## **Invited commentary**

## Validation of body composition methods and assumptions

The interesting paper by Wells *et al.* (2003) in the present issue of the *British Journal of Nutrition* addresses an important issue in body composition research, namely validation of methods and assumptions, and it highlights the difficulties that scientists often face when doing this type of study.

Since the early days of *in vivo* body composition research, the methods used rely on particular assumptions, most of them based on chemical analyses of carcasses (Mitchell *et al.* 1945; Forbes *et al.* 1953, 1956; Widdowson *et al.* 1951). Many of these assumptions or rules were later confirmed by *in vivo* methods (Heymsfield *et al.* 1989). For example, based on results from chemical analyses of carcasses, the density of the fat-free mass (FFM) can be calculated to be relatively constant at 1.100 kg/l; with a given density of the fat mass (FM) of 0.900 kg/l (Fidanza *et al.* 1953) and using water displacement or underwater weighing (UWW), body volume and body density can be determined (densitometry) and body fat calculated (Siri, 1961).

The two-component model of body composition (weight = FM + FFM) assumes a constant chemical composition and hence density of the FFM. It has long been recognised that the chemical composition of the FFM, although indeed relatively constant, can vary considerably between subjects, but also within subjects, throughout lifespan and/or during illness. When using UWW, measured body volume must be corrected for residual lung volume, ideally measured simultaneously with the UWW procedure. Correction for assumed intestinal gas space must also be made to account for the 'empty' spaces in the body (Roche *et al.* 1996).

With technical developments in the last few decades, it has become possible to go beyond the simple two-component model that forms the basis for densitometry. As the FFM consists of a few distinct chemical components (the main three: water, protein and minerals), today a four-component chemical model (weight = FM+water+ protein+minerals) is regarded as the method of reference (Baumgartner et al. 1991; Heymsfield & Waki, 1991; Pietrobelli et al. 2001). In this model, variations in the chemical composition of the FFM are taken into account and only some minor assumptions are made. In a four-component model, total body water (TBW) is measured by a dilution technique (for example using <sup>2</sup>H<sub>2</sub>O), minerals using dual-energy X-ray absorptiometry (DXA), protein by densitometry or by in vivo neutron activation analysis and the fourth component, fat, is calculated as the difference of weight from the sum of the three measured components. A slightly simpler, but also less accurate, three-component model (weight = FM + water + (dry FFM)) assumes a constant protein:minerals ratio (dry FFM), an assumption that might not be valid, especially in very young or old subjects.

Despite its limitations, densitometry (UWW) was long regarded as the method of reference for body composition. However, UWW can be quite demanding for subjects and might be even impossible in seriously ill patients; the air displacement plethysmography (ADP), commercially introduced about 10 years ago (Dempster & Aitkins, 1995), is certainly an easier procedure to carry out. When using ADP, lung volume is predicted and a correction for air trapped close to the body is also made (see Wells *et al.* 2003). Since its introduction in 1995, many studies in various populations have compared traditional UWW with ADP, unfortunately with slightly differing results. The ideal method of comparison should be straightforward: as both methods measure body volume, it is this body proportion that should be compared.

Body composition measurements in young children are challenging, as it is difficult to have the full cooperation of children. For this reason, UWW and ADP comparisons in children are difficult and it is not surprising that the results reported in the literature differ (Nuñez *et al.* 1999; Fields & Goran, 2000; Demerath *et al.* 2002).

The approach chosen by Wells et al. (2003) to overcome this problem, testing the hydration of the FFM with published reference values, is interesting, but it may still leave us with more questions. There is first the use of a three-component instead of a four-component model. Wells et al. (2003) assume the same protein: mineral ratio in young children compared with adults, and this may not be right. Their argument that DXA measurements for bone mineral content depend on the DXA machine used, although correct, would only cause a minor error in calculated body composition. The use of an 'adult' protein:mineral ratio in their model is likely to be the reason that the calculated density of the FFM is higher than the 'reference'. Second, as also stated by the authors, the calculated hydration fraction has TBW in the numerator and denominator and hence any error or deviation in TBW will be only partly reflected in the ratio. This means that the variation in hydration could be larger than suggested by the authors, and given the small number of children in the study, mean values could be easily affected as well. In validation studies, methods have to be independent of each other. Third, there is a comparison with 'reference' values for hydration of FFM in children. Although the authors discuss the use of these reference values, these values are rather theoretical and should not be used as absolute 'reference'.

P. Deurenberg

Apart from these methodological comments, the study highlights the need for further in-depth studies in small children in order to get more insight into changes in the chemical composition of the FFM during growth. Compared with some decades ago, many laboratories can now use models with more components, and with the use of ADP, body volume measurements in small children are not as challenging as UWW. For a proper validation of ADP, direct comparison with another method that determines body volume should be made and measured volumes should be compared.

The study of Wells *et al.* (2003) also highlights the shortcoming of ADP as it is conducted now, namely the prediction instead of the measurement of lung volume. This can lead to considerable error in individuals, as also shown by Wells *et al.* (2003).

It is to be hoped that the paper by Wells *et al.* (2003), as well as some other recent papers on the same topic, will stimulate further research in the area of validation of assumptions used in body composition research.

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