

**Body Composition Assessment in Early Infancy: A Review**

A White Paper

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by

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## Introduction

There is an increasing interest in the association between nutritional status during early infancy and childhood with the increased risks for adverse health effects as adults. For example, osteoporosis can be viewed, in part, as a 'pediatric disease' with its origins traced to less than optimal mineralization of the skeleton during growth. Likewise, there is increasing interest in the association between body fatness during infancy and the increasing incidence of childhood and adult obesity. In the past, basic anthropometry (the measurements of body weight, length, circumferences, and skinfold thickness) have been used to assess growth patterns during infancy. These indices, although useful on a population basis, are usually too crude to distinguish changes in the composition of weight. In recent years, a number of non-invasive techniques have been developed for the in-vivo assessment of human body composition [Ellis, 2000]. Although most of these techniques are intended for use in adults, several have been extended to the examination of pediatric populations.

Body composition refers to the tissue, organ, or physiological systems of the body that make up body weight. Both simple and complex models of body composition have been developed [Figure 1], based on chemical and molecular content, physiological function, and anatomical makeup. The simplest model, called the two-compartment (2-C) model, dividing weight into fat mass (FM) and fat-free mass (FFM). The more sophisticated models describe the subcomponents of the FFM, such as water, protein, and mineral. Some models describe

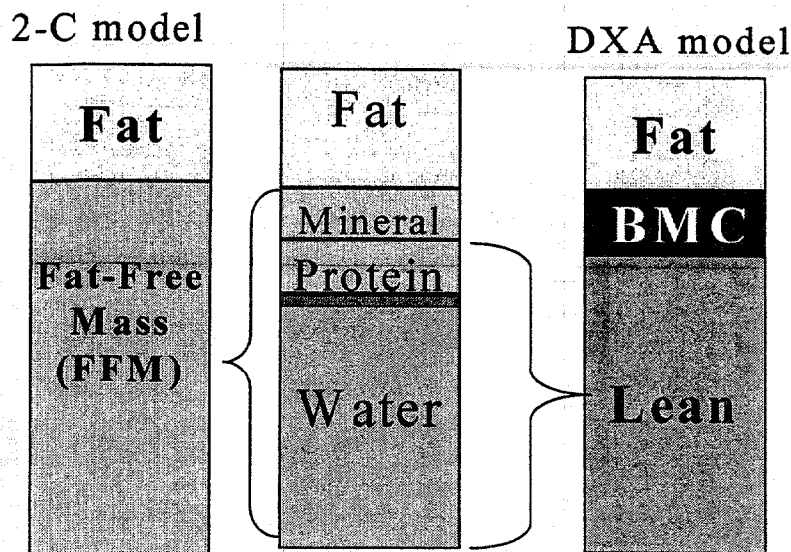


Figure 1: The classic two-compartment (2-C) model, multi-compartment model, and DXA-derived model of body composition. The body's water, glycogen, and protein mass make up the Lean mass obtained using dual-energy x-ray absorptiometry (DXA), while the fat-free mass is the compartment plus the body's mineral content.

functional components, such as body cell mass (BCM) which has no direct anatomical comparison. The reference infant model developed by Ziegler and Fomon is based on the measurements of total body potassium (TBK) and total body water (TBW), coupled with an estimate for bone mass. TBK, on the other hand, can be used to monitor BCM, which is considered to be the active metabolizing tissues of the body [Moore et al 1963]. There are also

body composition measurements techniques for the in-vivo assess of the mineral content of bone. The current technology is called dual-energy x-ray absorptiometry (DXA). Although these instruments have been built to assess bone mineral status of adults, specifically focusing on the detection of osteoporosis in older women, pediatric software continues to be developed. If the whole body is scanned, the DXA technique can also be used to provide estimates for the body's fat content, and non-bone soft tissue mass. Secondary assays, such as those based on the electrical properties of the body's wet tissues, have been developed in recent years, but these are not direct assays of body composition. The parameters measured by these techniques must be calibrated using a more direct assay such as those for TBW or BCM.

### **Body Composition Models and Methods**

The measurement of total body water (TBW), total body potassium (TBK), and body density (D) have been used with the two-compartment (2-C) model. That is, an estimate of FFM is first derived, so that FM is then be defined as Wt minus FFM. The major limitation with this approach is that the absolute error (in mass units) for the larger FFM is fully transferred to the smaller FM component. In the new-born infant, FM is about 13-15% of body Wt, so that an error of 3% for FFM becomes 17% when FM is calculated. Furthermore, it is known that the hydration content of the FFM does not remain constant during early infancy and may be altered by disease or medications. As noted previously, TBK is synonymous with BCM, but its contribution to the total FFM may not be constant, especially when the extracellular to intracellular water ratio is abnormal. Thus, the use of only TBW or TBK to estimate FFM (and FM indirectly) can be in error, which only amplifies the uncertainty of the FM estimate.

As an alternative to the isotope dilution technique to measure TBW, use of the body's electrical properties of the body have been investigated [NIH 1996]. These techniques include total body electrical conductivity (TOBEC) and bioelectrical impedance analysis (BIA). Both methods are based on the fact the electrolyte and water content of the lean tissues in the body. In the first method, when the body is passed through an external electro-magnetic field, the free charge particles in the body will align with the magnetic field causing a small perturbation in the original field, which is measured. For the second method, the body's resistance to a very weak electrical current (50 kHz, 800  $\mu$ Amp) is measured. Both are secondary assays, which means they must be calibrated using a more direct assay, such as TBW or TBK [Ellis 2000].

An alternate 2-C model of body composition, based on body density, has been used to estimate FFM and FM. For this model to be accurate, the density of FFM must be known very precisely. Thus this approach is limited by the fact that the density of FFM changes rapidly with its hydration during early infancy. Furthermore, the measurement technique requires that the subject be totally submersion in water, thus ruling out this assay for infants. This latter requirement may soon be eliminated if a technique called air-displacement plethysmography (ADP) can be used successfully with infants, but these evaluations are still in the development phase [Urlando et al 2002].

The best technique that is available for the measurement of bone mineral content (BMC) or bone mass is dual-energy x-ray absorptiometry (DXA). As the name implies, this technique uses x-rays to scan the body; the dose is very low, comparable to that received during a 3-hr commercial airplane flight. In order to provide a quantitative measure of BMC, the composition of the surrounding soft tissue must be known. This is achieved by using the non-bone pixels in the DXA scan in regions of only soft tissue. Thus, a whole-body DXA scan gives a 3-compartment (3-C) model of body weight: BMC, FM, and LTM (non-bone, non-fat lean tissue

mass). Most of the commercial instruments developed for human body composition measurements have focused on bone measurements in adults, the design of DXA scanners is no exception. These instruments do have optional pediatric software available, but the manufacturers' technical support and continued develop in this area will be slow until a clear market is identified.

If the study objective is to assess changes in body composition, then body composition should be used. Anthropometric indices of growth (weight, height, circumferences) are secondary to the study aim, but do provide comparisons with previous studies.

There are gender differences in the weight gain and composition [Rupich et al 1996].. Thus, the control and study groups should be matched for gender distribution ( it is assumed that these groups will also be matched for age). The differences in body composition and in the growth patterns requires larger sample sizes. BMI can be monitored, but this index is not an acceptable assay for body composition or nutritional status, except at the extremes of the BMI distribution.

If the target organ is bone mineral mass, then only DXA can be used. If it is FM or FFM, then several methods can be used. However, DXA also gives reliable estimates for FM and FFM, thus it tends to provide the best overall method for use with infants. Furthermore, this technique has probably been used the most in recent infant studies and, the accuracy of the technique continues to be improved for pediatric use, and it is the one method that is most readily available for multi-site studies compared with the alternate choices (TOBEC, TBK, TBW, BIA).

The excellent work by Pieltain et al [2001] shows that DXA has sufficient precision and sensitivity to detect differences in body composition at 3 wks related to the feeding choice. There are gender differences in body composition at birth, that tend to remain evident during the first 24 months [Butte et al 2000a]. What this does on a population basis is to increase the biological variability at baseline, and will average the gender differences in gain with aging. In statistical terms, these two effects result in the requirement of an increase sample size and/or longer time interval between measurements to detect the same difference within or between groups which have both gender. I am not suggesting that only one gender be examined, only that the inclusion of both gender increases the biological variability, which usually translates to higher sample sizes.

### **Selection of Method for Body Composition Assessment in Infants**

Body composition measurements during early infancy can provide more information about the nutritional status of infants than the simple measurements of body length or weight. It is reasonable to assume that the body composition assays that are presently available reflect functional tissues and energy deposition [Lapillonne et al, 1999]. A summary of the body composition methods current available for use in infants, the corresponding body composition compartment that is measured, and the precision and accuracy for infants are summarized in Table 1. In addition, the estimated minimal detectable change (MDC) between two measurements that would be statistical significant for an individual infant (clinical case) are included. To detect smaller changes in body composition as significant, population studies must be performed [Hassager et al 1995]. Alternatively, if the measurement precision can be improved or the biological variability of the population reduce, this will also increase the probability of detecting smaller changes in body composition as significant. A second consideration (see following section) relates to the time interval between repeat measurements.

Table 1. Precision and accuracy of different body composition methods and minimum detectable change for an infant\*

| Body Composition Compartment | Method <sup>a</sup>       | Measurement                |                           | Minimum Detectable Change <sup>d</sup> (infant) |
|------------------------------|---------------------------|----------------------------|---------------------------|---|
|                              |                           | Precision <sup>b</sup> (%) | Accuracy <sup>c</sup> (%) |   |
| TBW                          | D <sub>2</sub> O dilution | 1-2%                       | 2-4%                      | 100 ml ( 5% )                                   |
|                              | BIA /BIS                  | 2-4%                       | 3-7%                      | 200 ml (10%)                                    |
|                              | TOBEC                     | 2-3%                       | 4-6%                      | 150 ml (8%)                                     |
| BCM                          | TBK                       | 2-3%                       | 3-5%                      | 17 mEq (5%)                                     |
| FFM                          | DXA                       | 1.5%                       | 1-4%                      | 125 g (5%)                                      |
| FM                           | DXA                       | 2-3%                       | 3-5%                      | 50 g ( 9%)                                      |

<sup>a</sup>BIA=bioelectrical impedance analysis, BIS=bioelectrical impedance spectroscopy  
DXA = dual-energy x-ray absorptiometry

<sup>b</sup>Reproducibility for repeat measurements

<sup>c</sup>Accuracy error for absolute mass

<sup>d</sup>Percentage change based on 3.5 kg infant with 15% fat.

This is crucial in the study of infants, for if the time interval is too short, then the amount of change that would be needed to reach significance may not be physiologically possible (limited by the accretion rate). Likewise, if the time period becomes relative long, then normal growth and physiological changes in body composition may mask the amplitude of the effect being studied.

To assess the suitability of any body composition assay to detect longitudinal changes, the most important parameter is precision. The relation of MDC between two measurements (at 5% significance) with precision for an individual is given in Figure 2(a). If the precision is 1%, then MDC must be greater than 2.8%. If the precision is only 5%, then the MDC must be greater than 14%. That is, the poorer the precision, the greater must the change be to reach significance, which usually translates to a longer time interval between the two measurements. For population studies, both precision of the measurement and the biological variability within the population will define the sample size. The relation between sample size, precision, and biological variability (SD%) is shown in Figure 2b for the detection (5% significance level) of a MDC of 1%.

Pieltain et al [2001] used whole-body DXA to examine changes in body composition of preterm infants fed fortified human milk or a preterm formula. They calculated that a sample size of 20 infants per group would be sufficient to achieve a MDC at 3 weeks at a 5% significance level. For the longitudinal changes in the same infants, the minimal increases needed (to reach statistical significance) were 111 g lean tissue, 68 g fat, and 3.1 g bone mineral content. To achieve differences in each body composition compartment between the human milk and formula groups at discharge (3wks), the minimal group differences would have to be 160 g lean tissue, 86 g fat, and 4.1 g bone mineral, for a total weight gain difference of at least 250 g. If only body weight was used, and not body composition, then a 8% weight difference or 182 g would be significant. Wells [2001] and Lapillonne et al [1999] have also reviewed the use of body composition assessment as an indicator of nutritional status in infancy. These authors conclude that these techniques offer the best examination of the composition of weight gain.

Both a control group ( breast-fed or standard formula) and the new formula group should be compared for differences in outcome. If only FM and FFM are of interest, then TBW, TBK, TOBEC, or DXA can be used. If changes in bone mass is an outcome variable, then only DXA

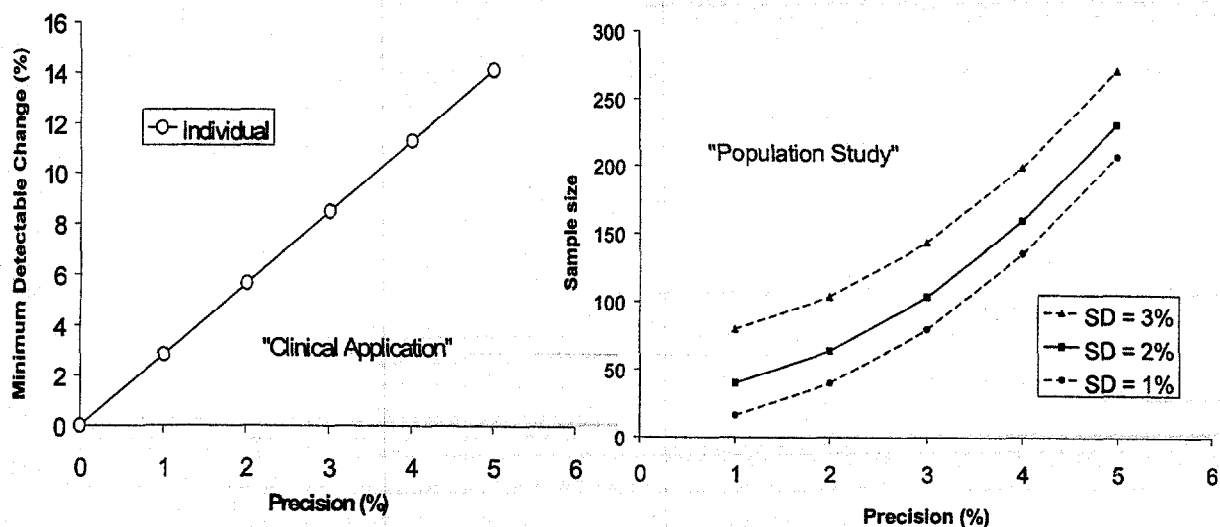


Figure 2: (a) Minimum detectable change for an individual as a function of the precision of the assay (5% significance level). (b) Sample size relation to precision and biological variance of the population to detect a minimum change of 1% (5% significance level).

can be used. However, DXA will also give simultaneous information about FM and FFM.

### Reference Body Composition Data for Infancy

As previously noted, most instruments have not been developed for use with infants, thus the manufacturers have not supplied any information related to a reference database at these ages. Many of the studies in early infancy have provided only group means at the beginning and end of the study period. However, there are several published paper with more detailed body composition information that could be considered as 'reference' data for infants [Butte et al 2000, Butte et al 2001, Koo et al 2000, Koo et al 2002, de Bruin et al 1996, Nyamugabo et al 1998, Rigo et al 1998]. These data have been derived using one or more of the following assays: TBW by D<sub>2</sub>O dilution, TBK by <sup>40</sup>K counting, TOBEC, BIA, and DXA. Of these assays, body composition data using DXA is the most prevalent. Although both cross-sectional and longitudinal data are available [Butte et al 2000, Butte et al 2001, Koo et al 2000, Koo et al 2002, de Bruin et al 1996, Nyamugabo et al 1998, Rigo et al 1998], most of the studies have been cross-sectional in design. The recent work of Butte et al [2000a, 2000b] provides an contemporary model of body composition using the criteria established by Fomon et al [1982]. A summary of the cross-sectional and longitudinal studies is provided in Table 2. These studies should not be construed as the only publications with sufficient body composition to develop a reference database for pre-term and full-term infant during early life. There are a number of infant studies that have reported mean values for body composition near birth and then a second time (usually 1-6 months later) following some form of feeding or intervention. These studies provide sufficient information to make reasonable estimates of the average expected rates of change (g/d) for FM and FFM for the time internal between the two measurements..

The longitudinal body composition data reported by Butte et al [2000a] was used to derive average accretions rates for Wt, FM, FFM, TBW, and BMC at different time periods over 24 months [see Figure 3 for boys]. In general, accretion rates are the highest at birth, and

Table 2. Studies with Reference Data for Body Composition in Infants

| Study Populations                          | Body Comp Assay                               | Longitudinal  | Reference             |
|--|---|---|-----------------------|
| 76 infants                                 | TBW,TBK,TOBEC,DXA                             | 0.5,3,6,9,12,18,24 mon                              |                       |
| 76 infants                                 | TBW,TBK,TOBEC,DXA                             | 0.5,3,6,9,12,18,24 mon                              | Butte et al 2000a     |
| 48 healthy full term<br>34 healthy preterm | whole-body DXA<br>whole-body DXA              | per month for 6 months<br>per 2 months for 6 months | Avila-Diaz et al 2001 |
| Study Populations<br>106 healthy infants   | Body Comp Assay<br>whole-body DXA             | Cross-Sectional<br>1-2 months                       | Rigo et al 1998       |
| 423 infants                                | TOBEC   | 2 wks - 12 months                                   | de Bruin et al 1996   |
| 163 healthy                                | BIA (phase angle)                             | 1-7 days  | Piccoli et al 2002    |
| 28 infants                                 | H <sub>2</sub> <sup>18</sup> O dilution & BIA | < 1 month   | Tang et al 1997       |
| 153 SGA                                    | Whole-body DXA                                | birth - 2 yr  | Ichiba et al 2001     |
| 64 infants                                 | Whole-body DXA                                | birth - 18 mon                                      | Rupich et al 1996     |
| 214 infants                                | Whole-body DXA                                | birth - 12 months                                   | Koo et al 2000        |

decrease with increasing age. A similar pattern was evident for girls (curves are not shown), but the rates are statistically different from those for boys. Thus, any study that includes both gender groups will need to adjust for these differences. That is, expected changes in body composition over a fixed time interval will be gender-specific, thus the study and control populations should at least be matched for gender distributions.

The expected changes in body Wt, FM, or FFM over a fixed time interval can be calculated by using the curves give in Figure 3 for boys or similar curves for girls. Since the accretion rates are decreasing with time, this means that to achieve the same mass change, the starting age will determine the required time interval. In addition, to detect statistical differences between a control (breast-fed or standard formula) and treatment (enriched formula) group, the biological variability (SD) of each group is important. In general, most cross-sectional studies of infants have indicated that the normal variations in the different body composition compartments is about 10-15% at ages less than 12 months, while it increases to about 15-18% by the age of 24 months.

The availability of acceptable assays for the multi-component composition of growth in infants is relatively new. As such, there are on-going studies that will provide sufficient reference data to define what is "expected" and what represents a "significant" risk. For adults, these limits tend to set a +/- 2 SD. It is reasonable to consider these limits for infants as well. There is sufficient body composition data to establish mean +/- SD values for several age groups up to 24 months.

### Study Design

In the design of any infant study, there tends to be two areas of concern: selection of an appropriate control group, and the time duration of the study. A reference dataset, if it is available, can be used as the control group. However, to insure that most external factors are controlled for, it may be better to monitor changes in body composition using a control group concurrent with the 'treated' group. When this is not practical or ethical, only then should an independent reference group be used for comparison. The reference group, however, does provide the information that is often needed to calculate the sample size for a study. This of course leads to the question: "What is the appropriate reference population?" Should the controls

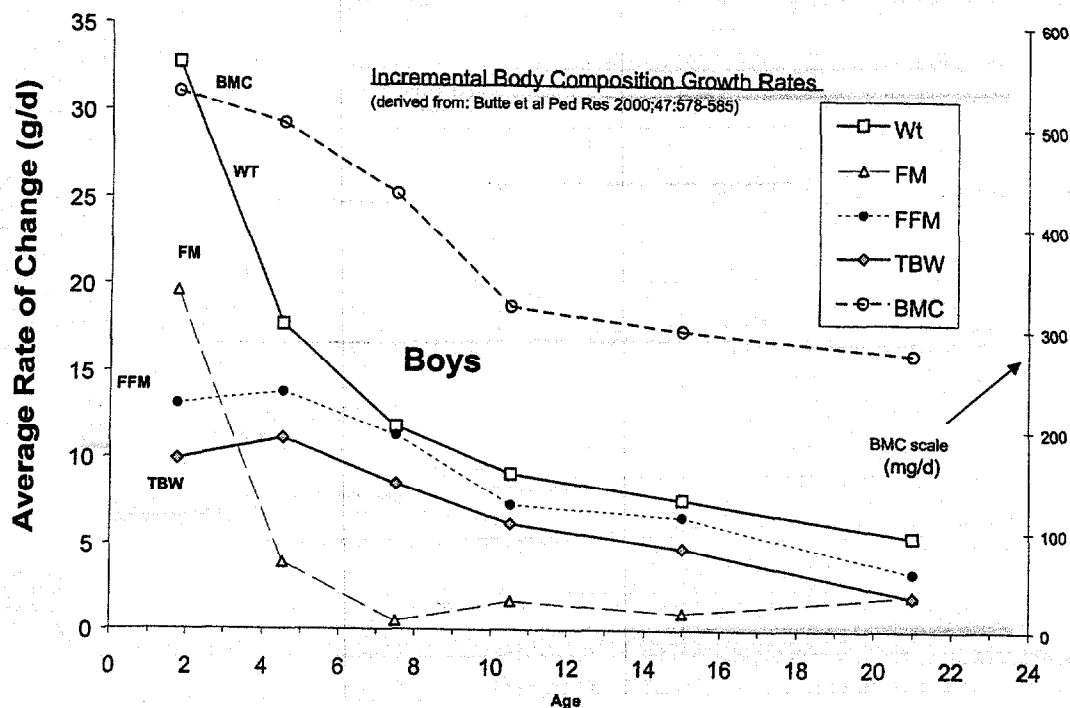


Figure 3: Changes in the Accretion Rates for Wt, FM, FFM, TBW, and BMC with Age (boys).

be breastfed or formula-fed? *This is not a new question.* There are known differences in body composition related to these feeding modes. Many factors, such as whether the infants are preterm, fullterm, AGA, or SGA, will influence the decision for the appropriate 'reference' group. Furthermore, the reference group will be defined by the target gains in body composition. For example, the NCHS/NHANES growth curves give national W-for-age, Ht-for-age, and BMI-for age percentiles for infants, but this is not a true measure of body composition. It would seem only logical to expect that nature knows the optimum feeding pattern for at least healthy infants of healthy mothers.

The outcome variable (FM, FFM, or BMC) will tend to dictate the choice of body composition assay. This, in turn, will define the length of the study period needed to achieve a measurable difference between control and treated groups. Younger infants have higher accretion rates (see Figure 2), thus a significant percentage changes in mass will occur in a shorter time interval than if the study were started late, for example, at 3 months. Hence, many clinical studies of infant feedings are performed while the infant is still in the hospital awaiting discharge. This will tend to limit the study to about 3-6 weeks. Caution must be used not to suggest that growth effects seen during this time period will continue with equal intensity after discharge simply because the accretion rates change dramatically.

The expected change in body composition will be dependent on both the starting age and the time interval of the study period. This can be calculated from the accretion rates [Butte et al 2000] illustrated in Figure 2. Pieltain et al [2001] shows that DXA could detect differences in body composition between groups at 3 wks corresponding a weight difference of 8%.



## Summary

Every method has its own set of advantages and disadvantages. There are reference FM and FFM percentiles for TOBEC, for example, but this instrument is used at only a few centers. Similar arguments can be found for whole-body counting of TBK and the dilution assay for TBW. For the TBW assay, the infant needs to ingest all of the deuterium tracer, the assay procedures tend to be labor intensive often delaying the results for weeks-months. BIA is cheap to perform, can be done quickly, but the body composition results have not been shown to be much better than using only weight and length measurements. Whole-body DXA give estimates for bone and the partition of the soft tissue into FM and lean mass. There were sufficient differences among these five assays for FM that it recommended that only one method should be used for any multi-site study [Butte et al 1999]. There may also be the need to consider geographical location as a co-factor when considering differences in FM [Galan et al 2001].

If the target outcome for body composition is bone, then the only technique currently available for use in infant is DXA. The pediatric software available for DXA instruments continues to be improved. Also, DXA is probably the one technique that is more available to most investigators. However, there are differences in the manufacturers analyzes for body composition, which are known to be instrument-, model-, and software-dependent. This means that if a multi-center study is proposed, all of the centers should use the same model of DXA instrument and the same software version. The one disadvantage of DXA is that a very small dose ( $< 10$  microSv, whole-body) is required. This dose, however, is well within the natural variation of the radiation background in the US and presents no measurable risk.

Accurate assessment of body composition in infancy and early growth is relatively new. The relation between 'normal' infant body composition and future outcome remains unknown. Any extrapolation from these ages to childhood or to adulthood should be done with caution. Several studies, however, have show correlations (limited predictive power) between infant feeding mode and childhood body size and the risk for obesity, hypertension, and diabetes as adults [Hediger et al 2001, Gillman et al 2001].

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