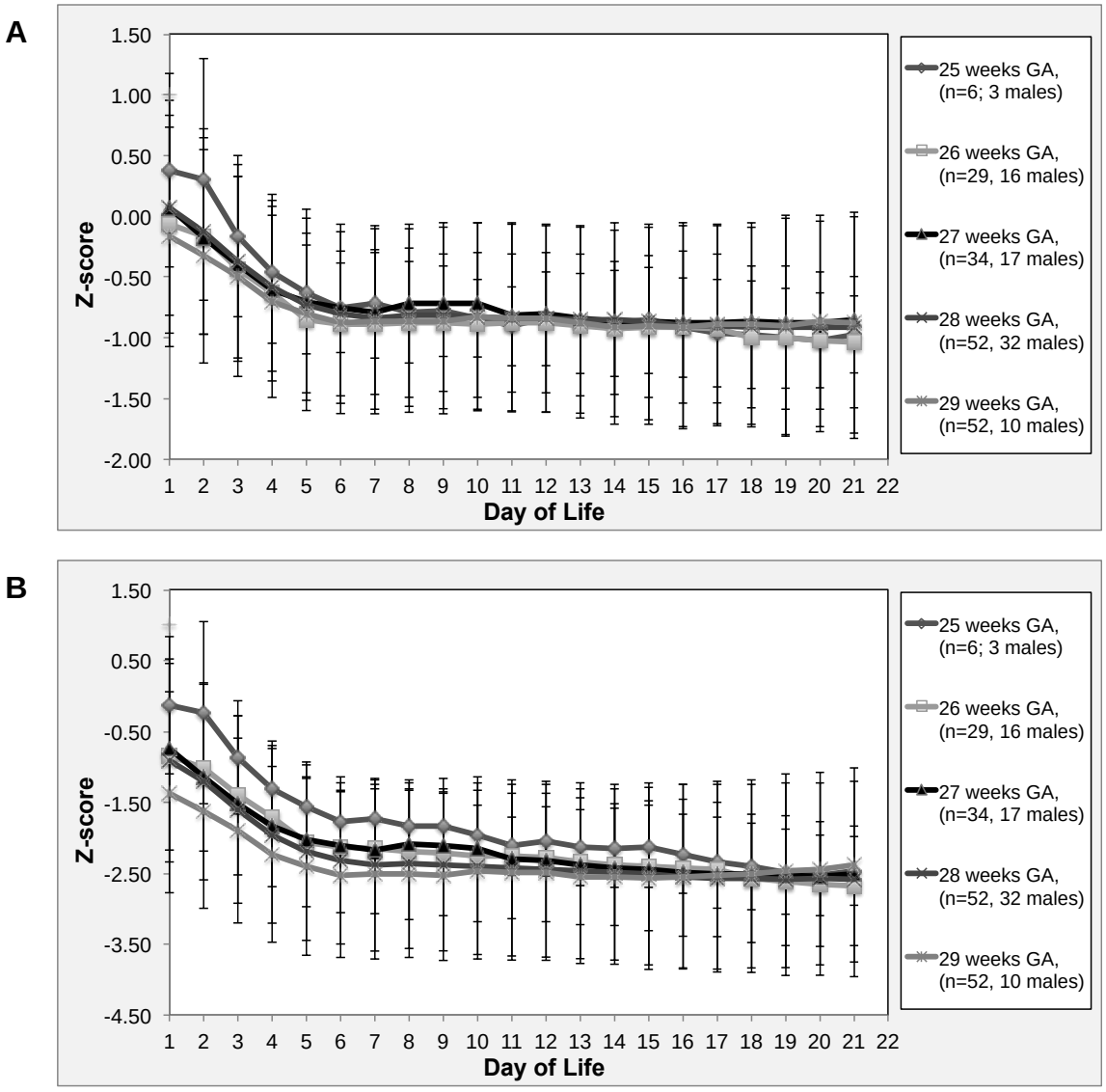


Figure 10: Z-scores of Group B infants (25 – 29 6/7 weeks GA) over first three weeks of postnatal life, using intrauterine growth references as recommended weight-for-age values. Z-scores were calculated on an individual-basis using day- and gender-specific reference intrauterine growth reference values obtained from (A) 50th BW%iles (Kramer *et al.* 2001), and (B) mean EFW values (Salomon *et al.* 2007). Z-scores were presented as mean ± standard deviation for each group of infants stratified by GA.

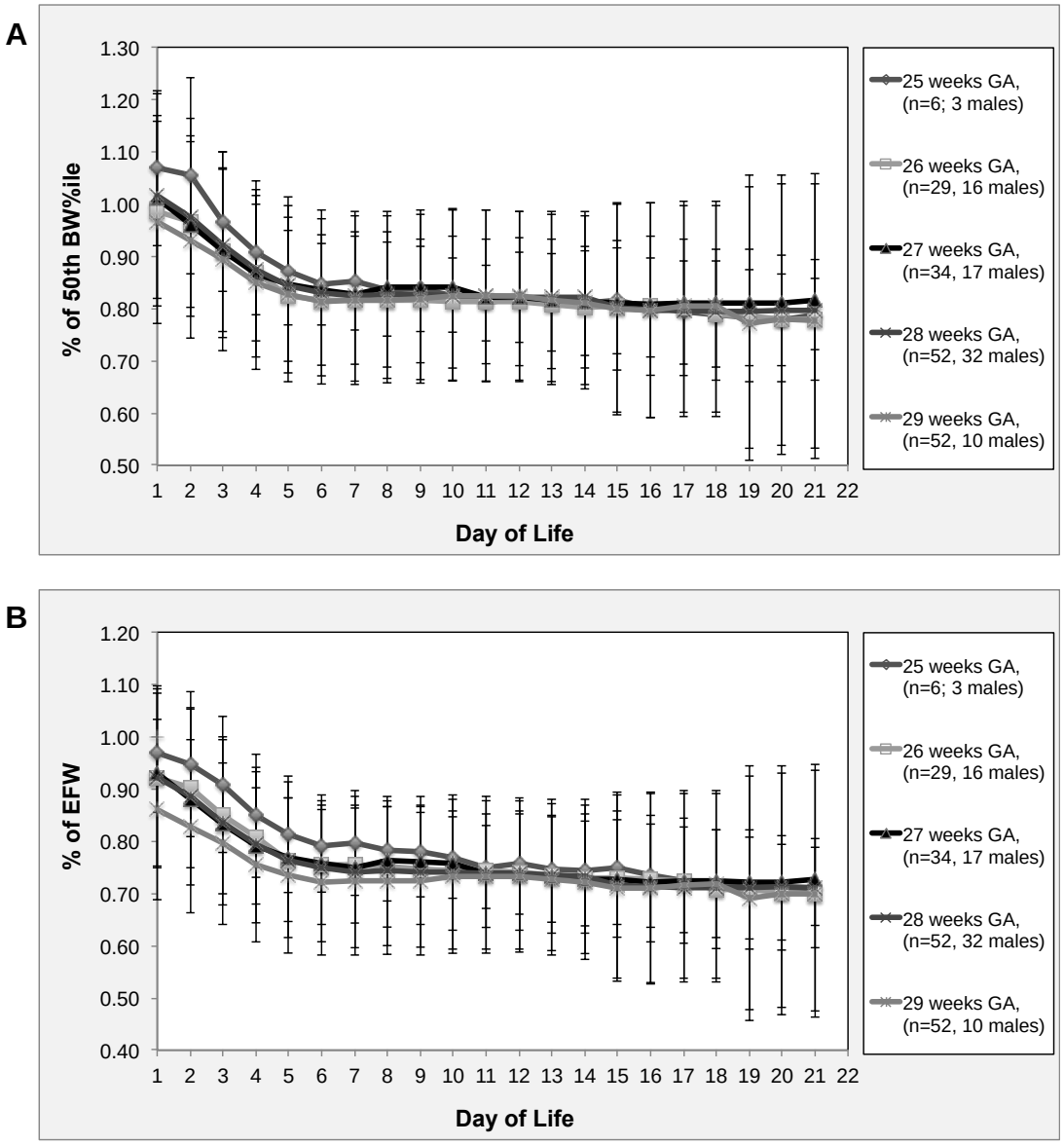


Figure 11: Day-specific body weights of Group B infants (25 – 29 6/7 weeks GA) over first three weeks of postnatal life expressed as percentages of recommended weight-for-age values. Percentages were calculated for each GA group and presented as mean ± standard deviation. Recommended weight-for-age values were represented using the gender-specific (A) 50th BW%iles from Kramer *et al.* 2001, and (B) mean EFW values from Salomon *et al.* 2007.

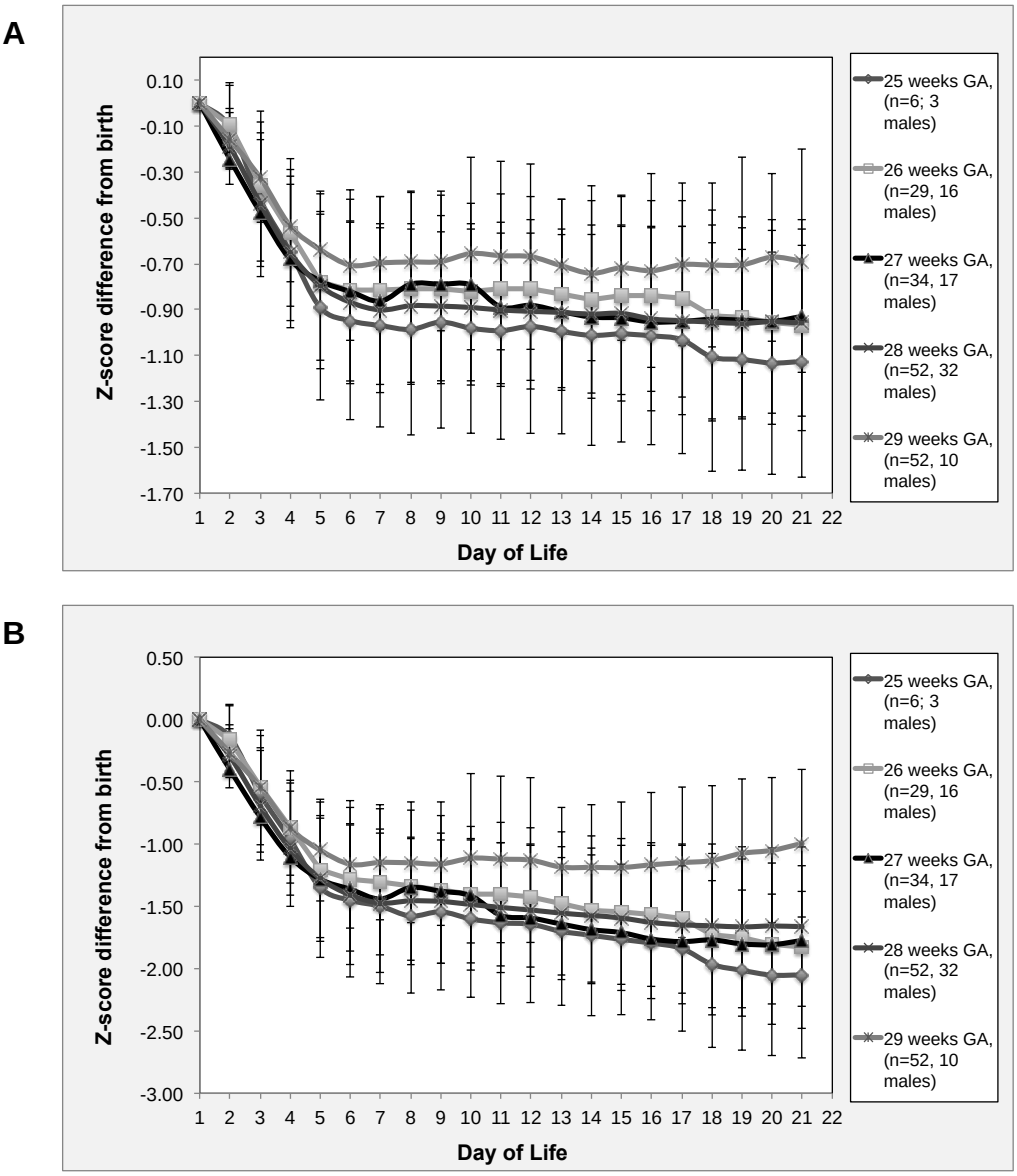


Figure 12: Z-score differences since birth of Group B infants (25 – 29 6/7 weeks GA) over first three weeks of postnatal life. Z-score differences were calculated by subtracting the mean z-score at each day from the z-score at birth, and were presented as mean ± standard deviation for each group of infants stratified by GA. Recommended weight-for-age values were represented using the gender-specific (A) 50th BW%iles from Kramer *et al.* 2001, and (B) mean EFW values from Salomon *et al.* 2007.

Assessment of study population characteristics and nutritional practices across hospital centres

Upon categorizing Group A infants by the hospital centre at which they were treated, distributions of BW **(Figure 13A)** and GA at birth **(Figure 13B)** were statistically different (Kruskal-Wallis test; p = 0.001, 0.005 respectively). Statistically significant differences in BW distributions were found primarily between MUMC/HUH/SMH and GUH/SJH (Mann-Whitney U Test), where the biggest difference was seen between MUMC and SJH (p < 0.001) **(Table 4).** Distributions of GA at birth over hospital centres were also very varied, with the biggest statistically significant difference seen between MUMC and GUH (p = 0.002) and MUMC and HUH (p = 0.004) **(Table 4)**.

The distribution of z-scores at birth of Group A infants between hospital centres was also analyzed to identify differences between BWs and weight-for-age recommendations that may have been hospital-specific **(Figure 14)**. It was observed that there were only statistically significant differences between SJH and HUH (p < 0.001) and SJH and MUMC (p = 0.047), where the distribution of z-scores at birth for SJH infants were closer to the recommended weight-for-age **(Table 4)**. By day 14, these differences between centres remained and additional differences between z-score distributions were observed between GUH and HUH (p = 0.003) and GUH and MUMC (p = 0.011) **(Table 4)**.

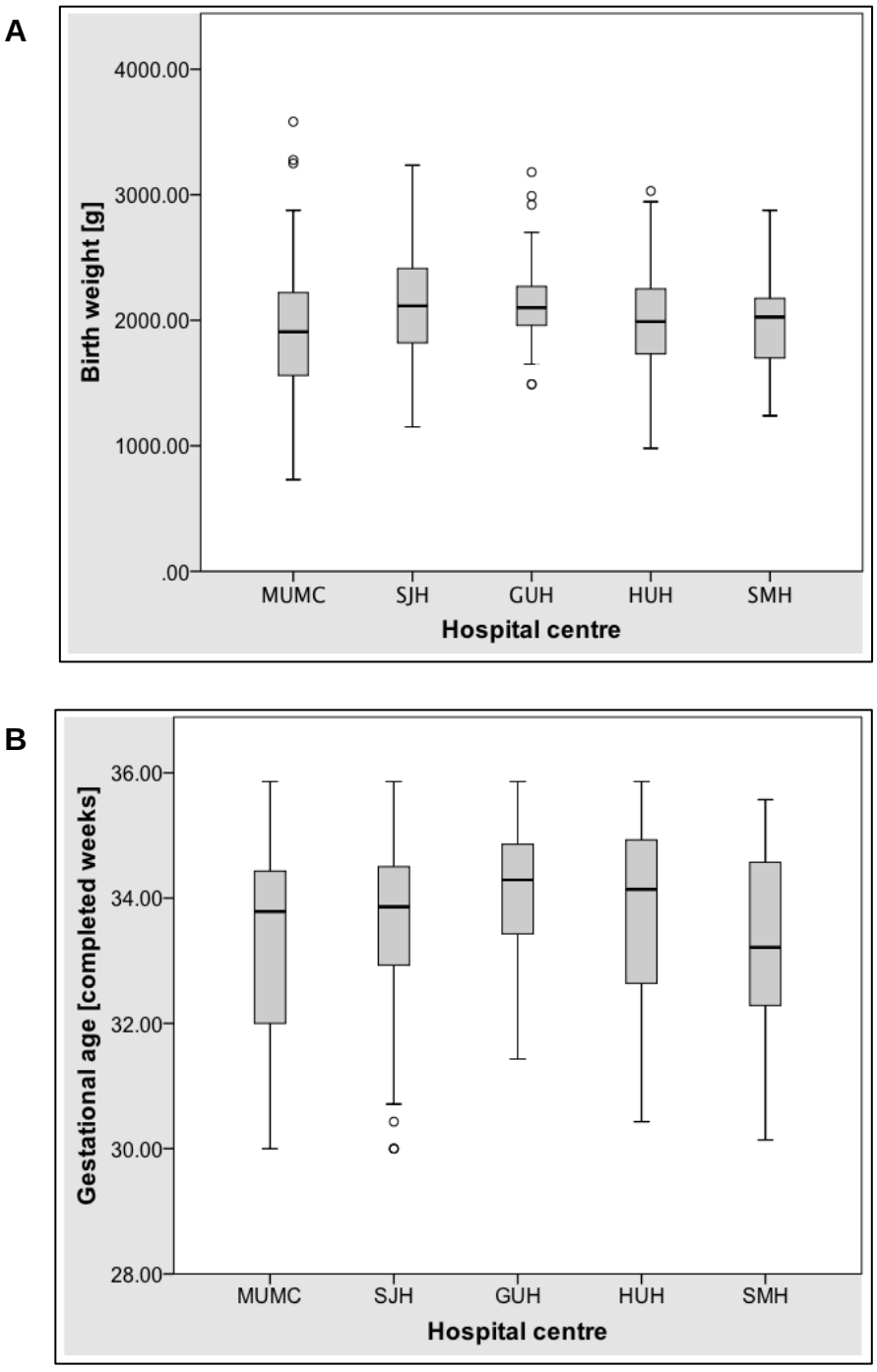


Figure 13: Distribution of (A) birth weights and (B) gestational ages of Group A infants, grouped by hospital centre.

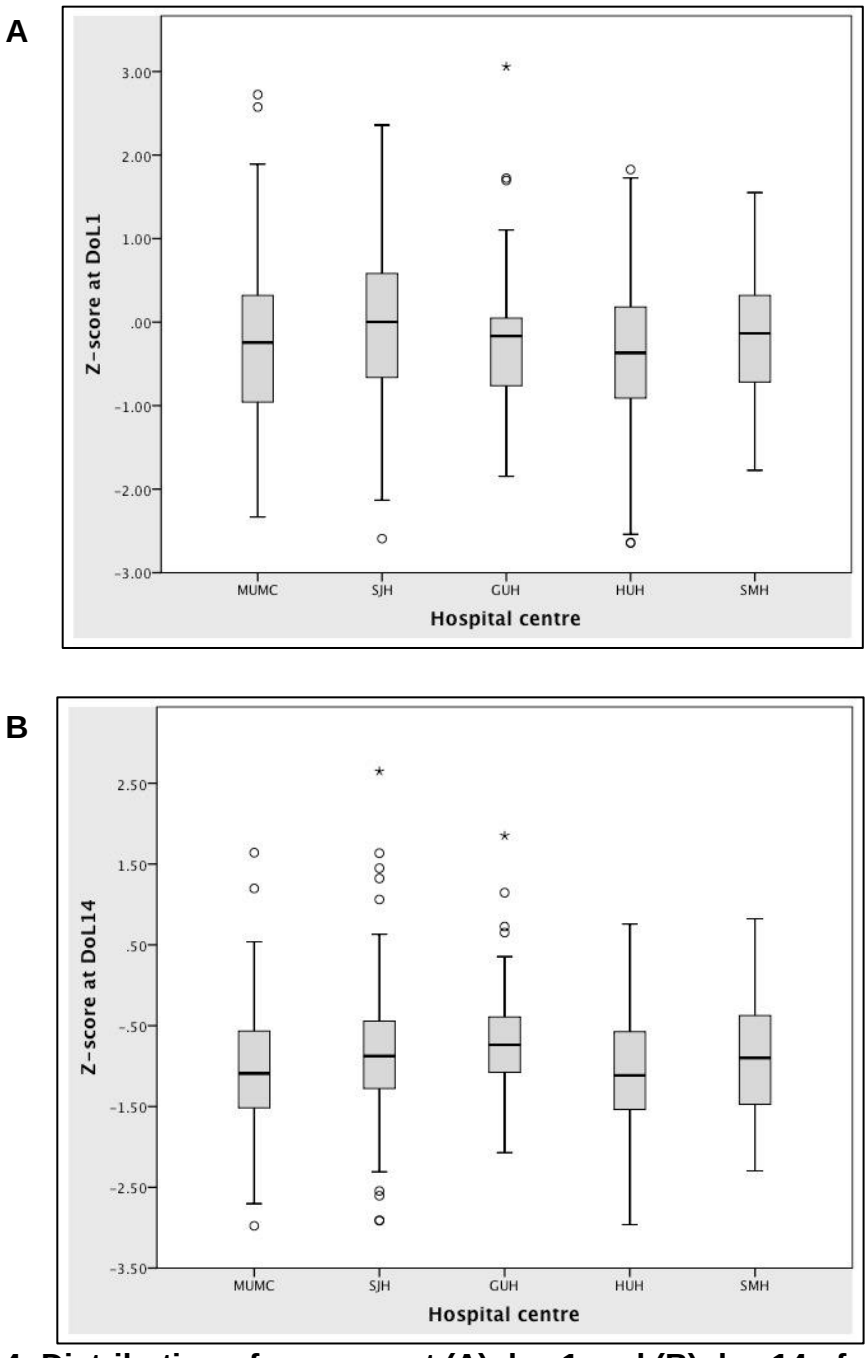


Figure 14: Distribution of z-scores at (A) day 1 and (B) day 14 of postnatal life of Group A infants by hospital centres. Z-scores were calculated using the 50th birth weight percentile as the reference weight (Kramer *et al.* 2001).

TABLE 4: Analyses of Group A distributions of birth weight, GA and growth characteristics across participating hospital centres. Z-scores were calculated using the 50th birth weight percentile as the reference weight-for-age. Mann-Whitney U Tests were used for distribution comparison across centres.

		MUMC	SJH	GUH	HUH
Birth weight	**SJH**	0.000*	-	-	-
	GUH	0.004*	0.816	-	-
	HUH	0.125	0.005*	0.045*	-
	SMH	0.240	0.045*	0.079	0.320

		MUMC	SJH	GUH	HUH
Gestational age	**SJH**	0.046*	-	-	-
	GUH	0.002*	0.038*	-	-
	HUH	0.004*	0.228	0.229	-
	SMH	0.685	0.178	0.008*	0.083

		MUMC	SJH	GUH	HUH
Z-score at birth	**SJH**	0.047*	-	-	-
	GUH	0.777	0.117	-	-
	HUH	0.195	0.000*	0.181	-
	SMH	0.578	0.312	0.709	0.082

		MUMC	SJH	GUH	HUH
Z-score at day 14	**SJH**	0.019*	-	-	-
	GUH	0.011*	0.312	-	-
	HUH	0.947	0.001*	0.003*	-
	SMH	0.361	0.525	0.207	0.283

* denotes a statistical difference at the 0.05 significance level

MUMC: McMaster University
SJH: St. Joseph's Healthcare Hamilton
GUH: Greifswald University Hospital
HUH: Heidelberg University Hospital
SMH: St. Michael's Hospital

In order to assess the differences in nutritional practices between centres, the distribution of the day of life when full enteral feeds (120 mL/kg/d) were first administered were compared across centres **(Figure 15)**. Significant differences were observed in the medians between MUMC/SJH and each of the remaining centres **(Table 5),** where MUMC and SJH administered full enteral feeds 1 – 2 days earlier in comparison to the other centres.

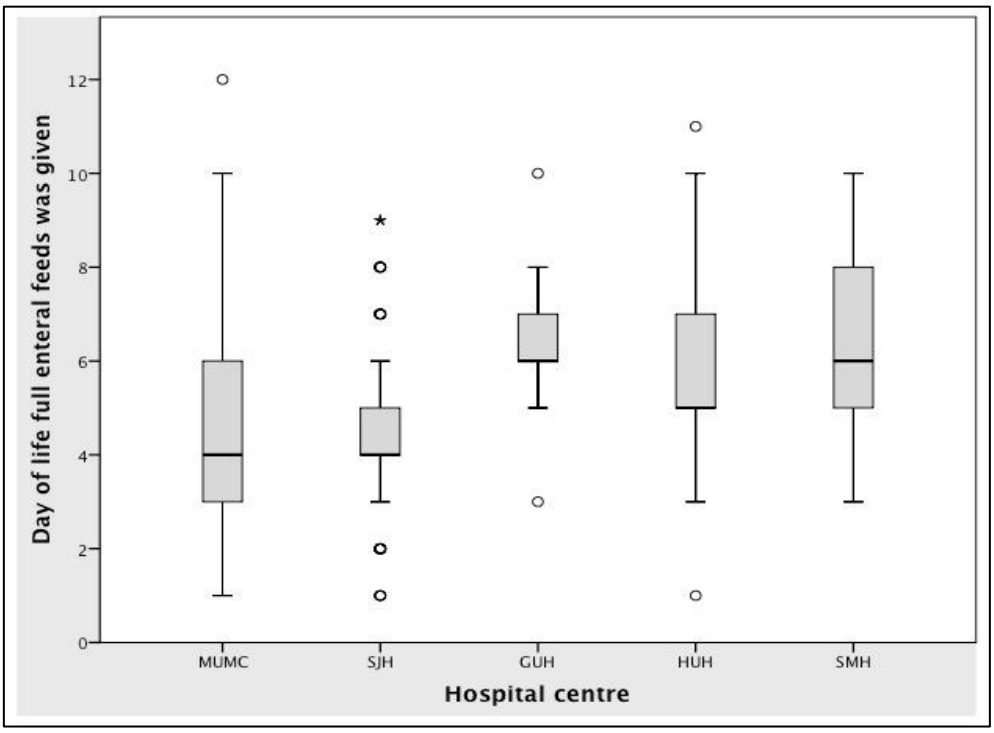


Figure 15: Comparison of nutritional strategies between participating hospital centres. The distribution of the day of life that full enteral feeds (120 mL/kg/d) were administered to Group A infants (30 – 35 6/7 weeks GA) is shown across hospital centres.

TABLE 5: Analyses of nutritional strategies across participating hospital centres in Group A infants. The median day of life that full enteral feeds (120 mL/kg/d) were administered was compared between centres using the Median Test.

Day of life full enteral feeds (120 mL/kg/d) were administered		MUMC	SJH	GUH	HUH
	SJH	0.929	-	-	-
	GUH	0.000*	0.000*	-	-
	HUH	0.005*	0.000*	0.091	-
	SMH	0.001*	0.000*	0.928	0.091

* denotes a statistical difference at the 0.05 significance level

Analysis of factors that affect postnatal growth

Z-score differences were used to represent postnatal growth after postnatal adaptation, because this accounted for the degree of prematurity and initial BW of the infant by relating the body weight to their weight-for-age recommended value. No correlation was found between the day of life full enteral feeds were attained and the z-score difference at day 14 (R^2 = 0.010). Similarly, there was no significant effect of the ethnic background of the infant on the z-score difference on day of life 14 (p = 0.100).

SECTION B: Summary of results

Group A and B preterm infants on average, had BWs that approximated the 50th BW%ile, and were approximately 1 SDS below EFW curves. On average, all infants (25 – 35 6/7 weeks GA) consistently adjusted to stable postnatal growth trajectories by day 14 of life. For Group A infants, stable growth was characterized as -0.97 SDS or 84% of the 50th BW%ile by days 14-21. For Group B infants, stable growth corresponded to trajectories that approximated -0.90 SDS or 80% of the 50th BW%ile by days 14-21. Differences were observed between Group A and Group B when postnatal growth was compared to EFW. Using EFW curves as the reference, Group A infants adjusted to stable growth that approximated -1.5 SDS or 86% of the EFW, while Group B infants adjusted to stable growth trajectories that approximated -2.5 SDS or 72% of EFW curves.

After stratification by hospital centre, it was observed that the distributions of BWs, GAs, z-scores and day of life full enteral feeds were administered (feeding tolerance) were significantly different between centres. SJH/GUH had higher BWs and MUMC/HUH/SMH had the widest range of GAs. By day 14, differences in growth were statistically significant between centres, where SJH/GUH infants adjusted to growth that was closer to the 50th BW%ile. Lastly, full enteral feeds (feeding tolerance) was attained significantly faster in MUMC/SJH than other centres, but similar to ethnic backgrounds, was found to have no statistically significant effect on day 14 z-score differences.

SECTION C: Development and selection of models to predict early postnatal growth in "low-risk" preterm infants

Models to predict body weights of "low-risk" preterm infants

Representing categorical variables as dichotomous variables

A step-wise forward selection procedure was used to produce predictive models of postnatal growth for "low-risk" preterm infants, where the dependent variable that produced the highest coefficient of determination was the raw body weight. The body weight model for days 7, 14 and 21 which produced the highest Pearson correlation coefficient incorporated the following independent variables: gestational age (GA, [weeks]), birth weight (BW, [g]) and hospital centre. The hospital centre variable was dichotomized according to the nutritional strategy used (C; MUMC/SJH = 0; GUH/HUH/SMH = 1) **(Table 6).** GA, BW and hospital centre were also significant predictors of body weight for day 14 **(Table 7)** and 21 **(Table 8)**. The following are the resulting predictive equations:

BODY WEIGHT [g] AT DAY 7 ($R^2 = 0.961$)

= -427.813 + (17.648) * GA + (0.868) * BW + (25.785) * C

BODY WEIGHT [g] AT DAY 14 ($R^2 = 0.930$)

= -604.210 + (27.738) * GA + (0.879) * BW + (42.407) * C

BODY WEIGHT [g] AT DAY 21 ($R^2 = 0.894$)

= -549.676 + (31.479) * GA + (0.895) * BW + (92.788) * C

TABLE 6: Model to predict body weight of "low-risk" preterm infants on day seven of life. Step-wise forward selection was used where additional independent variables were included in the model if the R^2 increased by at least 0.01 and the associated *p*-value was <0.001.

	Independent variable (s)	R^2	P-value
(1)	**Gestational age**	0.356	0.000
	Gestational age **Z score at birth**	0.942	0.000 0.000
(2)	Gestational age **Birth weight**	0.960	0.000 0.000
	Gestational age **% of 50th BW%ile at birth**	0.934	0.000 0.000
(3)	Gestational age Birth weight **Hospital centre, dichotomized by feeds**	0.961	0.000 0.000 0.000
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Gestation**	0.962	0.000 0.000 0.000 0.028
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Mode of delivery**	0.961	0.000 0.000 0.000 0.768
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Head circumference at birth**	0.959	0.000 0.000 0.000 0.071
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Body length at birth**	0.949	0.001 0.000 0.021 0.713

TABLE 7: Model to predict body weight of "low-risk" preterm infants on day fourteen of life. Step-wise forward selection was used where additional independent variables were included in the model if the R^2 increased by at least 0.01 and the associated *p*-value was <0.001.

	Independent variable (s)	R^2	P-value
(1)	**Gestational age**	0.372	0.000
	Gestational age **Z score at birth**	0.914	0.000 0.000
(2)	Gestational age **Birth weight**	0.928	0.000 0.000
	Gestational age **% of 50th BW%ile at birth**	0.906	0.000 0.000
(3)	Gestational age % of 50th BW%ile at birth **Hospital centre, dichotomized by feeds**	0.930	0.000 0.000 0.000
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Gestation**	0.932	0.000 0.000 0.000 0.655
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Mode of delivery**	0.941	0.000 0.000 0.000 0.537
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Head circumference at birth**	0.925	0.000 0.000 0.000 0.021
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Body length at birth**	0.919	0.000 0.000 0.000 0.546

TABLE 8: Model to predict body weight of "low-risk" preterm infants on day twenty-one of life. Step-wise forward selection was used where additional independent variables were included in the model if the R^2 increased by at least 0.01 and the associated *p*-value was <0.001.

	Independent variable (s)	R^2	P-value
(1)	**Gestational age**	0.350	0.000
	Gestational age Z score at birth	0.868	0.000 0.000
(2)	Gestational age **Birth weight**	0.881	0.000 0.000
	Gestational age % of 50th BW%ile at birth	0.863	0.000 0.000
(3)	Gestational age Birth weight **Hospital centre, dichotomized by feeds**	0.894	0.000 0.000 0.000
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Gestation**	0.890	0.000 0.000 0.000 0.653
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Mode of delivery**	0.901	0.000 0.000 0.000 0.092
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Head circumference at birth**	0.891	0.000 0.000 0.000 0.242
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Body length at birth**	0.870	0.000 0.000 0.000 0.674

Predictive models were also constructed using z-scores and percentage of the 50th BW%ile as the dependent variables, however were associated with lower Pearson correlation coefficients. Z-score models for days 7, 14 and 21 were associated with R^2 = 0.939, 0.889, 0.841. Percentage of the 50th BW%ile models for days 7, 14 and 21 were associated with R^2 = 0.936, 0.888, 0.836.

Representing categorical variables using "dummy" coding

Multiple linear regression models were also constructed with the replacement of the dichotomized hospital centre variable with the "dummy" coded hospital centre variable. Using the step-wise forward selection procedure, the same independent variables (GA, BW and hospital centre) were once again shown to be the lowest combination of variables to produce the highest Pearson correlation coefficient. The following were the resulting predictive equations with their associated Pearson correlation coefficients:

Body weight at DAY 7, $R^2 = 0.964$

$$= - 400.699 + 0.863*BW + 17.002*GA + 5.406*x_{SJH} + 111.629*x_{GUH} + 18.282*x_{HUH} + 15.401*x_{SMH}$$

Body weight at DAY 14, $R^2 = 0.934$

$$= - 567.729 + 0.873*BW + 26.559*GA + 19.926*x_{SJH} + 141.325*x_{GUH} + 47.524*x_{HUH} + 24.184*x_{SMH}$$

Body weight at DAY 21, $R^2 = 0.902$

$= - 466.248 + 0.891*BW + 28.447*GA + 39.034*x_{SJH} + 212.59*x_{GUH} + 113.26*x_{HUH} + 43.874*x_{SMH}$

Models to predict z-scores of "low-risk" preterm infants

Representing categorical variables as dichotomous variables

Using a step-wise forward selection procedure, the most accurate multiple linear regression model to predict z-scores when dichotomizing hospital centres by their feeding practices, resulted from the incorporation of the following independent variables: gestational age (GA, [weeks]), z-score at birth (Z_1) and hospital centre (C; MUMC/SJH = 0; GUH/HUH/SMH = 1) **(Table 9).** GA, Z_1 and dichotomized hospital centre continued to be significant predictors for z-scores at days 14 **(Table 10)** and 21 **(Table 11)**. The following are the resulting predictive equations:

Z-SCORE AT DAY 7 ($R^2 = 0.939$)

$= -0.214 – (0.016) * GA + (0.828) * Z_1 + (0.065) * C$

Z-score AT DAY 14 ($R^2 = 0.889$)

$= -0.689 - (0.005) * GA + (0.807) * Z_1 + (0.101) * C$

Z-score AT DAY 21 ($R^2 = 0.841$)

$= - 0.721 - (0.005) * GA + (0.787) * Z_1 + (0.216) * C$

TABLE 9: Model to predict z-score of "low-risk" preterm infants on day seven of life. Step-wise forward selection was used where additional independent variables were included in the model if the R^2 increased by at least 0.01 and the associated *p*-value was < 0.001.

	Independent variable (s)	R^2	P-value
(1)	**Gestational age**	0.036	0.000
(2)	Gestational age **Z score at birth**	0.937	0.006 0.000
	Gestational age **Birth weight**	0.917	0.000 0.000
	Gestational age **% of 50th BW%ile at birth**	0.930	0.000 0.000
(3)	Gestational age Z-score at birth **Hospital centre, dichotomized by feeds**	0.939	0.003 0.000 0.000
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Gestation**	0.939	0.037 0.000 0.000 0.022
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Mode of delivery**	0.939	0.003 0.000 0.000 0.862
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Head circumference at birth**	0.939	0.000 0.000 0.000 0.766
	Gestational age Z-score at birth Hospital centre, dichotomized by feeds **Body length at birth**	0.931	0.001 0.000 0.024 0.797

TABLE 10: Model to predict z-score of "low-risk" preterm infants on day fourteen of life. Step-wise forward selection was used where additional independent variables were included in the model if the R^2 increased by at least 0.01 and the associated *p*-value was < 0.001.

	Independent variable(s)	R^2	P-value
(1)	**Gestational age**	0.027	0.000
(2)	Gestational age **Z score at birth**	0.885	0.728 0.000
	Gestational age **Birth weight**	0.864	0.000 0.000
	Gestational age **% of 50th BW%ile at birth**	0.878	0.089 0.000
(3)	Gestational age Z score at birth **Hospital centre, dichotomized by feeds**	0.889	0.513 0.000 0.000
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Gestation**	0.887	0.848 0.000 0.000 0.607
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Mode of delivery**	0.906	0.165 0.000 0.000 0.583
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Head circumference at birth**	0.885	0.077 0.000 0.000 0.879
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Body length at birth**	0.888	0.071 0.000 0.000 0.862

TABLE 11: Model to predict z-score of "low-risk" preterm infants on day twenty-one of life. Step-wise forward selection was used where additional independent variables were included in the model if the R^2 increased by at least 0.01 and the associated *p*-value was < 0.001.

	Independent variable(s)	R^2	P-value
(1)	**Gestational age**	0.043	0.000
(2)	Gestational age **Z score at birth**	0.822	0.981 0.000
	Gestational age **Birth weight**	0.802	0.000 0.000
	Gestational age **% of 50th BW%ile at birth**	0.816	0.226 0.000
(3)	Gestational age Z score at birth **Hospital centre, dichotomized by feeds**	0.841	0.712 0.000 0.000
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Gestation**	0.829	0.702 0.000 0.000 0.732
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Mode of delivery**	0.850	0.599 0.000 0.000 0.136
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Head circumference at birth**	0.843	0.161 0.000 0.000 0.710
	Gestational age Z score at birth Hospital centre, dichotomized by feeds **Body length at birth**	0.834	0.498 0.000 0.000 0.639

Representing categorical variables using "dummy" coding

Similar to the body weight regressions, multiple linear regressions of z-scores for days 7, 14 and 21 were also re-constructed with the replacement of the dichotomized hospital centre variable with the "dummy" coded hospital centre variable. The following were the resulting predictive equations with their associated R^2 values:

Z-score at DAY 7, $R^2 = 0.945$

$= -0.09 + 0.823*Z_1 - 0.02*GA + 0.017*x_{SJH} + 0.275*x_{GUH} + 0.049*x_{HUH} + 0.04*x_{SMH}$

Z-score at DAY 14, $R^2 = 0.895$

$= -0.0541 + 0.802*Z_1 - 0.01*GA + 0.042*x_{SJH} + 0.327*x_{GUH} + 0.111*x_{HUH} + 0.051*x_{SMH}$

Z-score at DAY 21, $R^2 = 0.852$

$= -0.444 + 0.784*Z_1 - 0.014*GA + 0.066*x_{SJH} + 0.476*x_{GUH} + 0.252*x_{HUH} + 0.072*x_{SMH}$

Accuracy of predictive models

Representing categorical variables as dichotomous variables

For the day 7 body weight model, actual and predicted values were strongly correlated ($R^2 = 0.961$) and residuals ranged from -291 to 339 g **(Figure 16).** For the day 14 body weight model, actual and predicted values were also strongly correlated ($R^2 = 0.930$) and residuals ranged from -288 to 945 g **(Figure 17)**. For the day 21 body weight model, actual and predicted values were well correlated ($R^2 = 0.894$) and residuals ranged from -281 to 495 g **(Figure 18)**.

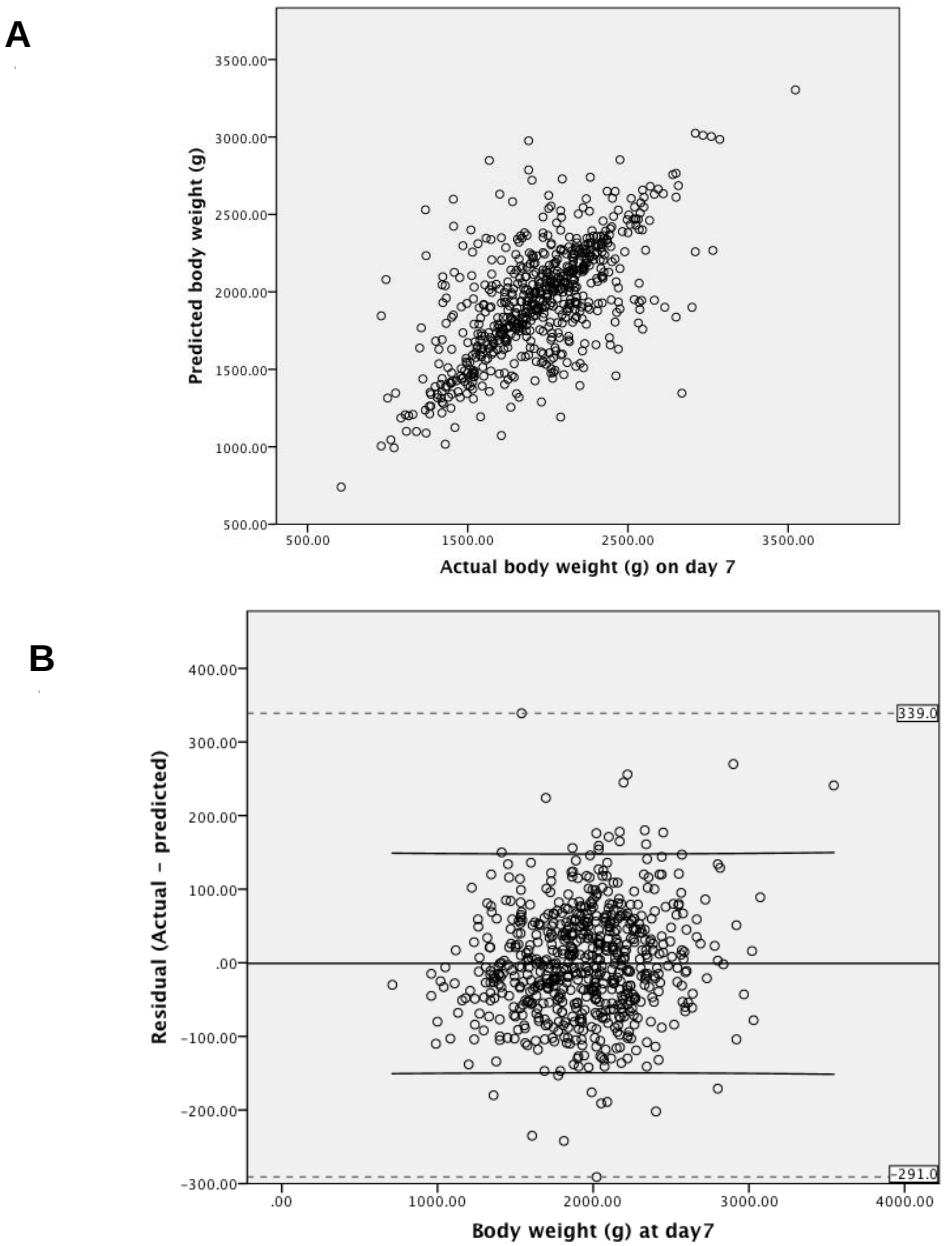


Figure 16: Correlation (A) and accuracy (B) of model to predict body weight at day 7 of life. Body weights were predicted using the following regression: -427.813 + (17.648)*GA + (0.868)*BW + (25.785)*C, where GA = gestational age at birth, BW = birth weight, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

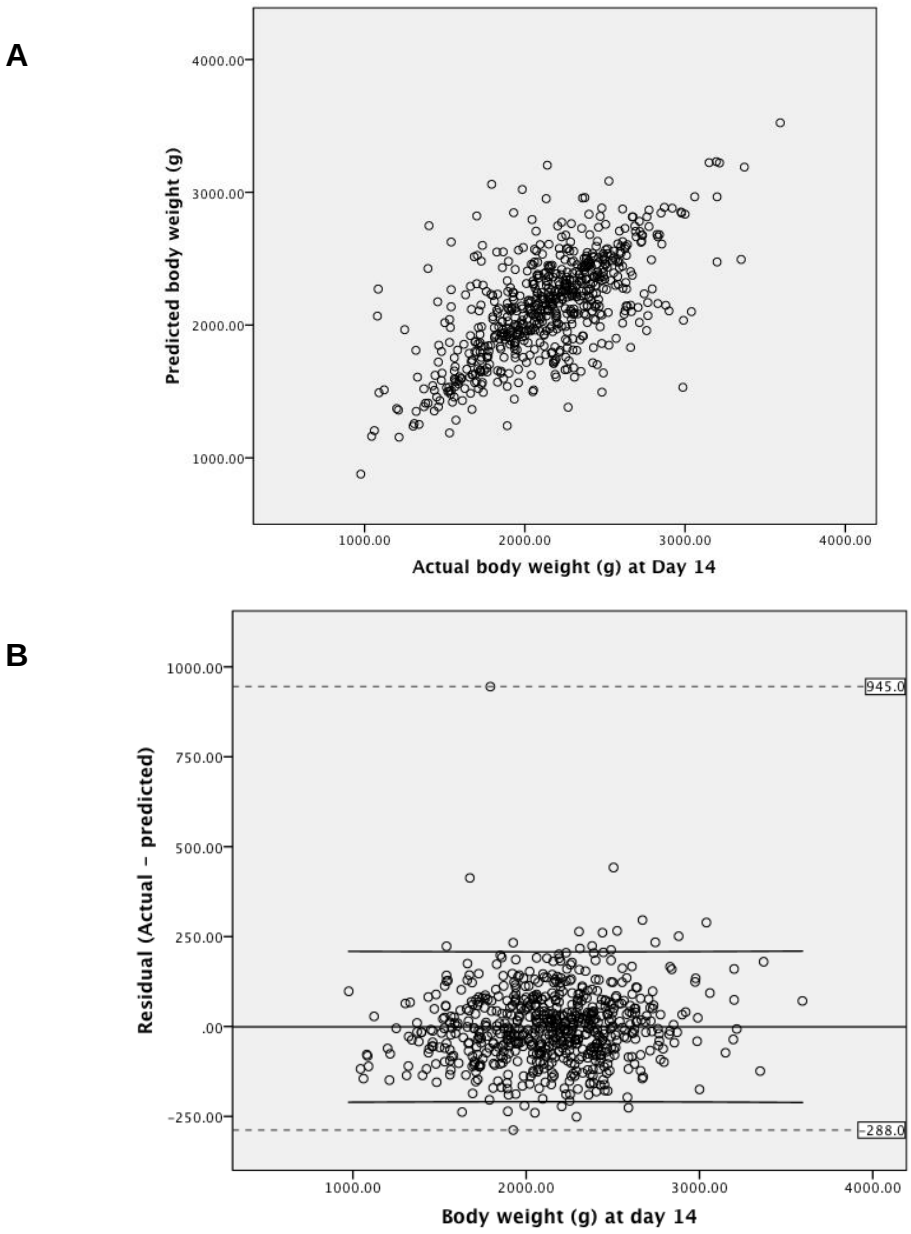
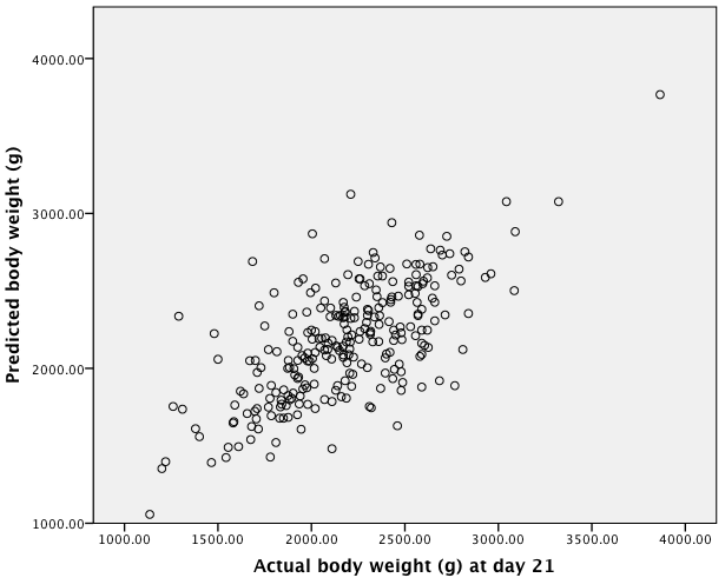


Figure 17: Correlation (A) and accuracy (B) of model to predict body weight at day 14 of life. Body weights were predicted using the following regression: -604.210 + (27.738)*GA + (0.879)*BW + (42.407)*C, where GA = gestational age at birth, BW = birth weight, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line.

A



B

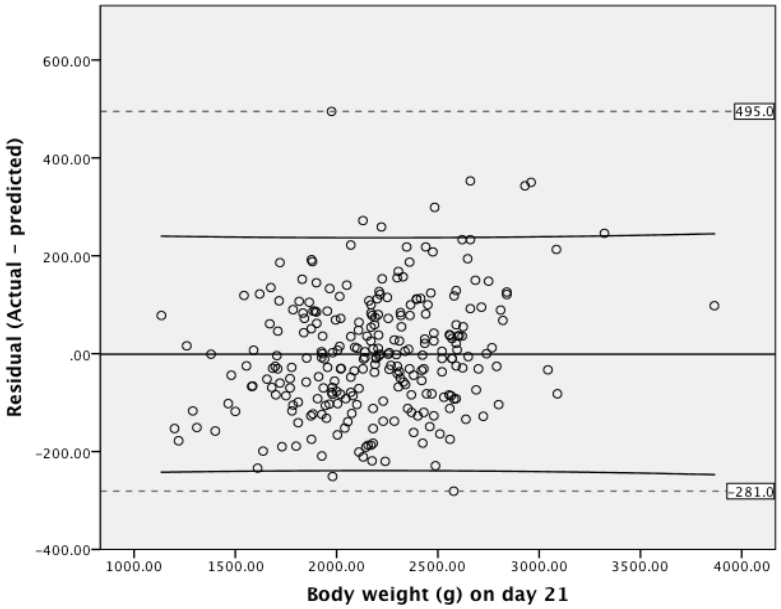


Figure 18: Correlation (A) and accuracy (B) of model to predict body weight at day 21 of life. Body weights were predicted using the following regression: -549.676 + (31.479)*GA + (0.895)*BW + (92.788)*C, where GA = gestational age at birth, BW = birth weight, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

For the day 7 z-score model, actual and predicted values were strongly correlated (R^2 = 0.939) and residuals ranged from -0.69 to 0.94 **(Figure 19).** In terms of body weight, this z-score residual range corresponded to a body weight residual range of -255 to 346 g (assuming 1 SDS = 368 g). For the day 14 z-score model, actual and predicted values were well correlated (R^2 = 0.889) and residuals ranged from -0.66 to 1.09 (-243 to 401 g) **(Figure 20)**. For the day 21 z-score model, actual and predicted values were also well correlated (R^2 = 0.841) and residuals ranged from -0.78 to 1.22 (-287 to 449 g) **(Figure 21)**. Overall, the residual range and confidence intervals were narrower for all of the z-score models than raw body weight models (days 7, 14 and 21), and thus all subsequent validation was only completed on the z-score models.

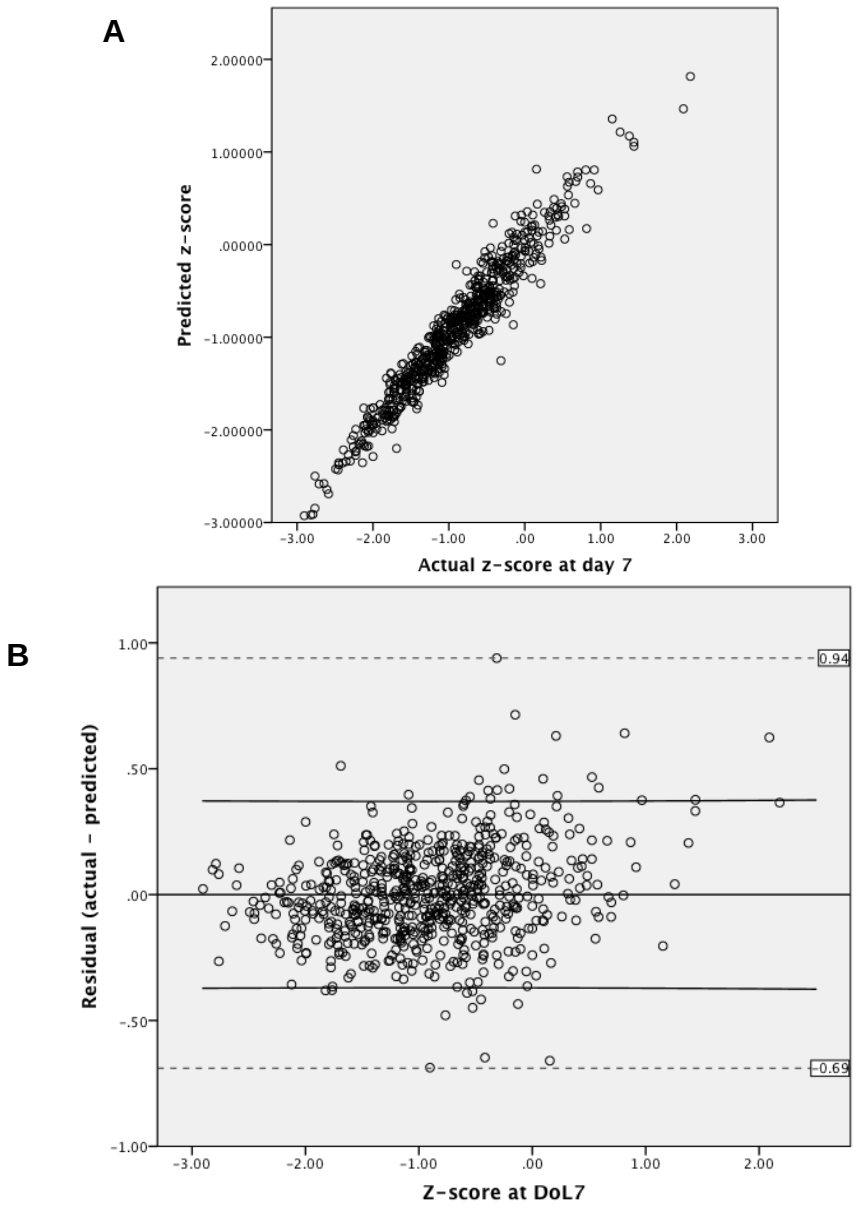


Figure 19: Correlation (A) and accuracy (B) of model to predict z-scores at day 7 of life. Z-scores were predicted using the following regression: -0.214 – (0.016) * GA + (0.828) * Z_1 + (0.065) * C, where GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

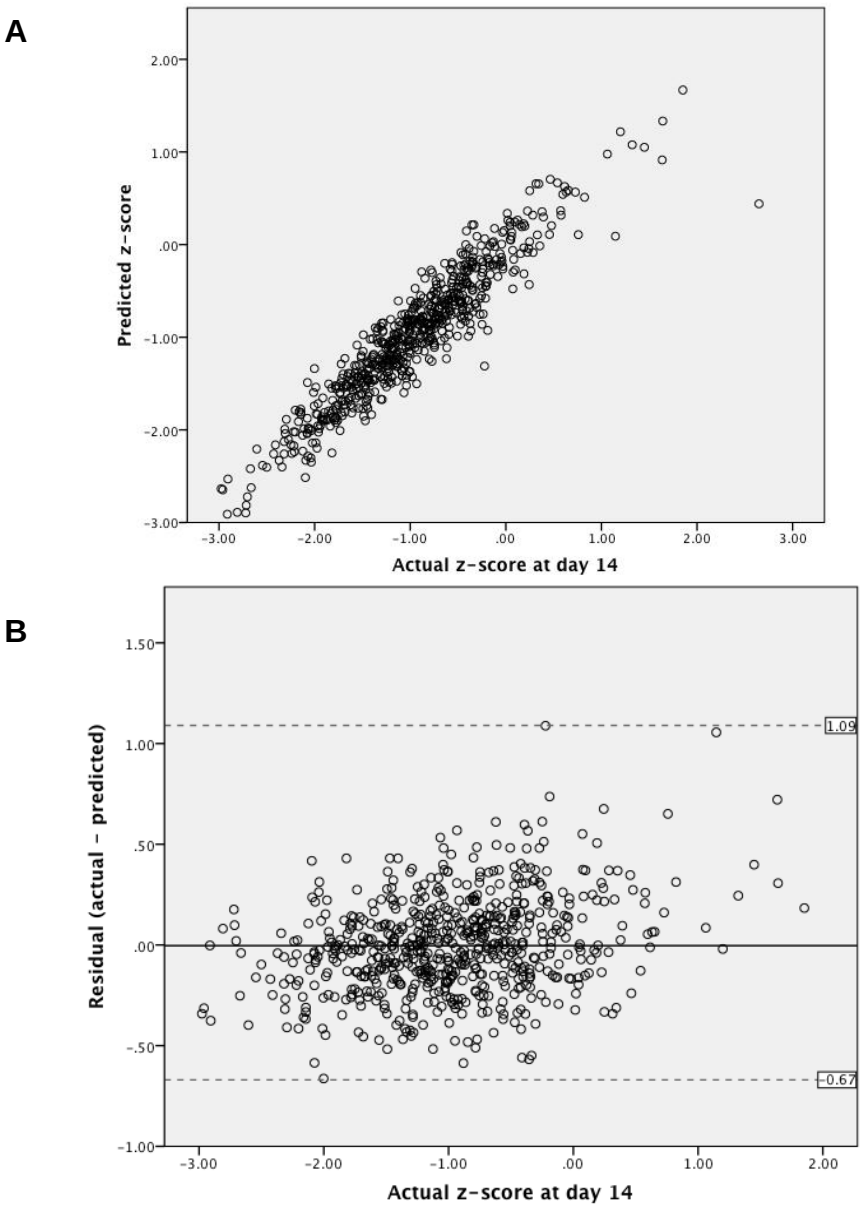


Figure 20: Correlation (A) and accuracy (B) of model to predict z-scores at day 14 of life. Z-scores were predicted using the following regression: -0.689 - (0.005)*GA + (0.807)*Z_1 + (0.101)*C, where GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

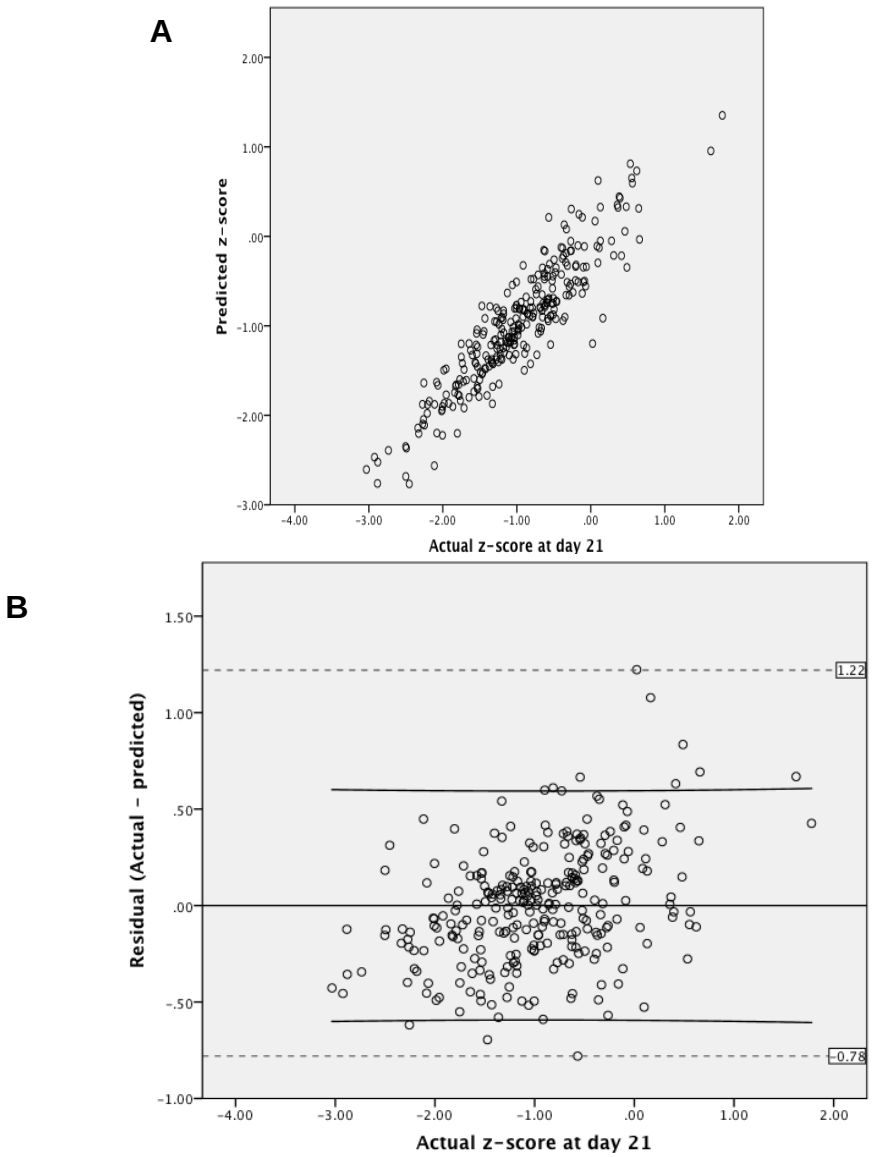
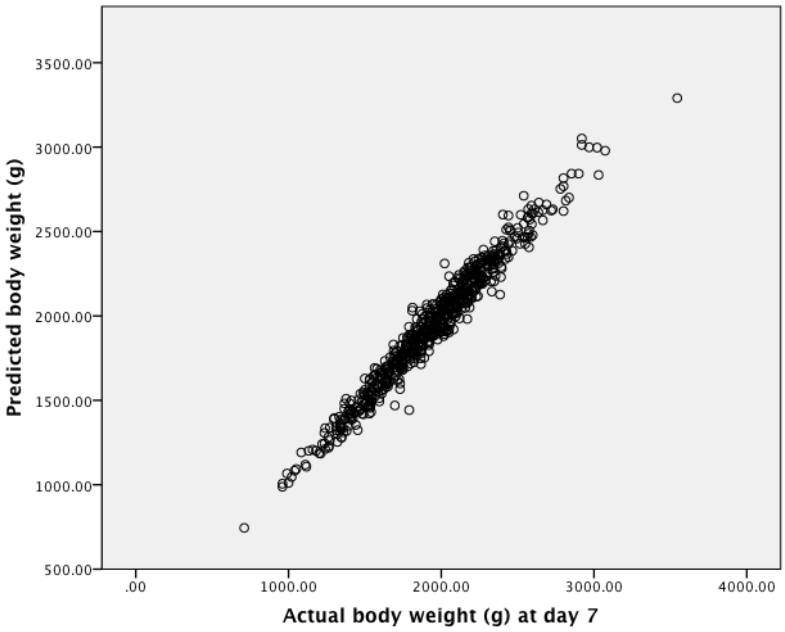


Figure 21: Correlation (A) and accuracy (B) of model to predict z-scores at day 21 of life. Z-scores were predicted using the following regression: - 0.721 - (0.005)*GA + (0.787)*Z_1 + (0.216)*C, where GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

Representing categorical variables using "dummy" coding

MUMC was again used as the reference centre for all regressions that used dummy coding for the hospital centre predictor variable. For the day 7 body weight model, actual and predicted values were strongly correlated (R^2 = 0.964) and residuals ranged from -289 to 347 g **(Figure 22).** For the day 14 body weight model, actual and predicted values were also strongly correlated (R^2 = 0.934) and residuals ranged from -290 to 948 g **(Figure 23)**. For the day 21 body weight model, actual and predicted values were well correlated (R^2 = 0.902) and residuals ranged from -268 to 493 g **(Figure 24)**. Overall, residual ranges were very similar but slightly narrower for body weight predictive models that treated the hospital centre variable as dichotomous.

A



B

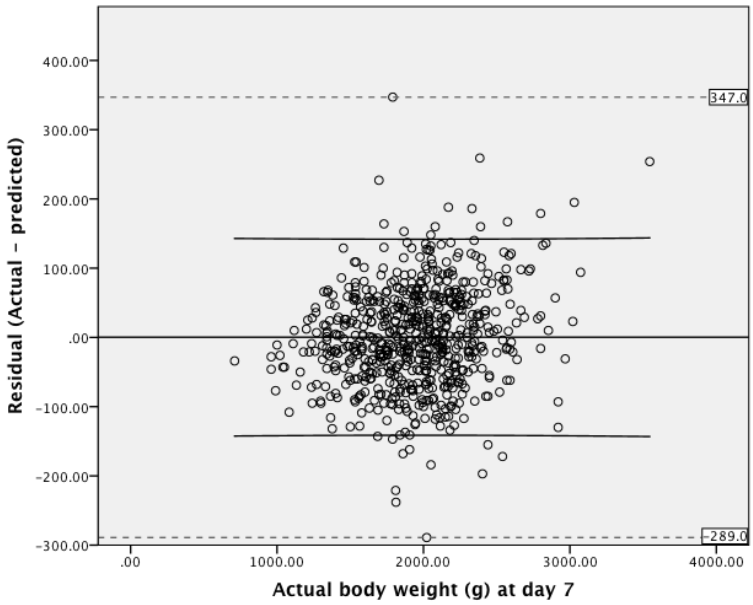
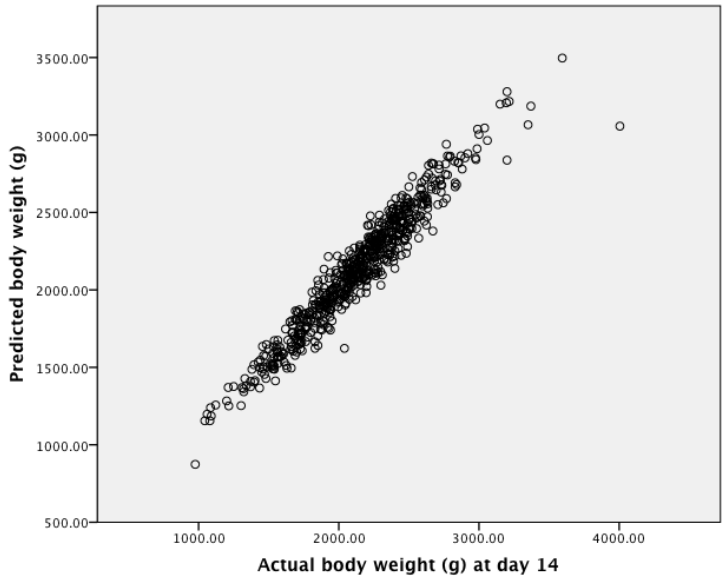


Figure 22: Correlation (A) and accuracy (B) of model to predict body weight at day 7 of life using the following regression: - 400.699 + 0.863*BW + 17.002*GA + 5.406*x_{SJH} + 111.629*x_{GUH} + 18.282*x_{HUH} + 15.401*x_{SMH}. GA = gestational age at birth, BW = birth weight, x_{centre} = 1 for corresponding hospital centre. Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

A



B

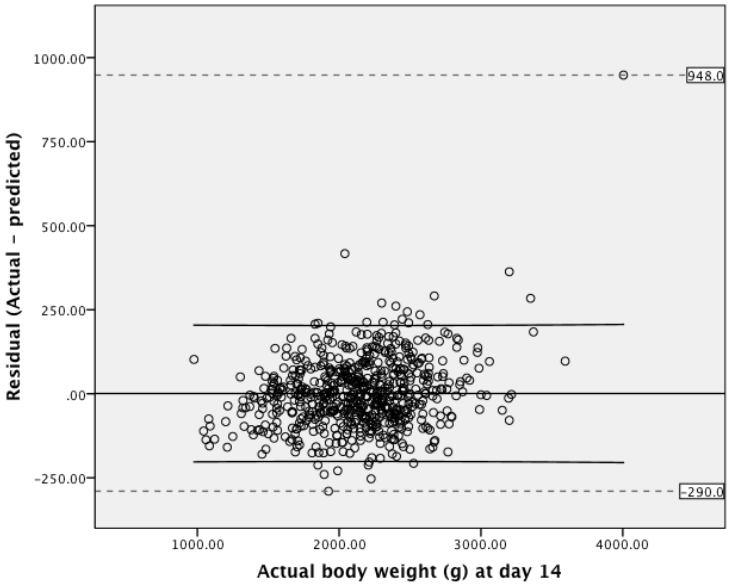


Figure 23: Correlation (A) and accuracy (B) of model to predict body weight at day 14 of life using the following regression: -567.729 + 0.873*BW + 26.559*GA + 19.926*x_{SJH} + 141.325*x_{GUH} + 47.524*x_{HUH} + 24.184*x_{SMH}. GA = gestational age at birth, BW = birth weight, x_{centre} = 1 for corresponding hospital centre. Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

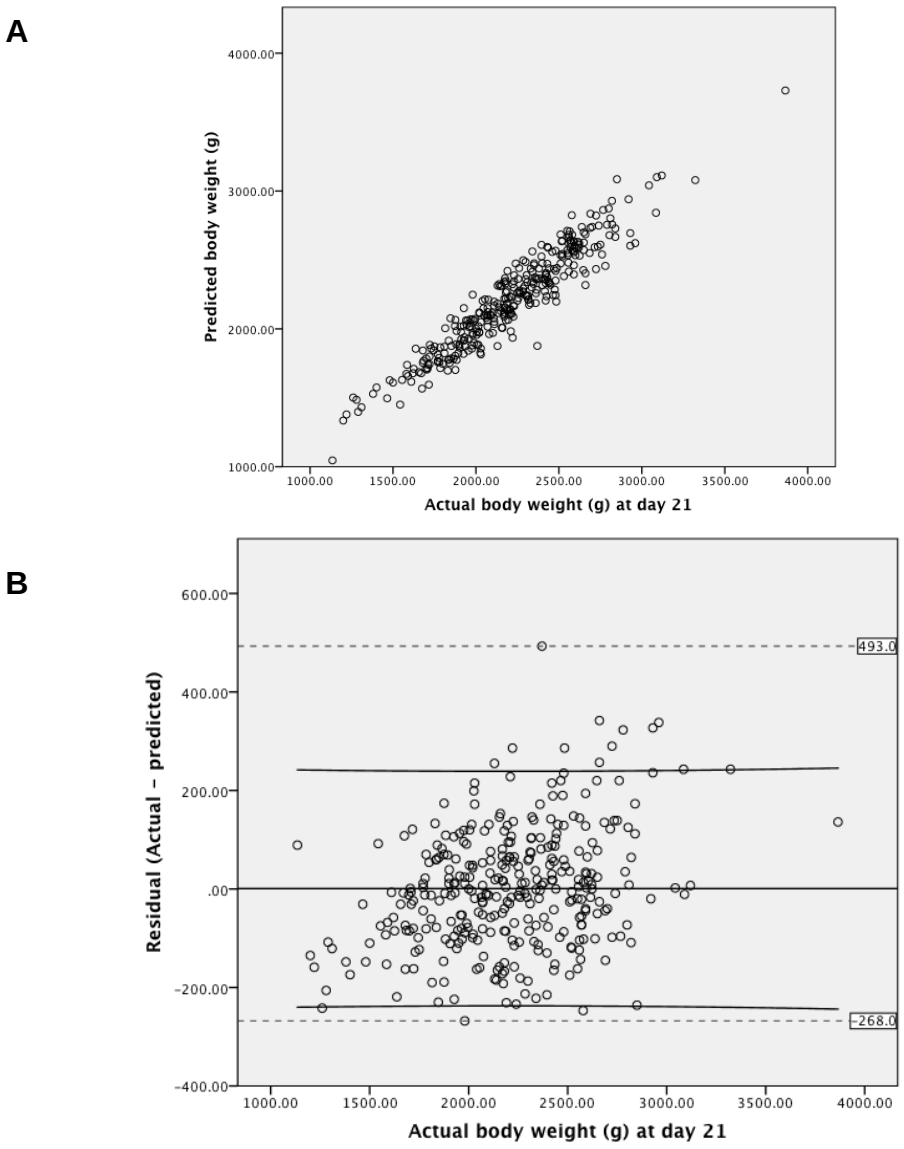


Figure 24: Correlation (A) and accuracy (B) of model to predict body weight at day 21 of life using the following regression: - 466.248 + 0.891*BW + 28.447*GA + 39.034*x_{SJH} + 212.59*x_{GUH} + 113.26*x_{HUH} + 43.874*x_{SMH}. GA = gestational age at birth, BW = birth weight, x_{centre} = 1 for corresponding hospital centre. Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

For the day 7 z-score model, actual and predicted values were strongly correlated (R^2 = 0.945) and residuals were shown to range from -0.68 to 0.93 **(Figure 25).** In terms of body weight, this z-score residual range corresponded to a body weight residual range of -250 to 342 g (assuming 1 SDS = 368 g). For the day 14 z-score model, actual and predicted values were well correlated (R^2 = 0.895) and residuals ranged from -0.64 to 1.08 (-236 to 397 g) **(Figure 26)**. For the day 21 z-score model, actual and predicted values were also well correlated (R^2 = 0.852) and residuals were shown to range from -0.69 to 1.2 (-254 to 442 g) **(Figure 27)**. For all days of life, the residual range and confidence intervals were narrower for all of the z-score models where the categorical predictor variable of hospital centre was “dummy” coded.

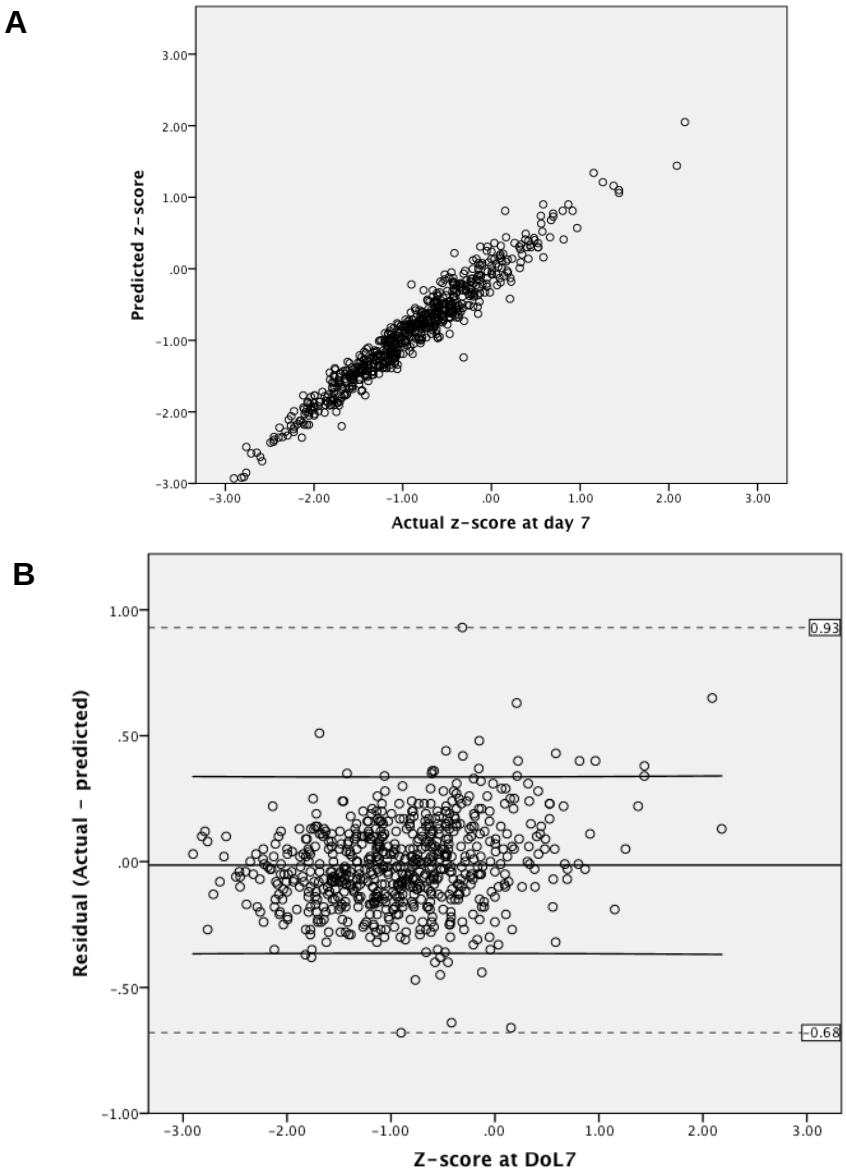


Figure 25: Correlation (A) and accuracy (B) of model to predict z-scores at day 7 of life using the following multiple linear regression: -0.09 + 0.823*Z_1 – 0.02*GA + 0.017*x_{SJH} + 0.275*x_{GUH} + 0.049*x_{HUH} + 0.04*x_{SMH}. GA = gestational age at birth, Z_1 = z-score at birth, x_{centre} = 1 for corresponding hospital centre. Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

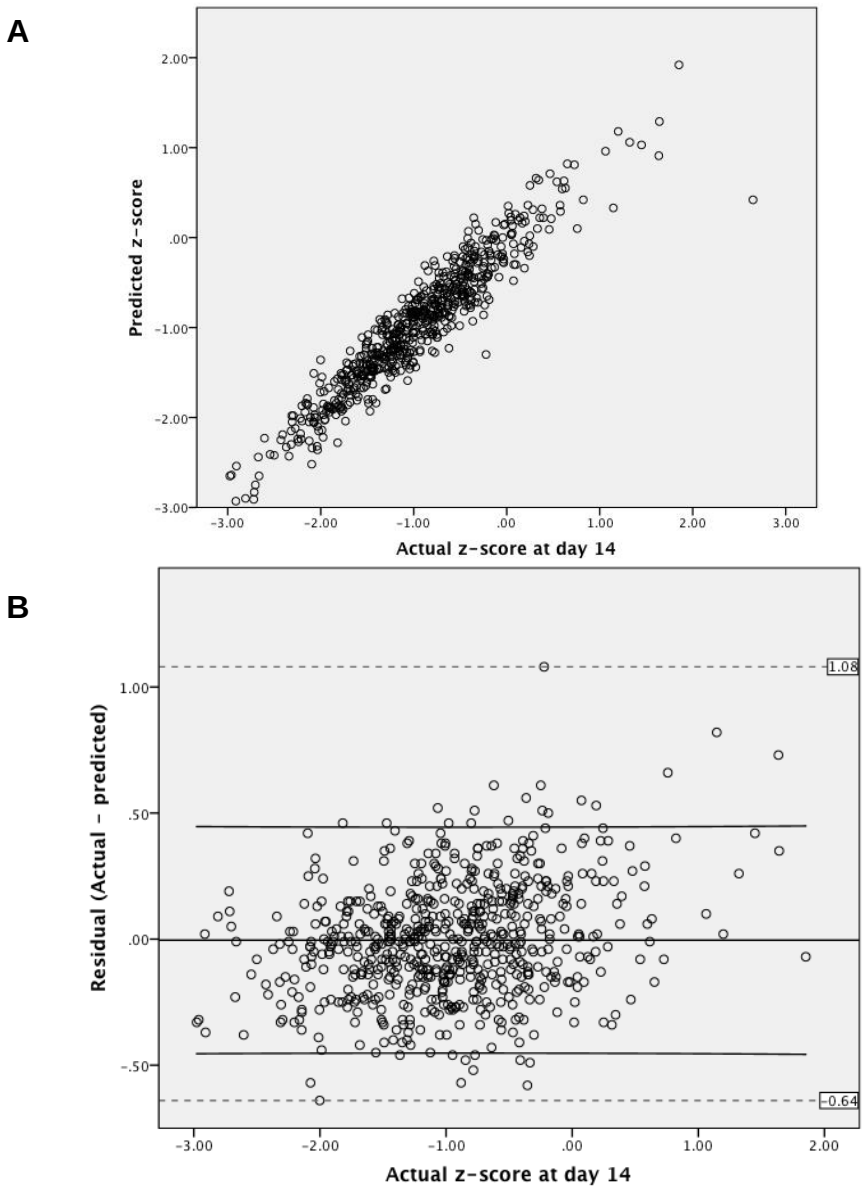


Figure 26: Correlation (A) and accuracy (B) of model to predict z-scores at day 14 of life using the following regression: $-0.0541 + 0.802*Z_1 - 0.01*GA + 0.042*x_{SJH} + 0.327*x_{GUH} + 0.111*x_{HUH} + 0.051*x_{SMH}$. GA = gestational age at birth, Z_1 = z-score at birth, x_{centre} = 1 for corresponding hospital centre. Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

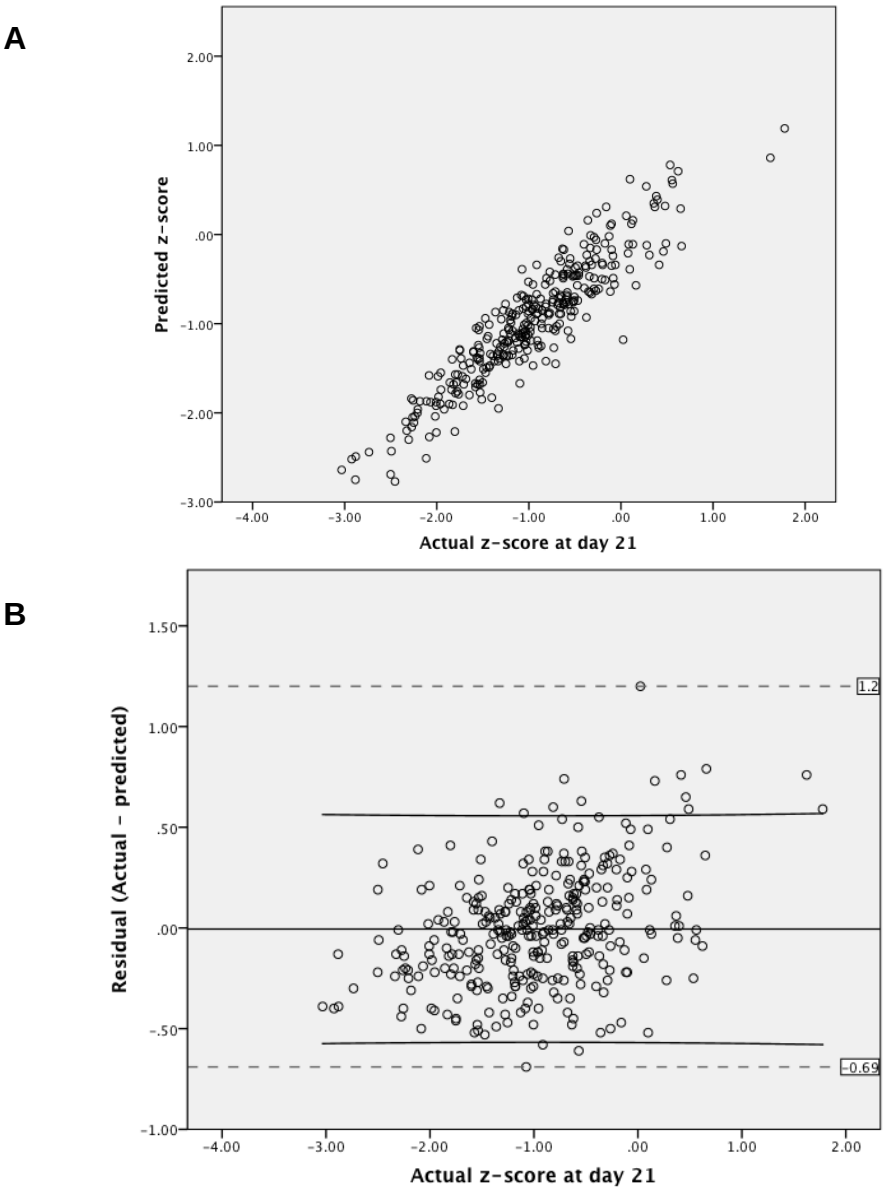


Figure 27: Correlation (A) and accuracy (B) of model to predict z-scores at day 21 of life using the following regression: $-0.444 + 0.784*Z_1 - 0.014*GA + 0.066*x_{SJH} + 0.476*x_{GUH} + 0.252*x_{HUH} + 0.072*x_{SMH}$. GA = gestational age at birth, Z_1 = z-score at birth, x_{centre} = 1 for corresponding hospital centre. Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

SECTION C: Summary of Results

Multiple linear regression models predicted body weight and z-scores at days of life 7, 14 and 21 accurately when the following independent variables were included in the models: GA at birth, BW/ z-score at birth and the hospital at which the infant was treated. The two models that produced the narrowest range of residuals and confidence intervals were (1) the z-score model that used the dichotomous coding for the categorical hospital centre variable, and (2) the z-score model that used the "dummy" coding for the categorical hospital centre variable.

SECTION D: Validation of predictive models

Apparent validation

Since the two z-score models for days 7, 14 and 21 were associated with the narrowest residual range, apparent validation was only conducted on these two models. There were no statistically significant differences between predicted and actual z-scores of Group A infants at days of life 7 (p = 0.994), 14 (p = 0.719) or 21 (p = 1.000) using the z-score models that coded hospital centres as a dichotomous variable. However, when the hospital centre variable was treated as a "dummy" variable, there was a statistically significant difference between predicted and actual z-scores of Group A infants at day 7 (p = 0.048), while no

significant differences between actual and predicted z-scores were observed on days 14 (p = 0.661) and 21 (p = 0.740).

Despite the evidently superiority of the z-score models for days 14 and 21 that coded the hospital centres as a "dummy" variable, we decided to progress to the validation stage with only the z-score models for days 7, 14 and 21 that coded hospital centres as a dichotomous variable based on feeding practices. This was decided because the difference between the two z-score models in terms of the range of the residuals was not clinically relevant and also because of the acknowledgement that a single dichotomous variable to code for hospital centres based on feeding strategies is more clinically feasible when considering the extension of the model to new hospital centres. Thus, all subsequent validation was only completed on the z-score models that coded hospital centre as a dichotomous variable according to feeding practices.

Internal validation

Z-score predictive models were internally validated using a split-sample validation technique. Group A subjects were randomly split into two groups: training set (n = 330) and validation set (n = 335), and z-score regressions were re-developed for each data set. The predictive equations for the training set were:

Z-score on day 7

= -0.332 – 0.013*GA + 0.815*Z + 0.047*C $R^2 = 0.930$

Z-score on day 14

= -0.651 – 0.006*GA + 0.770*Z + 0.113*C $R^2 = 0.886$

Z-score on day 21

$=-1.028 – 0.004*GA + 0.760*Z + 0.241*C$ $R^2 = 0.846$

The predictive equations for the validation set were:

Z-score on day 7

$= -0.099 – 0.020*GA + 0.844*Z_1 + 0.086*C$ $R^2 = 0.949$

Z-score on day 14

$= -0.681 – 0.005*GA +0.842*Z_1 + 0.101*C$ $R^2 = 0.896$

Z-score on day 21

$= -0.287 – 0.017*GA + 0.818*Z_1 + 0.195*C$ $R^2 = 0.841$

The relationships between the z-scores and the independent variables (z-score at birth and hospital at which the infant was admitted) remained significant in all three models based on both the training set and the validation set. Additionally, the Pearson correlation coefficients remained within 5% of the corresponding coefficient in the original predictive models that were based on the entire Group A population.

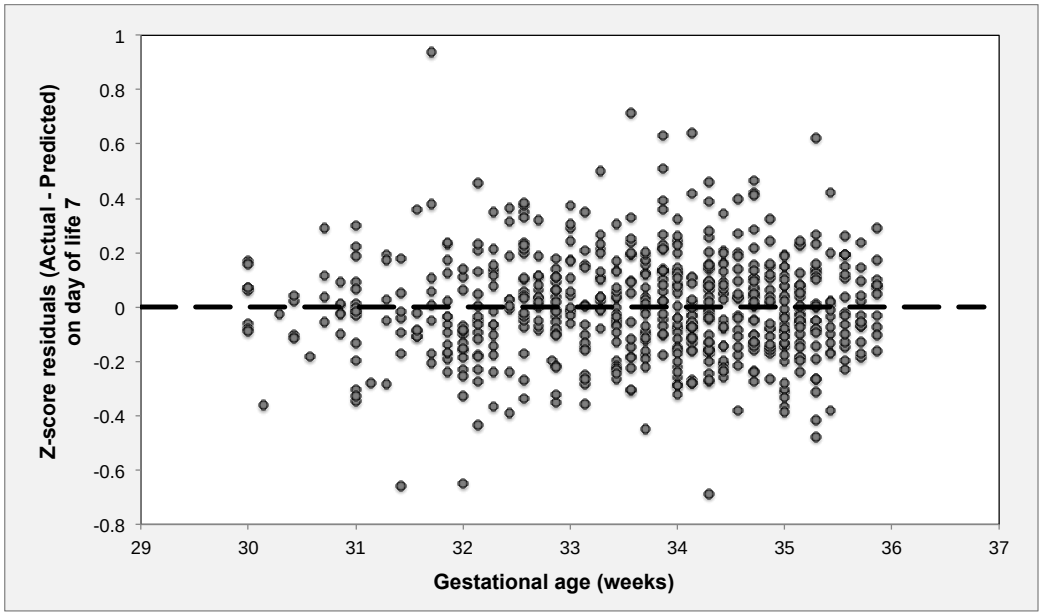
Day 7 and 14 z-scores that were predicted using the training and validation set equations were significantly different ($p < 0.001$), but very highly correlated ($R^2 = 1.000$). The mean difference in predicted day 7 z-scores was 0.01 ± 0.033 (4 ± 12.1 g), and was 0.02 ± 0.064 (7 ± 23.6 g) for day 14. Day 21 z-scores that were predicted using the training and validation set equations were also significantly different ($p < 0.001$), but showed a high correlation ($R^2 = 0.999$).

The mean difference between the two predicted values for day 21 z-scores was 0.27 ± 0.065 (99 ± 23.9 g).

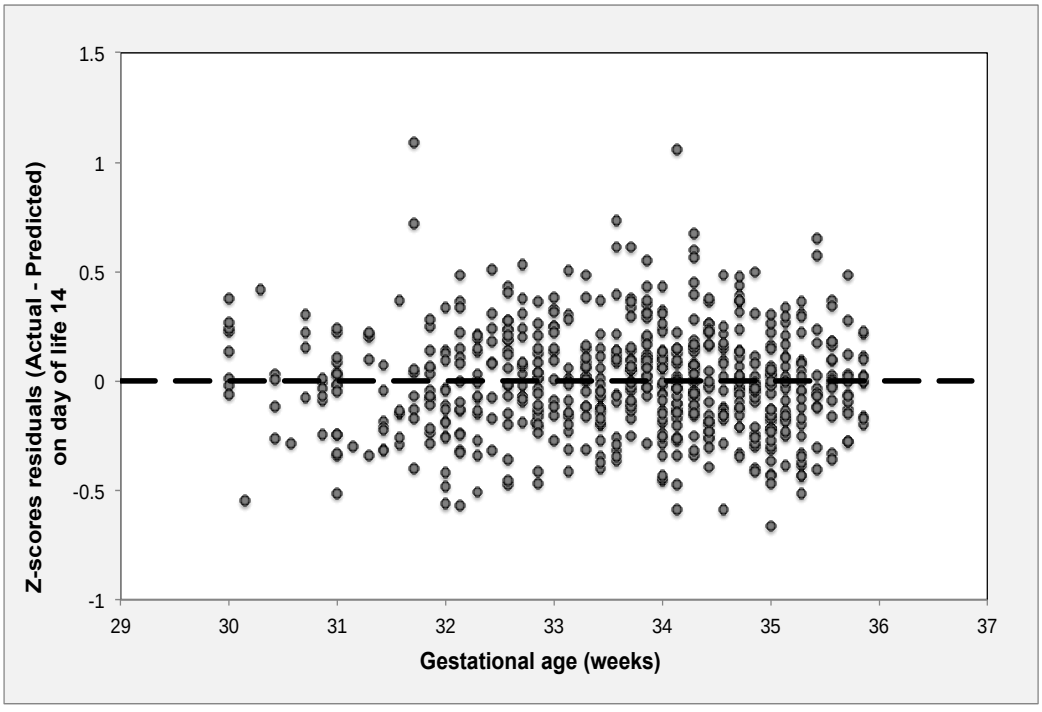
Analysis of the effect of factors on the accuracy of predictive models

There was no trend of higher magnitudes of residuals for specific GAs, genders or ethnicities for any of the z-score predictive models. The distribution of the residuals remained similarly dispersed across 30 – 35 completed weeks of gestation for day 7, 14 and 21 predictive models **(Figure 28)**. Similarly, no gender group **(Figure 29)** or ethnicity group **(Figure 30)** was shown to have a greater difference between actual and predicted z-score values. It was also qualitatively observed that absolute residuals were greater when actual z-scores of the infants >1.0.

A


Z-score residuals (Actual - Predicted) on day of life 7
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
29
30
31
32
33
34
35
36
37
Gestational age (weeks)

B


Z-scores residuals (Actual - Predicted) on day of life 14
1.5
1
0.5
0
-0.5
-1
29
30
31
32
33
34
35
36
37
Gestational age (weeks)

C

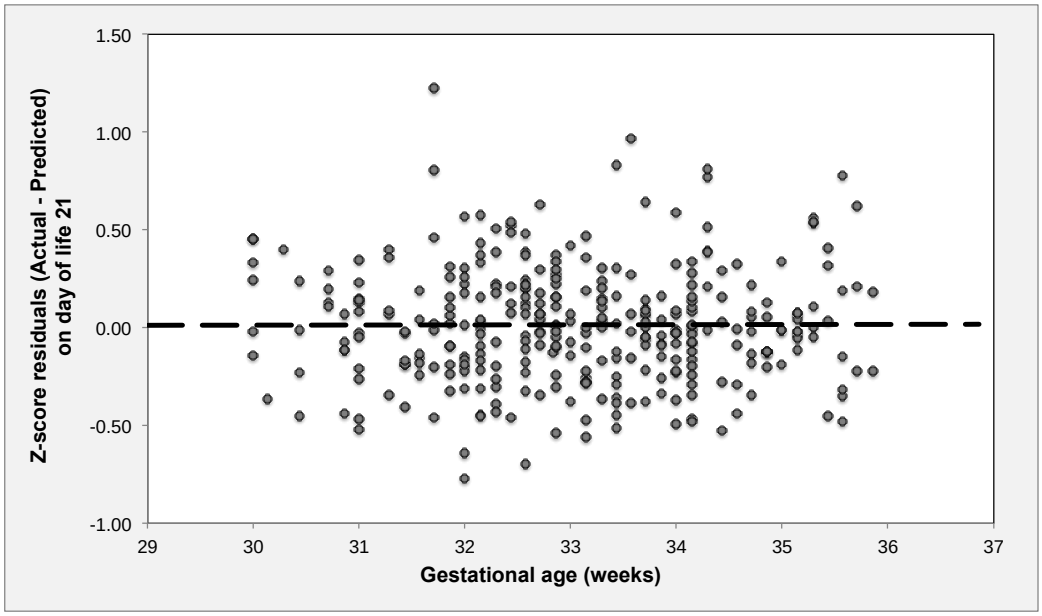
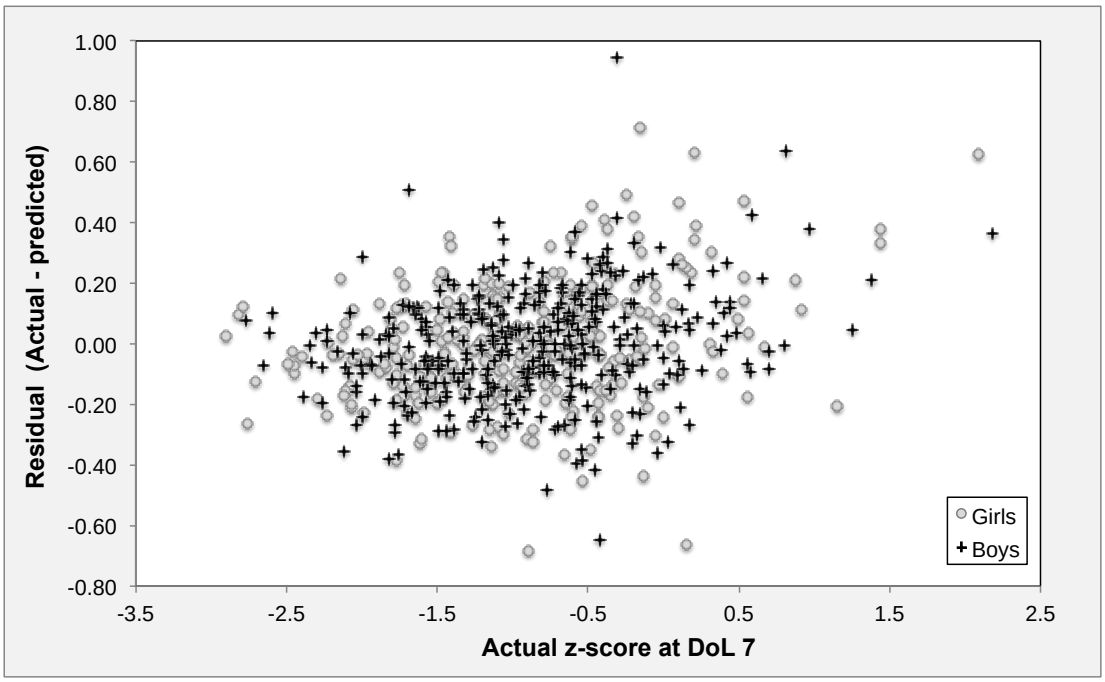
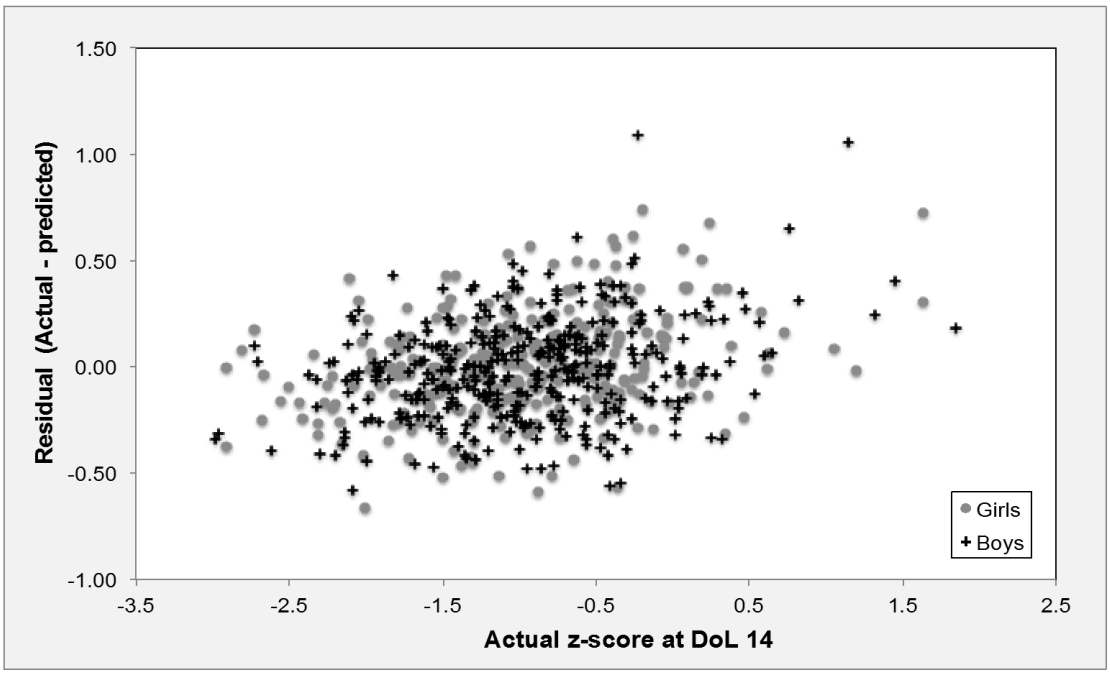


Figure 28: Effect of gestational age at birth on residuals of z-score models for (A) day 7, (B) day 14 and (C) day 21. Z-scores were predicted using the following multiple linear regressions: **Day 7 z score** = -0.214 – (0.016) * GA + (0.828) * Z_1 + (0.065) * C; **Day 14 z-score** = -0.689 - (0.005)*GA + (0.807)*Z_1 + (0.101)*C; **Day 21 z-score** = - 0.721 - (0.005)*GA + (0.787)*Z_1 + (0.216)*C. GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values of Group A infants and were plotted by GA at birth of the infant.

A


1.00
0.80
0.60
0.40
0.20
0.00
-0.20
-0.40
-0.60
-0.80
Residual (Actual - predicted)
-3.5
-2.5
-1.5
-0.5
0.5
1.5
2.5
Actual z-score at DoL 7
Girls
Boys

B


1.50
1.00
0.50
0.00
-0.50
-1.00
Residual (Actual - predicted)
-3.5
-2.5
-1.5
-0.5
0.5
1.5
2.5
Actual z-score at DoL 14
Girls
Boys

C

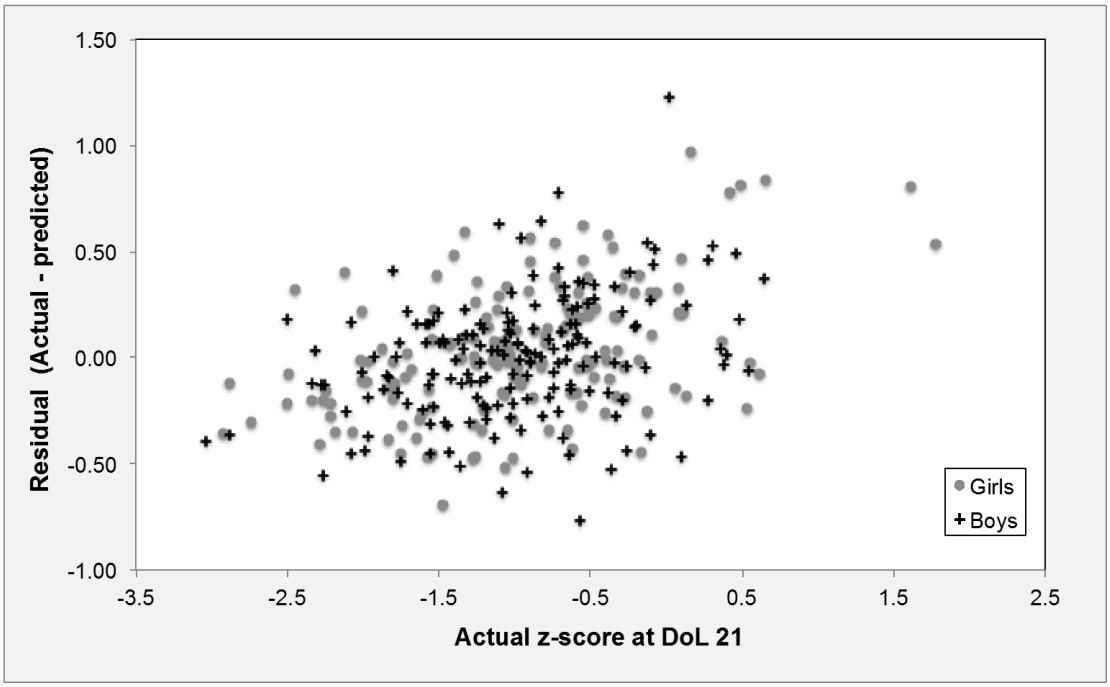
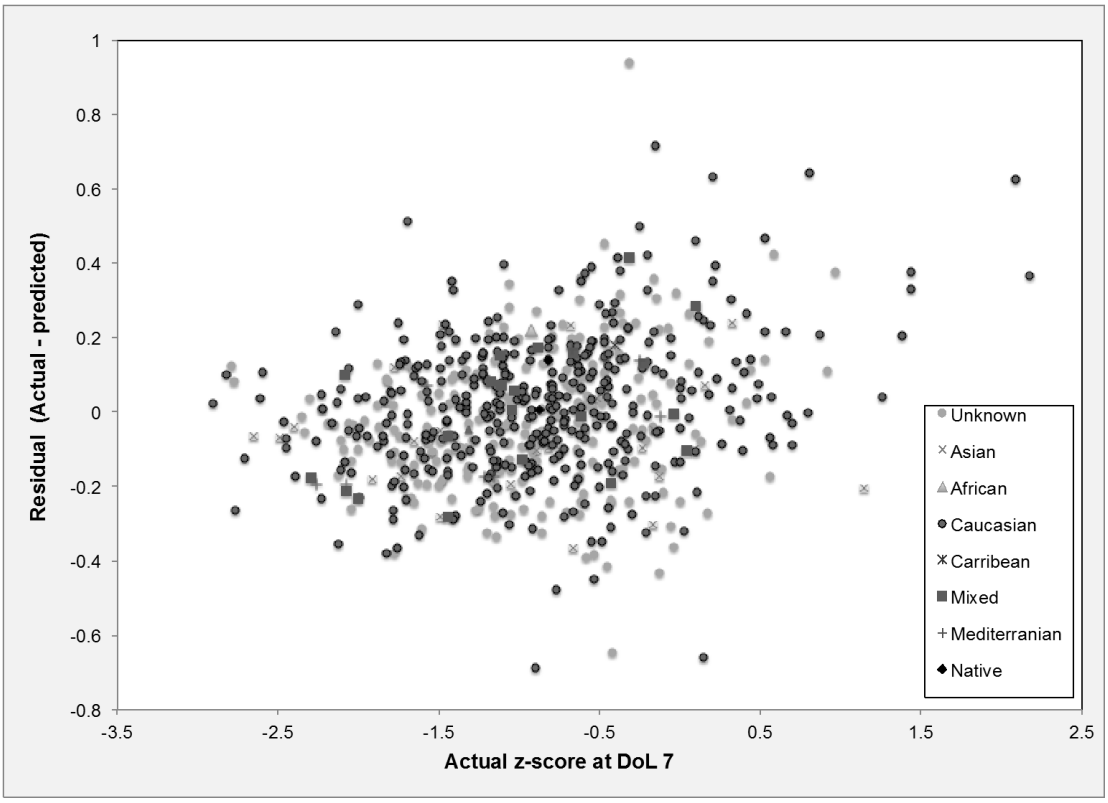
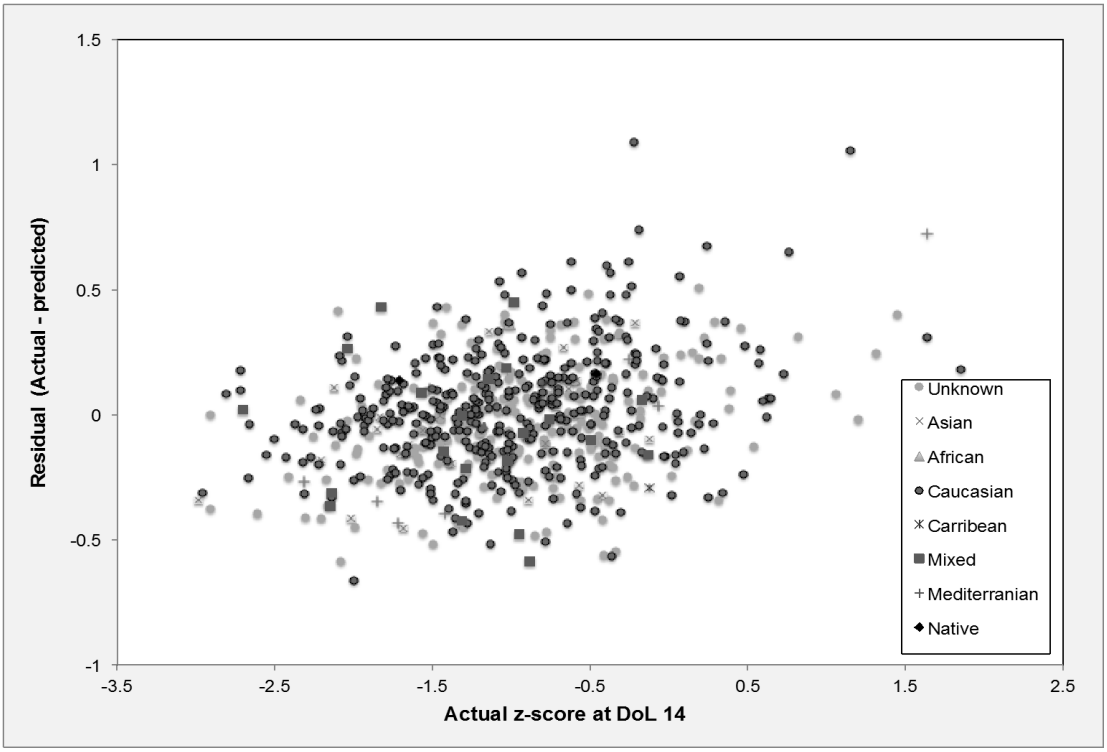


Figure 29: Validity of z-score predictive models for (A) day of life 7, (B) day 14 and (C) day 21 by gender. Z-scores were predicted for Group A infants using the following multiple linear regressions: **Day 7 z score =** -0.214 – (0.016) * GA + (0.828) * Z_1 + (0.065) * C; **Day 14 z-score =** -0.689 - (0.005)*GA + (0.807)*Z_1 + (0.101)*C; **Day 21 z-score =** - 0.721 - (0.005)*GA + (0.787)*Z_1 + (0.216)*C. GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were plotted by the actual z-score according to gender.

A


Residual (Actual - predicted)
Actual z-score at DoL 7
Unknown
Asian
African
Caucasian
Carribean
Mixed
Mediterranian
Native

B


Residual (Actual - predicted)
Actual z-score at DoL 14
Unknown
Asian
African
Caucasian
Carribean
Mixed
Mediterranian
Native

C

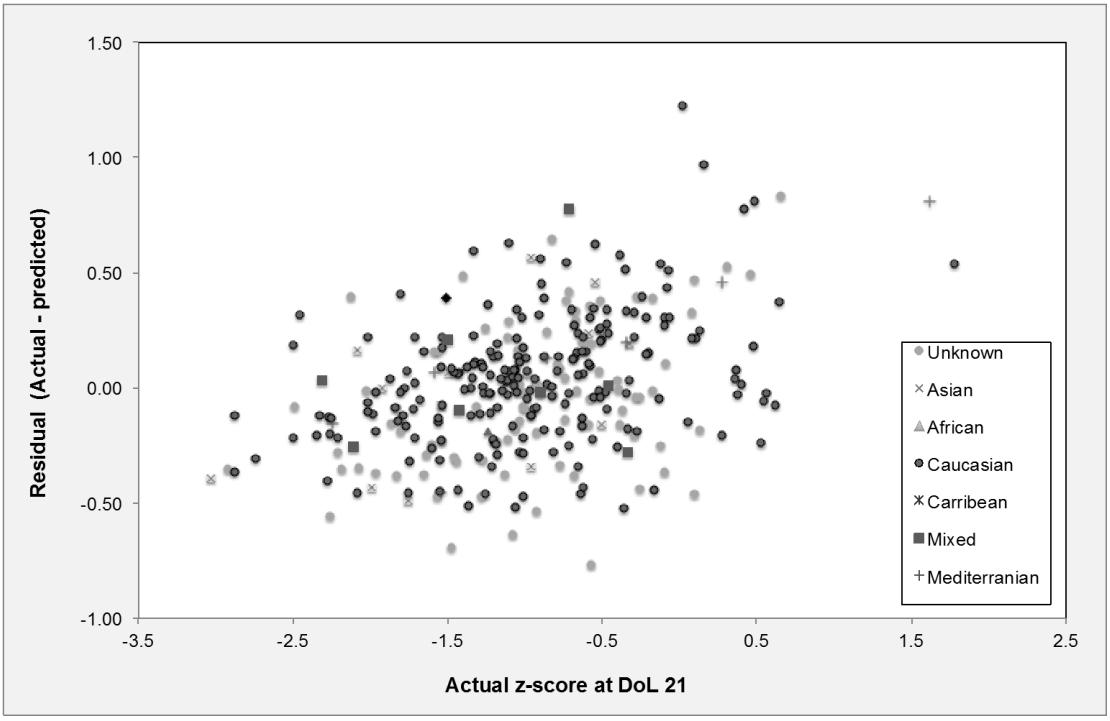


Figure 30: Validity of z-score predictive models for (A) day of life 7, (B) day 14 and (C) day 21 by ethnicity. Z-scores were predicted for Group A infants using the following multiple linear regressions: **Day 7 z score =** -0.214 – (0.016) * GA + (0.828) * Z_1 + (0.065) * C; **Day 14 z-score =** -0.689 - (0.005)*GA + (0.807)*Z_1 + (0.101)*C; **Day 21 z-score =** - 0.721 - (0.005)*GA + (0.787)*Z_1 + (0.216)*C. GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were plotted by the actual z-score according to ethnic background.

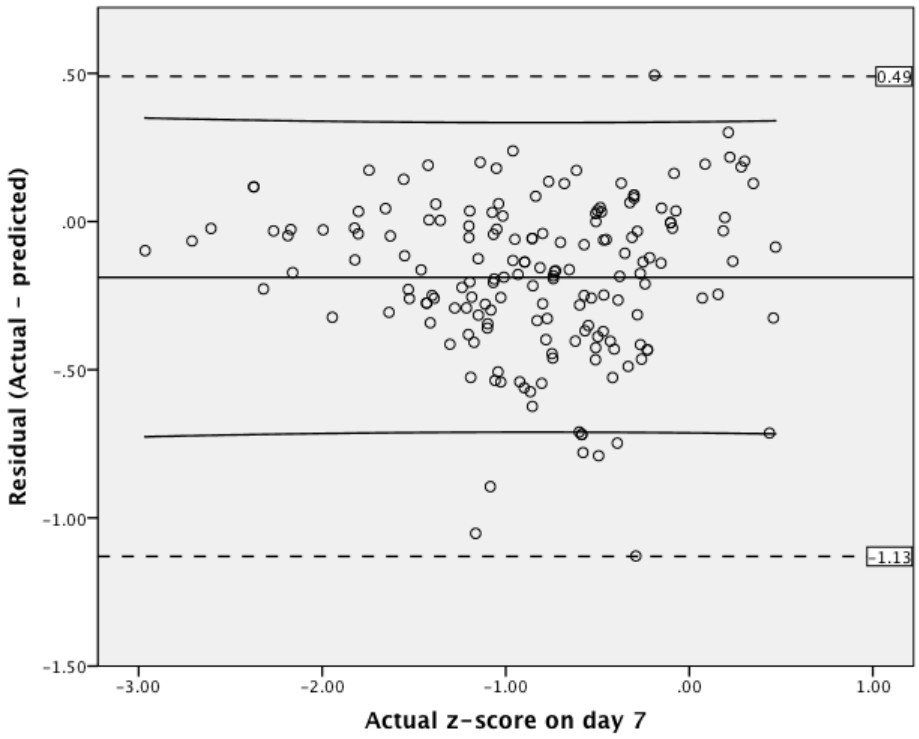
SECTION D: Summary of results

GA, z-score at birth and the hospital centre the infant was born/ admitted to (represented as a dichotomous variable) were significant predictors of day 7, 14 and 21 z-scores in the Group A study population. The described multiple linear regression models produced no statistically- or clinically-significant differences between observed and predicted z-scores. Day 7, 14 and 21 z-score models were also shown to have high reproducibility upon split-sample internal validation. Additionally, upon qualitative assessment, GA, gender nor ethnicity were shown to be factors that affect the accuracy of the z-score predictive models.

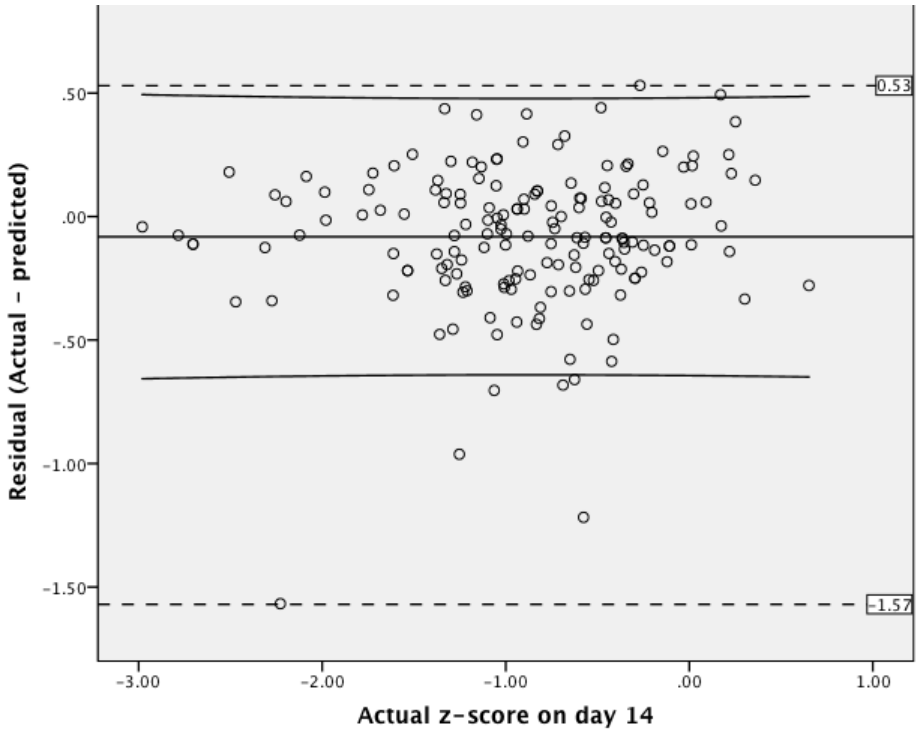
Section E: Extrapolation of predictive models to 25 weeks

Actual and predicted z-score values of Group B (25 – 29 6/7 week GA infants who required minimal postnatal support), were strongly correlated (R^2 = 0.930, 0.916, 0.873), but were significantly different ($p < 0.001$ for all three days). The average difference of actual and predicted z-score means of days 7, 14 and 21 were: 0.19 ± 0.264, 0.08 ± 0.283, and 0.13 ± 0.357. These values correspond to raw body weight values of: 47 ± 64.9, 20 ± 69.6, and 32 ± 87.8 (assuming 1 SDS = 246 g). Residual analyses showed that the residuals for day 7 z-scores of Group B infants ranged from -1.13 to 0.49 **(Figure 31A)**. Day 14 and 21 residuals ranged from -1.57 to 0.53 **(Figure 31B)** and -1.95 to 0.95 **(Figure 31C)**, respectively. Confidence intervals at a 95% confidence level were narrow, approximating 0.5 SDS for each day.

A


Residual (Actual - predicted)
.50
.00
-.50
-1.00
-1.50
0.49
-1.13
-3.00
-2.00
-1.00
.00
1.00
Actual z-score on day 7

B


Residual (Actual - predicted)
.50
.00
-.50
-1.00
-1.50
0.53
-1.57
-3.00
-2.00
-1.00
.00
1.00
Actual z-score on day 14

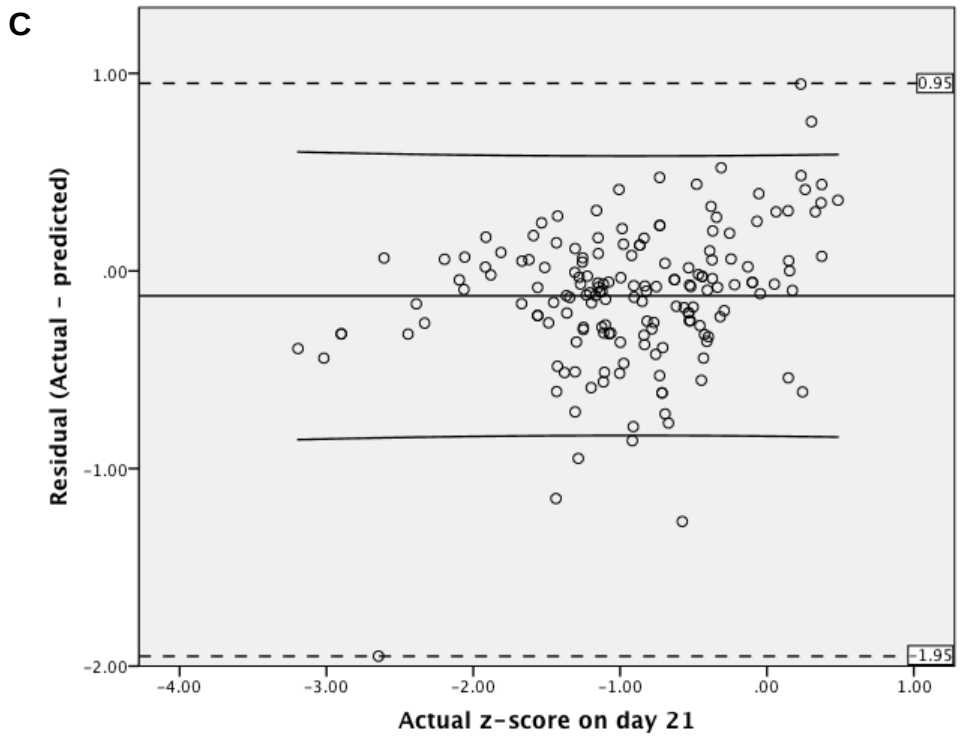


Figure 31: Accuracy of z-score predictive models for an independent 25 - 29 6/7 weeks GA preterm infants. Z-scores were predicted for Group B infants using the following regressions: **Day 7 z score =** -0.214 – (0.016) * GA + (0.828) * Z_1 + (0.065) * C; **Day 14 z-score =** -0.689 - (0.005)*GA + (0.807)*Z_1 + (0.101)*C; **Day 21 z-score =** - 0.721 - (0.005)*GA + (0.787)*Z_1 + (0.216)*C. GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Residuals were calculated as the difference between actual and predicted values and the range was indicated using a broken line. Mean of residuals and 95% confidence intervals are presented as solid lines.

SECTION E: Summary of results

The analyzed predictive models for z-scores at days 7, 14 and 21, which code hospital centre as a dichotomous variable, predicted z-scores of Group B infants (25 – 29 6/7 weeks GA) that were significantly different than Group B infants' actual z-scores. However, residual analyses and the narrow confidence interval at the 95% confidence level suggested the difference between actual and predicted means were not clinically relevant.

SECTION F: Combining Groups A and B to develop overall regressions

Using a step-wise forward selection procedure to develop multiple-linear regression models for z-scores at days 7, 14 and 21 as the dependent variable, it was once again found that the following independent variables were significant predictors of z-scores at days 7, 14 and 21: gestational age (GA, [weeks]), z-score at birth (Z_1) and hospital centre (C; MUMC/SJH = 0; GUH/HUH/SMH = 1). The resulting predictive equations are listed below:

Z-score AT DAY 7 ($R^2 = 0.923$)

$$= -1.058 + (0.008) * GA + (0.809) * Z_1 + (0.086) * C$$

Z-score AT DAY 14 ($R^2 = 0.887$)

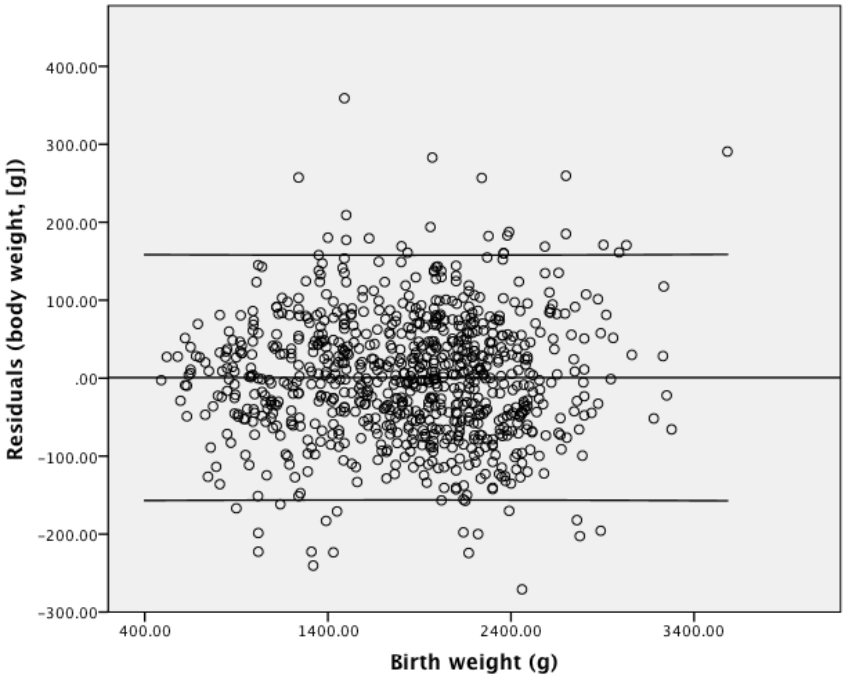
$$= -1.090 + (0.007) * GA + (0.783) * Z_1 + (0.118) * C$$

Z-score AT DAY 21 ($R^2 = 0.816$)

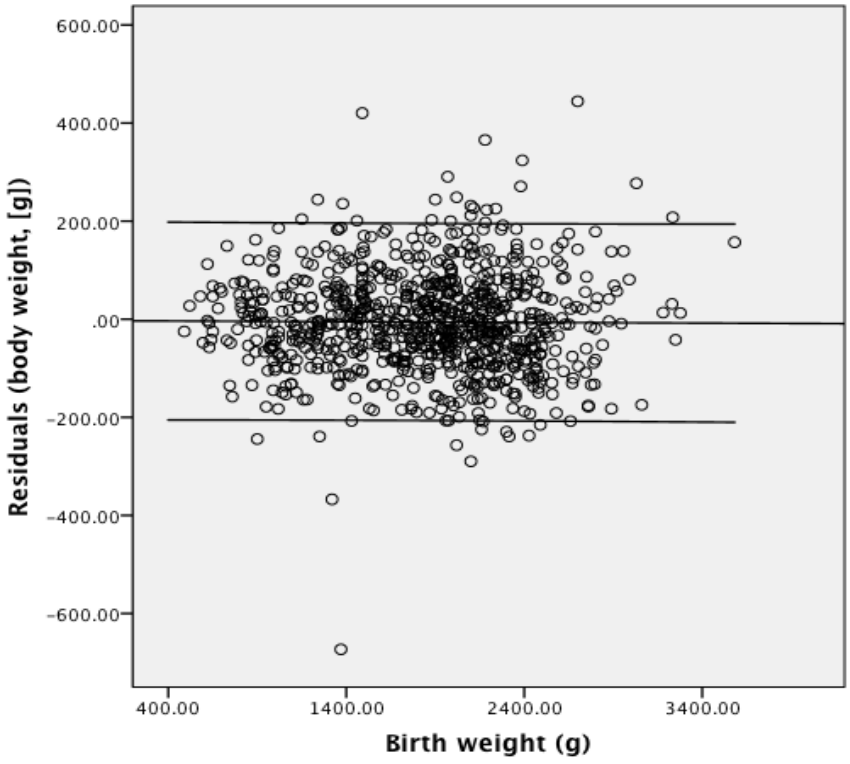
$= - 1.555 + (0.020) * GA + (0.765) * Z_1 + (0.221) * C$

Actual and predicted z-scores for Group A and Group B infants ($n_{total} = 838$) showed a high correlation on days 7, 14 and 21 ($R^2 = 0.961, 0.942, 0.903$) and all models predicted z-scores that were not significantly different from their actual value counter parts (day 7: $p = 0.889$, day 14: $p = 0.084$, day 21: $p = 0.462$). The residuals for the day 7 z-score model when applied to Group A and Group B infants (n = 838) ranged from -0.88 to 0.97, where the confidence interval approximated 0.4 SDS**.** The residuals for the day 14 z-score model ranged from -1.52 to 1.07, and the confidence intervals approximated 0.5 SDS. The residuals for the day 21 z-score model ranged from -1.86 to 1.25, and the confidence intervals approximated 0.5 SDS when the model was applied to Group A and Group B infants with growth data at day 21 (n = 502). Conversion of the residuals to a raw body weight value showed that the models were able to predict the body weight of infants within approximately 150 g of the observed z-score on day 7 **(Figure 32A)**, 200 g on day 14 **(Figure 32B)** and 250 g on day 21 **(Figure 32C),** at a 95% confidence level. Additionally, there was no significant correlation between residuals for day 7, 14 or 21 z-score regressions and BW (day 7: r = 0.017, p = 0.619; day 14: r = 0.007, p = 0.829; day 21: r = 0.007, p = 0.884) or GA (day 7: r = 0.028, p = 0.413; day 14: r = 0.022, p = 0.530; day 21: r = 0.008, p = 0.854).

A


Residuals (body weight, [g])
400.00
300.00
200.00
100.00
.00
-100.00
-200.00
-300.00
400.00
1400.00
2400.00
3400.00
Birth weight (g)

B


Residuals (body weight, [g])
600.00
400.00
200.00
.00
-200.00
-400.00
-600.00
400.00
1400.00
2400.00
3400.00
Birth weight (g)

C

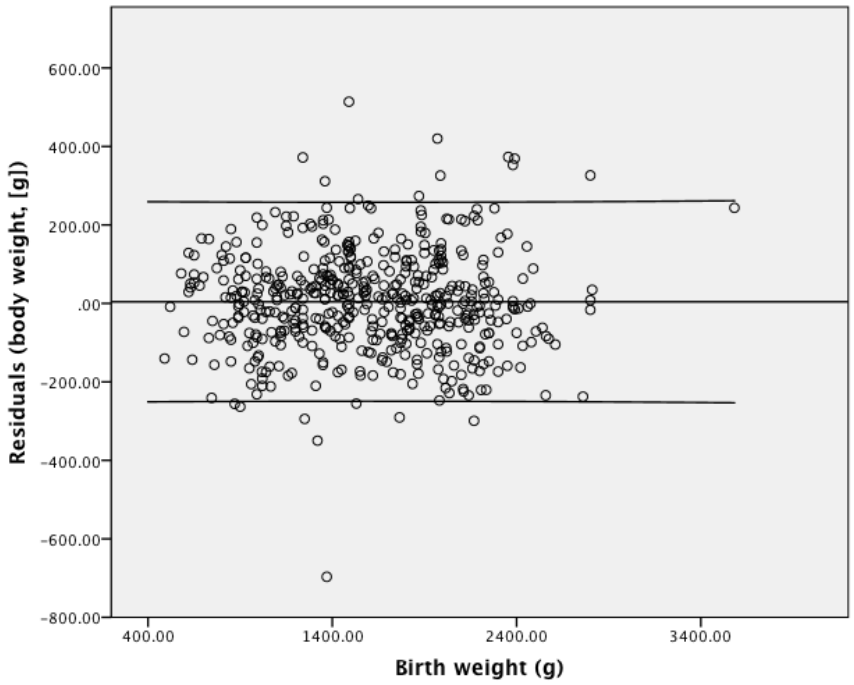


Figure 32: Accuracy of z-score predictive models based on Group A and Group B populations (25 – 35 6/7 week GA; n = 838). Residuals were calculated as the difference between actual and predicted z-scores and converted to a raw body weight value upon multiplying by the standard deviation that corresponded to the corrected age at time of measurement. Z-scores were predicted for n = 838 infants (25 – 35 6/7 weeks GA) for day 7 **(A)** and day 14 **(B)**, and for n = 502 (25 – 35 6/7 weeks GA) infants for day 21 **(C),** using the following regressions: **Day 7 z-score** = -1.058 + 0.008*GA + 0.809*Z_1 + 0.086*C; **Day 14 z-score** = -1.090 + 0.007*GA + 0.783*Z_1 + 0.118*C; **Day 21 z-score** = - 1.555 + 0.020*GA + 0.765*Z_1 + (0.221)*C. GA = gestational age at birth, Z_1 = z-score at birth, C = hospital centre (MUMC/SJH = 0; GUH/HUH/SMH = 1). Mean of residuals and 95% confidence intervals are presented as solid lines.

SECTION F: Summary of results

Upon application of the z-score predictive models that were developed using all Group A and Group B infant data (n = 838) on the same study population, there were no statistically significant differences between actual and predicted z-score values. The correlation and accuracy of days 7, 14 and 21 predictive models were very high, where z-scores were predicted within 0.5 SDS of their actual z-score and approximately 200 g of the actual weight (within a 95% confidence level). Further, no trends in inaccurate predictions were identified for BW or GA.

Discussion

The longitudinal, anthropometric data of the preterm infant who requires minimal postnatal support presented here showed that postnatal growth did not match the currently recommended intrauterine growth. We observed that the "low-risk" cohort of preterm infants in this study consistently adjusted to stable growth trajectories that were below *in utero* growth curves, despite achieving postnatal growth rates that were similar to intrauterine rates. We then used this data to develop multiple linear regressions by inputting information about the GA, z-score at birth and the hospital at which the infant was treated to predict the z-scores an individual low-risk preterm infant would adjust to over the first three weeks of life.

Postnatal growth during postnatal adaptation

In accordance to our hypothesis, the "healthy" subset of infants initially lost weight and began gaining weight attaining a stable growth trajectory that was lower than recommended intrauterine growth. Weight loss during postnatal adaptation occurred on average at day 5 of postnatal life for both groups of infants (25 – 35 completed weeks GA), requiring 11 days after birth for 30 – 35 completed week GA preterm infants and 12 days after birth for 25 – 29 completed week GA infants to regain their BW. These results are similar to findings by Horemuzova *et al.* (2011) who found that preterm infants < 26 weeks GA, experienced body weight nadir on day 6, and required 18 days to regain BW. The

greater number of days that the infants required to regain BW in the Horemuzova study may putatively be a result of the inclusion of preterm infants with major pathologies.

A more in depth look at the amount of weight loss seen within the first few days of life in the present study, showed that there was a significant negative correlation between the percentage of the birth weight lost and GA, which is consistent with literature (Wright *et al.* 1993). This offers an explanation of the difference in weight loss that was seen between the preterm infants in the present study (25 – 35 completed weeks GA) and those analyzed in the study led by Horemuzova *et al.* (< 26 weeks GA). In the present study, the maximal weight loss observed was 5.7% of the BW of 30 – 35 completed week GA infants and 9.4% of the BW of 25 – 29 complete week GA infants. However, Horemuzova *et al.* reported a maximal weight loss of 16% of the BW of infants of < 26 weeks GA.

The negative correlation between percentage of BW lost during postnatal adaptation and GA may be explained by the rearrangement of body composition that is known to occur during postnatal adaptation. The initial weight loss after birth is a result of the contraction of the extracellular fluid compartment during postnatal adaptation (Bauer *et al.* 1991). The contraction of the extracellular fluid compartment results in the irreversible loss of body water. It is also well established that more immature preterm infants have a greater percentage of body water content, approximating 80 – 90% of their body weight (Modi *et al.* 2004), in comparison to 10% in healthy 26 – 36 weeks GA neonates (Tang *et al.*

1997). Consequently, the greater postnatal weight loss seen in more premature infants may be due to the greater percentage of body water content of the more premature infant population. Further strengthening this postulate, we have observed weight losses in preterm infants that are similar to the body water reductions that have been previously reported (Bauer *et al.* 1991).

Comparison of postnatal growth to intrauterine growth references

Group A (30 – 35 6/7 GA infants) consistently adjusted to stable postnatal growth trajectories that were 0.97 SDS and 0.98 SDS below the 50th BW%ile by days 14 and 21 of postnatal life. Group B (25 – 29 6/7 GA) infants adjusted to stable postnatal growth trajectories at days 14 and 21 that were 0.88 SDS and 0.89 SDS below the 50th BW%ile. Thus as hypothesized, postnatal growth trajectories following postnatal adaptation were lower than BW%iles, however were shown to be higher than previously published postnatal growth trajectories. For example, it has been shown that a preterm infant population (<26 weeks GA) adjusted to a trajectory that was on average 2.8 SDS below the 50th BW%ile by 40 weeks corrected age (Horemuzova *et al.* 2011). This may be due to the selection of only the healthiest subset of preterm infants in the present study, whereas Horemuzova *et al*. had included all infants that had survived until discharge. Our results putatively suggest that “low-risk” preterm infants adjust to a postnatal growth trajectory that is closer to the recommended 50th BW%iles

than all surviving preterm infants, but that the postnatal growth trajectories still do not approximate intrauterine trajectories.

Upon comparison to EFW curves, the SDS of postnatal growth in relation to intrauterine growth was even greater than when compared to BW%iles during days 1 to 15. However, this gap began to diminish by day 21. This was a result of the difference between BW%iles and EFW curves; EFW curves (Salomon *et al.* 2007) represented higher body weights over the following GA periods: 23 to 27 weeks and 34 to 36 weeks than BW%iles (Kramer *et al.* 2001). Thus, at birth many of the study subjects were farther from the recommended intrauterine value represented by EFW than by BW%iles. Upon interpretation of these results it is important to consider the differences between using EFW and BW%iles to represent intrauterine growth.

The advantage of using the Salomon *et al*. (2007) EFW curves was that it provided measurements of a large number of fetuses (n = 9 577) that remained *in utero* for the length of a full-term pregnancy and who did not have a known abnormal karyotype or any congenital malformations. Thus, these references likely provide good insight into the weight-for-ages of fetuses of healthy pregnancies. The measurements were also obtained during a recent screening period (2002 – 2006), ensuring that the reported EFW references were reflective of recent clinical care. Additionally, the EFW references were estimated using the Hadlock formula (Hadlock *et al.* 1985), which has been shown to be one of the most accurate regressions to estimate fetal weight (Melamed *et al.* 2009).

However, the disadvantage of the use of EFW to represent a weight-for-age reference is that current techniques are indirect and rely on in accurate biometric measurements to predict fetal weight. Additionally, models have shown to underestimate weight for macrosomic fetuses but overestimate weight for smaller fetuses (Cohen *et al.* 2010; Goetzinger *et al.* 2013).

In contrast, BW%iles are constructed using postnatal measurements of fetuses that were born prematurely. Since preterm delivery is often a result of suboptimal intrauterine growth, such neonates are likely to have experienced growth restriction resulting in intrauterine growth curves that may be lower than healthy intrauterine growth. However, because BW%iles are constructed using accurate direct measurements of BWs from large cohorts instead of computing body weights of smaller populations of the fetus, we chose to incorporate z-scores based on the 50th BW%iles into the predictive models. This minimized the introduction of any systematic or random errors that would confound our results. Additionally, using the 50th BW%iles as our reference weight-for-age values when calculating z-scores, allowed all of the body weight data collected to be utilized, as opposed to using EFW values that did not span past 36 weeks corrected age.

Longitudinal assessment of postnatal growth

Z-scores are widely recognized as the best representation of anthropometric measures because they provide a value by which growth can be compared across genders and GAs (World Health Organization, 1995). However,

there are some inherent drawbacks when using z-scores to assess growth longitudinally. Z-scores are calculated by dividing the difference between observed and reference body weight values by the standard deviation of the reference population. Thus, as the variability in BWs of the reference population increases, standard deviations also increase, thereby reducing the calculated magnitude of the z-score for an individual infant. This may result in an artificially lower absolute z-score at a particular GA that is associated with a large variation in body weights in the reference population. When comparing this z-score with other infants of the same GA, the standard deviation does not pose a problem. However, when the z-score is compared to other GA z-scores with different standard deviations, the z-score no longer represents an objective evaluation of an individual infant's growth.

In light of the limitations listed above, we avoided the artefact by supplementing z-score based comparisons of postnatal growth and recommended intrauterine growth references with percentages that were not calculated using standard deviation values. At birth, group A infants (GA: 30 – 35 6/7 weeks) were on average 95% of the 50th BW%ile value, but had adjusted to a stable growth trajectory by day 14 - 21 of life that was 84% of the 50th BW%ile. Group B infants (GA: 25 – 29 6/7 weeks) had birth weights that were on par with the 50th BW%ile, but adjusted to stable postnatal growth trajectories that were approximately 80% of the 50th BW%ile. These findings paralleled the z-score results, suggesting that the described limitation of z-scores may be overlooked in

this case. This may have been due to the generally constant standard deviations of the BW%iles over the GAs of interest (standard deviations gradually increased from 306 to 443 g over the 30 to 37 week GA period, Kramer *et al.* 2001).

Next, growth rates of the study populations were calculated and compared to recommended intrauterine growth rates. In comparison to intrauterine rates of 16.0 g/kg/d during 30 – 35 6/7 weeks GA and 18.6 g/kg/d during 25 – 29 6/7 weeks GA (Kramer *et al.* 2001), the observed weekly average of postnatal growth rates of Group A preterm infants (30 – 35 6/7 weeks GA) ranged from 13.5 – 16.9 g/kg/d, and for Group B preterm infants (25 – 29 6/7 weeks GA) ranged from 10.3 to 20.0 g/kg/d. Thus overall, the observed postnatal growth rates approximated recommended intrauterine rates. The higher growth rates of the Group B preterm infants during 30 – 31 weeks, in comparison to Group A infants, may have been a result of aggressive nutritional strategies employed as part of clinical practice to promote catch-up growth in more premature infants, or may be an artefact of the different screening criteria that were used for the two study populations.

Unfortunately, our results of postnatal growth rates were difficult to compare with those presented in literature. This is due to the inconsistency in literature of how growth rates are calculated. Calculation methods include using linear models, exponential models and in some cases methods which have not been described. In the current study, an exponential growth model (Patel *et al.* 2005) was used to calculate the average growth velocity over a seven-day period following the attainment of a stable growth pattern. Postnatal growth rates were

calculated over a seven-day period, because day-to-day growth is highly variable and are a poor representation of the actual growth rate of the infant. The exponential growth model was used because it has been shown to be the most accurate model to calculate growth velocity (Patel *et al.* 2005), as it takes into account the relationship between weight at a certain time point and weight at a previous time point. Additionally, the starting point at which postnatal growth rates were measured in published studies varied and growth rates were often presented for infants grouped by BW (instead of GA), making comparison of postnatal growth rates difficult.

Analysis of factors that influence postnatal growth

In contrast to our hypothesis and findings in literature, our results suggest that the speed by which full enteral feeds were attained (a measure of feeding tolerance) had no effect on postnatal growth. Postnatal growth was measured by evaluating the z-score at day 14 of life. This result however, is difficult to interpret. This is because despite the speed by which full enteral feeds were administered, no consideration was taken of the nutrient accumulation of the infant. The data collected in this study did not include the type of nutrition, which is known to have a high impact on the rate of postnatal growth. For instance, it has been shown that early introduction of nutrition that is higher in amino acids relative to other macronutrient groups, results in more rapid postnatal growth of the preterm infant (Poindexter *et al.* 2006).

Further, considerations should be given to the extent of gut immaturity and metabolic function of more premature infants. In a study that compared the day of life full enteral feeds were attained in two groups of infants stratified by GA (<29 weeks GA vs. ≥29 weeks GA) it was shown that feeding tolerance was attained at a later date for the <29 weeks GA group (Leaf *et al.* 2012). In younger preterm infant populations, despite the speed by which feeding tolerance is achieved, it is likely that gut and metabolic function is not yet developed, and thus feeding tolerance may not be an appropriate indication of nutrient accretion. Although our results suggest that postnatal growth was not affected by feeding tolerance, the results should not be interpreted as a description of the effect nutrition has on postnatal growth, because intestinal function, nutritional substrate, nutrient balance and the microbial environments of the infants, which are key factors that impact nutrient accumulation (Leaf *et al.* 2013), were not considered.

Ethnicity of the infants was also shown to be an insignificant predictor of postnatal growth of preterm infants included in this study. These results parallel the findings of the Multicentre Growth Reference Study Group (2006), who found that there were no clinically relevant differences in linear growth of infants from various ethnic backgrounds and cultural centres (Brazil, Ghana, India, Norway, Oman and the USA). However, the impact of ethnicity on postnatal growth evaluated in the present study should be interpreted with caution, as ethnicity information was not available for 34% of the study population. Thus since the data was abstracted retrospectively, it is possible that there was a selection bias

of information-availability for certain groups. Also, the number of infants within each category was not evenly distributed, where some categories only included one infant. In order to evaluate the effect of ethnicity on postnatal growth, further studies are needed with appropriate sample sizes within each ethnic group.

Differences between centres

Since a multi-centre approach was used in this study to collect normative postnatal growth data, it was imperative to evaluate the differences between hospital centres. The distributions of BWs, GAs, growth outcomes on day 14 and the day of life full enteral feeds were attained were all significantly different across hospital centres. In general, SJH/GUH infants had higher BWs, and higher GA ranges that were more narrow than MUMC/HUH/SMH. It was also shown that SJH/GUH adjusted to growth that was closer to the 50th BW%ile by day 14. This is likely due to the overall greater maturity of the infants from these centres.

Since given nutrition can guide the growth of the preterm infant (Senterre *et al.* 2011, Rochow *et al.* 2012), we also considered the speed at which full enteral feeds were advanced at each centre. It was found that MUMC/SJH used more aggressive nutritional approaches, where full feeds were attained 1-2 days earlier than the other centres. Unfortunately, the differing BW and GA distributions between centres could not explain the differences in feeding tolerance that were observed; centres with BW and GA distributions that were skewed to lower values did not show trends of later introduction of full enteral

feeds. Thus, it is likely that the difference in feeding tolerance that was observed, although confounded by factors such as BW and GA, was a result of varying nutritional strategies between hospital centres. Thus, in order to account for these complex differences between centres, the categorical variable of hospital centre was incorporated into multiple-linear regressions as both a dichotomous variable (grouped according to the speed at which full enteral feeds were attained) and as "dummy" variables (which allowed a unique coefficient for each centre).

Development of predictive models

The consistency of the postnatal growth trajectory that the Group A infants (GA: 30 – 35 6/7 weeks) adjusted to, in relation to the 50th BW%ile, allowed for the development of accurate postnatal growth regressions. Different variations of multiple-linear regressions were evaluated for their accuracy, using z-scores, percentages of the 50th BW%ile and raw body weight values as dependent variables. GA at birth, z-score at birth and hospital centre were consistently significant predictors of postnatal growth at days 7, 14 and 21. However, contrary to our hypothesis, gestation, mode of delivery, head circumference and body length at birth were not significant factors.

Overall, the coefficient of determinations for body weight regressions were higher than those for z-score regressions, however residual ranges were more narrow for z-score models. The reported residual ranges of -255 to 346 g for day 7, -243 to 401 g for day 14 and -287 to 449 g for day 21 should be interpreted

with caution, because the conversion from z-scores to raw body weight values were based on the assumption that 1 SDS = 368 g. However, SDS is dependent on the gender and the GA of the infant, as a unique SDS is associated with each completed week and gender. For instance, the value of 1 SDS of a 30 week GA boy is 291 g, compared to the 434 g for a 35 6/7 GA boy. These values represent a maximum residual of approximately 18% of the body weight of the infant. The majority of predicted z-scores were clustered close to a residual value of zero and were associated with narrow confidence intervals that approximated 0.5 SDS. Further, upon comparison of the actual and predicted values of the Group A study population's z-scores at days 7, 14 and 21, it was seen that there was no statistically significant difference.

As previously mentioned, the independent variable of hospital centre that the infants were treated at were coded using two methods: dichotomous coding and dummy coding. Dichotomous coding was based on categorization of the centres according to the speed at which full enteral feeds were begun. Dummy coding allowed a specific variable to be assigned to each centre, where MUMC was considered the reference centre. Since it was shown that the average difference in advancement of feeds (1-2 days between centres) did not impact the postnatal growth of the infants and that there were significant differences in the distribution of BW and GA across centres, the theoretically superior method of coding the hospital centre variable was to use dummy coding. This is because this method of coding took into account all confounding differences between

centres (range of BWs, range of GAs, difference in nutritional and management practices). However, since the range of residuals was not clinically significant between resulting regressions using both coding methods, we decided to progress with the z-score models that coded for hospital centres as dichotomous variables, in order to easily incorporate additional hospital centres in future evaluations and applications of the predictive models. In this way, additional centres will be assessed for the rate at which they administer full enteral feeds and categorized into a group with SJH/MUMC (early feeds) or with GUH/HUH/SMH (later feeds), allowing the predictive models to immediately be evaluated using the new data.

In contrast to our hypothesis, our results suggested that gestation (single vs. multiple) was not a significant predictor of z-scores at days 14 and 21, but was a significant predictor of z-scores at day 7 ($p = 0.022$). This indicates that by the second week of life, the gestation of preterm infants is no longer a significant factor that influences the postnatal growth trajectory to which infants adjust. These results are not in line with literature, where it has long since been shown that multiple gestation infants grow on lower fetal growth trajectories than singletons (Iffy *et al.* 1983). Despite the lack of impact that gestation has on postnatal growth in the present study, it cannot be discounted that gestation may be a significant predictor of the quality of growth of the preterm infant in both short and long-term life. This postulate is in line with findings that have

associated increased adiposity in adult-life for twins when compared to singletons (Monrad *et al.* 2009).

Validation of predictive models

Internal validation of the day 7, 14 and 21 z-score regressions using a split-sample technique found that as expected, the predicted z-scores using the predictive and training set regressions were statistically different. However for days 7 and 14 the mean difference corresponded to clinically insignificant values of 4 and 7 grams. These results thus suggested that there was no selection bias in the preterm infant population used to develop the models, improving the confidence of generalizing the application of the presented regression models to other preterm infants who are shown to have unimpaired postnatal adaptation. Further, larger residuals were not able to be qualitatively associated with any of the following factors: z-score at birth, GA, gender or ethnicity, indicating that the regressions were accurate on an individual-infant basis. However, it is of valued to note that the number of infants within each ethnic category was low, making it difficult to draw conclusions regarding the effects of ethnicity on postnatal growth or the accuracy of the predictive models.

Lastly, upon extrapolating the predictive z-score models to 25 weeks of gestation, and assessing the accuracy of predictions using an independent 25 – 29 6/7 week GA infant population who also required minimal medical intervention during hospital stay (Group B infants), it was seen that the accuracy was

compromised. The actual and predicted z-scores of the Group B population were statistically different, where the 95% confidence intervals showed that predictions were up to 0.8 SDS of the actual z-scores at a 95% confidence level.

Predictive models based on 25 – 35 6/7 weeks GA preterm infants

Upon combining the data of all infants included in this study (Group A + Group B; n = 838), the same three independent variables (GA, z-score at birth and hospital centre) were shown to be significant factors that predicted z-scores at days 7, 14 and 21. Upon qualitative assessment, the coefficients remained similar to the regressions that were developed based only on the Group A population. Using apparent validation, by comparing actual and predicted z-scores of the same population used to develop the models, it was seen that there was no significant difference. This showed that there is a very high level of optimism for the overall predictive z-score models for days 7, 14 and 21. Further, the predicted z-score values were within approximately 0.5 SDS of the actual z-score value at a 95% confidence level, suggesting that deviations from the predicted value were not clinically relevant for most infants.

Limitations

As a result of the retrospective-nature of this study, many preterm infants in the later GA groups (34 – 36 week GA groups) were excluded from the study due to early hospital-transfer or discharge. Many of these preterm infants met our

rigorous screening criteria of requiring minimal medical intervention and having not been exposed to defined factors that are known to impact postnatal growth, but were excluded due to having <14 days of health records available. This introduced a selection bias that was very prominent for the 36 week GA group, where many of the “healthier” infants were excluded because of their short hospital stay. This resulted in lower weight-for-age values in the 34 – 36 week GA infants when compared to the more preterm infants and may have potentially compromised the degree of “healthiness” seen in the 34 - 36 week GA infant groups in our study. We acknowledged this selection bias, and as a result excluded the 36 weeks GA infant group from subsequent analyses. We also collaborated with SJH to allow inclusion of preterm infants from MUMC who had been transferred to SJH before 14 days of life, which allowed for the inclusion of 117 infants who would have otherwise not met the screening criterion of having a minimum of 14 days of data available.

Additionally, care was taken to include infants only within the 2008 to 2012 admission year range to reflect recent hospital care and preterm infant populations. However data from all years within this range were not abstracted from each of the participating hospital centres; SMH data was only collected for 2010 and 2011, and both SJH and HUH included only 2008 – 2011 admissions. Ideally, the regressions should have been developed based on data from each of the years in the screening period from each hospital centre. This however, was not possible to attain since research ethics approval was granted late into the

two-year study project, preventing enough time for full data-set abstraction. It is of value to note however, that no apparent trends were observed upon explorative analysis of the effect of the year of admission on residuals for day 7, 14 and 21 z-score models. This suggests that the above-mentioned artefact may have limited impact on our results.

Another limitation of the present study was that it did not consider maternal factors such as height, weight, BMI, age or gestational weight gain. Efforts were made to exclude infants exposed to maternal substance use or maternal diabetes mellitus, both of which have shown to affect growth, however other confounding factors were not corrected for. For instance, it has been shown that maternal height and weight (Gaillard *et al.* 2011), as well as primi- vs. multiparous mothers (Sankilampi *et al.* 2013) significantly influenced BW. Additionally, information was not collected regarding antenatal glucocorticosteroid use, which has recently been shown to leave a short-term impact on the whole-genome expression in preterm infants, with a particular effect on genes associated with inflammation and cancer (Saugstad *et al.* 2013). Thus, antenatal glucocorticosteroid use may impact the long-term health outcomes of preterm infants. Ideally, in light of the DOHaD hypothesis, each of these factors should be evaluated in future studies for their impact on predictive models of postnatal growth. It is of value to note however, that it has also been previously reported that the impact of maternal anthropometry on BW is minimal in comparison to GA and gender of the infant (Hutcheon *et al.* 2008).

The current study has also not focused on the type of nutrition administered to each infant. It has been shown that adjusting nutrition can dictate the postnatal growth trajectory of the infant (Rochow *et al.* 2012), making it a factor that should be considered in future predictive models. However, as previously discussed, the maturity of the infant gut, microbial milieu and type and balance of the nutritional substrate being introduced to the infants are key factors that affect nutrient availability for the preterm infant (Leaf *et al.* 2013). In the very preterm infant, the length of the gut and the number of microvilli are not as great as the more mature infant. Additionally, the production of digestive enzymes is also blunted (Kolacek *et al.* 1990). By not considering the GA of the infants when grouping by day of life full enteral feeds were attained, the maturity of the gut was not considered. The accrual of these factors thereby affects absorptive capacity in the very preterm infant, making feeding tolerance a crude measure of nutrient availability in the infant. Thus, the present study is limited in that these factors were not considered in the development of the predictive models.

Additionally, although extreme care was taken to select only the “healthiest” subset of preterm infants, the definition of “healthy” is by no means comprehensive. For example, it is possible that infants included in this study had abnormal body compositions that may have confounded our results. However, due to the retrospective nature of the study, it was not possible to have included body composition parameters in this study. Further, there are currently no normative body composition reference values for preterm infants to which this

data could be compared. Similarly, no long-term outcomes were measured of the preterm infants, so although we have attempted to select the healthiest subset of the preterm infants, we cannot be certain that these infants and their postnatal growth patterns will be associated with optimal long-term outcomes. In summary, we caution that the presented normative postnatal growth data are descriptive of a preterm infant population with minimal impairment of postnatal adaptation, rather than a prescriptive standard.

Strengths

The present study is one of few to assess postnatal growth trends using day-specific serial measurements of preterm infants. Other longitudinal studies (Wright *et al.,* 1993; Ehrenkranz *et al.* 1999) stratified preterm infants according to BW, which resulted in heterogeneous groups of infants, where larger immature infants were grouped with smaller more mature infants (Moutquin *et al.* 2003). Thus, it is a strength of the present study to have longitudinally assessed weight changes of infants that were included based on premature delivery rather than using low birth weight as a selection criterion.

Although there are currently two studies to our knowledge that assessed postnatal growth of preterm infants longitudinally and stratified by GA (Niklasson *et al.,* 2003; Horemuzova *et al.,* 2012), neither study have limited their study populations to preterm infants with no major neonatal complications, surgeries, and/or congenital malformations. The present study has attempted to stringently

select such infants, and have additionally also screened for defined factors that have been shown to impair growth (maternal diabetes mellitus, antenatal smoking/alcohol/ drug use or chorioamnionitis).

Additionally, the multi-centre approach used in this study, allowed the study populations, nutritional strategies and growth trends to be compared across centres because of the consistency in screening criteria of the population across centres. This information was then used when developing the multiple linear regression models that predicted z-scores during the first three weeks of postnatal life. Thus, the normative, longitudinal postnatal growth data for preterm infants who required minimal medical intervention during hospital stay that are presented here are not specific to a single centre and thereby biases introduced from a single hospital centre are reduced.

Lastly, all patient records were individually scrutinized as opposed to the use of a registry database, which allowed for careful selection of infants and quality checks of the day-specific postnatal growth measures that were recorded. Thus, although our results cannot be used as a prescriptive standard of postnatal growth for preterm infants, it does provide to our knowledge, the closest hypothesis of healthy postnatal growth currently available for preterm infants that are treated using current clinical practices.

Implications

We believe that the presented longitudinal, normative body weights of preterm infants who required minimal medical intervention and who were not exposed to defined factors that impact postnatal growth will provide the first step toward characterizing healthy postnatal growth for preterm infants. Characterizing healthy postnatal growth for preterm infants will guide clinicians with nutritional strategies that may help to minimize later-life morbidities and help to identify postnatal growth restriction. Such longitudinal, descriptive values presented here will help to form an individualized hypothesis of healthy postnatal growth for preterm infants that may be tested in future studies.

Future directions

In order to improve the assessment of postnatal growth, body composition studies would complement and provide depth to the presented results. Firstly, it would allow us to better evaluate the adaptations made during the postnatal adaptation period and identify infants with abnormal postnatal growth who may appear otherwise healthy. Secondly, body composition analyses will allow the presented data to be compared to fetal body composition, which is the current recommendation of growth for preterm infants (American Academy of Pediatrics, 1999). Thirdly, body composition analyses will allow for a more complete

understanding of healthy postnatal growth in preterm infants, by providing valuable information about the quality of growth.

Additionally, many of the confounding factors would also have to be controlled or accounted for when constructing predictive models, such as type of nutrition, maternal height, obesity, age, gestational weight gain and primi- vs. multiparous. Ethnicity and gestation may also prove to play roles in the postnatal growth of the preterm infants when body composition is analyzed. Thus, collecting more data about the ethnicity of the infants included in such studies may help to untangle some of the complex relationships between genetic factors, social factors and growth potential. In fact, a recent study has suggested that ethnicity impacts tissue development during gestation, and has postulated that this may account for the long-term morbidities associated with certain ethnicities (Lampl *et al.* 2012). Accounting for such factors in future studies will allow for a more comprehensive and individualized hypothesis of healthy postnatal growth for preterm infants.

In order to further strengthen the validity of the present study, data abstraction will continue at SJH, HUH and SMH centres to ensure complete data sets spanning 2008 to 2012 are obtained for all centres. This will also allow us to increase the robustness of our estimates of healthy postnatal growth by increasing the study population size.

Lastly, in order to ascertain that the described postnatal growth trends are reflections of optimal postnatal growth, long-term follow up of the included infants

would be needed. Long-term outcomes such as neurodevelopment, body composition and the development of cardiovascular disease risk factors should be assessed. Ideally, a randomized control trial that assesses the mentioned long-term outcomes would be needed to test the proposed hypothesis of healthy postnatal growth for preterm infants.

List of References

American Academy of Pediatrics Committee on Nutrition: Nutritional needs of low-birth weight infants. *Pediatrics* (1985); 75: 976.

Baker PN, Johnson IR, Gowland PA, Hykin J, Harvey PR, Freeman A, Adams V, Worthington BS, Mansfield P. Fetal weight estimation by echo-planar magnetic resonance imaging. *Lancet* (1994); **12**(343): 644-645.

Barker DJP, Harding JE, Owens JA, Robinson JS. Fetal nutrition and cardiovascular disease in adult life. *Lancet* (1993); 341: 938-941.

Barker DJ, Osmond C. Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales. *Lancet* (1986); 1:1077–1081.

Bauer K, Bovermann G, Roithmaier A, Götz M, Pröiss A, Versmold HT. Body composition, nutrition, and fluid balance during the first two weeks of life in preterm neonates weighing less than 1500 grams. *J Pediatr* (1991); 118: 615-620)

Bauer K, Cowett RM, Howard GM, VanEpp J, Oh W. Effect of intrauterine growth retardation on postnatal weight change in preterm infants. *J Pediatr* (1993); 123: 301-306.

Bertino, E., et al. Evaluation of Postnatal Growth of Preterm Infants. *J Matern Fetal Neonatal Med* (2011); 24(2): 9-11.

Bleeker SE, Moll HA, Steverberg EW, Donders AR, Derksen-Lubsen G, Grobbee DE, Moons KG. External validation is necessary in prediction research: a clinical example. *J Clin Epidemiol.* (2003); 56(9): 826-32.

Clark RH, Thomas P, Peabody J. Extrauterine growth restriction remains a serious problem in prematurely born neonates. *Pediatrics* (2003); 111: 986-990.

Clausson B, Gardosi J, Francis A, Cnattingius S. Perinatal outcome in SGA births defined by customized versus population-based birth weight standards. *Brit J Obstet Gynaecol.* (2001); 108: 830-834.

Cooke RW. Conventional birth weight standards obscure fetal growth restriction in preterm infants. *Arch Dis Child Fetal Neonatal Ed* (2007); 92: 189–192.

Cooke, R. J. Postnatal Growth and Development in the Preterm and Small for Gestational Age Infant. *Nestle Nutr Workshop Ser Pediatr Program* (2010); 65: 85-95

Dudley NJ. A systematic review of the ultrasound estimation of fetal weight. *Ultrasound Obstet Gynecol* 2005; 25:80-89.

Ehrenkranz RA, Younes N, Lemons JA, Fanaroff AA, Donovan EF, Wright LL, Katsikiotis V, Tyson JE, Oh W, Shankaran S, Bauer CR, Korones SB, Stoll BJ, Stevenson DK, Papile LA. Longitudinal growth of hospitalized very low birth weight infants. *Pediatrics* (1999); 104(2): 280-9.

Ehrenkranz, R. A., et al. Growth in the Neonatal Intensive Care Unit Influences Neurodevelopmental and Growth Outcomes of Extremely Low Birth Weight Infants. *Pediatrics* (2006); 117(4): 1253-61.

Fenton TR. A new growth chart for preterm babies: Babson and Brenda's chart updated with recent data and a new format. *BMC Pediatr* (2003); 16(3): 13.

Figueras F, Figueras J, Meler E, Eixarch E, Coll O, Gratacos E, Gardosi J, Carbonell X. Customized birth weight standards accurately predict perinatal morbidity. *Arch Dis Child Fetal Neonatal Ed* (2007); 92(4):277-280.

Gardosi J. Customized fetal growth standards: Rationale and Clinical Application. *Seminars in Perinatol.* (2004); 28(1): 33-40.

Gluckman PD, Hanson MA, Beedle AS. Early Life Events and Their Consequences for Later Disease: A Life History and Evolutionary Perspective. *Am J Human Biol* (2007); 19:1-19.

Goetzinger KR, Odibo AO, Shanks AL, Roehl KA, Cahill AG. Clinical accuracy of estimated fetal weight in term pregnancies in a teaching hospital. *J Maternal-Fetal Neonatal Med.* (2013); 1476-5954

Hadlock, FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body and femur measurements: a prospective study. *Am J Obstet Gynecol* (1985); 151:333-337.

Hales CN, Barker DJ, Clark PM, Cox LJ, Fall C, Osmond C, Winter PD. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. (1991); 303:1019–1022.

Ho MY, Yen YH, Hsieh MC, et al. Early versus late nutrition support in premature neonates with respiratory distress syndrome. *Nutrition* (2003); 19:257-60.

Hutcheon JA , Zhang X , Cnattingius S , Kramer MS , Platt RW. Customised birthweight percentiles: does adjusting for maternal characteristics matter? *BJOG*. (2008); 115: 1397 – 404.

Iffy L, Lavenhar MA, Jakobovits A, Kaminetzky HA. The rate of early intrauterine growth in twin gestation. *Am J Obstet Gynecol* (1983); 146: 970–972.

Jain V, Singhal A. Catch up growth in low birth weight infants: Striking a healthy balance. *Rev Endocr Metab Disord (*2012); 13(2): 141-147.

Kolacek S, Puntis JW, Lloyd DR, Brown GA, Booth IW. Ontogeny of pancreatic exocrine function. *Arch Dis Child* (1990); 65:178-81.

Kramer MS, Platt RW, Wen SW, Joseph KS, Allen A, Abrahamowicz M, Blondel B, Bréart G; Fetal/Infant Health Study Group of the Canadian Perinatal Surveillance System. A New and Improved Population-Based Canadian Reference for Birth Weight for Gestational Age. *Pediatrics* (2001); 108(2): 35.

Latal-Hajnal B, von Siebenthal K, Kovari H, et al. Postnatal growth in VLBW infants: significant association with neurodevelopmental outcome. *J Pediatr* (2003); 143: 163-70.

Leaf A, Dorling J, Kempley S. Early or delayed enteral feeding for preterm growth-restricted infants: a randomized trial. *Pediatrics* (2012);129:e1260e8.

Leaf A. Introducing enteral feeds in the high-risk preterm infant. *Seminars Fetal Neonatal Med* (2013): 1-5.

Lucas A, Morley R, Cole TJ. Randomised trial of early diet in preterm babies and later intelligence quotient. *Br Med J* 1998; 317: 1481–7**.**

Ma Y-N, Wang J, Dong G-H, Liu M-M, Wang D. Predictive Equations Using Regression Analysis of Pulmonary Function for Healthy Children in Northeast China. *PLoS ONE* (2013); 8(5): e63875.

Mark J, Goldberg MA. Multiple regression analysis and mass assessment: A review of the issues. *Appraisal J* (2001); 89-109.

Martin CR, Brown YF, Ehrenkranz RA, O'Shea TM, Allred EN, Belfort MB, McCormick MC, Leviton A. *Pediatrics* (2009); 124:649-657.

Melamed N, Yogev Y, Meizner I, Mashiach R, Bardin R, Ben-Haroush A. Sonographic fetal weight estimation: Which model should be used? *J Ultrasound Med* (2009); 28:617-629.

Min JW, Kong KA, Park BH, et al. Effect of postnatal catch-up growth on blood pressure in children at 3 years of age. *J Hum Hypertens* (2007); 21(11): 868–74.

Modi N. Management of fluid balance in the very immature neonate. *Arch Dis Fetal Neonatal Ed* (2004); 89: 108-111.

Monrad RN, Grunnet LG, Rasmussen EL, Malis C, Vaag A, Poulsen P. Age-dependent nongenetic influences of birth weight and adult body fat on insulin sensitivity in twins. *J Clin Endocrinol Metab* (2009); **94:** 2394–2399.

Mourad M, Bertand-Krajewski, Chebbo G. Calibration and validation of multiple regression models for stormwater quality prediction: data partitioning, effect of data sets size and characteristics. *Water Sci Technol*. (2005); 52(3):45-52.

Moutquin, J. M. Classification and Heterogeneity of Preterm Birth. *BJOG* (2003); 110(20): 30-3.

Multicentre Growth Reference Study Group. Assessment of differences in linear growth among populations in the WHO multicentre Growth Reference Study. *Acta Pediatr.* (2006); 56-65.

Niklasson, A., et al. Growth in Very Preterm Children: A Longitudinal Study. *Pediatr Res* (2003); 54(6) 899-905.

Nutrition Committee, Canadian Paediatric Society. Nutrient needs and feeding of premature infants. *CMAJ* (1995);152:1765–85.

Patel AL, Engstrom JL, Meier PP, Kimura RE. Accuracy of methods for calculating postnatal growth velocity for extremely low birth weight infants. *Pediatrics* 2005; 116(6):1466-1473.

Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series No. 854. Geneva: World Health Organization, 1995.

Poindexter BB, Langer JC, Dusick AM, Ehrenkranz RA. Early provision of parenteral amino acids in extremely low birth weight infants: Relation to growth and neurodevelopmental outcome. *J Pediatr* (2006); 148: 300-5.

Reeves S, Bernstein IM. Optimal growth modeling. *Semin in Perinatol* (2011); 32:148-153.

Rochow N, Fusch G, Muhlinghaus A, Niesytto C, Straube S, Utzig N, Fusch C. A nutritional program to improve outcome of very low birth weight infants. *Clinical Nutr* (2012); 31:124-131.

Rochow N, Raja P, Straube S, Voigt M. Misclassification of newborns due to systematic error in plotting birth weight percentile values. *Pediatrics* (2012); 130(2): e347-51.

Saigal S, Stoskopf B, Streiner D, Paneth N, Pinelli J, Boyle M. Growth trajectories of extremely low birth weight infants from birth to young adulthood: A longitudinal, population-based study. *Pediatric Research* (2006); 60(6): 751-758.

Salomon LJ, Bernard JP, Ville Y. Estimation of fetal weight: reference range at 20 – 36 weeks' gestation and comparison with actual birth-weight reference range. *Ultrasound Obstet Gynecol* (2007); 29: 550-555.

Sankilampi U, Hannila ML, Saari A, Gissler M, Dunkel L. New population-based references for birth weight, length and head circumference in singletons and twins from 23 to 43 gestation weeks. *Annals of Med.* (2013); 1-9.

Saugstad OD, Kwinta P, Wollen EJ, Bik-Multanowski M, Madetko-Talowski A, Jagla M, Tomasik T, Pietrzyk JJ. Impact of antenatal glucocorticosteroids on whole-genome expression in preterm infants. *Acta Paediatr* (2013); 102(4): 349-355.

Senterre T, Rigo J. Optimizing early nutritional support based on recent recommendations in VLBW infants allows abolishing postnatal growth restriction. *J Pediatr Gastroenterol Nutr* (2011); 53(5): 536-42.

Senterre T, Rigo J. Reduction in postnatal cumulative nutritional deficit and imrovemnet of growth in extremely preterm infants. *Acta Paediatrica.* (2011); 101:e64-e70.

Singhal A, Fewtrell M, Cole TJ, et al. Low nutrient intake and early growth for later insulin resistance in adolescents born preterm. *Lancet* (2003); 361:1089–97**.**

Tang W, Ridout D, Modi N. Influence of respiratory distress syndrome on body composition after preterm birth. *Archives of Disease in Childhood.* (1997); 77:F28-31.

Thureen, P. J. The Neonatologist's Dilemma: Catch-up Growth or Beneficial Undernutrition in Very Low Birth Weight Infants-What Are Optimal Growth Rates? *J Pediatr Gastroenterol Nutr* 45 Suppl 3 (2007): S152-4.

Trevisanuto D, Peruzzetto C, Cavallin F, Vedovato S, Cosmi E, Visentin S, Chiarelli S, Zanardo V. Fetal placental inflammation is associated with poor neonatal growth of preterm infants: a case-control study. *J Matern Fetal Neonatal Med* (2013): 1-7.

Uhing MR, Das UG. Optimizing growth in the preterm infant. *Clinics in perinatology* (2009); 36(1):165–76.

Velaphali S. Nutritional requirements and parenteral nutrition in preterm infants. *S Afr J Clin Nutr* (2011); 24(3): 27-31.

Villar J, Knight HE, de Onis M, Bertino E, Gilli G, Papageorghiou AT, Ismail C, Barros FC, Bhutta ZA. Conceptual issues related to the construction of prescriptive standards for the evaluation of postnatal growth of preterm infants. *Arch Dis Child* (2010); 95: 1034-1038.

Volpe JJ. Neurology of the newborn. Chapter 11, 5th edition. Philadelphia: Saunders-Elsevier (2008); 517-588.

Woo JS, Wan CW, Cho KM. Computer-assisted evaluation of ultrasonic fetal eight prediction using multiple regression equations with and without the fetal femur length. *J Ultrasound Med* (1985); 4:65-67.

Wright K, Dawson JP, Fallis D, Vogt E, Lorch V. New postnatal growth grids for very low birth weight infants. *Pediatrics* (1993); 91(5): 922–6.

Ziegler EE, Thureen PJ, Carlson SJ. Aggressive nutrition of the very low birthweight infant. *Clin Perinatol* (2002); 29: 225–44**.**