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Application of air displacement plethysmography to the study of body composition in late preterm and term newborns

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Udine, December 2011.

Abstract

Background: nutrition in early life, growth, and subsequent health over a lifetime are significantly interrelated. Data on body composition of newborns are sparse, due to the long lasting lack of appropriate methods for body composition measurement in infancy.

Methods: we analyzed body composition (fat mass, fat free mass) by means of air displacement plethysmography at birth, during the physiological weight loss and in early life up to 3 months of age in 5 categories of newborns: term appropriate for gestational age (AGA), late preterm, small (SGA) and large for gestational age (LGA) and twins.

Results: AGA infants resulted to have a similar body composition than those of another Northern Italian population at birth and their % fat mass increased significantly from birth to 3 months of age. During the physiological weight loss, fat free mass was the compartment mostly affected, and this was reproduced in all categories of babies. No difference emerged in body composition between babies born from vaginal delivery or caesarean section or between boys and girls. Late preterm infants, although leaner at birth, resulted to be have a higher fat mass content than term infants at term-equivalent age. SGA infants were smaller and with less fat mass at birth but gained more fat than both AGA and LGA infants in the first month of life. Conversely, LGA infants had a higher fat content at birth but did not differ from AGA infants at one month of life. Body composition of AGA and SGA twins did not differ from that of AGA and SGA singletons, respectively, at birth or in the first three months of life.

Conclusions: the application of a novel method for body composition measurement in term and late preterm infants provided novel insights into the study of normal values of body composition in newborns at birth and in early life and of how these components are modified by different patterns of fetal growth and neonatal characteristics. These notions may constitute the basis for the elaboration of individualized feeding strategies aimed at a balanced growth of the various body components, with the final perspective of optimizing the infants' chances of a better later health.

Sommario

Introduzione: la nutrizione dei primi mesi di vita, la crescita e la salute nelle età successive sono elementi strettamente correlati. I dati sulla composizione corporea dei neonati sono scarsi, a causa della mancanza per lungo tempo di metodi appropriati alla misurazione della composizione corporea nella popolazione neonatale.

Metodi: è stata analizzata la composizione corporea (massa grassa, massa magra) mediante pletismografia ad aria alla nascita, durante il fisiologico calo ponderale e nelle prime settimane di vita fino ai 3 mesi in 5 categorie di neonati: neonati a termine di peso appropriato alla nascita (AGA), neonati "late preterm", neonati di peso basso (SGA) e di peso alto per età gestazionale (LGA) e neonati da gravidanza gemellare.

Risultati: i neonati a termine AGA sono risultati avere una composizione corporea alla nascita paragonabile a quella riportata in un'altra popolazione neonatale del Nord Italia. La percentuale di massa grassa è aumentata nei primi mesi di vita. Durante il calo ponderale fisiologico, la massa magra è stato il compartimento maggiormente interessato dal calo, e questo risultato è stato riscontrato in tutte le categorie di neonati analizzati. Non è stata riscontrata alcuna differenza nella composizione corporea dei neonati nati da cesareo o da parto vaginale. I neonati "late preterm", nonostante risultassero più magri alla nascita, sono risultati avere significativamente più massa grassa dei neonati a termine a comparabile età post-menstruale. I neonati SGA erano più piccoli e con meno massa grassa alla nascita, ma successivamente la loro massa grassa è andata incontro a un incremento più marcato di quella dei neonati AGA e LGA nel primo mese di vita. Invece i neonati LGA sono risultati avere un maggior contenuto di grasso alla nascita, ma questa differenza non è stata più riscontrata rispetto ai neonati AGA a un mese di vita. I neonati da gravidanza gemellare, AGA e SGA, sono risultati avere la stessa composizione corporea alla nascita e nelle età successive dei neonati singoli rispettivamente AGA e SGA.

Conclusione: l'utilizzo di un nuovo metodo per la misurazione della composizione corporea in neonati a termine e "late preterm" ha fornito novità riguardanti i valori normali della composizione corporea nei neonati alla nascita e nei primi mesi di vita e di come essi si modificano in caso di diversi percorsi di crescita fetali e di diverse caratteristiche neonatali. Ciò potrà costituire la base per elaborare strategie di alimentazione personalizzate che consentano una crescita bilanciata delle varie componenti corporee.

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Introduction

Part 1. Growth, body composition and later health

In the 1990s a theory has been elaborated that related small size at birth with subsequent risk of coronary disease, stroke and metabolic syndrome[1,2]. The so-called "Barker hypothesis" or "fetal origin of adult disease" hypothesis stemmed from the initial observation that areas of Britain with the highest rates of neonatal mortality (and, by inference, of impaired fetal growth) early in the 20th century tended to have the highest rates of coronary heart disease later in the century[1]. Subsequently, epidemiological, mainly retrospective, studies confirmed these findings, implying that babies who were born small had an increased risk of hypertension, diabetes type 2 and coronary disease in later life[3].

This hypothesis has subsequently been challenged by the observation that the association between size at birth and subsequent diseases was significant only after adjustment for current body size, implying that it was probably the postnatal change in size (and, especially, postnatal percentile crossing) rather than fetal biology that was implicated[4]. The highest risk of adverse health outcomes was seen in adults who were born small and who subsequently underwent a rapid early postnatal growth. An excessive growth rate in the first periods of life has been associated with later obesity in infants small for gestational age[5,6], with coronary disease[7] , type 2 diabetes[8] and hypertension[9,10] . Other authors have emphasized the role of postnatal growth versus size at birth in predisposing to subsequent metabolic and cardiovascular diseases[11].

Although it is difficult to gauge from the literature the relative contribution of fetal versus early infancy growth on subsequent health, it is clear that nutrition in early life, growth, and subsequent health over a lifetime are significantly interrelated.

This notion has been confirmed in several animal studies. Since McCance's observations in the 1960s on the long term effects of early nutrition in rats[12], nutrition in infancy or fetal life has been shown in animals to induce lifetime effects on metabolism, growth, and neurodevelopment and on major disease processes such as hypertension, diabetes, atherosclerosis, and obesity[4].

A better understanding of the associations of early infant nutrition and growth with adult health requires accurate assessment of body composition in infancy. This should bring to the notion of what normal values of body composition components are at birth and in early life and how they are modified by different patterns of fetal growth, pregnancy complications,

neonatal characteristics and postnatal events. Once these data and their relationship with later outcomes are known, an accurate, qualitative evaluation of the growth of infants implemented in clinical practice could be the basis for the elaboration of individualized feeding strategies aimed at a balanced growth of the various body mass components, with the final perspective of optimizing the infants' chances of a better later health.

Part 2. Growth and body composition of singleton newborns

Human growth during pregnancy and the first months of life involves not only quantitative changes in body size, but also qualitative changes in body composition.

During pregnancy, both early and late fetal growth patterns appear to be linear, beginning at approximately 20 weeks' gestation and lasting until 38 weeks; thereafter, the rate of weight gain begins to decline[13](Figure 1). After birth, growth again assumes the intrauterine rate.

As regards body composition, one general trend includes a decrease of total body and extracellular water content as the fetus and infant mature. Simultaneously, there is an increment of body protein and fat content. The increase of tissue protein is gradual during development, instead the increment of fetal fat mass is delayed until the third trimester. In the third trimester, once initiated, the deposition of body adipose tissue is more rapid than the rate of protein accumulation[14] (Figure 2).

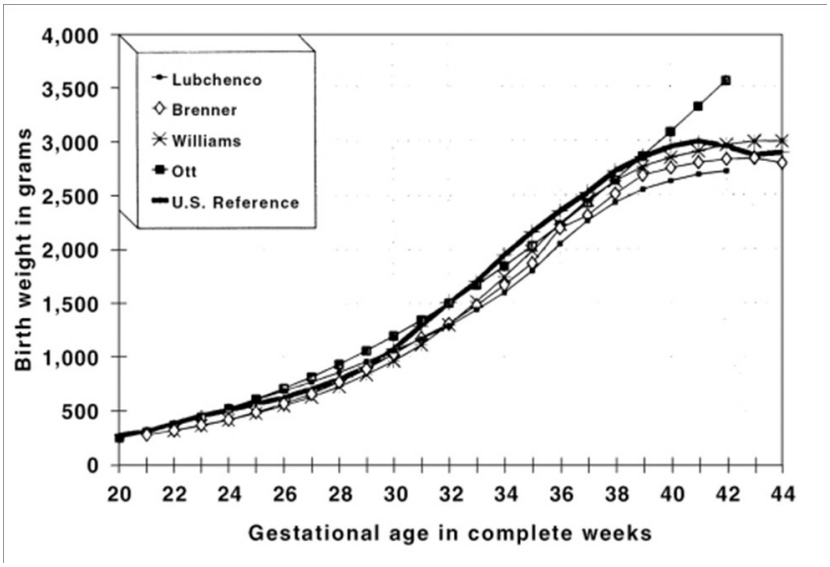


Figure 1. fetal weight as a function of gestational age[13]

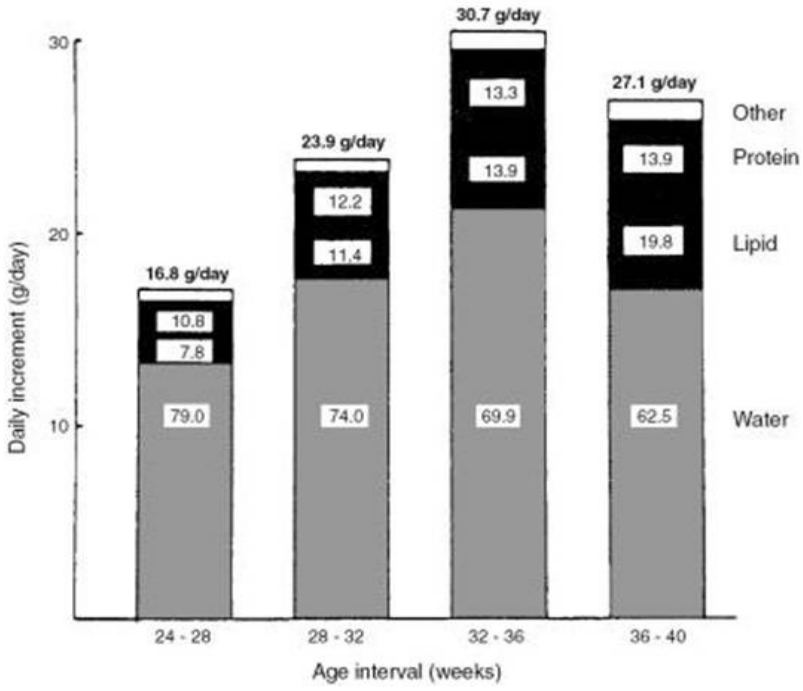


Figure 2. Composition of fetal weight gain[14]

These data on the variation of body composition according to gestational age have been obtained initially by cadaver analysis and have been confirmed by subsequent studies employing in-vivo methods for body composition assessment, such as dual energy X-ray absorptiometry (DEXA)[15] (Figure 3). The direct relationship between fat mass and gestational age follows logically the notion that fat mass accumulates in the third trimester of pregnancy.

Both fat mass and fat free mass at birth have a direct relationship with birth weight in appropriate for gestational age infants[14] (Figure 4).

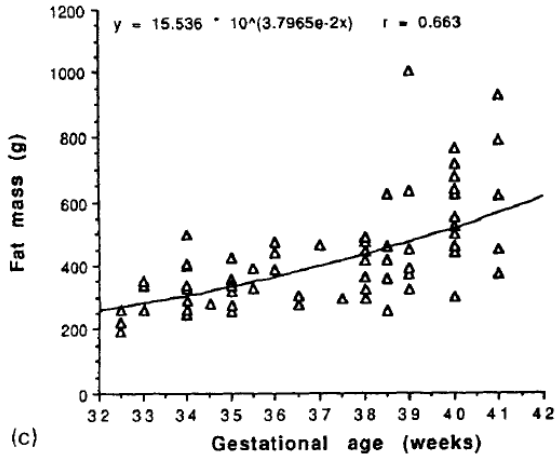


Figure 3. Correlation between gestational age and fat mass measured by dual energy x-ray absorptiometry (DEXA) in 70 appropriate for gestational age infants[14]

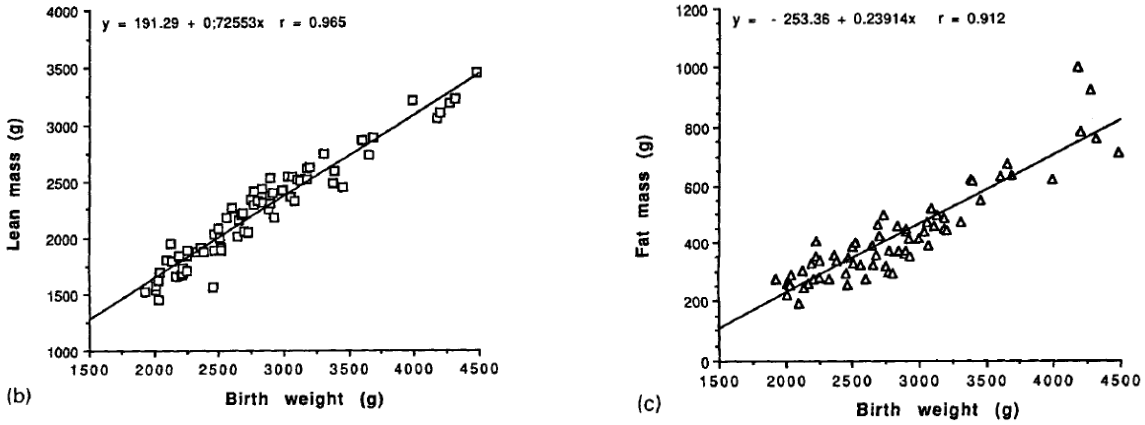


Figure 4. Correlations between birth weight and fat free mass (b, left) and between birth weight and fat mass (c, right) measured by DEXA in 70 appropriate for gestational age infants[14]

After birth, newborns undergo a decrease in their birth weight which averages 6% and lasts approximately 7 days, the so-called "physiological weight loss"[16,17]. Afterwards, growth resumes. Newborns gain approximately 1 Kg per month in the first 3 months of life and double their birth weight by 4 months of life[18].

Historical seminal work on body composition in newborns after birth have shown that fat mass gradually increases in postnatal life up to approximately 6 months of life[19,20] (Figure 5). The same trend has been shown in several other studies. A study aimed at elaborating body

composition percentiles using air displacement plethysmography (ADP) at birth, 6 weeks, 3 and 4.5 months in healthy term newborns born from normal weight mothers reported that the percentage of fat mass doubled between birth and 6 weeks of life[21]. An Italian study that evaluated both cross-sectionally and longitudinally the body composition of term, exclusively breast fed infants monthly up to 6 months of life confirmed the progressive accumulation of fat mass from birth to 6 months[22] (Figure 5). In this population, fat mass doubled from birth to 1 month and tripled from birth to 3 months. This study employed ADP for the measurement of body composition as well.

	N	%FM	FM (g)	FFM (g)
Girls				
Birth	23	8.69 ± 3.09	260 ± 120	2710 ± 380
2 wk	30	12.16 ± 3.60	410 ± 170	2910 ± 360
1 mo	35	16.12 ± 5.22	640 ± 290	3180 ± 410
2 mo	35	22.42 ± 3.97	1090 ± 300	3700 ± 380
3 mo	32	25.95 ± 3.72	1440 ± 330	4050 ± 380
4 mo	28	27.95 ± 4.96	1730 ± 490	4350 ± 330
5 mo	23	29.50 ± 3.27	1920 ± 310	4570 ± 370
6 mo	12	25.58 ± 4.51	1730 ± 390	4990 ± 270
Boys				
Birth	17	8.94 ± 2.78	290 ± 90	2910 ± 260
2 wk	24	12.44 ± 3.57	460 ± 150	3200 ± 300
1 mo	23	18.82 ± 3.77	840 ± 200	3610 ± 400
2 mo	19	24.69 ± 3.99	1360 ± 270	4130 ± 340
3 mo	23	27.25 ± 4.16	1740 ± 340	4620 ± 430
4 mo	20	28.28 ± 4.47	1940 ± 360	4900 ± 410
5 mo	10	27.47 ± 2.06	2020 ± 230	5320 ± 280
6 mo	8	28.08 ± 3.59	2180 ± 410	5410 ± 30

Figure 5. Fat mass and fat free mass measured by ADP at each study point[22]

Growth differs according to gender. Boys are heavier and longer than girls at birth and in the first months of life[18]. Body composition at birth does not seem to differ significantly between girls and boys[22-24] even if a trend towards a higher fat mass in girls has been reported by other authors[19,21,25] . In the first months after birth, evidence indicates a higher adiposity of girls vs boys[19], at 1 month[24], and 4.5 months[21], even if the results were not consistent among all studies[22] (Figure 6).

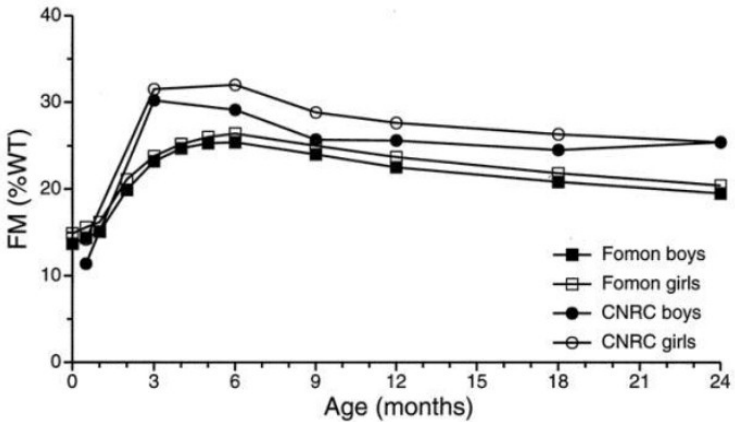


Figure 6. Percent of fat mass in boys and girls in the first 24 months after birth[20].

Appropriateness of fetal intrauterine growth is traditionally evaluated comparing anthropometric measures at birth, namely birth weight, with percentiles obtained from a reference population. In this way, infants are categorized as appropriate for gestational age (AGA) if their birth weight falls between the 10th and the 90th percentile of weight of the reference population; small for gestational age (SGA) if their weight is below the 10th percentile and large for gestational age if their birth weight is higher than the 90th percentile.

SGA infants have been shown to have a lower percentage of fat mass versus AGA infants of the same gestational age at birth[15].

On the contrary, LGA infants appear to be fatter at birth than AGA infants[26]. This difference seems to be accentuated in macrosomic infants born from mothers with gestational diabetes[27]. Indeed, SGA and LGA infants have to be considered a heterogenic group. In fact, weight is an imprecise indicator of the nutritional status of newborns as it does not differentiate between two situations: that is, whether a low or high birth weight according to percentiles is the result of an abnormal intrauterine growth pattern or simply of the achievement of the infant's genetic potential. In this regard, interestingly, a study found a poor correlation between the classification of infants into AGA, SGA or LGA and fat mass percentiles at birth[28]. In particular, a large overlap of % fat mass between the three growth groups was observed: infants born AGA had %fat mass both lower than 10% and higher than 20%; infants born SGA or LGA had %fat mass between 10% and 20%.

Parental size and infant birth weight are related through genetic and environmental mechanisms, with the stronger relationship between maternal and fetal size believed to represent contributions of the intrauterine environment[29]. Body composition of newborns at birth is influenced by maternal nutritional status. In particular, it is dependent on maternal pregravid BMI and on maternal weight gain during pregnancy.

Seventy-two neonates of singleton pregnancies with normal glucose tolerance had their bodyweight and body composition assessed by air-displacement plethysmography at 19 days of age. Infants of obese mothers (BMI 31.8) did not differ in birth weight or birth length from infants of normal weight mothers (BMI 21.7), but had significantly more fat mass and higher %fat mass (13.6% vs 12.5%) than the latter[30].

The relationship between maternal pregravid BMI and neonatal adiposity resulted to be independent from glucose control during pregnancy, parity, and other potential confounders[31,32]. Infants of obese mothers not only have a higher percentage of body fat at birth, but they also show biochemical signs of insulin resistance in the cord blood[32,33].

The effect of maternal obesity seems to have lasting effects on offspring body composition[34]. In a prospective study that correlated children adiposity by DEXA at 9 years of life with perinatal factors showed that maternal pregravid BMI and a family history of diabetes mellitus were the only variables associated with later adiposity. Mothers with a pregravid BMI of > 30 were 5.4 times as likely than a mother with a BMI < 30 to have children in the upper tertile for percentage body fat at 9 years[33].

High pregnancy weight gain is directly associated with large size at birth[35]. By altering the intrauterine environment, pregnancy weight gain not only influences fetal growth, but also alters body composition. Greater pregnancy weight gain was associated with greater neonatal fat mass (0.10 SD per 5 kg weight gain (0.04, 0.15), *P*=0.0004) measured by means of DEXA in a British cohort[36]. Offspring of women who gained more than recommended[35] weight during pregnancy showed not only an increased adiposity later in life, but also signs of metabolic disregulation and biochemical cardiovascular risk factors[37].

The rate of macrosomia, defined as a weight > 90° percentile, is 8-14% in normal pregnancies and 25-45% in pregnancies complicated by gestational diabetes[38]. An impaired glucose tolerance during pregnancy, even not achieving the diagnosis of diabetes, is associated with an increased neonatal adiposity, independently from macrosomia and other potential confounders[39]. Increasing glucose concentrations less severe than diabetes were associated with fetal overgrowth, specifically adiposity, measured by skin fold thickness. Odds ratios

ranged from 1.35 to 1.44 for the measures of adiposity for fasting, 1-h, and 2-h plasma glucose higher by 1 standard deviation. Furthermore, neonatal adiposity correlated with cord blood C-peptide levels[39]. This finding corroborated the so-called Pedersen hypothesis, according to which the increased growth and fat deposition in infants of mothers with impaired glucose tolerance is mediated by the enhanced fetal insulin production induced by fetal hyperglicemia secondary to the transplacental crossing of maternal glucose[40].

The quality of postnatal feeding has been studied as a factor possibly influencing body composition in early life. Breast milk is recommended as the ideal nutrition for newborns by the World Health Organization. Breast milk induces different metabolic responses than formula, such as a weaker stimulus to insulin production, which induces fat deposition and the production of adipocytes[41]. The amount of energy metabolized and the protein intake of breastfed children have been shown to be considerably below the intake of infants who are fed formulas[42]. Weight gain is slower in breastfed versus formula fed infants[43]. Breast feeding seems to protect against overweight and obesity later in life in comparison with formula, but experimental evidence is not conclusive in this regard[44].

Body composition of infants fed with breast milk has been reported to differ from that of infants fed with formula. Infants fed exclusively with breast milk for the first 4 months of life had a higher fat mass and percentage of fat mass at 3 and 6 months in comparison to infants fed with formula. These differences did not persist after 1 year of age[43]. A trend towards a higher adiposity at 3 months of age in healthy term breastfed infants versus infants fed with mixed breast and formula milk was also found in a small study in which body composition was assessed with air displacement plethysmography[45]. Other reports have not confirmed the presence of differences in body composition according to the type of feeding in the first months of life[46].

Part 3.Growth and body composition of twins

Twin deliveries have been rising considerably in recent years, due to assisted reproduction technologies and to increased maternal age[47]. In the US, from 1998 the number of twins has continued to rise at a rate of approximately 3% per year[48].

Twins have an increased risk of being born preterm than singletons. Approximately 57% of twins are born before 37 gestational weeks and 12% before 32 weeks[49]. The average duration of pregnancy is 40 weeks for singletons, 36 weeks for twins and 32 weeks for triplets[50] (Figure 7)

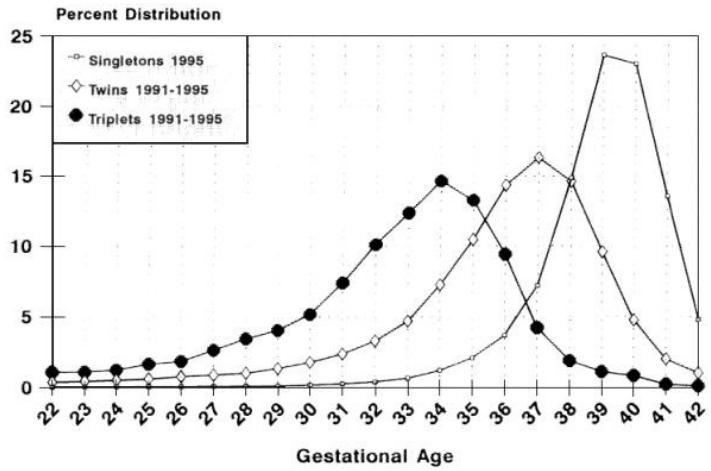


Figure 7. Gestational age distribution by singletons, twins, and triplets (U. S. resident live births)[50]

Twins are smaller than singletons at birth, not only because of shorter gestation. During fetal life, twins have a different fetal growth pattern that deviates from that of singletons from approximately around 30 to 32 gestational weeks[51] (Figure 8). Whether intrauterine growth restriction in twins depends on limited placental supply or limited uterine size is a matter of debate. Whatever the cause, twins on average are more than 500 g lighter than singletons at birth; even when allowance is made for the shorter gestation a discrepancy remains[51,52]. It has been estimated that approximately 60% of twins are born with a birth weight inferior to 2500 g[49].

Therefore, all twins born after 32 gestational weeks have to be considered growth restricted in comparison with singletons, even if at birth they do not result to be small for gestational

age. Or, from another point of view, they may be considered “growth adapted”, meaning that that a fetus in a twin pregnancy might be small because of a “physiological” adaptation to the limited uterine environment[53].

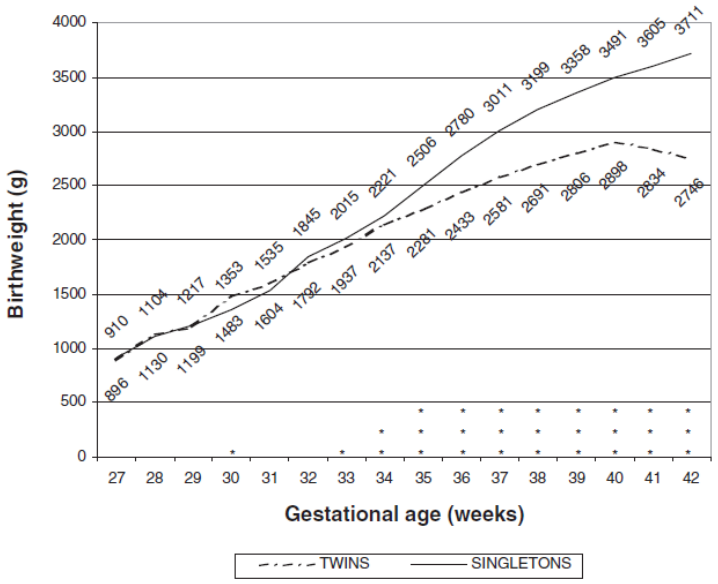


Figure 8. Mean birthweight in relation to gestational age for twins and singletons. Difference between twins and singletons:*P<0.05; **P< 0.01; ***P <0.001[51]

Other factors influence the growth of twins during gestation. There are often discrepancies in growth within twins, as frequently one twin grows more than the other. Moreover, fetal growth varies according to zygosity and chorionicity. The mean birth weight of a male twin is 2538 g is he is dyzigotic, 2453 g is monozygotic-dichorionic and 2423 g if monozygotic-monochorionic. Umbilical cord insertion may influence fetal growth, as a peripheral umbilical cord insertion is associated with a lower birth weight[51].

Body composition of twins at birth has been assessed in two studies, by the same group of investigators.

Body composition measurements of 96 twin infants were compared to those of 76 appropriate for gestational age (AGA) singletons[54]. Each AGA twin was matched as closely as possible for birth weight to an AGA singleton; each small for gestational age (SGA) twin was matched with two cohorts of AGA singleton infants: one with similar birth weight and another with similar gestational age. DEXA scan were performed at a median of 2.5 days of life, with most subjects studied in the first week of life. Between AGA twins and AGA weight-matched

singletons there were no differences either in anthropometric measures or in body composition components measurements. SGA twins had a lower weight and absolute lean mass than gestational age-matched AGA singletons. However, they had similar proportions of fat mass, lean mass and bone mineral content expressed as percentage of weight than both gestational age-matched and weight-matched AGA singleton infants. The conclusion was that in normally grown infants, body composition components are similar regardless of whether they are twins or singletons. In contrast, growth-restricted twins have lower lean mass, and possibly lower fat mass and bone mineral content, when compared to singletons matched for gestational age (Figure 9).

	Bone mineral content (%)	Fat mass (%)	Lean mass (%)
AGA twin versus AGA singleton ($n=76$ per group)			
Twin	1.71 ± 0.18	10.7 ± 2.7	87.5 ± 2.9
Singleton	1.76 ± 0.18	10.4 ± 2.1	87.9 ± 2.2
SGA twin versus AGA singleton ($n=20$ per group)			
Twin	1.76 ± 0.23	10.6 ± 3.2	87.6 ± 3.4
Singleton matched for birth weight	1.71 ± 0.14	10.1 ± 2.5	88.2 ± 2.6
Singleton matched for gestation	1.78 ± 0.19	10.9 ± 1.8	87.3 ± 1.9

AGA, appropriate for gestational age; SGA, small for gestational age.
[a]Comparison of twins with matched singleton groups. No significant difference in any comparisons between groups or within each body composition component. Values are expressed as mean ± SD.

Figure 9. Dual energy X-ray absorptiometry measured total body bone mineral content, fat and lean mass, reported as percentage of total weight, of twins versus singletons, matched for birth weight or for gestational age[54]

Fetal growth discrepancy has a reported incidence of 10 to 30% of twin pregnancies[55]. It is defined as a difference in 15-30% of weight (expressed as a percentage of the larger twin weight) between the larger and the smaller twin[55]. It has been associated with an increased rate of both short and long-term adverse outcomes[56], especially if the smaller twin is also small for gestational age at birth[57].

One study assessed interpair differences in the body composition of twins[58]. Koo and collaborators studied 48 pair of twins with birth weights from 976 g to 3135 g and a gestational age ranging from 30 to 40 weeks (Koo 2002). Body composition was studied by dual energy X-ray absorptiometry (DEXA). Scans were performed at 3.8 ± 3.2 days of life. In individual infants, both DEXA and anthropometric measurements were higher with higher gestational age. When considering body composition components as percentage of body weight, there was an increase in bone mineral content and fat mass, but a decrease in lean mass with increasing gestational age. With regard to interpair differences a difference in

weight, although correlated with, was not proportional to differences in body composition components.

Twins have a slower long-term growth than singletons. According to the data from the Netherlands twin registry, enrolling over 4000 twin infants, twins are born smaller than singletons and subsequently, in the first 2 years of life, they show a catch-up growth but do not reach the body size of singletons. These differences were only in part attributable to the difference in gestational age at birth, as twins were born more premature than singletons, as they persisted in the first 2.5 years of life in spite of correction for gestational age[59].
Twins resulted to be shorter and considerably lighter than singletons at 9 years of age in a study including over 1500 twins. Weight difference was higher in males than in females. Girls had a weight z-score value of 0.11 less than singletons and boys 0.22 less ($p=0.01$ and<0.005, respectively). Parental height accounted for the height difference in twins, but only in part. Monozygotic twins were more affected by growth restriction than dizygotic twins. In conclusion, twins are smaller and thinner than singletons during the first decade of life[60].
When followed to adulthood, 270 Japanese twins had a shorter final height, on average of 2.6 cm smaller than singletons, mainly because of lower growth velocity between birth and prepuberty[61]. Moreover, twins seem to have slightly lower weight than singletons in adulthood[62].There appears to be a significant correlation between birth weight and body composition components in twins aged between 18 and 80 years old. DXA measurements performed on 2228 dizygotic and 842 monozygotic female twins aged between 18 and 80 y showed that a 1-kg increase in birth weight was associated with a 1.72-kg increase in lean mass, a 0.25-kg increase in fat mass, and a 0.05-unit increase in the lean:fat mass ratio. The authors concluded that a higher birth weight was associated with a higher proportion of lean to fat mass as adults. This association was not determined by individual specific factors in utero (eg, fetal nutrition) but through factors shared in the common environment of the twins. This study highlights the fact that a correlation between birth weight and body composition components persists well into adulthood[63].

Part 4. Techniques for measuring body composition

Anthropometric measures (weight, length, cranial circumference) are the most commonly used indicators of growth in infants. They are non invasive and easy to obtain. Plotting these measures on growth charts or comparing them with percentiles obtained from a reference population provide information about the adequacy of growth. However, weight is an imperfect parameter of growth. In fact, an abnormal pattern of intrauterine growth that does not result in an abnormal birth weight according to reference percentiles may not be recognized. Or, on the other side, babies that have achieved their genetic growth potential in utero but that fall in the extreme tails of the normal birth weight distribution may be classified as abnormally grown[64]. Thus, the ability of birth weight to indicate fetal growth experience is crude and cannot indicate the extent to which genetic growth potential has been achieved[3]. Furthermore, weight does not provide information on the quality of growth, that is on the relative contribution of fat mass, which is essentially proportional to caloric intake, and fat free mass, which describes protein storage. These both are key indicators of optimal nutritional care.

The ponderal index (weight/length^3) and the body mass index (BMI, i.e. weight/length^2) have been used to overcome some of these limitations. However, they are poor indicators of body composition and adiposity. For instance, children with the same gender and age have a twofold range of body fat mass for a given BMI value[65] (Figure 10). Some children with a high BMI have a high muscle mass rather than a high fat mass, while other children with a normal BMI have high fat mass and relatively low muscle mass[3].

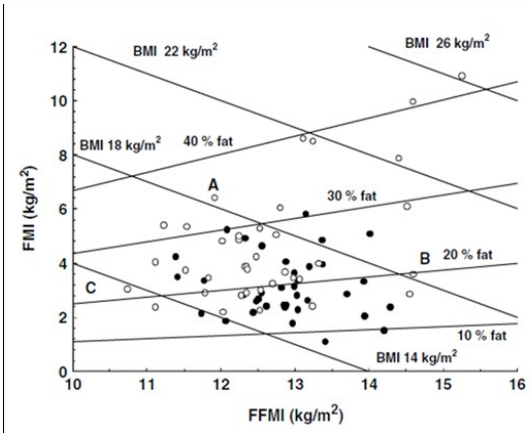


Figure 10. Hattori graph for children aged 8 years showing fat mass adjusted for height (fat mass index; FMI) v. lean mass adjusted for height (fatfree mass index, FFMI). The individuals A and B represent two girls with similar BMI (approx 18 kg/m2), but with A having twice the FMI of B. The individuals B and C have similar percentage fat, but very different BMI and FFMI[65]

Skinfold thickness is another anthropometric measure that has been used to determine body fat. It is a simple, quick non invasive technique and it has shown a good correlation with other methods of measurement of body composition, such as total body electrical conductivity (TOBEC)[66], dilution methods and DEXA[67]. However, this method is based on the unproven assumptions that the thickness of the subcutaneous adipose tissue reflects a constant proportion of the total body fat and that the sites selected for measurement represent the average thickness of the subcutaneous adipose tissue[68]. Moreover, there are many sources of potential errors when skinfold thickness is measured in infants. These are related to the use of adult-designed calipers, to the lack of rigorously validated formulas to derive the fat and lean tissues from anthropometric measurements and to observer's error[67].

Direct chemical analysis of cadavers is theoretically the gold standard of assessing body composition. Data have been obtained in newborns[14]. However, post-mortem body analysis underestimates the water content because of the loss of water that occurs from death until the time of the chemical analysis[69]. Furthermore, the causes of death may have adversely affected body composition.

Therefore, more accurate and objective methods have been elaborated to provide an indirect in vivo measurement of body composition. Unfortunately, technological and practical limitations have hampered the success of these new methods of body composition measurement in newborns, to the point that body composition assessment in infants is still rarely performed.

The body composition model that is usually used in newbors is the simplest one, the bicompartimental model. According to this model, the body weight is divided into two compartments: fat mass (FM) and fat free mass (FFM). The bicompartimental model is focused principally on the assessment of body fat mass, which is the parameter more subjected to variability according to the nutritional status[68]. Another model that has been used in infants is the tricompartimental model, in which the body mass is divided into three compartments: fat mass, fat free mass and minerals. This model is relevant in particular for the study of osteopenia.

The isotope dilution assay, the bioelectrical impedence analysis (BIA) and the total body electrical conductivity (TOBEC) methods are based on the direct (dilution assay) or indirect (BIA, TOBEC) measurement of total body water (TBW), the principal component of fat free mass. According to the bicompartimental model, fat mass is derived by subtraction of FFM, calculated from TBW content, from birth weight. One main theoretical limitation of the TBW-

based methods is that they rely on the assumption that the TBW content of FFM is constant. This is usually true in healthy adults (TBW =73.2%±3% of FFM), but not in newborns that are subjected to a constant decrease of extracellular water content and thus of FFM hydration after birth and to steep, erratic alterations in FFM water content in the course of acute illnesses[70].

To estimate TBW with the isotope dilution assay, deuterium ($^2H_2$) or O^{18} are administered orally to infants. Isotope concentration is then measured via mass or infrared spectroscopy in urine samples collected before and at several time intervals after the isotope has been given to the baby. This method is complex, time-consuming and requires expertise and elaborate analysis, so eventually it is impractical for routine use in clinical and research setting. Furthermore, repeated measurements are possible only after the complete clear out of the previous isotope has taken place, which may take 10-14 days in premature babies[70].

Bioelectrical impedence analysis (BIA) and total body electrical conductivity (TOBEC) make use of the electrical conductivity of water to estimate TBW content.

The principle underlying TOBEC is that lean tissue creates a greater electromagnetic disturbance than fat when a weak homogeneous magnetic field is applied[71]. This method has been validated and reference values have been published for infants from birth to 1 year of age[72]. However, it is not reliable in premature newborns due to excessive estimation error, it requires immobility and the equipment is heavy, expensive and out of production.

BIA is based on the observation that a tissue rich in water and electrolytes is much more resistant to the passage of an electrical current compared with adipose tissue[73]. It is easy to use, has a light, cheap equipment which can be used in several settings, and that requires little collaboration from infants. Repeated measurements are easily performed. Unfortunately, results are confounded by the variability of FFM hydration, and BIA precision and accuracy have been questioned[74].

Dual energy x-ray absorptiometry (DEXA) uses low-intensity collimated X-ray beams at two energies to scan the whole body or at specific bone sites, such as the spine and hip. Basically, this assay has become the de facto standard for detection of low bone mineral density in older children and adults. For the whole body scan, values for body fat and non-fat soft tissue mass are also obtained[70]. Reference values for newborns have been published[75]. The main limitations of this method are that it requires immobility and that results change according to the type of DEXA device and software used, thus hindering the generalizability of the reference values. Furthermore, although radiation dosage is low (<0.3 mrem), it is not suitable for repeated measurements, especially in vulnerable infants[67].

Additional methods to measure adipose tissue in newborns are magnetic resonance (MRI)[76] and limb ultrasonography[77]. Both these methods share the problem of extrapolating composition of cross-sectional slices from a part of the body to whole body composition. However, limb ultrasound measurements of muscle and fat correlate well with regional and whole body DEXA[78]. MRI has the advantage of measuring regional, and in particular intraabdominal, fat. Its limitations are the limited availability of the equipment and the poor comparability of the results with those obtained with other techniques[73].

Part 5. Air displacement plethysmography

Air displacement plethysmography (ADP) is a novel method for the measurement of body composition. It has been first introduced in the mid 1990s[79].

The term plethysmography refers to the measurement of size, usually volume[80]. In principle, the functioning of ADP is similar to underwater weighing.
ADP measures the volume and the mass of the subject, then from these two parameters the whole body density is calculated according to the equation:

$$D = M/V$$

where M is mass (i.e. what we commonly call weight) and V is volume.
Once the body density of the subject is known, it is possible to calculate fat mass and fat free mass (knowing the density of fat mass and fat free mass) solving the two following equations for Mf:

$$M/D = Mf/Df + Mfm/Dfm$$

$$\%fat = (Mf/Mb) * 100\%$$

where Mf is fat mass, Df is fat density, Mfm is fat-free mass equal to Mb - Mf, and Dfm is fat-free mass density.
The reference data for the densities of fat mass and fat free mass of infants have been previously published. Two models are available[19,20], that differ in the fat free mass

composition. Recently, the Fomon fat free mass model has been shown to provide a more accurate estimate of fat free mass density[81].

In ADP, the volume of an object is measured indirectly by measuring the volume of air it displaces inside an enclosed chamber (plethysmograph). Thus, human body volume is measured when a subject is placed inside the chamber and displaces a volume of air equal to his or her body volume.

Body volume is calculated indirectly by subtracting the volume of air remaining inside the chamber when the subject is inside from the volume of air in the chamber when it is empty. The air inside the chamber is measured by applying physical gas laws, that is Boyle's Law and Poisson's Law[80].

Boyle's Law states that, at a constant temperature, volume (V) and pressure (P) are inversely related:

$$P1/P2 = V2/V1$$

where P1 and V1 are pressure and volume at an initial condition and P2 and V2 are pressure and volume at a final condition.

Therefore, when a constant temperature is maintained (isothermal conditions), Boyle's Law can be applied. Most early plethysmographs required temperature-controlled surroundings and isothermal conditions within the test chamber. This presented burdensome requirements for testing conditions, which restricted practical implementation of air-displacement plethysmography.

When air is allowed to change temperature in response to volume changes (adiabatic conditions), Poisson's Law expresses its behavior as follows:

$$P1/P2=(V2/V1)^{\square}$$

For air, $\square$ is 1.4.

A consequence of these two equations is that equal volume changes result in different pressure changes for air under isothermal and adiabatic conditions[82]. In air, for small volume changes, P2 under adiabatic conditions is always approximately 40% larger than P2 under isothermal conditions. Air under isothermal conditions is therefore 40% "easier" to compress than air under adiabatic conditions[79].

The mass of the infant is measured by a high precision electronic scale (number 8 on Figure 11).

For the purpose of volume measurement the infant is placed inside a test chamber (number 1 on Figure 11) by means of a sliding tray. The chamber is heated to 30°C, is aerated by a continuous air flow and is equipped by a CO_2 detector that measures CO_2 continuously. The test chamber is connected to a reference chamber of known volume (number 5 on Figure 11) through a diaphragm (number 3 on Figure 11). During the measure, the diaphragm oscillates and thus determines sinusoidal volume changes that are equal in magnitude but opposite in sign in the two chambers. From the changes in pressure in the two chambers provoked by these small volume changes it is possible to measure the volume of the infant, according of the laws of gases previously described. The magnitude and frequency of the volume perturbations are 35 mL and 6 Hz, respectively. Pressure changes resulting from the volume perturbations are below 0.5 cm H_2O[82].

To allow for a precise quantification of the amount of air behaving adiabatically in the test chamber, the amount of air behaving isothermally (that results in a 40% overestimation of volume) in the proximity of the hair, of body surface and in the lungs must be accounted for.

Hair has to be smoothed down before the baby enters the test chamber with the use of baby oil or a cap. The surface area artefact (SAA) is subtracted to the raw volume. The SAA is obtained by multiplication of body surface area (BSA), calculated from body weight and length by means of the Boyd formula[83], with a constant k derived by testing aluminum sheets with known volumes and areas. This constant was derived so that the result of its multiplication by the surface area of the object being tested would equal the difference between the volume measured by the system and the object's actual volume, a negative value. Lung volume is calculated as functional residual capacity (FRC) plus approximately half tidal volume. FRC is predicted from body weight and length by an equation derived by plethysmographic assessment of FRC in infants[84] as follows[82]:

$$FRC(mL) = 2.36 * Length(cm)^{0.75} * Weight(kg)^{0.63}$$

Then, the body volume (BV) is calculated (in liters) from directly measured raw body volume (BVr), SAA and lung volume (Vl) as follows:

$$BV = BVr - SAA + 40\% Vl$$

Note that SAA is by definition a negative value.

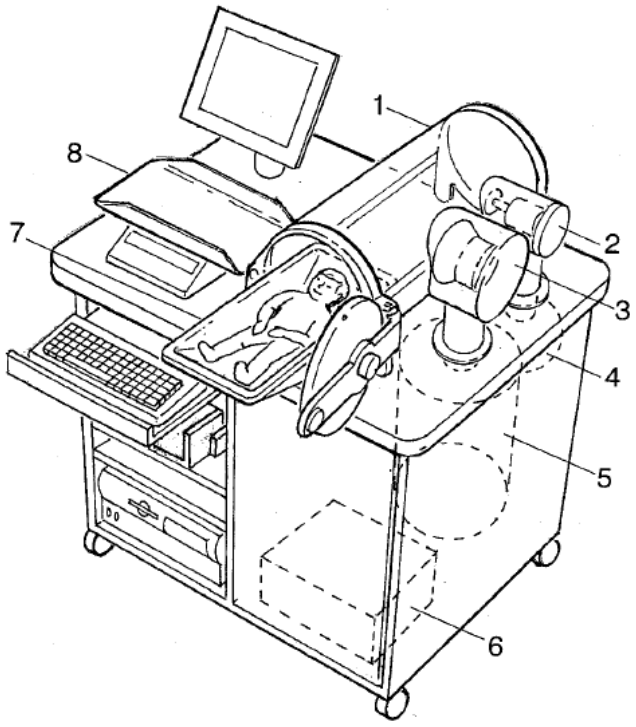


Figure 11. The prototype version of the Pea Pod, Body Composition System, used in this study. Numbers refer to the test chamber (1), calibration valve (2), diaphragm (3), calibration volume (4), reference chamber (5), electronics (6), sliding tray (7), and scale (8)[82]

The ADP system has a good precision and accuracy in the measure of both mass and volume[82,85]. It has been validated for body composition assessment in relation to total body water measured by a dilution method[86] and to a DEXA four-compartment reference model[87]. It has been shown to have a minimal between-day and within-day variability. Moreover, the measurements are not significantly influenced by the infant behavioral state[87,88].

The Pea Pod system has a high interdevice reliability. This is crucial for determining overall validity and has practical implications for multicenter studies.

Materials and methods

Part 1. Objectives of the study

The objectives of this study were:

1.To analyse body composition at birth, during the physiological weight loss and up to 3 months of life in appropriate for gestational age singleton term infants

2.To compare body composition at birth, during the physiological weight loss and in early life in late preterm versus term infants

3. To compare body composition at birth, during the physiological weight loss and in early life among appropriate for gestational age, small for gestational age and large for gestational age singleton term infants

4. To compare body composition at birth, during the physiological weight loss and in early life between twin and singleton newborns

Part 2. Design of the study

This study was an observational cohort study with two components:

- prospective longitudinal
- transverse.

Part 3. Population of the study

Inclusion criteria

The study included healthy newborns born at the IRCCS "Burlo Garofolo" of Trieste from January 2011 with the following characteristics:

Gestational age at birth > 34 gestational weeks: that is, late preterm[89] (from 34 gestational weeks 0 days to 36 weeks and 6 days) or term newborns (born at 37-41 gestational weeks)

AND

- singletons or twins
- appropriate for gestational age, small or large for gestational age

Exclusion criteria

Infants were not included if they needed intensive care support (mechanical ventilation, central line, and so on) or any form of intravenous therapy, intravenous hydration or oxygen supplementation, or if their conditions were not stable. Infants with congenital malformations or chromosomal anomalies and those whose parents did not agree to participate were also excluded.

Part 4. Methods

1.Measurement techniques

Anthropometric measures were taken at birth, at 15 days and 1 and 3 months of life. All infants were weighted in the delivery room on the scale included into the Panda Warmer (GE Healthcare, Finland), with an accuracy of 10 g or within the first half an hour of life on the NICU scale (Seca gmbh & co. Kg, Hamburg, Germany) with an accuracy of 10 g. Recumbent length was measured at birth, at 15 days, 1 and 3 months of life on the Seca device (Seca gmbh & co. Kg, Hamburg, Germany) to the nearest 0.2 mm.

Cranial circumference was measured by the investigator to the nearest 1 mm with a graduated, non stretchable, flexible tape measure which was positioned on the front 1-2 cm above the eyes, on the temporal lobe just above the ears and on the most prominent part of the occipital bone.

The anthropometric measures were compared with percentiles obtained from a reference North-Eastern Italian population (SMILA Neonatal Standards for North-East Italy. Montecatini Sep 1996)[90]. Infants whose birth weight was between the 10th and the 90th percentile were defined as appropriate for gestational age (AGA); infants whose birth weight was < 10th percentile were classified as small for gestational age (SGA), while those who were > 90th percentile of birth weight were defined large for gestational age (LGA). Gestational age was calculated according to the last menstrual period and corrected if a better estimate was available from the ultrasound done in the first gestational weeks.

Body composition measurements were taken with the air displacement plethysmography system previously described (Pea Pod Infant Body Composition System, Life Measurement Inc., Concord, CA). Measurements were performed in the first 24 hours of life, at 48 to 72 hours of life (3rd day of life), at 2 weeks, 1 month and 3 months of life.

The Pea Pod Infant Body Composition System has been designed specifically to assess body composition in infants up to 8 Kg of weight. It provides a quick measurement of both weight (6-20 seconds) and volume (2 minutes). The test chamber is heated and comfortable. Immobility is not required, so the infant is free to move inside the chamber. No sedation is needed. Through a window on the top of the chamber the infant can be closely watched by

parents and by the investigator during the test. The chamber door opens automatically in case excessive CO_2 levels are detected by the CO_2 sensor.

As previously described, hair had to be smoothed down before the baby enters the test chamber with the use of baby oil or a cap. Furthermore, the scale had to be calibrated for feeding tubes, umbilical cord plugs, nasal prongs or any other device that the baby was wearing. Infants were measured naked and all foreign bodies were accounted for during the measurements.

The PeaPod system that was employed in this study uses the Boyd formula to estimate body surface area[83], the Fomon model[19] as a reference for fat mass and fat free mass density and the Stocks thoracic gas volume model[84]. Each time a measurement was performed, the gestational age, length and sex of the infants had to be entered into the computer in order to adjust the body composition measures according to these equations. For mass measurement, the precision is 0.1 g of standard deviation. For body composition measurement, the precision is 0.7% fat (standard deviation), and the accuracy is 3.3% fat (standard error of the estimate).

Figure 12. Left: ADP system monitor showing the initial screen (top) with required fields and the output screen (bottom) with body composition measurements. Right: a baby and her mother during volume measurement.

2.Feeding practices

Term and late preterm infants born with a birth weight higher than 2500 g and who did not require any respiratory support or other forms of intensive care were admitted at the Newborn Nursery. Here they were managed with a rooming-in program. Breastfeeding was strongly encouraged by appropriate trained nurses. No feeding other than breast milk was given to the infants as long as their weight loss was not higher than 10% of birth weight and breastfeeding was proceeding well.

Infants whose birth weight was lower than 2500 g were admitted at the Neonatology Department and were started on an enteral feeding protocol for the prevention of early hypoglycemia. This protocol was standardized and consisted in a continuous enteral nutrition with: dextrose 8% 15 ml/Kg for the first 4 hours of life, then breast or formula milk 25 ml/Kg for the next 8 hours. In the subsequent 12 hours, enteral nutrition was given via drip or by mouth with 40 ml/Kg of breast or formula milk subdivided into 4 meals. According to this protocol, all babies less than 2500 g at birth received 80 ml/Kg of feeding in the first 24 hours of life.

3.Maternal data

Data regarding maternal pregravid health, pregnancy and pregnancy complications were obtained from maternal charts. Pregravid weight, height and weight increase during pregnancy were self-reported (most cases) or derived from medical charts as well.

Maternal BMI was calculated according to the formula: weight (Kg)/height $(m)^2$. Gestational diabetes was defined according the recently published international guidelines[91].

4.Statistical methods

Variables were expressed as mean (standard deviation) in case of normal distribution and median (interquartile range) in case the distribution was non-normal. Normality was assessed with the D'Agostino-Pearson test for Normal distribution. For the transverse cohort study, the independent sample t-test was used to compare data obtained from different subjects if normally distributed and with equal variances; in case of unequal variance, the Welch test was used; in case of non-normal distribution, the Mann-Whitney test was used. For the longitudinal part of the study, data obtained from repeated measurements in the same

subject were compared using the paired sample t-test or, if not normally distributed, the Wilcoxon test. To compare multiple variables, an ANOVA one way analysis of variance was used.

The association between two continuous variables was determined by means of linear regression analysis. To test the independent effect of multiple variables on a dependent continuous variable, a multiple regression analysis was employed. To account for the clustering of data regarding twins, a cluster correction was included in the multiple regression analysis. Statistical significance was assumed at $p<0.05$. Statistical analyses were performed using MedCalc software rel. 9.3.9.0 (MariaKerke, Belgium) and STATA (StataCorp LP, Texas, US).

From the initial data obtained, it was calculated that the variance of % fat mass was equal to 12. The total sample size required to detect a 2.5% of difference in % fat mass resulted to be of 30 infants, with a power of the study of 70% and an alpha error of 0.05[92].

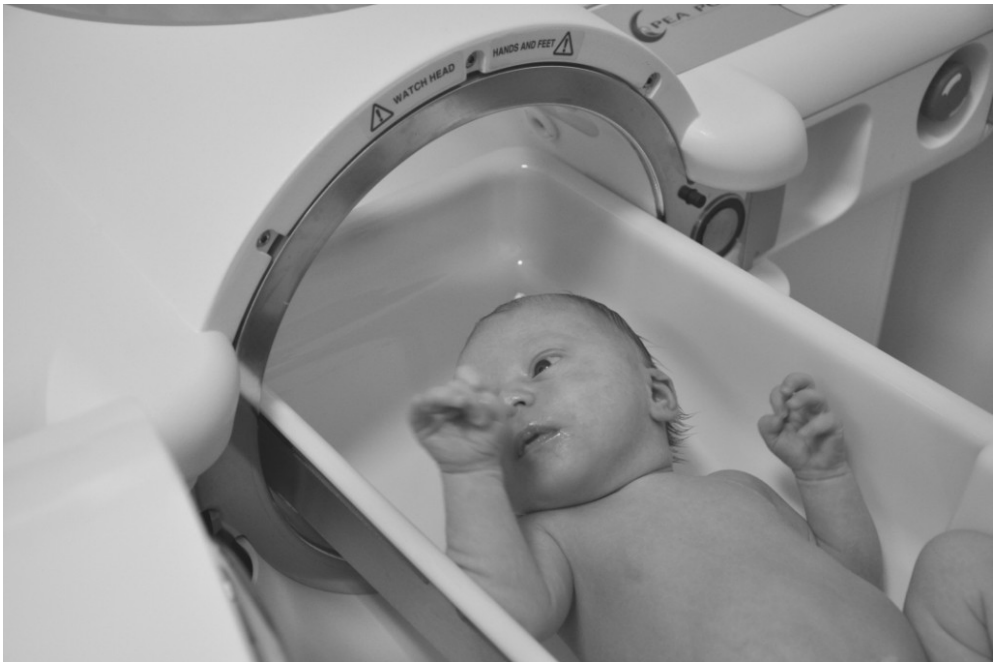


Figure 13. A baby exiting the test chamber of the Pea Pod after volume measurement.

Results

Part 1. Body composition of term appropriate for gestational age (AGA) infants.

Forty term appropriate for gestational age infants were included in the study, 18 boys and 22 girls. All infants were exclusively breastfed. The clinical characteristics of the infants at birth and their body composition measurements in the first 24 hours of life are shown in Table 1.

Characteristics of AGA term infants at birth	
Birth weight (g)	3323.4 (374.0)
Gestational age (weeks)	39.5 (1.1)
Male:female	18:22
Maternal pregravid BMI	22.9 (3.9)
Weight increase during pregnancy (Kg)	13.6 (3.7)
Length at birth (cm)	49.6 (1.8)
Cranial circumference at birth (cm)	34.4 (1.0)
C-section (n)	16
Breast milk feeding (n)	40
Maternal diabetes (n)	2
Other pregnancy complications	none
Apgar score at 5 minutes	10
Body composition on DOL 1	
Body mass (g)	3192.0 (377.5)
% fat mass	7.85 (4.29)
Fat mass (g)	257.5 (152.9)
Fat free mass (g)	2935.2 (315.5)

Table 1. Clinical, demographic characteristics and body composition of 40 term AGA at birth and on day of life (DOL) 1.

The percentage of fat mass at birth did not correlate with birth weight, body mass on the first day of life (DOL 1), maternal pregravid BMI, weight increase during pregnancy or maternal diabetes.

To evaluate the effect of physiological weight loss on body composition components, body composition on DOL 1 was compared with body composition on the 3rd day of life (DOL 3). The results are shown in Table 2.

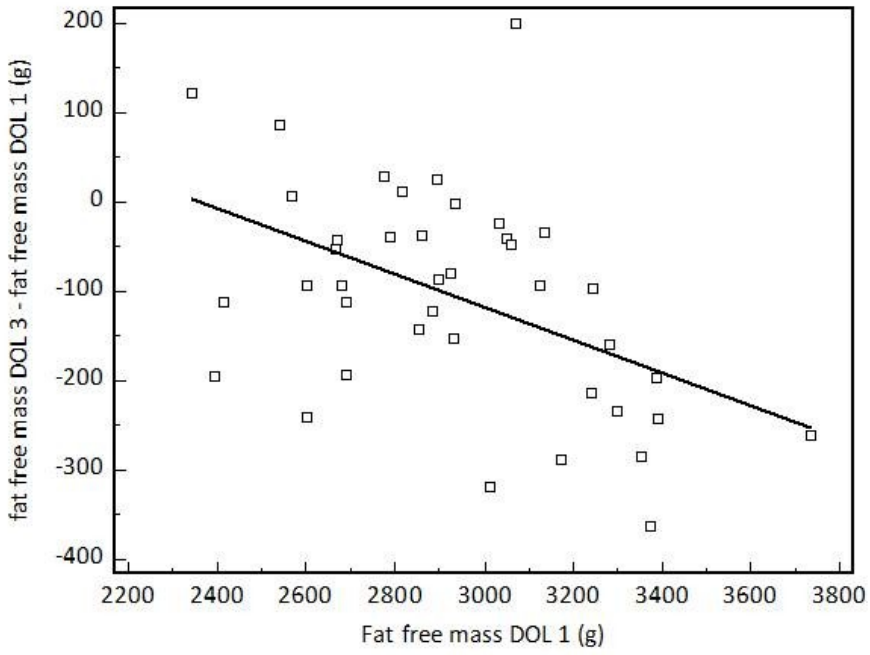
40 term AGA infants	DOL 1	DOL 3	P
Hours of life at measurement	20.7 (6.0)	59.8 (11.3)	< 0.0001
Body mass (g)	3192.0 (377.5)	3115.4 (361.2)	< 0.0001
% fat mass	7.85 (4.29)	8.97 (3.98)	0.01
% fat free mass	92.14 (4.30)	91.03 (3.98)	0.01
Fat mass (g)	257.5 (152.9)	287.6 (144.6)	0.03
Fat free mass (g)	2935.2 (315.5)	2830.0 (279.3)	< 0.0001

Table 2. Body composition changes during physiological weight loss in 40 AGA term infants.
DOL: day of life.

From the first to the third day of life, infants lost -2.34% (2.35) of their body mass measured on DOL 1. The DOL 1 body mass, measured at a mean of 20.7 (6.0) hours of life, was significantly lower than infants' weight measured immediately after birth (3192.0 g (377.5) vs 3323.4 g (374.0), p<0.0001), accounting for an additional -4.00% (1.73) of birth weight lost in the course of the first hours of life. Total birth weight loss, from birth to 59.8 (11.3) hours of life (DOL 3), was -6.27% (2.16) of birth weight. No infant lost more than 10% of birth weight.

Body mass measured on the 3rd day of life did not differ from the lowest recorded weight after birth (3115.4 (361.2) vs 3230.0 g (359.4), p=0.2).

The percentage of body mass lost from DOL 1 to DOL 3 did not correlate with body mass on DOL 1. The decrease in fat free mass from DOL 1 to DOL 3 was inversely correlated with fat free mass on DOL 1 (Graph 1), but not with birth weight or with body mass on DOL 1.



Graph 1. Inverse association between the quantity (g) of fat free mass lost from DOL 1 to DOL 3 and fat free mass on DOL 1 in AGA term infants (n 40). R^2= 0.22, p=0.002. Intercept: coefficient 0.43 (standard error (SE) 0.16), p=0.01. Slope: coefficient -0.18 (SE -0.05), p=0.002.

We compared data on body composition according to the mode of delivery. Twelve caesarean section were performed electively for breech presentation (11) or maternal indication (1) before the onset of labor or membrane rupture; in two cases caesarean section was medically indicated because of complications occurring in the course of labor (cardiotocographic abnormalities, lack of progress).

No difference was found at DOL 1, DOL 3 and 2 weeks of age between babies born from vaginal delivery and those born from c-section (Table 3).

In both groups of babies, those vaginally born and those born from caesarean section, body mass and the absolute amount of fat free mass decreased from DOL 1 to DOL 3 (Table 3). The entity of fat free mass decrease from DOL 1 to DOL 3 was similar between the two groups (Δ fat free mass vaginal birth vs Δ fat free mass c-section: -100.1 (134.3) g vs -114.7 (102.4) g, p=0.7).

Mode of delivery	vaginal birth	cesarean section
	n 26	n 14
Maternal pregravid BMI	22.6 (3.4)	23.4 (4.8)
Weight increase during pregnancy (Kg)	14.2 (2.9)	12.5 (4.9)
Gestational age (weeks)	39.6 (1.1)	39.2 (1.3)
Birth weight (g)	3355.4 (372.0)	3263.9 (384.4)
Cranial circumference (cm)	34.3 (1.1)	34.6 (0.9)
Length (cm)	49.7 (1.9)	49.3 (1.7)
DOL 1		
Body mass (g)	3231.2 (371.6)*	3119.1 (391.6)*
%fat mass	7.38 (3.95)	8.74 (4.92)
Fat mass (g)	242.4 138.6)	285.5 (178.7)
Fat free mass (g)	2988.4 (333.0)*	2836.4 (262.8)*
DOL 3		
Body mass (g)	3158.0 (354.8)*	3036.1 (372.7)*
%fat mass	8.25 (3.98)	10.30 (3.77)
Fat mass (g)	268.0 (143.4)	324.0 (150.8)
Fat free mass (g)	2888.3 (276.5)*	2721.7 (260.2)*
2 weeks	n 25	n 9
Body mass (g)	3611.0 (384.7)	3561.9 (447.0)
%fat mass	12.13 (4.12)	13.44 (4.65)
Fat mass (g)	441.0 (164.2)	491.1 (206.5)
Fat free mass (g)	3182.5 (355.6)	3073.9 (305.4)

Table 3. Comparison of body composition measured on DOL 1, DOL 3 and at 2 weeks of age in infants born from vaginal delivery versus infants born from caesarean section. DOL: day of life. *significant difference ($p<0.05$) of the variable measured on DOL 1 versus the same variable measured on DOL 3.

The results of the longitudinal analysis of growth and body composition in AGA term exclusively breastfed infants from DOL 1 to 3 months of age are shown in Table 4, divided by gender. Boys resulted to be longer and with a higher content in fat free mass than girls at several time points. No significant difference in the percentage of fat mass or in the absolute amount fat mass was found between boys and girls.

Body composition changes during the physiological weight loss were similar in boys and girls, even if the degree of fat free mass decrease from DOL 1 to DOL 3 was more pronounced in males. In fact, males lost almost double grams of fat free mass in comparison to girls, even if this difference was not statistically significant (boys vs girls: -139.9 (96.4) g vs -76.8 (13.6) g, p=0.1).

The percentage of fat mass increased significantly from birth to 3 months of age in both girls ($p<0.001$) and boys ($p<0.001$) (p refers to comparisons between all time points).

	n	%Fat mass	Fat mass (g)	Fat free mass (g)	Body mass (g)	Length (cm)	CC (cm)
Boys							
DOL 1	18	7.25 (3.80)	247.0 (141.3)	3077.7 (303.2)*	3321.4 (354.2)*	50.4 (1.8)*	34.7 (1.0)
DOL 3	18	8.32 (3.31)	276.4 (134.0)	2937.8 (260.9)	3216.8 (363.5)		
2 weeks	16	11.79 (4.10)	445.3 (183.7)	3296.3 (307.0)*	3722.9 (363.8)*	52.6 (1.8)*	36.8 (0.9)*
1 month	5	12.94 (4.00)	539.3 (182.8)	3586.0 (142.9)*	4060.8 (298.5)	55.5 (1.6)*	38.2 (0.4)*
3 months	3	24.87 (4.78)	1682.0 (500.5)	4919.9 (868.7)	6601.8 (1526.4)	61.9 (2.7)	41.0 (1.0)

	n	%Fat mass	Fat mass (g)	Fat free mass (g)	Body mass (g)	Length (cm)	CC (cm)
Girls							
DOL 1	22	8.35 (4.69)	266.1 (164.7)	2818.6 (280.6)	3086.0 (370.2)	48.9 (1.6)	34.2 (1.1)
DOL 3	22	9.50 (4.46)	296.7 (158.7)	2741.8 (267.8)	3032.3 (354.4)		
2 weeks	18	13.15 (4.37)	463.4 (171.2)	3017.9 (324.0)	3479.7 (398.9)	50.8 (1.7)	35.3 (1.2)
1 month	7	16.31 (5.38)	634.6 (228.9)	3226.3 (282.1)	3860.9 (327.6)	53.7 (1.6)	36.1 (0.6)
3 months	5	27.30 (6.84)	1624.1 (618.0)	4143.2 (327.6)	5749.3 (812.2)	60.4 (1.4)	39.7 (1.35)

Table 4. Longitudinal study of the growth and body composition of 18 term, AGA infant boys (top) and 22 term, AGA infant girls (bottom) from birth to 3 months of age. DOL: day of life. CC: cranial circumference. *boys > girls, $p < 0.05$

Part 2. Body composition of late preterm infants

Ten late preterm infants were included in the study, 6 boys and 4 girls. Four babies were born from c-section, performed in two cases because of spontaneous onset of labor in the presence of breech presentation, in one case because of severe maternal preeclampsia and in one case because of abnormalities in fetal umbilical artery flow velocity.

Pregnancy was complicated by gestational diabetes in 2 cases and by preeclampsia in 2 cases. Maternal BMI of late preterm infants did not differ from maternal BMI of the 40 term, AGA infants of the control group (23.5 (7.9) vs 22.9 (3.9), p=0.7).

In the first day of life, 7 babies received the continuous enteral feeding protocol for the prevention of hypoglycemia and 3 were fed with breast milk and formula milk. At 3 days of life 7 infants out of 10 were exclusively breastfed and at 2 weeks and later they were all exclusively fed with breast milk.

At birth, late preterm infants were younger, lighter, shorter and with a smaller cranial circumference than term infants. All their body composition components, both fat mass and fat free mass, were lower than those of the control group. However, at 2 weeks and at 1 month of age their % fat mass did not differ significantly from the % fat mass of the control group anymore, even if late preterm babies continued to be shorter, lighter and with a lower content in fat free mass (Table 5).

Body mass of late preterm infants decreased from DOL 1 to DOL 3, while % fat mass and fat free mass did not change significantly (Table 4). Fat mass increased significantly from DOL 1 to 2 weeks to 1 month of age ($p<0.001$ for all comparisons).

At 1 month of age, late preterm infants' postmenstrual age did not differ from term infants' gestational age at DOL 1 (40.4 (39.7-41.0) weeks vs 39.6 (38.8-41.3) weeks, p=0.2). At this term-equivalent postmenstrual age, body mass of late preterm infants was similar to that of term infants (3167.5 (323.1) vs 3192.0 (377.5), p=0.9), but late preterm infants had more fat mass, both as a percentage (16.40% (2.61) vs 7.85% (4.29), $p<0.0001$) and as an absolute value (517.3 g (93.6) vs 257.5 g (152.9), p=0.002) and less fat free mass (2643.2 g (291.4) vs 2935.2 g (315.5), p=0.04) than term infants.

	late preterm infants	term infants	p
At birth	n 10	n 40	
Gestational age (weeks)	36.2 (0.7)	39.5 (1.1)	< 0.0001
Weight (g)	2370.5 (364.4)	3323.4 (374.0)	0.0003
Length (cm)	45.6 (2.5)	49.6 (1.8)	< 0.0001
Cranial circumference (cm)	32.9 (1.8)	34.4 (1.0)	< 0.0001
DOL 1	n 10	n 40	
Body mass (g)	2312.6 (364.6)*	3192.0 (377.5)	< 0.0001
%fat mass	5.47 (2.65)	7.85 (4.29)	0.04
Fat mass (g)	146.5 (70.3)	257.5 (152.9)	0.05
Fat free mass (g)	2213.0 (307.3)	2935.2 (315.5)	< 0.0001
DOL 3			
Body mass (g)	2252.0 (315.6)*	3115.4 (361.2)	< 0.0001
%fat mass	6.31 (3.18)	8.97 (3.98)	0.05
Fat mass (g)	145.0 (85.9)	287.6 (144.6)	0.0009
Fat free mass (g)	2142.3 (313.3)	2830.0 (279.3)	< 0.0001
2 weeks	n 7	n 34	
Length (cm)	45.8 (1.5)	51.7 (1.9)	<0.0001
Cranial circumference (cm)	33.3 (0.4)	35.7 (1.3)	0.02
Body mass (g)	2555.7 (302.7)	3591.8 (391.5)	<0.0001
%fat mass	10.47 (2.44)	12.76 (4.44)	0.09
Fat mass (g)	268.2 (78.2)	462.7 (178.5)	0.01
Fat free mass (g)	2287.5 (272.8)	3138.7 (346.4)	<0.0001
1 month	n 7	n 12	
Length (cm)	49.2 (1.8)	54.4 (1.7)	<0.0001
Cranial circumference (cm)	34.3 (1.5)	36.7 (1.2)	0.01
Body mass (g)	3167.5 (323.1)	3944.2 (318.6)	0.0002
%fat mass	16.40 (2.61)	14.91 (4.96)	0.5
Fat mass (g)	517.3 (93.6)	592.9 (207.7)	0.4
Fat free mass (g)	2643.2 (291.4)	3376.2 (291.8)	<0.0001

Table 5. Growth and body composition of late preterm infants vs term infants at various study time points. DOL: day of life. *significant difference ($p<0.05$) between the variable measured on DOL 1 and the same variable measured on DOL 3.

Part 3. Body composition of small for gestational age and large for gestational age term infants

The study included 19 SGA and 15 LGA term infants.

Mother prepregnancy BMI and weight increase during pregnancy did not differ between SGA, LGA and AGA infants. Preeclampsia complicated 4 pregnancies of SGA infants. Two mothers of LGA infants developed gestational diabetes. Five SGA infants were born from caesarean delivery, 4 because of complications of intrauterine growth restriction and 1 electively. Two LGA infants were born from elective caesarean section.

Twelve out of 19 SGA infants were males, with the boys to girls ratio not different from the control AGA group. In the LGA infants group there were significantly more males than in the control group (13/15 vs 18/40, p=0.02).

After birth, all except for 2 SGA infants were fed in the first 24 hours of life according to the enteral feeding protocol for the prevention of hypoglycemia. At 2 weeks of life, they were all breastfed. All except for one LGA infants were exclusively breastfed at all time points. Small for gestational age infants were approximately 1 week younger than the other 2 groups at birth (Table 6).

At birth, on DOL 1 and on DOL 3 SGA infants and LGA infants resulted, respectively, smaller and bigger than control AGA infants in all parameters measured. The percentage of fat mass also increased significantly according to the birth weight category, as well as body mass, fat mass and fat free mass. The percentage of fat mass on DOL 1 was significantly correlated with DOL 1 body mass in the whole group of infants (n 74) (Graph 2).

The % fat mass on DOL 1 was higher in LGA versus AGA infants even after LGA infants born from diabetic mothers were excluded from the analysis (12.41 (3.29) vs 7.85 (4.29) , p=0.004).

From DOL 1 to DOL 3, body mass and fat free mass decreased in LGA infants. The % fat mass increased in SGA infants, the fat free mass decreased and there was no significant difference in body mass (Table 6). From birth to DOL 3, SGA infants lost significantly less weight than LGA or AGA infants (SGA vs AGA: -3.2% (4.1) vs -6.3% (2.1), p=0.008. The percentage is calculated on birth weight.).

	SGA	AGA	LGA	p
	n 19	n 40	n 15	
Gestational age at birth (weeks)	38.1 (1.2)"	39.5 (1.1)	39.7 (0.9)	"
Birth weight (g)	2347.9 (257.3)	3323.4 (374.0)	4265.7 (129.1)	< 0.001
Length at birth (cm)	45.7 (1.9)	49.6 (1.8)	52.7 (1.5)	< 0.001
Cranial circumference at birth (cm)	32.5 (1.3)	34.4 (1.0)	35.9 (0.7)	< 0.001
DOL 1	n 19	n 40	n 15	
Body mass (g)	2307.1 (246.5)	3192.0 (377.5)*	4135.3 (182.9)*	< 0.001
% fat mass	3.96 (2.67)*	7.85 (4.29)*	12.35 (3.33)	< 0.001
Fat mass (g)	91.4 (63.1)	257.5 (152.9)*	505.5 (135.5)	< 0.001
Fat free mass (g)	2212.7 (245.8)*	2935.2 (315.5)*	3595.0 (210.2)*	< 0.001
DOL 3	n 19	n 40	n 15	
Body mass (g)	2263.1 (218.1)	3115.4 (361.2)*	3971.8 (198.0)*	< 0.001
% fat mass (g)	5.05 (3.06)*	8.97 (3.98)*	11.75 (2.50)	< 0.001
Fat mass (g)	110.5 (76.9)	287.6 (144.6)*	504.1 (176.8)	< 0.001
Fat free mass (g)	2163.5 (232.6)*	2830.0 (279.3)*	3503.3 (177.9)*	< 0.001
2 weeks	n 14	n 34	n 15	
Length (cm)	48.9 (3.4)	51.7 (1.9)	55.6 (1.5)	< 0.001
Cranial circumference (cm)	34.6 (2.0)	35.7 (1.3)	38.0 (0.8)	< 0.001
Body mass (g)	2704.0 (291.0)	3591.8 (391.5)	4504.5 (196.7)	< 0.001
% fat mass (g)	8.96 (3.01)"	12.76 (4.44)	13.70 (3.29)	"
Fat mass (g)	242.3 (83.5)	462.7 (178.5)	656.5 (163.5)	< 0.001
Fat free mass (g)	2472.1 (281.6)	3138.7 (346.4)	3869.2 (168.1)	< 0.001
1 month	n 12	n 12	n 9	
Length (cm)	50.3 (2.3)	54.4 (1.7)	58.0 (1.7)	< 0.001
Cranial circumference (cm)	35.4 (0.9)	36.7 (1.2)	38.5 (0.9)#	#
Body mass (g)	3295.5 (352.3)	3944.2 (318.6)	538.0 (515.4)	< 0.001
% fat mass (g)	17.30 (4.32)	14.91 (4.96)	20.1 (4.9)	0.2
Fat mass (g)	577.2 (177.6)	592.9 (207.7)	1149.7 (330.5)#	#
Fat free mass (g)	2714.3 (245.4)	3376.2 (291.8)	4120.5 (254.9)	< 0.001

Table 6. Anthropometric measures and body composition in SGA, AGA and LGA term infants. The p value refers to the difference between each of the three groups (ANOVA). " SGA < AGA and SGA < LGA, p=0.001. # LGA > SGA and LGA > AGA, p=0.001 (ANOVA). *significant difference ($p<0.05$) between the variable measured on DOL 1 and the same variable measured on DOL 3.

After the physiological weight loss, growth resumed in all three groups of babies. The % fat mass increased significantly from birth to 3 months of life in SGA ($p<0.001$) and in LGA infants ($p<0.001$) (p refers to comparisons between all time points). The % fat mass at 3 months of life was 24.80 (3.17) in SGA (n 3) and 25.5 (6.48) in LGA infants (n 6).

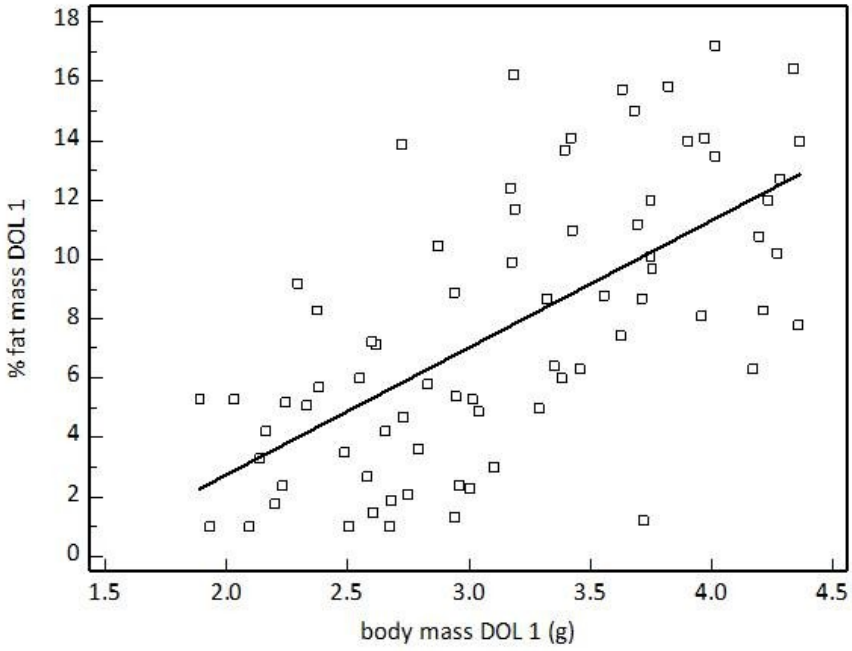
The difference in all anthropometric measures and body composition components persisted among the three groups of babies at all time points, SGA being smaller and LGA bigger than AGA infants. Two exceptions were the % fat mass at 1 month of life which was similar in the three groups, and the absolute value of fat mass at 1 month that was comparable in SGA and AGA newborns. Only LGA infants had significantly higher fat mass than the other two groups at 1 month of life.

When growth from day 1 to day 30 was analyzed in the subgroup of infants followed up longitudinally from birth to 1 month (n 31), SGA infants resulted to gain significantly more weight (as a % of body mass on DOL 1) and more % fat mass than both AGA and LGA infants (Table 7). This result was independent from gestational age at birth in multiple regression analysis.

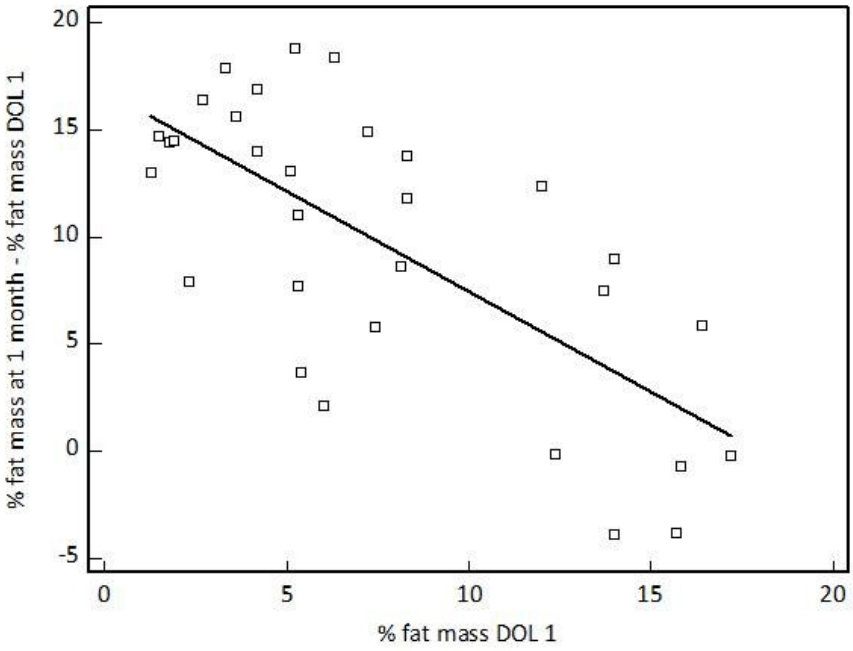
In the whole group of babies (n 31), a significant inverse relationship was noted between % fat mass on DOL 1 and % fat mass increase from DOL 1 to 1 month of life (Graph 3).

Growth from DOL 1 to 1 month of age	**SGA**	**AGA**	**LGA**	**p**
	n 10	n 12	n 9	
Δ body mass (g)	1068.8 (250.2)	882.7 (347.2)	1079.0 (494.2)	NS
Δ length (cm)	5.5 (1.2)	5.4 (1.9)	5.2 (1.3)	NS
Δ % fat mass	14.16 (3.22)”	8.18 (6.86)	6.81 (7.24)	"0.03
Δ fat mass (g)	524.3 (144.8)	376.9 (259.9)	505.7 (417.2)	NS
Δ fat free mass (g)	548.3 (168.6)	529.3 (151.6)	569.5 (217.3)	NS
% body mass increase	47.4 (11.3)”	30.0 (13.6)	26.3 (11.9)	"0.002
% length increase	12.1 (2.6)	11.0 (4.1)	9.9 (2.4)	NS

Table 7. growth and body composition changes from DOL 1 to 1 month of life in AGA, SGA and LGA term infants. “ SGA > AGA and SGA > LGA (ANOVA). % body mass and % length increases are calculated on DOL 1 body mass and DOL 1 length respectively.



Graph 2. Correlation between % fat mass on DOL 1 and body mass on DOL 1 in 74 term newborns. R^2= 0.41, p<0.001. Intercept: coefficient -5.77 (standard error (SE) 1.93), p=0.004. Slope: coefficient 4.27 (SE -0.59), p<0.001.



Graph 3. Inverse relationship between % fat mass increase from DOL 1 to 1 month and % fat mass on DOL 1 in 31 term newborns. R^2= 0.49, p<0.001. Intercept: coefficient 16.84 (standard error (SE) 1.59), p<0.001. Slope: coefficient -0.94 (SE -0.17), p<0.001.

Part 4. Body composition of twins

Twenty couples of twins were included in the study. They were all dizygotic twins except for one pair. There were no significant pregnancy complications. Maternal pregravid BMI was normal, 21.9 (2.8). Only one mother was moderately overweight (BMI=26) before delivery. Weight increase during pregnancy was 16.3 (6.7) Kg. Five couples were born from vaginal delivery and the others from caesarean section. Eighteen infants were small for gestational age, there were no LGA; twenty-one twin infants were boys (52.5%).

The gestational age at birth of twins as a group was 37.2 (1.7) weeks, their birth weight was 2566.6 (416.4) g, their length 47.3 (1.9) cm, and their cranial circumference 32.9 (1.5) cm. Gestational age at birth was shorter and all anthropometric measures at birth were significantly smaller than those of the 40 AGA term infants ($p<0.0001$ in all comparisons). Despite these differences, there was only a trend towards a lower % fat mass in twins vs AGA term infants at birth (6.26 (3.56) vs 7.86 (4.3), $p=0.07$). Twins were leaner than AGA term infants at 2 weeks of age (% fat mass: 9.60 (3.91) vs 12.76 (4.45), $p=0.005$), but this result was not significant anymore after controlling for gestational age and birth weight in multiple regression analysis.

In view of the heterogeneity of the group of twins in terms of birth weight category, and considering the dramatic difference in both birth weight and gestational age between twins and AGA term singletons, the analyses were conducted as follows: AGA twins (n 22) versus AGA term and late preterm singletons (n 51); SGA twins (n 18) vs SGA singletons (n 19).

Maternal pregravid BMI did not differ neither between SGA twins and SGA singletons (21.5 (3.1) vs 23.3 (5.3), $p=0.3$) nor between AGA twins and AGA singletons (22.2 (2.5) vs 22.9 (3.9), $p=0.3$). Weight increase during pregnancy of the mothers of SGA twins was similar to that of the mothers of SGA singletons (13.0 (6.3) vs 10.6 (5.5), $p=0.5$). Mothers of AGA twins gained significantly more weight during pregnancy than mothers of AGA singletons (18.5 (5.5) vs 13.6 (3.7), $p=0.004$).

After birth, 3 AGA twins were fed only with breast milk, 2 exclusively with formula and the others with mixed breast and formula milk. Sixteen out of 18 SGA twins were fed with the continuous enteral feeding protocol for the prevention of hypoglycemia, in comparison to 17 SGA singletons ($p=0.95$). At 2 weeks of age, 2 SGA twins were fed with their mothers' milk, 7

with mixed breast and formula and 3 with formula only. The corresponding figures were 2, 4, 4 infants at one month.

	AGA twins	AGA singletons	p
At birth	n 22	n 51	
Gestational age (weeks)	37.3 (1.5)	38.3 (1.8)	0.03
Birth weight (g)	2791.4 (396.1)	3104.3 (561.9)	0.02
Length (cm)	48.2 (1.7)	48.8 (2.5)	0.3
Cranial circumference (cm)	33.1 (1.7)	34.0 (1.5)	0.03
DOL 1	n 22	n 51	
Body mass (g)	2696.8 (392.5)*	3013.8 (514.7)*	0.02
% fat mass	7.84 (3.46)	7.30 (4.16)*	0.6
Fat mass (g)	209.6 (96.7)	234.9 (149.2)*	0.5
Fat free mass (g)	2487.7 (380.8)	2801.5 (419.3)*	0.006
DOL 3	n 22	n 51	
Body mass (g)	2616.1 (403.5)*	2940.1 (495.7)*	0.02
% fat mass	8.47 (3.12)	8.42 (3.94)*	0.9
Fat mass (g)	226.8 (102.3)	258.6 (147.4)*	0.4
Fat free mass (g)	2411.9 (368.5)	2692.1 (394.6)*	0.01
2 weeks	n 14	n 41	
Length (cm)	50.4 (2.4)	50.8 (2.7)	0.6
Cranial circumference (cm)	35.3 (1.7)	35.3 (1.6)	0.9
Body mass (g)	3021.9 (441.9)	3415.4 (541.1)	0.02
% fat mass	10.76 (2.26)	12.24 (4.38)	0.1
Fat mass (g)	329.5 (95.5)	426.0 (185.0)	0.02
Fat free mass (g)	2692.4 (374.1)	2997.4 (456.3)	0.03
1 month	n 10	n 19	
Length (cm)	52.0 (1.7)	52.5 (2.9)	0.6
Cranial circumference (cm)	36.1 (1.6)	36.3 (0.8)	0.7
Body mass (g)	3609.1 (397.3)	3662.0 (485.2)	0.7
% fat mass	36.3 (0.8)	36.1 (1.6)	0.7
Fat mass (g)	18.08 (3.27)	15.44 (4.18)	0.1
Fat free mass (g)	647.7 (122.0)	566.8 (173.9)	0.2

Table 8a. Comparison of growth and body composition between AGA singletons and AGA twins from birth to 1 month of life. *significant difference ($p<0.001$) between the variable measured on DOL 1 and the same variable measured on DOL 3.

AGA twins' gestational age at birth was one week shorter and they were smaller than AGA singletons at birth. The percentage of fat mass resulted to be comparable in the two groups at all time points. Body mass and fat free mass resulted to be lower in twins versus singletons at the measurements done on DOL 1, DOL 3 and 2 weeks; fat mass resulted to be lower in twins than in singletons at 2 weeks of life. However, all these differences were no more significant after controlling for gestational age at birth in multiple regression analysis (Tables 8a and 8b).

From DOL 1 to DOL 3, body mass in AGA twins decreased significantly, as noted in AGA singletons. The mean values of % fat mass and absolute fat mass increased and mean fat free mass decreased in AGA twins, but these changes were not significant (Table 8a).

	AGA twins	AGA singletons	p
3 months	n 6	n 10	
Length (cm)	61.0 (59.3-62.5)	60.1 (59.0-61.5)	0.4
Cranial circumference (cm)	40 (40-40.3)	40 (37.9-41)	0.7
Body mass (g)	5881.2 (5620.5-6012.6)	5602.5 (5198.7-6478.7)	0.8
% fat mass	27.3 (23.8-28.8)	25.4 (21.3-31.5)	0.9
Fat mass (g)	1395.5 (1161.8-1688.6)	1419.0 (1148.7-2210.0)	0.8
Fat free mass (g)	4155.3 (4024.8-4323.9)	4181.8 (3797.3-4617.7)	0.6

Table 8b. Comparison of growth and body composition between AGA singletons and AGA twins at 3 months of life. Results are presented as median (interquartile range). Mann-Whitney was used for comparisons.

Growth and body composition of SGA twins did not differ from those of SGA singletons at all time points, despite the shorter gestational age at birth. There was a trend towards a slightly lower fat mass at 1 month of life in twins vs singletons (Table 9).

SGA twins did not show any significant weight loss from DOL 1 to DOL 3; body composition did not change as well (Table 9).

When compared to SGA twins, AGA twins and singletons were heavier, longer and had higher body mass, % fat mass, grams of fat mass and of fat free mass at all time points ($p < 0.05$ in all comparisons).

The percentage of fat mass increased significantly from birth to 3 months of life in both SGA twins ($p<0.001$) and LGA twins ($p<0.001$) (Table 8b). The p value refers to comparisons

between all time points. The % fat mass in SGA twins at 3 months of life was 24.80 (3.18) (n 3).

	SGA twins	SGA singletons	p
At birth	n 18	n 19	
Gestational age (weeks)	36.8 (1.6)	38.1 (1.2)	0.01
Birth weight (g)	2291.9 (242.6)	2347.9 (257.3)	0.5
Length (cm)	46.3 (1.7)	45.7 (1.9)	0.3
Cranial circumference (cm)	32.7 (1)	32.5 (1.3)	0.7
DOL 1	n 18	n 19	
Body mass (g)	2194.1 (223.0)	2309.2 (238.7)	0.2
% fat mass	4.33 (2.68)	3.96 (2.52)*	0.7
Fat mass (g)	98.7 (61.4)	91.2 (59.5)	0.7
Fat free mass (g)	2121.7 (238.2)	2215.5 (240.7)*	0.3
DOL 3	n 18	n 19	
Body mass (g)	2138.3 (234.2)	2263.1 (218.1)	0.2
% fat mass	4.73 (3.32)	5.05 (3.06)*	0.8
Fat mass (g)	99.7 (68.1)	110.5 (76.9)	0.7
Fat free mass (g)	2050.5 (203.9)	2163.5 (232.6)*	0.2
2 weeks	n 12	n 14	
Length (cm)	48.0 (3.6)	48.9 (3.4)	0.5
Cranial circumference (cm)	34.3 (0.9)	34.6 (2.0)	0.7
Body mass (g)	2478.6 (322.4)	2731.1 (291.0)	0.05
% fat mass	8.25 (4.99)	8.96 (3.01)	0.7
Fat mass (g)	213.8 (136.4)	244.8 (83.5)	0.5
Fat free mass (g)	2279.2 (236.0)	2472.1 (281.6)	0.08
1 month	n 10	n 12	
Length (cm)	49.9 (2.1)	50.3 (2.3)	0.7
Cranial circumference (cm)	35.4 (0.6)	35.5 (0.9)	0.9
Body mass (g)	3142.5 (312.8)	3295.5 (352.3)	0.3
% fat mass	14.23 (2.61)	17.30 (4.32)	0.06
Fat mass (g)	450.1 (116.0)	577. 2 (177.6)	0.07
Fat free mass (g)	2692.1 (237.3)	2714.3 (245.4)	0.8

Table 9. Comparison of growth and body composition between SGA singletons and SGA twins. *significant difference ($p<0.001$) between the variable measured on DOL 1 and the same variable measured on DOL 3.

Discussion

In this study we report the body composition data for five categories of newborns: term appropriate for gestational age, late preterm, small for gestational age, large for gestational age and twins. Body composition was assessed at birth, during the physiological weight loss and in early life up to 3 months of age, and was measured by means of air displacement plethysmography (ADP).

The study of body composition in newborns has long been hindered by the poor applicability of methods designed for adults to the newborn population. Air displacement plethysmography is a relatively new method for measuring body composition in newborns. It has been introduced in clinical practice and research no more than 7 years ago, is currently found in approximately 60 centers worldwide (2 in Italy) and has generated relatively few publications to date. The ADP system is easy to use, quick and comfortable for the babies. The measures are accurate and can be compared with those obtained by other ADP devices. The availability of this technique has offered new opportunities to explore body composition in newborns.

The body composition of forty AGA term exclusively breastfed infants was studied at a mean of 20.7 (6) hours of life and at 59.8 (11.3) hours of life. Of these, 34 were followed up at 2 weeks, 12 at 1 month and 8 at 3 months of age. The high rate of babies lost at follow up at 3 months is mostly related to the fact that the measurements at 3 month of age were introduced after enrollment had already begun.

Body composition percentiles for newborns at birth and in the first months of life are currently being developed by many centers worldwide[93]. In an Italian study by the group of Milan (Roggero et al.), 40 (23 girls and 17 boys) term, AGA, exclusively breastfed infants were measured at birth (third day of life) and a subgroup of them was followed up longitudinally with monthly measurements up to 6 months of age. Only a few babies were measured at 5 and 6 months of life, mainly because of the 8-Kg of weight limit of the Pea Pod[22].
In comparison to the data reported by Roggero et al, the % fat mass we measured was slightly lower in boys and slightly higher in girls at all time points. However, the differences were very small except for the % fat mass of boys at 1 month, which was much lower in our sample, but which was also calculated on a smaller number of babies. In both studies, the % fat mass increased significantly from birth to 3 months of age. Body mass, length and cranial circumference were also similar in boys, except for the lower body mass of the boys of our

sample at 1 month. Girls in our study were modestly heavier than those measured in Milan; the other measures were almost identical. Girls had a higher % fat mass than boys in both populations, even if this difference was not statistically significant. In both studies boys were heavier and with higher fat free mass.

The marked similarities between these two populations can be well explained by the common genetic background, by the similar nutritional and socioeconomic conditions of Eastern and Western Northern Italy, and by the fact that mothers in both studies were non-smokers, prevalently or exclusively non diabetic, and with comparable pregravid BMIs and weight increases during pregnancy, which were both in the normal range.

The absence of these factors and the differences in both genetic backgrounds and nutritional conditions may help explain the differences found with body composition data of newborns from other populations.

Forty-five term Australian babies were studied by Carberry with the ADP system at birth and followed up to 4.5 months of age[21]. In this group, the % fat mass at birth was higher in boys and girls and both males and females were approximately 200 g heavier at birth than in our population, notwithstanding the similar gestational age. Mothers' pregravid BMI was similar to that of our study and was in the normal range, and 98% of the population was Caucasian. Nevertheless, differences in the mothers' nutritional status or in the genetic background might explain the different growth and body composition of babies at birth. The % fat mass was slightly lower in the Australian study at 3 months. Forty percent of infants were fed with formula, and the remainder was fed either with either exclusive or partial breast milk, without any distinction. This might be relevant in accounting for the different postnatal growth[43].

In a Swedish study by Eriksson[23], the Pea Pod system was used to measure body composition in 53 girls and 55 boys at 1 and 12 weeks of age. Mothers had a higher BMI (23.4), gained more weight during pregnancy (14.4 Kg) and babies were ~500 g heavier at birth than in other sample, even if they were born at the same gestational age. The % fat mass at 1 week of age was higher than the one we measured at 2 weeks of age in both boys and girls, while the % fat mass at 3 months was similar. Differences in maternal nutrition might explain the higher fat mass at 1 week of age in Eriksson's study, while similarities in feeding practices (85% infants were exclusively and 12% prevalently breastfed) might account for the similar body composition observed at 3 months. In the Swedish population, the fat free mass was significantly higher in boys than in girls, similarly to our findings. Girls resulted to be fatter than boys at both time points.

In a study by Fields[24], in which the Pea Pod system was also employed, the % fat mass obtained at 20 days of life in 117 US infants (64 girls and 53 boys) was similar to the one we measured at 1 month of life in both boys and girls. In this study, mothers had a higher pregravid BMI (26.2), and this might explain the result. Girls resulted to have a higher %fat mass than boys.

Older studies reported body composition data for newborns that were even more dissimilar from ours than the ones we previously mentioned. Reasonably, part of these differences might also be attributed to the different techniques used for body mass measurements.

In a seminal work by Fomon[19], both boys and girls had higher fat mass at birth than in our study. Fat mass was lower (~23%) in both males and females at 3 months of life than in our sample. Different types of postnatal feeding might account for these differences, as babies in Fomon's were exclusively formula-fed.

With respect to our study, fat mass was comparable at 2 weeks of age and significantly higher (~ 30%) at 3 months of age in a study by Butte[20] including 76 healthy term infants. Fat mass and fat free mass contents were estimated from a multicomponent model. Infants were partly (40/76) exclusively breastfed. In another study by Butte[43], exclusively breastfed infants resulted to have higher % fat mass at 3 months than formula fed babies. Both these groups had higher % fat mass than our babies.

Fat mass at 3 (2.8) days of life resulted to be higher than in our population in a study by Koo[94] including 74 AGA infants, despite the fact that their gestational age was shorter (35.9 weeks) and their birth weight lower (2410 g). Mean % fat mass resulted to be ~ 12%. Body mass measurements were performed by DEXA. No data regarding maternal weight were included.

Fat mass measured by DEXA at 4 days of life in 53 healthy term infants in the study by Rigo[25] was higher than in our sample. The study included also 106 healthy, moderately preterm newborns (gestational age 33.3 (2.4) weeks). Rigo elaborated percentile curves for fat mass, expressed as % body weight, related to body weight. In these curves, the 50th percentile of fat mass for a 3.5 Kg baby was ~ 16%. In the regression curve we elaborated from 74 term infants, the corresponding figure was ~ 9% (Graph 2). An even higher fat mass content than the one reported by Rigo was found in 70 AGA term or late preterm infants in the first 48 hours of life by Lappillonne[15], who used a DEXA device with a different software than the one used by Rigo. In Lappillonne's study, a 3500 g term newborn resulted to have ~ 17% fat mass.

In our population of AGA term infants, fat mass at birth did not correlate with maternal BMI or with weight gain during pregnancy. Mothers with higher BMI have children with higher fat mass[30], as well as mothers who gain more weight during pregnancy[36,37]. The lack of correlation found in our study is probably due to the fact that all mothers had a normal BMI and a ponderal increase within the norm and that both had a narrow range of values.

Weight loss after birth is an universal phenomenon that occurs in both term and preterm infants. However, it has been little studied, and debate still exists on what the composition of the amount of weight that is lost is. Term babies lose on average 6 to 6.8% of their birth weight in the first 3 days of life[16,17,95].

In our study, we found a significant decrease in body mass from DOL 1 to DOL 3 in AGA, term, exclusively breastfed newborns. This decrease was accounted for exclusively by the loss of fat free mass, shown also by the concomitant decrease in fat free mass expressed as a percentage of birth weight. Fat mass did not decrease, rather, it increased slightly from DOL 1 to DOL 3. LGA exclusively breastfed infants showed the same pattern of body composition changes.

These results contrast with those reported by two recent studies. Rodriguez[96] studied 43 term, AGA, exclusively breastfed children (18 males and 25 females) in the first 3 days of life with bioelectrical impedance and found that during weight loss there was a decrease in both total body water and solids. Roggero[97] measured body composition with the Pea Pod system longitudinally in 28 newborns (13 males and 15 females) and found that weight, fat free mass but also fat mass decreased from DOL 1 (12.8 hours of life) to DOL 3 (58.2 hours of life). Fat mass continued to drop also on DOL 4, and increased only afterwards, after 82 hours of life. Interestingly, the increase in fat free mass seemed to anticipate by one day that of fat mass in this sample. Similar results were obtained in the cross-sectional part of this study, in which fat mass decreased steadily from birth to its nadir at 63 hours of life.

It must be noted that, in our population, most of the weight loss occurred before the first measurement. In fact, of the mean 6.27% (2.16) of birth weight that was lost overall, only one third (-2.34%) occurred from DOL 1 to DOL 3. Therefore it may be argued that the decrease in fat mass might have already happened in the first hours of life and that what we noticed was the post-loss regain of fat. However, this explanation seems unlikely. Previous studies conducted on premature infants have shown that weight loss consisted essentially in extracellular fluid loss, especially in the first hours of life[98]. Then, the above mentioned

studies reported that solids or fat mass decreased progressively from birth to 60 hours or to the fourth day of life, changing minimally in the first day[96,97].
We speculate that the effort put by the Nursery personnel of our Institute in counselling mothers about breastfeeding might have, as a result, determined an earlier production of breast milk in our mothers, thus preventing newborns from losing their fat mass reserves as a consequence of a lengthy weight loss. One hint to this is the fact that the weight loss that we observed at ~ 60 hours of life corresponded to the nadir. In the study by Roggero, infants were still losing weight at 87 hours (4th day) of life.

Changes in body mass composition from DOL 1 to DOL 3 in late preterm infants and in SGA infants were similar to those of AGA term infants. Nevertheless, in late preterm infants the decrease in fat free mass was not significant, probably because of the paucity of samples. In SGA infants the % fat mass increased significantly, reasonably as a result of the decrease in fat free mass, as there was no significant increase in the absolute amount of fat mass. SGA infants lost significantly less weight from birth to the third day of life, and there was no significant difference in body mass from DOL 1 to DOL 3. It is known that the amount of physiological weight loss depends on birth weight, being less pronounced in small babies[95]. We demonstrated that the amount of fat free mass lost from DOL 1 to DOL 3 is proportional to the initial amount of fat free mass (Graph 1). Being fat free mass the main component of the amount of weight that is lost, this finding well correlates with the clinical observation that infants who look edematous at birth lose more weight, while “dry” babies, such as SGA infants, lose less.
In late pretem and SGA infants, body composition changes during the physiological weight loss may have been influenced by the effect of the continuous enteral feeding protocol, that was given to the majority of infants. It is hard to distinguish the effect of feeding from that of a genuinely “physiological” weight loss. However, exclusively “physiologically” breastfed late preterm or SGA infants are infrequently seen in clinical practice. In fact, in these babies the sole breastfeeding is often insufficient for an adequate nutrition in the first days of life because of the immaturity of oropharyngeal coordination, poor suckling autonomy, feeding difficulties, hypoglycemia or other complications[89,99,100] . From another perspective, the body composition changes we presented may be considered somehow independent from the variability associated with individual modalities of feeding, as the feeding regimen was standardized and the same for most of the infants.

In any case, the results of our study show that in the first hours after birth body composition is subjected to significant changes, that may affect all components (fat mass, fat free mass, body mass), but that essentially consist in a decrease in fat free mass.

We found no difference in body composition between AGA term infants born from vaginal delivery and those born from caesarean section neither at birth nor in the first 3 months of life. Infants born from caesarean section have a higher rate of transient tachypnea of the newborn[101] and of other respiratory complications[102] that can be related to an excess in pulmonary fluid. In fact, the absorption of the fluid that fills the lungs during fetal life is started before birth, because ion channels change their action from secretion to absorption in late gestation, is greatly enhanced by stress hormones (cathecolamines and cortisol) secreted during labor, and is completed in the course of vaginal delivery[101]. It is therefore possible to hypothesize that babies born from c-section have a higher fat free mass (i.e. water content) than babies born from vaginal birth. In fact, an historical study found a higher amount of total body water measured by a dilution method in 17 babies born from caesarean section versus 16 vaginally delivered children at birth. This difference was related to a 20% higher intracellular body water content. Babies born from caesarean section had also a less pronounced decrement of intracellular water in the first hours after birth than vaginally delivered children[103]. Interestingly, labor did not influence body water content. Our findings did not replicate such results. Babies born from caesarean section lost the same amount of fat free water in the first 3 days of life than infants vaginally born.

Late preterm infants had a lower body mass and fat mass content at birth than AGA term infants. This result is not surprising, given that the % fat mass increases with birth weight (Graph 2) and with gestational age[15], as fat accumulates in the course of the last trimester of pregnancy. Interestingly, this initial disadvantage was compensated very quickly. In fact, the % fat mass of late preterm infants did not differ anymore from that of AGA term infants at 2 weeks and 1 month of life. Conversely, the absolute amount of body mass remained inferior to that of AGA term infants both at 2 weeks and at 1 month of age. Moreover, late preterm infants at term-equivalent gestational age, that is at 1 month of life, resulted to be significantly fatter than AGA term infants at birth, even if their body weights were similar. Although not statistically significant, the rate of % fat mass increase in postnatal life seemed to be higher in late preterm infants, as fat mass doubled at 2 weeks and tripled at 1 month,

while in AGA term infants the % fat mass doubles at 1 month, as shown in ours and in other populations[22]. Even if these data should be interpreted with caution because of the paucity of samples in the late preterm group, they are consistent with previous reports of a greater fatness in preterm infants at term-equivalent gestational age versus term infants at birth[104]. While these latter findings referred to more premature babies (in the study of Roggero, mean gestational age at birth was 29.9 (2.3) weeks), similar results have never been found in late preterm infants.

Small for gestational age term singleton infants were smaller at birth, at DOL 1, DOL 3, 2 weeks and 1 month of life than both AGA and LGA infants. However, their % fat mass showed a rapid catch up growth and did not differ from the % fat mass of AGA and LGA infants at 1 month of life. Actually, the rate of fat mass accumulation from birth to 1 month of life was significantly higher in SGA infants than in both AGA and LGA infants. Also the rate of weight gain was more rapid in SGA infants, but this was not sufficient to compensate for the initial gap, so SGA infants were still lighter than the other groups at 1 month.

A very quick growth of fat mass was found in premature SGA infants after birth. In one study, SGA premature infants (31.4 (2.2) gestational weeks at birth), though born with less fat mass than AGA preterm babies, achieved similar % fat mass as AGA preterm babies by 3 months of corrected age[105]. In other reports, preterm SGA infants at term-equivalent age were found to be fatter than SGA term infants[106], although their weights were similar, and also to be fatter, even if with a lower weight, than AGA term infants[107].

This rapid gain in fat mass is concerning, as SGA infants are at an increased risk of central adiposity in later life[108], and as, in infants small at birth, a rapid catch up growth in early life has been associated with increased fatness in later life[6, 109], metabolic[110] and cardiovascular diseases[9]. However, from another point of view, growth has to be pursued. SGA infants who do not catch up with their peers by 2 years of age, especially in terms of height, are at increased risk of short stature in adult life[111], and, in this regard, early nutrition and growth are relevant, as most of the 90% of SGA infants who achieve a normal height at 2 years of age accomplish most of their increase in height in the first 2 months of life[112].

Growth retardation in utero determines complex modifications of several endocrine and metabolic systems (for instance, induction of insulin resistance[113] that have been advocated as the mechanisms through which SGA infants tend to accumulate fat and have an increased risk of metabolic syndrome as adults[3].

In our sample, the weight growth achieved in the first month of life in SGA infants consisted mainly in an increase in fat mass. Therefore, an accurate monitoring of the quality of growth even from the very first weeks of life seems to be pivotal in SGA infants, with the perspective of finding and implementing feeding strategies aimed at achieving an adequate growth without an excessive increase in fat mass. Interestingly, most SGA infants were exclusively breastfed at 2 week and 1 month of life.

Large for gestational age infants were bigger at all time points than both AGA and SGA infants. The % fat mass resulted to be higher than the other two groups on DOL 1 and DOL 3. The higher % fat mass of LGA at birth was an expected result, in view of the direct relationship between birth weight and the percentage of fat mass[25] and of data previously reported on large infants[26]. LGA infants however are a heterogeneous group, and it has been reported that large infants of mothers with gestational diabetes mellitus have increased fat mass and decreased lean body mass compared with infants of mothers with normal glucose tolerance levels[26,27]. Only two infants in our sample were born after pregnancies complicated by gestational diabetes. Even when the analysis was repeated with the exclusion of these two babies, % fat mass was still higher in LGA infants.

The quality of growth of large infants in early life has been poorly studied. In our study, LGA infants seemed to grow on average less than AGA and SGA infants in the first month of life, in terms of both % body mass and % fat mass. In fact, despite the initial differences in % fat free mass, there were no differences in % fat mass measure between LGA and AGA infants at 2 weeks or 1 month of age.

In the whole sample of AGA, LGA and SGA babies followed up longitudinally from birth to 1 month, we found an interesting inverse relationship between the % fat mass 1-month increase and % fat mass on DOL 1, meaning that lean babies put up more fat mass early after birth, and large babies less. This behaviour seems to be consistent with the clinical evidence that large for gestational age babies are not at risk of cardiovascular disease in later life, despite their higher BMI, and they actually have a more abundant fat free mass in later life[114]. It must also be noted that all LGA infants that were followed up to 1 month in our study were born from mothers with a normal glucose control during gestation. Large for gestational age infants born from diabetic mothers might show a different pattern of postnatal growth.

Body composition of AGA and SGA twins did not differ from that of AGA and SGA singletons, respectively, neither at birth nor in the first 3 months of life. Anthropometric measures were also similar. SGA twins resulted to be smaller in all anthropometric measures and to have significantly less fat mass, fat free mass and % fat mass than both AGA twins and singletons.

Body composition of twins at birth has been investigated in two studies by the same group of investigators, and, to our knowledge, has never been studied in the first months of life after birth. Our results are consistent in with those of the previous study by Demarini[54], that did not find any significant difference in body composition between AGA twins and AGA singletons. In this study, body composition measurements were done by DEXA at a median of 2.5 days of life, with most of the babies studied in the first week of life. Differently from what we found, SGA twins also had a similar % fat mass than AGA singletons, even if their birth weight and fat free mass were significantly lower. As in another study from the same group of authors[94], AGA infants had a slightly higher % fat mass than AGA babies of our population (10.4 (2.1)%). Instead SGA infants, both twins and singletons, had a % fat mass that was 2.5 times higher than that of our SGA babies (10.6% (3.2) vs 3.96% (2.52)), although their weight was inferior (1886 (495) g vs 2309.2 (238.7) g) and they had a shorter gestational age. We wonder whether different techniques for body composition measurement used in these studies are the sole explanation of these marked discrepancies.

Although the growth of twins in utero is slower than the one of singletons from about 30 gestational weeks, the results of our and previous studies[54] seem to demonstrate that this does not result in an aberrant body composition, neither at birth nor in the first months of life. This might support the hypothesis that the reduction in fetal growth during twin gestation has the scope of sparing maternal and placental resources and saving them for the growth of both twins, as in a sort of "physiological" adaptation[53].

If growth retardation in utero results in small for gestational age twins, they do not seem to be dissimilar or more severely affected than singleton SGA infants, as regards body composition. On the opposite, as body composition of SGA twins resulted to be similar to that of SGA singletons that were ~ 1 week older, it might be postulated that both weight and % fat mass could be even higher than those of SGA singletons in SGA twins of comparable gestational age. Postnatal growth of SGA twins followed substantially the path of SGA singletons in the first 3 months.

In conclusion, we hereby report the following main results. Appropriate for gestational age infants were found to have a similar body composition than those of another Northern Italian population and their % fat mass increased significantly from birth to 3 months of age. During the physiological weight loss, fat free mass was the compartment most affected, and this was reproduced in all categories of babies, term appropriate for gestational age, late preterm, small and large for gestational age singletons and twins. No difference emerged in body composition between babies born from vaginal delivery or caesarean section or between boys and girls. Late preterm infants, although leaner at birth, resulted to be have a higher fat content than term infants at term-equivalent age. Small for gestational age infants were smaller and with less fat mass at birth but gained more fat than both AGA and LGA infants in the first month of life. Conversely, LGA infants had a higher fat content at birth but did not differ from AGA infants at one month of life. Body composition of AGA and SGA twins did not differ from that of AGA and SGA singletons, respectively, at birth or in the first three months of life.

These results show that body composition at birth and in early life can differ significant in different categories of babies and according to several factors (type of pregnancy, nutritional status at birth, gestational length,...). Assessing body composition in newborns from the first months of life can provide the basis to follow up their growth not only quantitatively but qualitatively and to elaborate personalized feeding strategies aimed at achieving a balanced growth of the various components of body mass, with the final perspective of maximizing their chances for future health.

Bibliography

1. Barker DJP, Osmond C. Infant mortality, childhood nutrition and ischaemic heart disease in England and Wales. Lancet 1986;i: 1077-81.

2. Barker DJ. The fetal and infant origins of adult disease. BMJ. 1990 Nov 17;301(6761):1111.

3. Wells JC, Chomtho S, Fewtrell MS. Programming of body composition by early growth and nutrition. Proc Nutr Soc 2007;66(3):423-34.

4. Lucas A, Fewtrell MS, Cole TJ. Fetal origins of adult disease-the hypothesis revisited. BMJ 1999;319(7204):245-9.

5. Singhal A, Kennedy K, Lanigan J, Fewtrell M, Cole TJ, Stephenson T, Elias-Jones A, Weaver LT, Ibhanesebhor S, MacDonald PD, Bindels J, Lucas A. Nutrition in infancy and long-term risk of obesity: evidence from 2 randomized controlled trials. Am J Clin Nutr 2010;92(5):1133-44.

6. Ong KK, Ahmed ML, Emmett PM, Preece MA, Dunger DB. Association between postnatal catch-up growth and obesity in childhood: prospective cohort study. BMJ. 2000 Apr 8;320(7240):967-71

7. Barker DJ, Osmond C, Forsén TJ, Kajantie E, Eriksson JG. Trajectories of growth among children who have coronary events as adults. N Engl J Med. 2005 Oct 27;353(17):1802-9.

8. Eriksson JG, Osmond C, Kajantie E, Forsén TJ, Barker DJ. Patterns of growth among children who later develop type 2 diabetes or its risk factors. **Diabetologia. 2006** Dec;49(12):2853-8.

9. Singhal A, Cole TJ, Lucas A. **Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials. Lancet**. 2001 Feb 10;357(9254):413-9.

10. Eriksson JG, Forsén TJ, Kajantie E, Osmond C, Barker DJ. Childhood growth and hypertension in later life. Hypertension. 2007 Jun;49(6):1415-21.

11. Singhal A, Lucas A. Early origins of cardiovascular disease: is there a unifying hypothesis? Lancet. 2004 May 15;363(9421):1642-5.

12. McCance RA. Food, growth and time. Lancet 1962;2:2712.

13. Alexander GR, Himes JH, Kaufman RB, Mor J, Kogan M. A United States national reference for fetal growth. Obstet Gynecol 1996;87(2):163-8.

14. Ziegler EE, O'Donnell AM, Nelson SE, Fomon SJ. Body composition of the reference fetus. Growth. 1976 Dec;40(4):329-41

15. Lapillonne A et al. Body composition in appropriate arid in small for gestational age infants. Acta Pediatr 1997;86:196-200.

16. Maisels MJ, Gifford K: Breast feeding, weight loss and jaundice. J Pediatr 1983; 102: 117-118.

17. Maisels MJ, Gifford K, Antle CE, Leib GR. Jaundice in healthy newborn infant: a new approach to an old problem. Pediatrics 1988;81: 505–511.

18. WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: Methods and development. Geneva: World Health Organization, 2006.

19. **Fomon SJ**, **Haschke F**, **Ziegler EE**, **Nelson SE**. Body composition of reference children from birth to age 10 years.**Am J Clin Nutr 1982** May;35(5 Suppl):1169-75.

20. Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. Body composition during the first 2 years of life: an updated reference. Pediatr Res 2000;47:578–85.

21. Carberry AE, Colditz PB, Lingwood BE. Body composition from birth to 4.5 months in infants born to non-obese women. Pediatr Res 2010;68(1):84-8.

22. Roggero P, Giannì ML, Orsi A, Piemontese P, Amato O, et al. Quality of growth in exclusively breast-fed infants in the first six months of life: an Italian study. Pediatr Res 2010;68(6):542-4.

23. Eriksson B, Löf M, Forsum E. Body composition in full-term healthy infants measured with air displacement plethysmography at 1 and 12 weeks of age. Acta Paediatr 2010;99(4):563-8.

24. Fields DA, Krishnan S, Wisniewski AB. Sex differences in body composition early in life. Gend Med 2009;6(2):369-75.

25. Rigo J et al. Reference values of body composition obtained by dual energy X-ray absorptiometry in preterm and term neonates. J Pediatr Gastroenterol Nutr 1998;27(2):184-90.

26. Hammami M, Walters JC, Hockman EM, Koo WW. Disproportionate alterations in body composition of large for gestational age neonates. J Pediatr. 2001 Jun;138(6):817-21.

27. Durnwald C, Huston-Presley L, Amini S, Catalano P. Evaluation of body composition of large-for-gestational-age infants of women with gestational diabetes mellitus compared with women with normal glucose tolerance levels. Am J Obstet Gynecol. 2004 Sep;191(3):804-8.

28. Schmelzle HR, Quang DN, Fusch G, Fusch C. Birth weight categorization according to gestational age does not reflect percentage body fat in term and preterm newborns. Eur J Pediatr. 2007 Feb;166(2):161-7.

29. Griffiths LJ, Dezateux C, Cole TJ. Differential parental weight and height contributions to offspring birthweight and weight gain in infancy. Int J Epidemiol. 2007 Feb;36(1):104-7.

30. Hull HR, **Dinger MK**, **Knehans AW**, **Thompson DM**, **Fields DA**. Impact of maternal body mass index on neonate birthweight and body composition. **Am J Obstet Gynecol 2008**;198(4):416.e1-6.

31. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. **Fetuses of obese mothers develop insulin resistance in utero. Diabetes Care** 2009;32(6):1076-80.

32. HAPO Study Cooperative Research Group. Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Study: associations with maternal body mass index. BJOG 2010;117:575–584.

33. Catalano PM, Farrell K, Thomas A, Huston-Presley L, Mencin P, et al. Perinatal risk factors for childhood obesity and metabolic dysregulation. Am J Clin Nutr 2009;90:1303–13.

34. Whitaker RC. Predicting preschooler obesity at birth: the role of maternal obesity in early pregnancy. Pediatrics 2004;114:e29 –e36.

35. Institute of Medicine. Weight gain during pregnancy: reexamining the guidelines. The National Academies Press; Washington, DC: 2009.

36. Crozier SR, Inskip HM, Godfrey KM, Cooper C, Harvey NC, Cole ZA, Robinson SM, and SWS Study Group. Weight gain in pregnancy and childhood body composition: findings from the Southampton Women's Survey. Am J Clin Nutr. 2010 June ; 91(6): 1745–1751.

37. Fraser A, Tilling K, Macdonald-Wallis C, Sattar N, Brion MJ, Benfield L, Ness A, Deanfield J, Hingorani A, Nelson SM, Smith GD, Lawlor DA. Association of maternal weight gain in pregnancy with offspring obesity and metabolic and vascular traits in childhood. Circulation. 2010 Jun 15;121(23):2557-64.

38. Lindsay CA. Pregnancy complicated by diabetes mellitus. In: Martin RJ, Fanaroff AA, Walsh MC. Neonatal-perinatal medicine, 8th edition, Mosby Elsevier 2006.

39. The HAPO Study Cooperative Research Group. Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Associations With Neonatal Anthropometrics. Diabetes 2009;58:453–459.

40. Pedersen J. The pregnant diabetic and her newborn: problems and management, 2nd Ed. Baltimore, Williams & Wilkins, 1977.

41. Lucas A, Sarson DL, Blackburn AM, Adrian TE, Aynsley-Green A, Bloom SR. Breast vs bottle: endocrine responses are different with formula feeding. Lancet 1980;1:1267–9.

42. Heinig MJ, Nommsen LA, Peerson JM, Lonnerdal B, Dewey KG. Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING Study. Am J Clin Nutr 1993;58:152–61.

43. Butte **NF**, **Wong WW**, **Hopkinson JM**, **Smith EO**, **Ellis KJ**. Infant feeding mode affects early growth and body composition. **Pediatrics. 2000** Dec;106(6):1355-66.

44. Beyerlein A and von Kries R. Breastfeeding and body composition in children: will there ever be conclusive empirical evidence for a protective effect against overweight? Am J Clin Nutr 2011;94(suppl):1772S–5S.

45. **Anderson AK**. Association between Infant Feeding and Early Postpartum Infant Body Composition: A Pilot Prospective Study. **Int J Pediatr**2009;2009:648091.

46. De Curtis M, Pieltain C, Studzinski F, Moureau V, Ge´rard P, Rigo J. Evaluation of weight gain composition during the first 2 months of life in breast- and formula fed term infants using dual energy X-ray absorptiometry. Eur J Pediatr 2001;160:319–320

47. Astolfi P, Ulizzi L, Zonta LA. Changes in twinning rate: Italy 1950-1996. Hum Reprod. 2003;18(1):207-11

48. Martin JA, Kung HC, Mathews TJ, Hoyert DL, Strobino DM, Guyer B, Sutton SR: Annual summary of vital statistics: 2006. Pediatrics 2008; 121: 788–801.

49. Chauhan SP, Scardo JA, Hayes E, Abuhamad AZ, Berghella V. Twins: prevalence, problems, and preterm births. Am J Obstet Gynecol 2010;203(4):305-15.

50. Alexander GR, Kogan M, Martin J, Papiernik E. What are the fetal growth patterns of singletons, twins, and triplets in the United States? Clin Obstet Gynecol 1998;41(1):114-25

51. Loos RJ, Derom C, Derom R, Vlietinck R. Determinants of birthweight and intrauterine growth in liveborn twins. Paediatr Perinat Epidemiol 2005;19 Suppl 1:15-22.

52. Naeye RL, Benirschke K, Hagstrom JW, Marcus CC. Intrauterine growth of twins as estimated from liveborn birth-weight data. Pediatrics 1966;37:409–416.

53. Blickstein I. Is it normal for multiples to be smaller than singletons? Best Pract Res Clin Obstet Gynaecol 2004 ;18(4):613-23.

54.Demarini S, Koo WW, Hockman EM. Bone, lean and fat mass of newborn twins versus singletons. Acta Paediatr. 2006 May;95(5):594-9.

55. Kingdom JCP, Nevo O and Murphy KE. Discordant growth in twins. Prenat Diagn 2005; 25: 759–765.

56. Blickstein I, Lancet M. The growth discordant twin. Obstet Gynecol Surv. 1988 Sep;43(9):509-15.

57. Yinon Y, Mazkereth R, Rosentzweig N, Jarus-Hakak A, Schiff E, Simchen MJ.Growth restriction as a determinant of outcome in preterm discordant twins. Obstet Gynecol 2005;105:80–84.

58. Koo B, Walters J, Hockman E, Koo W. Body composition of newborn twins: intrapair differences. J Am Coll Nutr 2002;21(3):245-9.

59. van Dommelen P, de Gunst M, van der Vaart A, van Buuren S, Boomsma D. Growth references for height, weight and body mass index of twins aged 0-2.5 years. Acta Paediatr 2008;97(8):1099-104.

60. Buckler JM, Green M. The growth of twins between the ages of 2 and 9 years. Ann Hum Biol. 2008 Jan-Feb;35(1):75-92.

61. Ali MA, Ashizawa K, Kato S, Kouchi M, Koyama C, Hoshi H. Biological variables in height growth of Japanese twins: a comparison with those of singletons. Ann Hum Biol 2007;34: 283–295.

62. Andrew T, Hart DJ, Snieder H, de Lange M, Spector TD, MacGregor AJ. Are twins and singletons comparable? A study of disease-related and lifestyle characteristics in adult women. Twin Res 2001;4(6):464-77.

63. Skidmore PM, Cassidy A, Swaminathan R, Richards JB, Mangino M, Spector TD, MacGregor AJ. An obesogenic postnatal environment is more important than the fetal environment for the development of adult adiposity: a study of female twins. Am J Clin Nutr 2009;90(2):401-6.

64. McMillen C, Robinson JF. Developmental origins of the metabolic syndrome: prediction, plasticity, and programming. Physiol Rev 2005;85:571–633.

65. Wells JCK. A Hattori chart analysis of body mass index in infants and children. International Journal of Obesity 2000;24:325–329.

66. Catalano PM, Thomas AJ, Avallone DA, Amini SB. Anthropometric estimation of neonatal body composition. Am J Obstet Gynecol 1995;173(4):1176-81.

67. Koo W. Body composition measurements during infancy. Ann N Y Acad Sci 2000;904:383-92.

68. Rigo J, de Curtis M, Pieltain C. Nutritional assessment in preterm infants with special reference to body composition. Semin Neonatol 2001;6(5):383-91.

69. Fomon SJ, Nelson KM. Body composition of the male and female reference infants. Ann Rev Nutr 2002;22:1–17.

70. Ellis KJ. Evaluation of body composition in neonates and infants. Semin Fetal Neonat Med 2007;12:87-91.

71. Rigo J. Body composition during the first year of life. Nestle Nutr Workshop Ser Pediatr Program 2006;58:65-76.

72. de Bruin NC, van Velthoven KA, Stijnen T, et al. Body fat and fatfree mass in infants: new and classic anthropometric indexes and prediction equations compared with total-body electrical conductivity. Am J Clin Nutr 1995;61:1195–205.

73. Roggero P et al. Measuring the body composition of preterm and term neonates: from research to clinical application. J Pediatr Gastroenterol Nutr 2007;45:S159–S162.

74. Lafeber HN. Nutritional assessment and measurement of body composition in preterm infant. Clin Perinatol 1999;26:997–1005.

75. Rigo J et al. Reference values of body composition obtained by dual energy X-ray absorptiometry in preterm and term neonates. J Pediatr Gastroenterol Nutr 1998;27(2):184-90.

76. Uthaya S, Bell J, Modi N. Adipose tissue magnetic resonance imaging in the newborn. Horm Res 2004;62 (Suppl 3):143–8

77. Scholten RR, Pillen S, Verrips A, Zwarts MJ. Quantitative ultrasonography of skeletal muscles in children: normal values. Muscle Nerve. 2003;27(6):693-8.

78. Ahmad I, Nemet D, Eliakim A, Koeppel R, Grochow D, Coussens M, Gallitto S, Rich J, Pontello A, Leu SY, Cooper DM, Waffarn F. Body composition and its components in preterm and term newborns: A cross-sectional, multimodal investigation. Am J Hum Biol. 2010;22(1):69-75.

79. Dempster P, Aitkens S. A new air displacement method for the determination of human body composition. Med Sci Sports Exerc 1995;27:1692–7.

80. Fields DA, Goran MI, McCrory MA. Body-composition assessment via air-displacement plethysmpgraphy in adults and children: a review. Am J Clin Nutr 2002;75:453–67.

81. Eriksson B, Löf M, Eriksson O, Hannestad U, Forsum E. Fat-free mass hydration in newborns: assessment and implications for body composition studies. Acta Paediatr. 2011 May;100(5):680-6.

82. Urlando A, Dempster P, Aitkens S. A new air displacement plethysmograph for the measurement of body composition in infants. Pediatr Res 2003;53:486–92.

83. Boyd E. Experimental error inherent in measuring growing human body. Am J Phys Anthropol 1930 13:389–432

84. **Stocks J**, **Quanjer PH**. Reference values for residual volume, functional residual capacity and total lung capacity. ATS Workshop on Lung Volume Measurements. Official Statement of The European Respiratory Society. **Eur Respir J. 1995** Mar;8(3):492-506.

85. Sainz RD, Urlando A. Evaluation of a new pediatric air-displacement plethysmograph for body-composition assessment by means of chemical analysis of bovine tissue phantoms. American Journal of Clinical Nutrition 2003;77:364-370.

86. Ma G, Yao M at al. Validation of a new pediatric air-displacement plethysmograph for assessing body composition in infants. Am J Clin Nutr 2004;79:653-60.

87. Ellis KJ, Yao M, Shypailo J, et al. Body-composition assessment in infancy: air-displacement plethysmography compared with a reference 4-compartment model. Am J Clin Nutr 2007;85:90-5.

88. Yao M, Nommsen-Rivers L, Dewey K, Urlando A. Preliminary evaluation of a new pediatric air displacement plethysmograph for body composition assessment in infants. Acta Diabetol 2003;40:S55–S58.

89. Raju TN, Higgins RD, Stark AR, Leveno KJ. Optimizing care and outcome for late-preterm (near-term) infants: a summary of the workshop sponsored by the National Institute of Child Health and Human Development. Pediatrics. 2006;118(3):1207-14.

90. SMILA Neonatal Standards for North-East Italy. Montecatini Sep 1996

91. International Association of Diabetes and Pregnancy Study Groups. Recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care 2010; 33: 676–682.

92. Cleophas TJ, Zwinderman AH, Cleophas TF. Statistics applied to clinical trials. 3rd Ed. Springer ed. 2006.

93. Fields DA, Gilchrist JM, Catalano PM, Giannì ML, Roggero PM, Mosca F. Longitudinal body composition data in exclusively breast-fed infants: a multicenter study. Obesity (Silver Spring). 2011 Sep;19(9):1887-91.

94. Koo WW, Walters JC, Hockman EM. Body composition in neonates: relationship between measured and derived anthropometry with dual-energy X-ray absorptiometry measurements. Pediatr Res. 2004 Nov;56(5):694-700.

95. Macdonald PD, Ross SR, Grant L, Young D. Neonatal weight loss in breast and formula fed infants. Arch Dis Fetal Neonatol 2003;88:F472–F476.

96. Rodriguez G, Ventura P, Samper MP, Moreno L, Sarría A, Pérez-González JM: Changes in body composition during the initial hours of life in breast-fed healthy term newborns. Biol Neonate 2000; 77:12–16.

97. Roggero P, Giannì ML, Orsi A, Piemontese P, Amato O, Moioli C, Mosca F. Neonatal period: body composition changes in breast-fed full-term newborns. Neonatology. 2010;97(2):139-43.

98. Shaffer SG, Meade VM. Sodium balance and extracellular volume regulation in very low birth weight infants. J Pediatr 1989;115:285–90.

99. Wang ML. Clinical Outcomes of Near-Term Infants. Pediatrics 2004;114;372-376.

100. Escobar GJ, Gonzales VM, Armstrong MA, Folck BF, Xiong B, Newman TB. Rehospitalization for neonatal dehydration: a nested case-control study. Arch Pediatr Adolesc Med. 2002;156(2):155-61.

101. Yurdakök M. Transient tachypnea of the newborn: what is new? J Matern Fetal Neonatal Med. 2010 Oct;23 Suppl 3:24-6.

102. **Zanardo V**, **Padovani E**, **Pittini C**, **Doglioni N**, **Ferrante A**, **Trevisanuto D**. The influence of timing of elective cesarean section on risk of neonatal pneumothorax. **J Pediatr.** 2007;150(3):252-5.

103. Cassady G. Effect of Cesarean Section on Neonatal Body Water Spaces. N Engl J Med 1971; 285:887-891

104. Roggero P, Giannì ML, Amato O, Orsi A, Piemontese P, Morlacchi L, Mosca F. Is term newborn body composition being achieved postnatally in preterm infants? Early Hum Dev 2009;85(6):349-52.

105. Roggero P, Giannì ML, Liotto N, Taroni F, Orsi A, Amato O, Morlacchi L, Piemontese P, Agosti M, Mosca F. Rapid recovery of fat mass in small for gestational age preterm infants after term. PLoS One. 2011 Jan 5;6(1):e14489.

106. Giannì ML, Roggero P, Taroni F, Liotto N, Piemontese P, Mosca F. Adiposity in small for gestational age preterm infants assessed at term equivalent age. Arch Dis Child Fetal Neonatal Ed. 2009 Sep;94(5):F368-72.

107. Yau K-IT , Chang M-H. Growth and body composition of preterm, small-for-gestational-age infants at a postmenstrual age of 37–40 weeks. Earl Hum Develop 1993; 33(2):117-131.

108. Labayen I, Ruiz JR, Vicente-Rodríguez G, Turck D, Rodríguez G, Meirhaeghe A, Molnár D, Sjöström M, Castillo MJ, Gottrand F, Moreno LA; Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study Group. Early life programming of abdominal adiposity in adolescents: The HELENA Study. Diabetes Care. 2009 Nov;32(11):2120-2.

109. Ekelund U, Ong K, Linne Y, et al: Upward weight percentile crossing in infancy and early childhood independently predicts fat mass in young adults: The Stockholm weight development study. Am J Clin Nutr 83:324-330, 2006

110. Morrison JL, Duffield JA, Muhlhausler BS, Gentili S, McMillen IC (2010) Fetal growth restriction, catch-up growth and the early origins of insulin resistance and visceral obesity. Pediatr Nephrol 25: 669–677.

111. **Albertsson-Wikland K**, **Boguszewski M**, **Karlberg J**. Children born small-for-gestational age: postnatal growth and hormonal status. **Horm Res.** 1998;49 Suppl 2:7-13.

112. Karlberg JP, Albertsson-Wikland K, Kwan EY, Lam BC, Low LC. The timing of early postnatal catch-up growth in normal, full-term infants born short for gestational age. Horm Res. 1997;48(suppl 1):17–24

113. Hattersley AT, Tooke JE. The fetal insulin hypothesis: an alternative explanation of the association of low birthweight with diabetes and vascular disease. Lancet. 1999 May 22;353(9166):1789-92

114. Singhal A, Wells J, Cole TJ, Fewtrell M, and Lucas A. Programming of lean body mass: a link between birth weight, obesity, and cardiovascular disease? Am J Clin Nutr 77: 726–730, 2003.