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# SOME RECENT TECHNIQUES IN BODY COMPOSITION ASSESSMENT: COMPARISON WITH TRADITIONAL METHODS

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## Summary

The major techniques of body composition are briefly reviewed with particular emphasis on the propagated errors evident in the use of indirect estimates of body compartments. Error propagation is firstly examined from first principles by consideration of both measurement and biological components of the residual errors in regression relationships. Next the techniques for the measurement of total body fat are described and compared in the context of error propagation and it is shown that the so called 'difference' techniques are more precise than the traditional method of skinfold anthropometry. Finally the measurement of other body compartments is considered and some of the technologies used are briefly described.


## I. INTRODUCTION

Empedoceles of Acragas (c 5th century BC) considered the universe and all who live within it as comprising four basic elements, namely water, fire, earth and air. Allowing for a little philosophical license this turns out to be not a bad model for body composition, requiring only the renaming of three of the 'elements' as follows; protein for fire, minerals for earth and energy stores for air. Indeed almost all of the many thousands of reported human body composition studies have been directed at the measurement or derivation of one or more of these four elements (compartments). Furthermore, in normal people water, protein and minerals are present in more or less constant relative proportions and it is frequently considered convenient to group these into an entity known as the fat-free mass (FFM) or, sometimes, as the lean-body mass (LBM). Because of the confusion in the literature between the FFM and LBM (Hill and Beddoe 1988) the term FFM is unambiguously defined and used in this paper as body mass minus the ether-extractable substances in the body. Most body composition studies are concerned with measuring either the total fat compartment or the FFM. This paper therefore concentrates on the measurement of these two compartments although reference is made in the context of wasting disease to the measurement of the other nutritionally important compartments, water and protein.

Total body fat (TBF) has been measured by five general approaches, including densitometry (usually by under-water weighing) skinfold anthropometry, imaging techniques (X-ray radiometry, computerized tomography or ultrasonography and dual photon absorptiometry), electrical conductivity or impedance measurement and finally by measurement of other major compartments and subtraction of these from measured body mass (the so-called 'difference' methods). All five approaches have one thing in common (except perhaps some of the imaging techniques), namely that they all involve indirect techniques of measuring fat. Because densitometry was invented first (Welham and Behnke 1941) it has tended to become the gold standard by which other newer techniques are compared. This is perhaps unfortunate because densitometry is particularly dependent on critical assumptions which limit the precision and accuracy not only of the technique itself but also of

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techniques standardised against it, a situation which progressively worsens as one moves from normal individuals through the range of depletive to critical illness. Any discussion of techniques would be incomplete without at least a cursory examination of the sources of error or uncertainty that limit their usefulness, especially in body composition studies of the very sick. It is not necessary to resort to sophisticated statistical arguments (which too often only cloud the issue) but to examine our measurements critically from first principles.

## II. ERROR PROPAGATION

Indirect techniques generally depend on being able to develop a predictor relationship between a readily measured quantity (say x) and a not-so-readily measured quantity (say y). To generate the relationship both x and y must first be measured in a representative sample of the applicable population and a relationship imposed between the two quantities. Assuming for the sake of argument that the relationship imposed is linear then the residual error of y upon x, $\sigma_r$, can be considered as made of a measurement component, $\sigma_m$, and a biological component, $\sigma_b$, such that

$$\sigma_r^2 = \sigma_m^2 + \sigma_b^2$$

The biological component simply is a measure of the refusal of nature to conform to the imposed relationship. In the field of body composition research, especially clinical situations, $\sigma_b >> \sigma_m$. Subsequent measurement of x in another population (or subpopulation) gives rise to several classes of propagated error in the prediction of the true value of y. Firstly it can be shown (Burkinshaw 1967) that the true value of y depends only on the biological component of the residual error in the originally derived relationship. For a given individual one has no a priori knowledge of the magnitude of this biological error. It not only affects the accuracy of each individual estimate to an unknown extent but also the precision of an estimate of the mean value of y from several measured values of x in different subjects. Because the error is systematic in origin and subject specific it does not affect the precision of an estimate from a number of measurements in the same individual, which is why its presence is frequently missed or ignored.

On the assumption that $\sigma_b >> \sigma_m$ Table 1 shows the types of error which arise from using predictor relationships derived from normal studies in both individual subjects and groups of subjects for both normals and depleted patients.

Table 1. Systematic and random errors in y arising from measurements of x in normals and depleted patients

| Subjects | Error type | Presence of error In individuals | In groups |
|---|---|---|---|
| Normals | Systematic | Yes | No |
| | Random | No | Yes |
| Depleted Patients | Systematic | Yes | Yes |
| | Random | No | Yes |

It can be seen that in groups of patients both systematic errors and random errors are in evidence. This is because the regression relationships used in patient studies are usually derived from studies of normal populations. Not only are depleted patients (with a range of clinical conditions and nutritional states) more heterogeneous than a group of normals, thus giving rise to greater biological residual error, but the true relationship between x and y in a patient group is systematically different from that derived from normal studies.

What is the implication of the above on body composition research based on indirect measurements? For clinical research four conclusions can be drawn as follows:

(i) It is not advisable to use predictor relationships derived from studies of normals to studies of any other population.

(ii) To reduce the effect of the biological component of the residual error large numbers of subjects (say >50) must be studied if precise estimates of population means are to be determined.

(iii) Longitudinal studies must be treated with caution because the true regression relationships might change.

(iv) Studies on individuals can be expected to have large systematic errors not because of measurement imprecision but because of biological departure from imposed regression lines.

Unfortunately it is apparent from the literature that many authors are unaware of either the magnitude of the biological component of the residual error or its effects on estimates of the true values of predicted quantities. For example, to quote a distinguished authority in the body composition field (Garrow 1982) 'The problems associated with skinfold measurements concern interobserver error and the impossibility of obtaining skinfolds at the prescribed sites in very obese subjects'. Would that this were true. Results will be presented below which show that measurement or technical error has a relatively small effect on estimates of body fat from skinfold measurements.

## III. MEASUREMENT OF TBF OR FFM

For historical reasons it is important to look first at densitometry as well as the most widely used technique in body composition assessment, skinfold anthropometry.

### (a) Densitometry

The use of densitometry (usually by underwater weighing) to estimate body fat depends first on the fact that fat is less dense than other compartments but also on the assumption that the FFM has a constant density, D, such that

$$\frac{TBF}{M} \propto \frac{1}{D}$$

where M is body mass. The most commonly used relationship is that suggested by Siri (1956) namely

$$\frac{TBF}{M} = \frac{4.95}{D} - 4.50$$

which assumes densities of 0.90 and 1.10 $gcm^{-3}$ for fat and FFM respectively. This indirect means of measuring TBF has a total precision in

normal man of around 0.0063 $gcm^{-3}$ of which 0.006 $gcm^{-3}$ is due to biological causes (Siri 1961) and 0.002 $gcm^{-3}$ to measurement precision (Buskirk 1961). Clearly the biological component of the residual error is the critical factor. In addition one cannot rule out the possibility of systematic errors arising from the underwater technique including the problem of measuring residual lung volume (Streat et al. 1985).

(b) Skinfold anthropometry

Skinfold anthropometry is perhaps the cheapest and easiest method of determining TBF. It depends on two assumptions (i) that the ratio of subcutaneous fat to TBF is constant and (ii) that measurements at several sites are representative of total subcutaneous fat. Even in normals the proportion of subcutaneous fat varies from 20% to 70% of TBF (Lohman 1981). In sick patients the relationship is not yet known, but will probably vary significantly depending on the extent of depletive illness or trauma.

Skinfold anthropometry is usually calibrated by densitometry and several investigators have derived relationships between sums of skinfold thicknesses and body density. Perhaps the best known is that reported by Durnin and Womersley (1974) where the general form of the relationship is given by

$$D = c - m \log_{10} \sum_i s_i$$

where $s_i$ is the mean measured skinfold thickness at site i and where c and m are constants derived from linear regression. Using densitometry these authors demonstrated residual errors for the sum of three skinfolds between 0.008 and 0.012 $gcm^{-3}$ for females, with no obvious trend with age and between 0.006 and 0.010 $gcm^{-3}$ for males with an increased trend for age. On the assumption that any error in the estimate of TBF is only dependent on the residual error in the relationship between density and sums of skinfolds it can be shown, for example, that the residual error for 20 - 29 year-old-men (0.0087 $gcm^{-3}$) propagates to a $\pm$ 24% error in the measurement of body fat, which implies that approximately two thirds of this subpopulation of males will have TBF estimates up to $\pm$ 24% in error and one third with corresponding errors greater than $\pm$ 24%. This computation assumes that the relationship between density and TBF (Siri's equation) is free from biological imprecision which was shown above to be incorrect. It is difficult to assess the effects of the combined variances because there is almost certainly covariance present. However it is clear that the derivation of body density as an intermediary step is probably counter-productive if only some other means could be found to correlate skinfold measurements with TBF, though the problem of the variability of the ratio of subcutaneous fat to TBF remains. Finally, if typical errors of the order of 24% are observed in a relatively homogeneous and healthy group it can be appreciated that even larger errors will occur in studies of older, more heterogeneous and sicker subjects.

(c) Difference methods

The difference methods depend on the well known Newtonian concept that the whole is equal to the sum of its parts. Unlike TBF the other major compartments, protein, water and minerals, are readily measured by technologies developed over the last 20 years.

(i) Isotopic measurement of total body water (TBW). Since TBW can be measured with precision of the order of 1.5% by $^3H_2O$, $^2H_2O$ and $H_2{}^{18}O$ dilution and since it is the largest compartment, TBF can in

principle be obtained from the relation

$$TBF = M - TBW/f$$

where f is the assumed fraction of the FFM that consists of water, usually taken as 0.73 (Pace and Rathbun 1945) but more recently shown to range from 0.69 to 0.75 with means of 0.710 and 0.726 for normal males and females respectively (Beddoe et al. 1985). The range of f in depleted patients is greater with a tendency for many patients to develop increased hydration up to 0.80 or more. The range of f therefore renders the method unsatisfactory in principle although it is much better than skinfold anthropometry with an overall precision (including biological) of the order of 8.0% in normals. Like anthropometry, however, it cannot be used for depleted patient studies unless the mean and range of hydration ratios is predetermined and the increased imprecision is understood. There is also the problem of non-aqueous exchangeable hydrogen in both normals and patients, though this is a more or less constant systematic error estimated by Schoeller and Jones (1987) to be ~5% overestimate of the water space by $^{2}H$ or $^{3}H$ dilution and 1% by $H_2{}^{18}O$ dilution. Results can of course be corrected for this.

(ii) Isotopic measurement of water and in vivo neutron activation analysis (IVNAA). Since the second largest compartment in the FFM is total body protein (TBP) some improvement could be obtained if TBP were to be measured. This can be done by IVNAA (Cohn et al. 1981) using measurements of total body nitrogen (TBN) and the assumption TBP = 6.25TBN. IVNAA can also be used to measure the total body mineral compartment (TBM) (Cohn and Dombrowski 1971) although because it is a small compartment which does not change markedly in nutritional illness (in the sense of affecting the accuracy of estimates of TBF) it is sufficient to estimate TBM based on anthropometric principles. A

Table 2. Comparison of total precisions achievable with three methods of measuring TBF in normals

| Technique | Random error (CV) | Based on |
|---|---|---|
| Skinfold anthropometry[1] | 24% | Durnin and Womersley's formulae together with Siri's equation |
| Tritium dilution[2] | 8.0% | M - TBW/0.73 |
| IVNAA/tritium dilution[3] | 6.5% | M - (TBW+TBP+TBM+TBG) |

[1]The random error for skinfold anthropometry excludes any error propagated via the Siri equation.

[2]The random error for the tritium dilution technique assumes a 2% s.d. (Streat et al. 1985) variation in the ratio TBW/FFM which propagates to an 8.0% error in the measurement of water (includes the biological variation in TBW/FFM ratio).

[3]The random error for the IVNAA/tritium dilution method assumes random measurement or prediction precisions of 1.5% for TBW, 4.2% for TBP, 10% for TBM and 20% for TPG. No account for variations in the relation TBP = 6.25TBN are included because of the unknown variability of this ratio. However measurements of TBN/TBP in two cadavers (one of which was extremely cachectic) did not reveal significant departure from 6.25 (Knight et al. 1986).

combined IVNAA/tritium dilution technique has been reported by Beddoe et al. (1984) which enables TBF to be estimated in a typical normal with an overall precision of around 6.5%. This technique does not depend on a priori assumptions about body composition (other than about TBM and total glycogen), so is in principle applicable to studies of the very sick as well as normals.
(iii) Comparison of above difference techniques with skinfold anthropometry. Table 2 summarises the typical precisions for estimates of TBF obtainable with the techniques discussed above. It is emphasised that these precisions include the biological component of the residual error discussed earlier (where applicable).

A further illustration of the limitation of skinfold anthropometry can be seen in Figure 1. Histograms of the ratio TBW/FFM are plotted for both normals and patients as obtained both by the IVNAA/tritium dilution technique and by skinfold anthropometry (FFM = M-TBF). Clearly anthropometry yields values of TBW/FFM that are outside of accepted biological limits and this is especially apparent with the depleted patient group, reflecting both the increased heterogeneity and inapplicable regression relationship used for this group.

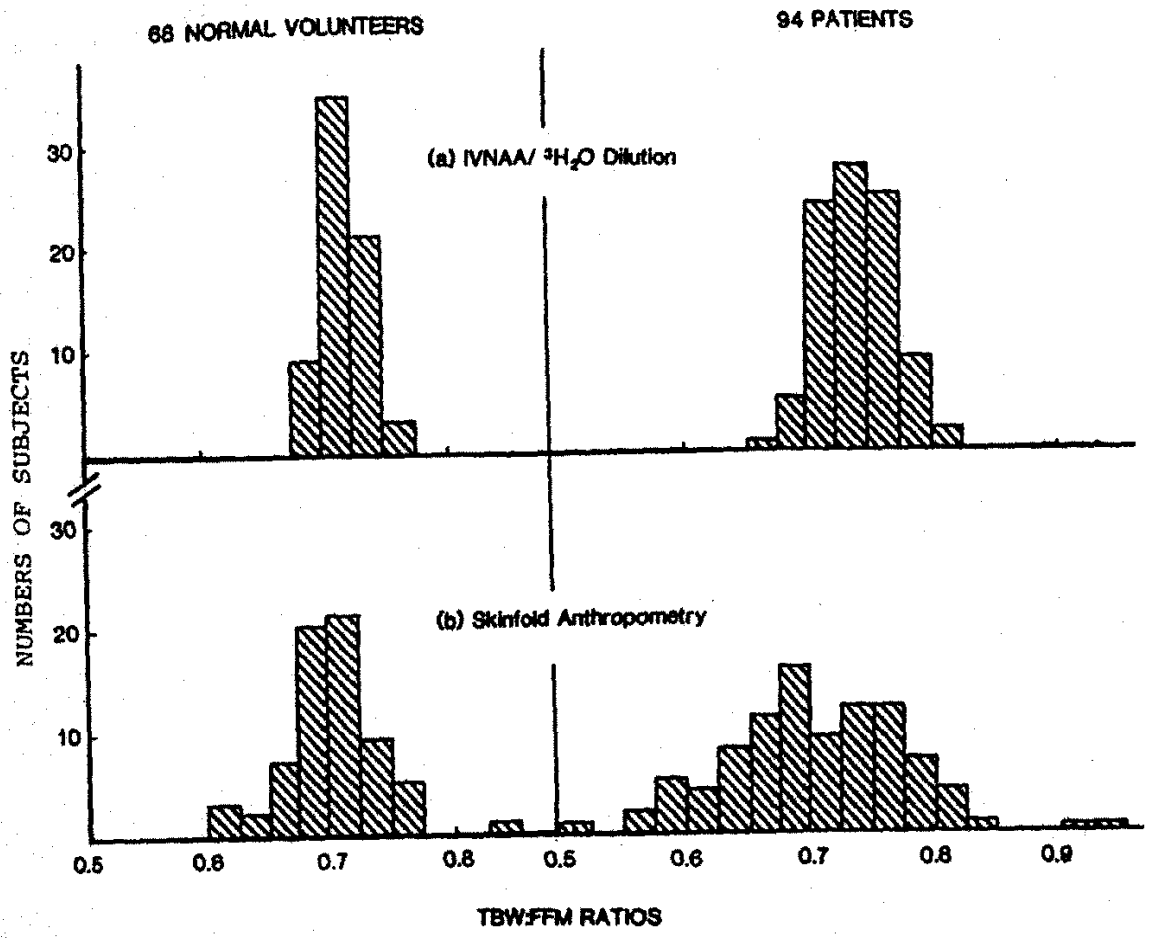


Figure 1. Histograms of the TBW:FFM ratio for 68 normal volunteers and 94 patients presenting for nutritional support. TBW measured by $^3H_2O$ dilution. FFM measured by IVNAA/$^3H_2O$ method in (a) and by skinfold anthropometry in (b).

(d) Electrical conductivity

In 1982 a new technique was reported by Harrison and Van Itallie based on the differential conductivity of fat and lean tissue. Measurement of total body electrical conductivity (TOBEC) can be achieved by subjecting a person to a 5MHz radio frequency power source, the measured impedance being a function of FFM. The method is also indirect which means that it must be calibrated against another method - to the author's knowledge this has only been carried out with densitometry (Presta et al. 1983). However TOBEC is rapid, safe, non-invasive and claimed to be reproducible and is in principle applicable to many classes of hospitalized patients.

(e) Imaging techniques

X-ray imaging allows the volume of fat over a given site to be determined. Conventional radiography (or radiometry) is limited to measuring fat at subcutaneous sites in the limbs and simply replaces calipers. In the last decade computerized tomography has been used to obtain fat at any site in the human body and is particularly suited to differentiating subcutaneous and intra-abdominal fat (Borkan et al. 1982). In principle the method is almost a direct means of measuring TBF (or at least total adipose tissue) with sufficient numbers of CT scans, although it is of limited application due to cost and radiation dose. Errors do arise at muscle/adipose tissue interfaces and the accuracy is critically dependent on an accurate calibration of fat in terms of CT numbers.

The most recent technique which shows great promise is that of dual photon absorptiometry (DPA). The DPA technique has been used for mineral estimation for 20 years but the advancement of computing over the last decade has enabled rapid total body analyses to be performed with 1% CV for whole body radiation dose equivalents of 0.01-0.02mSV (Heymsfield et al. 1989). These authors have correlated TBF measured by DPA in 13 normal subjects (24-94 years) with TBF measured by other techniques including densitometry and a difference method (M-6.25TBN-TBW-TBM). Correlation coefficients for the two methods were comparable (0.94 and 0.95 respectively) and standard errors of the estimates were 1.82 kg and 1.68 kg which, expressed as a percentage of mean TBF (as measured by DPA) are equivalent to 10.9% and 10.1% respectively. The authors claim that no a priori assumptions about the composition of the FFM are made so that the technique is not dependent on nutritional status, that is it is applicable to the whole range of clinical conditions. The same claim can be made about IVNAA. Nevertheless DPA, like IVNAA, is dependent on accurate calibration. For example, accurate knowledge of photon attenuation coefficients in a range of adipose and lean tissues is required. Do these remain constant over all diseased states? The answers are not yet known.

## IV. MEASUREMENT OF OTHER COMPARTMENTS

In discussing the difference technique for measurement of fat, estimates of other total body compartments were used. In the context of depletive illness TBP and TBW are particularly important, the latter because it accompanies the capillary leak syndrome. Several physically-based techniques are discussed below.

(a) Dilution methods

The dilution principle is remarkable for its simplicity, the volume of a given organ (space) being equal to the volume of labelled compound (radioactive or stable isotope) injected into the organ (space) multiplied by the ratio of injected to sampled concentrations. Dilution is not normally

instantaneous and equilibration must be achieved before sampling can be carried out. Several other rules apply. The volume of injected labelled compound must be small compared to volume of organ (space), and the latter should remain constant over the equilibration period. Equilibration time should be short compared to biological turnover or excretion (both physical and metabolic) and, most importantly, the tracer must not distribute to other organs (pools).

Mention has already been made of the measurement of TBW by $^{2}H_2O$, $^{3}H_2O$ and $H_2{}^{18}O$ dilution. The water space can be further partitioned into extra- and intra-cellular water by also measuring the bromide space (McMurray et al. 1958) or by the $^{22}Na$ early distribution volume (Shizgal 1983). There is considerable interest in the partitioning of the water space because of the redistribution of intra- and extra-cellular water in depletive illness (Beddoe et al. 1985) and in critical illness (Streat et al. 1985a).

Table 3 summarises the use of isotope dilution techniques to measure a range of total body compartments. It is not intended to be exhaustive. Of particular interest in the nutritional context is the so called body cell mass (BCM), since it is this which provides an entity which should be related to the total metabolic energy expenditure (Moore et al. 1963). Furthermore since 98% of the body's potassium (TBK) is intra-cellular, a measure of TBK would furnish an estimate of BCM, $^{42}K$ being normally used. Note that Shizgal et al. (1977) estimate TBK from the 24 hour distribution of $^{24}Na$.

Table 3. Body composition determined by isotope dilution

| Compositional entity | Dilution method |
|---|---|
| Red cell volume (RCV) | $^{51}Cr$ labelled red cells |
| Plasma volume (PV) | $^{125}I$ labelled human serum albumin |
| Blood volume | RCV + PV |
| TBW | $^{3}H_2O$, $^{2}H_2O$, $H_2{}^{18}O$ |
| Extra-cellular water (ECW) | $^{82}Br$ |
| | $^{22}Na$ (early distribution volume) |
| Total body sodium (TBNa) | $^{22}Na$ |
| Intra-cellular water | TBW - ECW |
| Total body potassium | $^{42}K$ |
| | $\propto$TBW - TBNa (Shizgal et al. 1977) |
| FFM | TBW/f (already discussed) |
| TBFat | M-FFM |
| Body cell mass | 0.0833 TBK (Moore et al. 1963) |

Isotopic dilution techniques are often less accurate when used with critically ill patients, partly because compartment sizes do not remain constant during the equilibration process. If the patient is not haemodynamically stable, is receiving intravenous fluids or is gaining (or losing) water it is wise to look at alternative techniques where possible.

(b) Measurement of TBK using a whole body counter

An alternative to using $^{42}K$ or $^{22}Na$ dilution is to measure the naturally occurring isotope of potassium $^{40}K$ which occurs as a fixed proportion of total body potassium (0.01%). The 1.46 MeV ray can readily be measured with an array of sodium iodide detectors placed above and below the patient in a massive lead - and/or steel-lined room. $^{40}K$ can also be measured by a shadow shield counter. Either technique is totally non-invasive and capable

of providing estimates of TBK with precisions around 2% (sd) in 10 to 30 min. Calibration of such a facility can be achieved with $^{42}K$ either given to volunteers of various shapes and sizes or to a range of anthropomorphic phantoms. The technique is suitable for the whole range of depleted patient.

(c) In vivo neutron activation analysis

Since nitrogen is uniquely associated with protein a measure of TBN furnishes an estimate of TBP, and TBN can only be measured by IVNAA. While the measurement of nitrogen was first reported by Palmer et al. in 1968 the method was not applied to nutritional or metabolic studies until the latter half of the seventies (Hill et al. 1978). TBN can be measured either by delayed gamma IVNAA (via the $^{14}N(n,2n)^{13}N$ reaction with fast neutrons) or by prompt gamma IVNAA (via the $^{14}N$ $(n,\gamma)^{15}N$ reactions with thermal neutrons). For the advantages and disadvantages of the two techniques the reader is referred to reviews by Cohn (1981) and Beddoe and Hill (1985). Prompt gamma IVNAA is now the preferred method for reasons of cost, accuracy and radiation dose.

The advantage of IVNAA over nitrogen balance is that it enables absolute estimates of body protein to be determined which in turn can be used to determine a patient's protein index (Beddoe et al. 1985) and also that IVNAA is logistically more suitable for long term studies. As indicated earlier, in combination with the $^{3}H_2O$ dilution technique it is possible to derive the nutritionally important compartments TBP, TBW and TBF, the latter by the difference technique, (Beddoe et al. 1984; Cohn et al. 1981). Further division of body protein is possible, firstly into muscle and non-muscle components using a model proposed by Burkinshaw, Hill and Morgan (1979) and into the actively metabolizing body cell mass and the slowly metabolizing structural protein (Cohn et al. 1983).

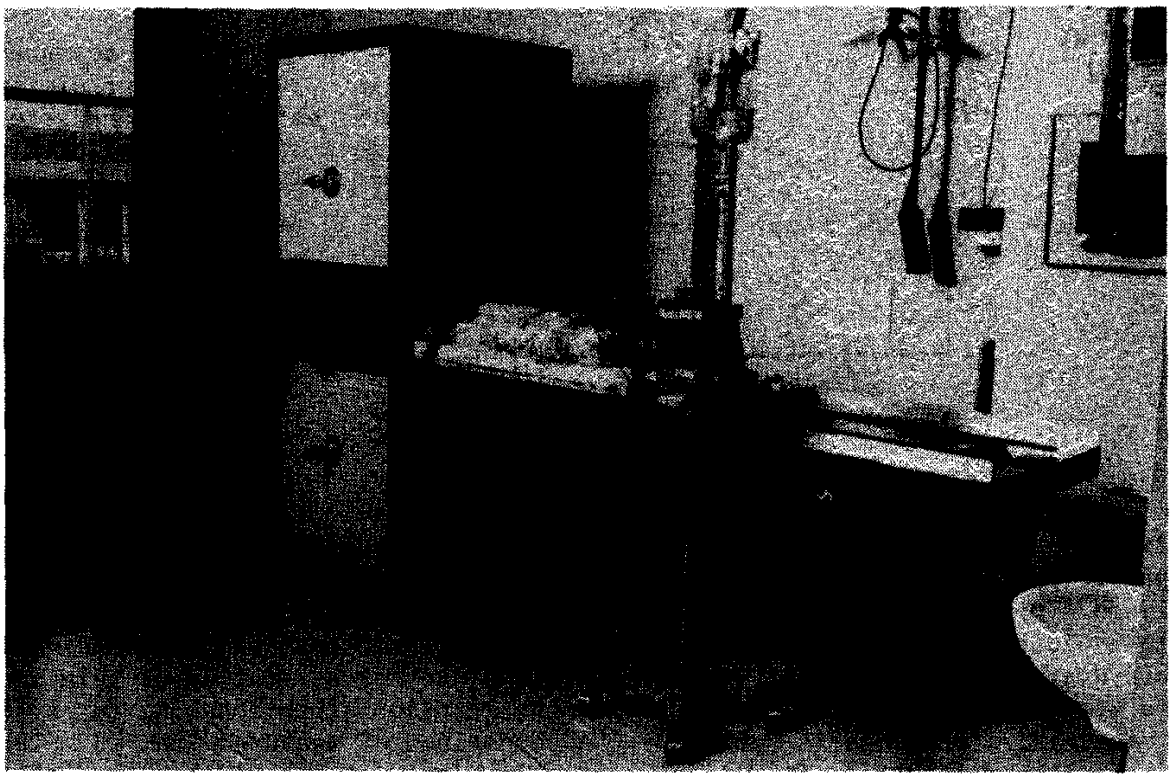

Figure 2. The prompt gamma IVNAA facility at Auckland.

The advantage of IVNAA over other traditional body composition techniques (and over most of the newer ones, with the exception of DPA) is that it is not necessary to make a priori assumptions about the ratios of major compartments, in particular that the density, composition or gross chemical composition of these compartments need be constant. However there are still some assumptions which need to be made, one of the most significant of which is that TBP:TBN is a constant ratio, 6.25. Perhaps one should simply refer to the 6.25N space.

A prompt gamma IVNAA facility was designed and built in the Department of Surgery, University of Auckland in 1981/82 (Beddoe et al. 1984), as illustrated in Figure 2. Validation was carried out by several approaches including comparison with other centres using published predictor relationships relating TBN to age, height, weight and sex in normal individuals. In Figure 3 measured protein is compared to predicted protein, using predictor equations developed by Burkinshaw et al. (1981). The ratio of TBP (measured) to TBP (predicted) in this group was 0.986 ± 0.110 for 35 females and 1.033 ± 0.118 for 33 males. The contribution of measurement errors to these ratios (compounded from both centres) is of the order of 6% with the remaining imprecision (around 9.7%) being due to the biological range of protein mass in people of a given height, weight, age and sex.

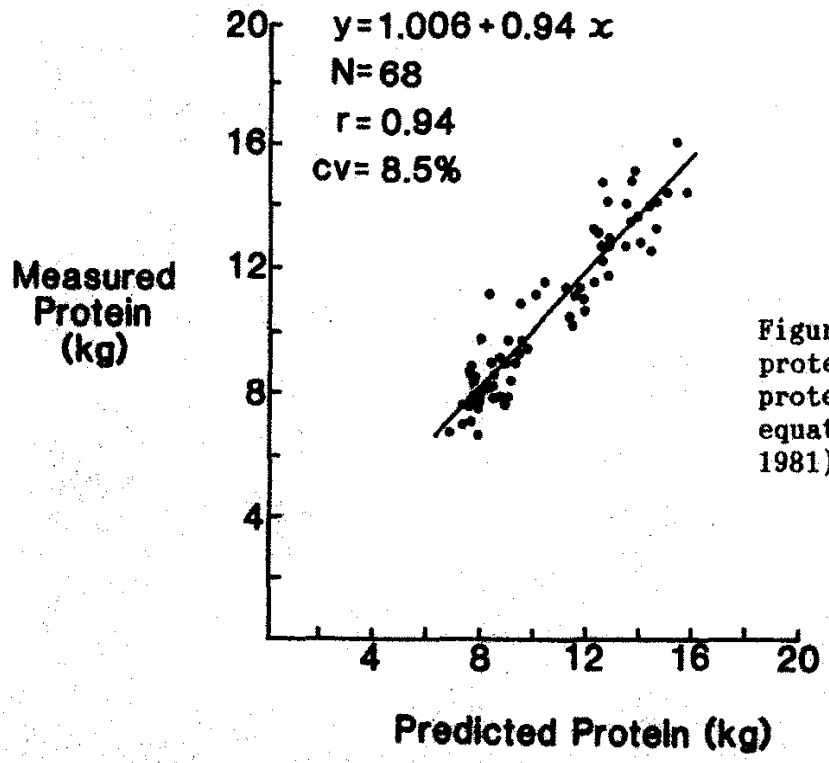


Figure 3. Plot of measured protein versus predicted protein using Leeds predictor equations (Burkinshaw et al. 1981).

The ultimate form of validation must always be by whole cadaver analysis. This was attempted in Auckland by homogenizing two cadavers (separately) after performing IVNAA. Aliquots of the homogenates were analysed by freeze drying (for water) ether extraction for fat, a micro-kjeldahl technique for nitrogen, by subtracting minerals and carbohydrate from fat-free, freeze-dried residue for estimating protein and by a 2, 4, 6 - (2-pyridyl) -1, 3, 5-triazine reaction after leaching fat free dry material for measuring chloride. A comparison of measured TBN, TBP, TBF and total body chlorine (TBCl) is shown in Table 4. It should be noted that

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because it is not possible to apply the $^3H_2O$ dilution technique to cadavers the estimates of TBF by the difference method use the chemically determined TBW. Clearly the results, at least for these two cadavers, show relatively good agreement between the conventional chemical technique and whole body neutron activation.

Table 4. Measured composition of two cadavers by whole cadaver neutron activation and by chemical analysis (sem in parenthesis)

| Cadaver | Analysis | TBN (kg) | TBF (kg) | TBP (kg) | TBCl (g) |
|---|---|---|---|---|---|
| 58.6 kg male | Chem | 1.51 (.03) | 10.48 (.15) | 9.56 (0.21) | 147 (2) |
| | NAA | 1.47 (.02) | 10.71 (.13) | 9.21 (0.13) | 144 (4) |
| 25.9 kg female | Chem | 0.572(.014) | 7.71 (.17) | 3.66 (.09) | 25.0 (1.4) |
| | NAA | 0.576(.008) | 6.77 (.05) | 3.60 (.05) | 22.7 (1.7) |

## CONCLUSION

Someone once said that most science is a process of diminishing deception. The science of body composition measurement is surely of this ilk. As the century advances new technologies are introduced and gradually body composition assessment becomes more precise and, hopefully, more accurate.

## ACKNOWLEDGEMENTS

I wish to acknowledge my colleagues in the Department of Surgery, University of Auckland who had a major impact on the work referenced and/or described in the text; especial thanks to Prof. Graham Hill, Dr. Stephen Streat, Dr. Grant Knight and Mr. Herman Zuidmeer.

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