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From chemical analysis of the body . . . to metabolic insights provided by the new methodology

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It is by no means an exaggeration to say that Dr Widdowson is one of the pioneers of body composition research and her studies in the 1940s and 1950s have laid the foundations of body composition science today. These have included both animal and human studies, although this paper will focus only on the latter. Together with Professor McCance and Christine Spray she was responsible for the analysis of three entire adult human cadavers and that of a 4-year-old child (Widdowson *et al.* 1951). Further work with Dr Dickerson explored the composition of specific tissues and organs of the body (Widdowson & Dickerson, 1964). These studies provide some of the best direct data on human body composition. Moreover the findings from this work form the basis of the indirect techniques which are used so widely today.

REFERENCE METHODS

Direct analysis has demonstrated that although there may be gross differences between individuals in the proportions of fat and fat-free mass, the composition of the fat-free compartment is relatively constant (Widdowson & Dickerson, 1964). From this basis it is possible to estimate the size of the fat-free compartment based on the measurement of some physical properties of the tissues: body density, hydration fraction or K concentration. Hence we have the three classical methods to measure body composition: densitometry, total body water and total body K. Each represents a two-compartment analysis of the body in which fat is estimated as the difference between fat-free mass and body weight (Coward *et al.* 1988).

These methods have provided the mainstay of body composition research until the last 15 or 20 years and remain some of the best methods available. Many of the recent advances in this field are simply new technological developments to measure the same physical properties of the body, rather than truly novel approaches. A good example is the advent of systems to measure body volume based on air displacement rather than classical underwater weighing. This makes the method quicker, simpler and more easily applied to a wider range of subjects by avoiding the need for total submergence under water (Dempster & Aitkens, 1995; McCrory *et al.* 1995).

However, in the search for ever greater accuracy we have come to recognize the importance of some of the cautionary notes expressed by Dr Widdowson and her colleagues at the time of their original analyses; despite a relative constancy in the composition of fat-free tissue there are important inter-individual differences, which are exacerbated in pathological conditions (Widdowson *et al.* 1951). A recent study showed the mean hydration of fat-free tissue to be 73·8 (SD 2·13) % (Fuller *et al.* 1992*a*). Thus the measurement of fat mass in a 70 kg man with 40 litres of total body water can range from 14·2 to 17·3 kg. Similarly assumptions regarding the intracellular K concentration, which in men ranges from 2·46 g/kg (Behnke, 1974) to 2·66 g/kg (Forbes *et al.* 1961) will give a

difference in the absolute estimate of fat-free mass of approximately 7·5 %. Most importantly, when measuring changes in body composition any deviations in the proportional composition of fat-free tissue, which it is then assumed remains constant, can obscure important underlying changes in composition.

In recognition of this there is now a drive to quantify each of the principal compartments notably water, mineral and fat, leaving a smaller and more homogeneous remainder, dry, soft, fat-free tissue, which is predominately protein. This has led to so-called three- or four-compartment models where water and/or mineral concentration are measured independently in addition to the overall measurement of body density (Jebb & Elia, 1995). Water is typically measured using deuterium and bone mineral by dual-energy X-ray absorptiometry (DXA). The remaining compartment, principally fat plus protein, can then be divided by assuming the densities of fat and protein, typically 0·9 and 1·34 kg/l respectively. In this way body fat mass can be calculated with a precision of $\pm 0{\cdot}75$ kg fat (Fuller *et al.* 1992*b*).

Measurements of this kind allow us to examine individual differences in the mineral and hydration fractions of fat-free mass and this has confirmed the large inter-individual variability in the composition of fat-free mass (Friedl *et al.* 1992). Moreover the error associated with traditional two-compartment models becomes apparent (Jebb & Elia, 1995). In the future, as more data from multi-compartment models accumulate, it may be possible to refine the assumptions of two-compartment models; for example, to allow for changes in the composition of fat-free mass which are associated with ageing or obesity.

Alongside this refinement of classical methods and compartmental analysis new technologies have also emerged, particularly in the field of medical physics. *In vivo* neutron activation analysis (IVNAA) probably represents the ultimate compartmental analysis of the body at a molecular level. Most IVNAA systems involve prompt γ neutron activation (Beddoe & Hill, 1985). The patient is first injected with tritium to measure H and then exposed to γ radiation emitted by radionuclides specific for various elements. The complex spectrum of radiation from the patient is measured and analysed to determine N, H, C, Cl and in some systems Ca, P, Mg and Na. This allows the measurement of fat, protein, mineral and water. The total radiation dose is approximately 50 mSv per patient, approximately six times that of a cardioangiogram. The quoted precision of measurements of both total body N (for the estimation of protein) and C (used in the derivation of body fat mass) is approximately 3 % (Heymsfield *et al.* 1993). Comparative studies between IVNAA and chemical dissection in two cadavers have shown good agreement (Knight *et al.* 1986).

IVNAA has been used most widely in clinical studies, including the compositional changes during protein–energy malnutrition and subsequent repletion, and studies of nutritional status in patients with a range of disorders including cancer, renal dysfunction, CHD, hypertension, obesity and anorexia nervosa (Beddoe & Hill, 1985; Hill, 1992). A small series of subjects has been studied to examine body composition during ageing (Heymsfield *et al.* 1993). Future developments are seeking to minimize the radiation dose whilst maintaining or improving the precision of the estimate.

Imaging techniques have taken a more anatomical approach to body composition analysis. Computed tomography (CT) and more recently magnetic resonance imaging (MRI) allow the examination of the composition of the body by tissue, organ or region. With sufficient time, resources and patience whole body analyses can also be performed. The most comprehensive work has measured the composition of twelve main volumes of tissues, organs and gas within the body and numerous sub-fractions (Chowdhury *et al.* 1994) (Fig. 1). These data were collected from twenty-six cross-sectional scans, taking 90

min and with a total radiation dose of up to 5 mSv per subject. Subsequent data analysis took three working days per subject. Both intra- and inter-observer errors were very small, with overall precision for total adipose tissue volume of $< 0{\cdot}5\,\%$ and visceral organs $< 2\,\%$. This is perhaps the closest we can get *in vivo* to the detail provided by classical dissection studies. The outstanding ethical dilemma is the radiation exposure which is much too high for routine use.

Alternatively MRI can be used in a similar manner but without the radiation risks. Instead, the difficulties relate to the cost and availability of machines. The subject is placed in a strong magnetic field and irradiated with radiofrequency pulses. The signal intensity is determined by the concentration and relaxation properties of water and fat in the tissues being studied. Adipose tissue has a much shorter relaxation time than other tissues and can be accurately identified. Methods have been described for the measurement of both total and regional adipose tissue (Ross *et al.* 1992) and skeletal muscle (Ross *et al.* 1995). The precision of the measurement of fat or lean tissue in a single slice is $< 3\,\%$ (Ross *et al.* 1995) and the mean difference for repeated whole-body fat measurements in animals is approximately 4 % (Ross *et al.* 1992). As the opportunities to use MRI increase it will be possible to measure individuals throughout the age range and in a variety of circumstances, giving enormous potential for the study of many facets of metabolic regulation in a non-invasive manner.

IVNAA and imaging are highly sophisticated research techniques for the measurement of body composition which are only available in specialist centres. However another novel approach, DXA, has become commonplace in many medium-to-large hospitals as a routine method for the clinical measurement of bone-mineral density (Prentice, 1995). The technique can also be used to measure the composition of soft tissue and thus a much wider range of researchers now has access to relatively sophisticated body composition analysis. DXA uses X-rays at two energies which are passed through the body in a whole-body scan.

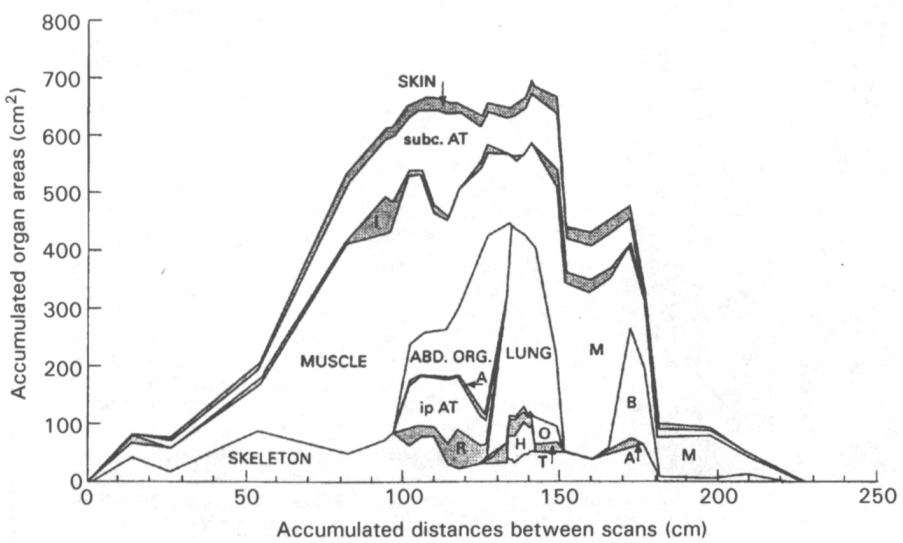
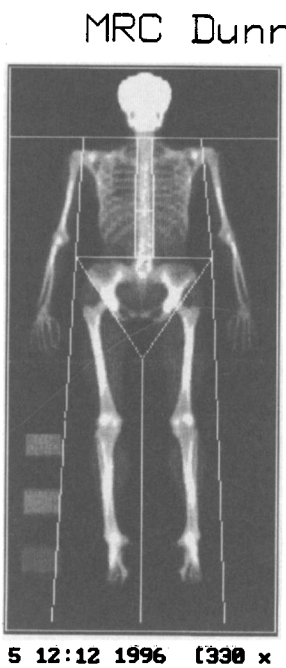


Fig. 1. Detailed body composition analysis by computed tomography. Subc. AT, subcutaneous adipose tissue; ABD. ORG., retro- plus intraperitoneal abdominal organs excluding adipose tissue; ip AT, intraperitoneal adipose tissue; R, retroperitoneal adipose tissue; H, heart muscle; O, thoracic tissues other than lung, heart and adipose tissue; T, thoracic adipose tissue; A, air; B, brain; M, skeletal muscle. (From Chowdhury *et al.* 1994, with permission.)

Knowing the differential attenuation of the beams by bone and soft tissue it is possible to calculate the relative mass of each component. Further analysis of the soft-tissue compartment allows its sub-division into fat and fat-free compartments (Jebb, 1997) (Fig. 2). The single greatest attraction of DXA is the excellent precision, with a CV of $< 2\,\%$ for fat mass (Tothill *et al.* 1994).

In addition to the measurement of gross body composition it is possible to analyse specific regions of the body, e.g. arms, legs and trunk (Mazess *et al.* 1990). With the current interest in abdominal fat as a site of particular metabolic importance, some investigators use DXA to define a specific abdominal site, usually between L2 and L4 (van der Kooy & Seidell, 1993). This measures both the intra-abdominal and subcutaneous fat within this region. If subcutaneous fat around the abdomen is estimated from simple anthropometric measures of the sagittal diameter and abdominal skinfold thickness it is possible to estimate intra-abdominal fat (Svendsen *et al.* 1993). Inevitably the precision of these further estimates of specific fat regions is less good than whole-body measurements, with typical estimates for the CV of 5 % for regional analyses including total abdominal fat and 10 % for intra-abdominal fat (Jebb, 1997).

A variety of other techniques have been employed from time to time and by different groups of investigators. Total body electrical conductivity (TOBEC) was widely promoted in the 1980s, but its use has been almost entirely confined to a few centres in the USA. This method uses a solenoidal coil driven by an oscillating radio-frequency current which induces an electrical current in the body's conductive tissues when placed within the field.



MRC Dunn Nutrition Unit.

A08259303 Wed Aug 25 09:04 1993
Name: SAJ
Comment: Comparison
I.D.: Sex: F
S.S.#: - - Ethnic:
ZIPCode: Height: 163.00 cm
Scan Code: SAJ Weight: 48.00 kg
BirthDate: 08/29/64 Age: 28
Physician:
Image not for diagnostic use
TBAR227
F.S. 68.00% 0(10.00)%
Head assumes 17.0% brain fat
LBM 73.2% water

Region	Fat (grams)	Lean+BMC (grams)	% Fat (%)
L Arm	543.8	1639.1	24.9
R Arm	712.1	1696.9	29.6
Trunk	2235.4	19618.9	10.2
L Leg	2168.4	6457.0	25.1
R Leg	2341.5	6747.3	25.8
SubTot	8001.2	36159.2	18.1
Head	650.1	3536.8	15.5
TOTAL	8651.3	39695.9	17.9

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Hologic QDR-1000/W (S/N 971 P)
Enhanced Whole Body V5.61P

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Fig. 2. An example of the information obtained from whole-body soft-tissue analysis of dual-energy X-ray absorptiometry using the Hologic QDR-1000W machine.

A measurement is made of the coil impedance when empty and when the subject is passed through. This creates a phase curve representing the interaction of the magnetic field with the geometric shape and the distribution and amount of fat-free tissue. Body composition can be estimated based on the differences in electrical conductivity and dielectric properties of the fat and fat-free compartments (Presta *et al.* 1983). However considerable difficulties have been encountered due to differences in body geometry between individuals. Although still in use its growth has been stalled by the development of other more useful techniques.

PREDICTION METHODS

As the value of measurements of gross body composition and fat distribution is recognized there has been an increasing demand for simpler techniques for use at a clinical or public health level. To a large extent these have taken the form of methods to predict the outcome of the classical two-compartment techniques, most notably the measurement of skinfold thicknesses to predict body density (Durnin & Womersley, 1974), bioelectrical impedance to estimate total body water (Hoffer *et al.* 1969) and more recently multifrequency impedance to measure extracellular water (Cornish *et al.* 1995). In the case of fat distribution the waist : hip ratio (Ashwell *et al.* 1985), or more recently waist circumference alone, have been employed (Seidell *et al.* 1994). These prediction methods are quick, simple and cheap, practical for almost all individuals in almost all circumstances. However undoubtedly accuracy is compromised for convenience. It is important to note that such methods carry all the assumptions of the method from which they were derived in addition to their own inherent errors.

Other methods have been used to estimate the size of particular body compartments e.g. creatinine (Forbes & Bruining, 1976) or 3-methyl-histidine excretion (Lukaski & Mendez, 1980) to estimate muscle mass. Although the analytical precision is high, both methods suffer from problems relating to the effect of recent dietary intake on excretion, the need for complete 24 h urine collections and inherent inter-individual variation in excretion of each metabolite.

APPLICATIONS

To a large extent the quantification of body composition serves to satisfy human curiosity. However advances in our understanding of body composition have underpinned conceptual advances in human physiology. Again Dr Widdowson led the way. One of her most important findings was the documentation of changes in the composition of the body during the development of the fetus and infant (Widdowson & Spray, 1951). The fat-free mass of a fetus weighing 10 g is over 90 % water, yet by term this has decreased to approximately 83 % and during infant life it progressively falls to the adult value of about 73 %. This is accompanied by an increase in the proportion of protein (Fig. 3).

Reference datasets which define normal or optimal body composition can be used to identify individuals showing abnormal or suboptimal composition during normal growth and development, including pregnancy, or to monitor changes in response to pathological conditions. Dr Widdowson's studies of the body composition of undernourished subjects in which she measured both total and extracellular water, demonstrated that the major change in undernutrition is the expansion of the extracellular water compartment, visible as the 'famine oedema' (Widdowson, 1951). Indeed she noted that the body composition of undernourished children reverted to a composition which was similar to that of a new-born

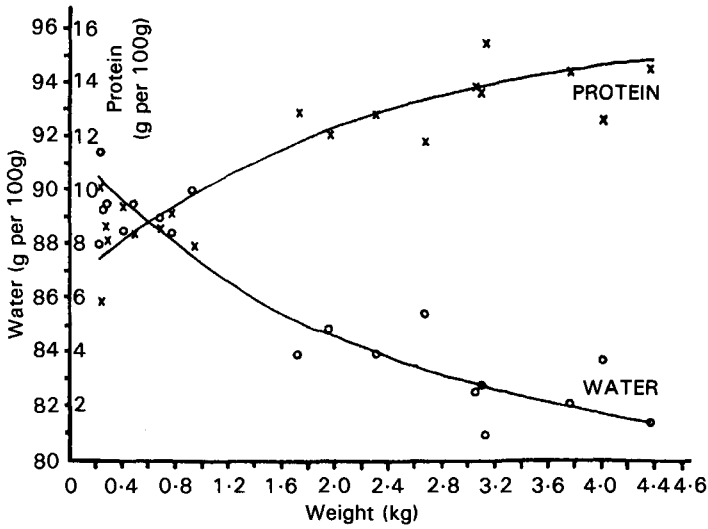

Fig. 3. Changes in the proportions of water and protein in the human body during development from fetus to infant. (Reproduced from Widdowson & Spray, 1951, with permission.)

infant with a considerably increased hydration fraction of fat-free tissue. The precise aetiology of this condition remains a topic of great debate. Early theories that a dietary protein deficiency led to low plasma albumin concentrations and low plasma osmotic pressure have given way to the suggestion that free radicals may damage the cell membranes. However in each case the final step is the leakage of plasma fluid into the interstitium leading to an enlarged extracellular water compartment.

We now recognize many typical changes in body composition associated with a variety of catabolic diseases. The studies of Hill and co-workers have been particularly notable in this field, mostly using IVNAA (Beddoe & Hill, 1985). In general disease-associated weight loss is characterized by an excessive loss of lean tissue, possibly mediated by cytokine action. To a large extent recent studies have confirmed the early observations of Dr Widdowson who asserted that: 'when a person becomes seriously undernourished... the cell mass and fat decrease and extracellular fluids come to occupy an abnormally large part of the body. Plenty of good food leads to an increase in cell mass and fat and to a decrease in the volume of extracellular fluids. The person gains weight because the increase in cell mass and fat exceeds the loss of extracellular fluids', (McCance & Widdowson, 1951). One of the main differences we see in clinical practice is that very often 'good food' has been replaced by artificial nutrition! Progress which has been made in our understanding of the effect of disease on body composition can largely be attributed to the enhanced precision of our measurements which allows the detection of more subtle changes. In this way it is possible to make an earlier diagnosis of malnutrition and to instigate more careful monitoring and evaluation of treatment interventions. For example it can be clearly seen that growth hormone has a powerful anabolic effect which enhances the repletion of fat-free tissue (Mulligan *et al.* 1993). This is important because therapies to restore body weight in sick patients need also to restore an appropriate body composition. This requires proportionally greater increases in fat-free tissue than are typically associated with dietary interventions alone.

Regional measurements have also provided an insight into disease processes. For example, in a recent study body composition was measured by DXA in patients with

Cushing's disease and weight-matched obese patients (Wajchenberg *et al.* 1995). Total fat and lean masses were broadly similar in both groups but patients with Cushing's disease had a reduced muscle mass in the arms and legs and the intra-abdominal fat mass was increased, relative to the obese controls. These changes are now recognized as typical of hypercortisolism.

Observed changes in the composition of fat-free tissue have also adjusted our concepts of energy metabolism in disease. It is frequently observed that patients with cancer who have lost weight have an increased BMR. This has been interpreted as an increase in energy expenditure per cell and has precipitated an intensive search for 'hypermetabolic' mediators. However detailed body composition measurements have revealed that despite overall losses of fat-free mass, visceral organs are relatively well preserved in patients with cancer (Heymsfield & McManus, 1985). Body organs have a relatively high metabolic rate per unit weight relative to skeletal muscle, so if energy expenditure is expressed per unit fat-free mass this will lead to apparent hypermetabolism, whereas in fact the energy expenditure per cell has remained unchanged.

It has also become apparent that changes in metabolic rate during growth and development can be attributed to changes in the relative contribution of different body organs to body mass. Liver, brain, kidneys and heart account for 18 % body weight at birth but only 6 % in adult man. When metabolic rate is expressed in relation to body weight or surface area there is more than a threefold decrease from birth to adulthood. But since organs account for over 60 % of resting metabolic rate, changes in the proportional contribution of these organs to body mass can have a profound effect on energy expenditure at rest. If we account for the changes which occur in organ size during growth and development the changes in metabolic rate per kg can be ameliorated (Elia, 1992*b*) (Fig. 4).

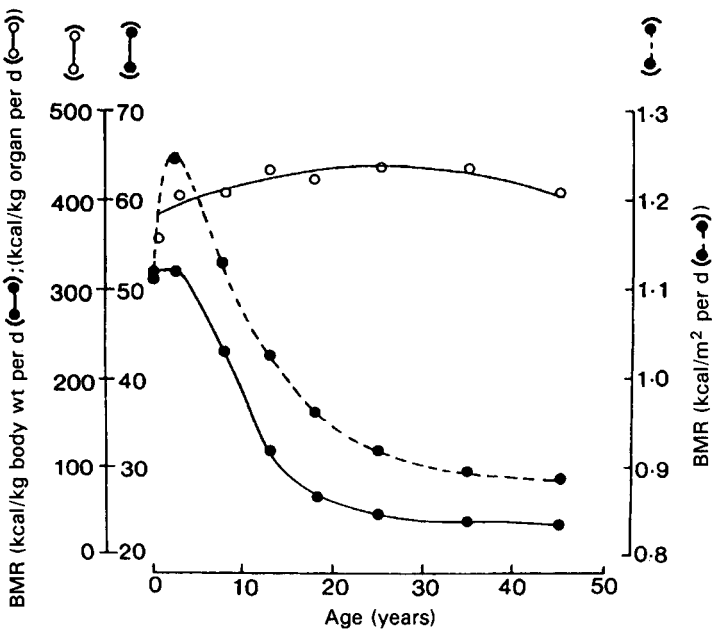

Fig. 4. Changes in BMR during growth and development expressed in relation to body weight, surface area and organ size. (Reproduced from Elia, 1992*b*, with permission.)

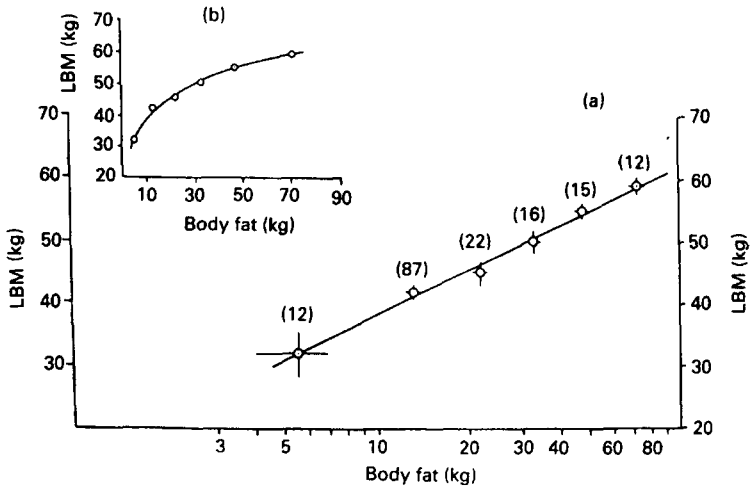

Fig. 5. (a) Plot of lean body mass (LBM, kg) *v.* $\log_{10}$ body fat (kg) for female subjects grouped according to body fat mass. (b) Plot of LBM *v.* fat from the same data. (Reproduced from Forbes, 1987, with permission.)

The effect of weight change on the relative proportions of fat and fat-free mass is an area of particular research interest. By examining body composition of individuals of different body weight, Forbes (1987) has demonstrated a so-called 'companionship' of fat and lean tissue. A plot of lean body mass *v.* body fat gives a curvilinear relationship which becomes linear on log transformation. This in itself demonstrates that, as weight is progressively gained, the composition shifts from a high proportion of lean tissue to proportionally more fat and vice versa (Fig. 5). Obesity and slimming provide a practical example of these changes. Obese individuals have more fat and more lean tissue than their lean counterparts. As predicted from Forbes' (1987) model, those with greater initial body fat stores will tend to lose a greater proportion of their weight as fat than leaner individuals. Furthermore there is some evidence that greater losses of lean tissue occur with greater energy deficits, whilst the addition of exercise to a weight loss programme can help to preserve fat-free mass (Prentice *et al.* 1991). Some adaptation also occurs as the period of undernutrition progresses, particularly in obese subjects, who show a progressive decrease in N excretion (Elia, 1992*a*). Thus the largest proportional losses of fat will occur in obese subjects who lose weight gradually through a combination of modest energy restriction and increased physical activity. Conversely individuals who are not particularly overweight, but who try to lose weight rapidly, by severe dieting over short periods of time, are liable to lose excessive quantities of lean tissue rather than fat. In this way detailed measurements of body composition during voluntary energy restriction have demonstrated important principles regarding the composition of weight loss which have helped to refine our concepts of appropriate weight loss and mould intervention strategies for the treatment of obesity.

The composition of weight change in other circumstances has also been investigated. Typical changes in body composition during the ageing process include a progressive decrease in lean tissue and acquisition of fat which occurs more rapidly with advancing age (Borkan & Norris, 1977). IVNAA studies reveal a selective loss of muscle mass with relative preservation of visceral organs and blood proteins (Cohn *et al.* 1980). The mechanism underlying these changes is unclear but is likely to include hormonal and

cytokine mediators (Roubenhoff & Rall, 1993) in addition to a decline in physical activity. In direct contrast accurate measurements have demonstrated that intensive physical activity leads to a loss of body fat and modest increases in fat-free mass (Forbes, 1992). Studies are now underway to examine the effect of different types of exercise, not only on gross changes in body composition but also on fat distribution. A 1-year programme of resistance training in subjects aged 50–70 years has shown increases in body density which represent an increase in fat-free mass and decrease in fat relative to a control group who did not take part in the exercise programme (Nelson *et al.* 1996). Regional CT scans were able to identify a statistically significant increase in thigh muscle mass. In this way it will be possible to identify the most effective interventions to reduce or prevent the age-related decrease in muscle mass. Further studies need to assess whether this can fulfil the theoretical benefits of improved health and well-being in later life.

Indeed the major interest in body composition and fat distribution stems from a growing appreciation of the links between body fat mass and long-term health. The relationship between excess weight and morbidity or premature death is well known (Bray, 1985), but we are only just beginning to refine this to a link between excess fatness and the sites of excess fat deposition (Seidell, 1992). A recent examination of 10-year mortality following the 1984–5 *Heath and Lifestyle Survey* of British adults has shown that the waist : height ratio (an index of central obesity) is a better predictor of mortality than the BMI in both men and women (Cox *et al.* 1996). Increased visceral fat leads to reduced hepatic insulin clearance which leads on to hyperinsulinaemia, glucose intolerance and ultimately diabetes, whilst increased hepatic VLDL production leads to increased triacylglycerols and decreased HDL, thus increasing the risk of IHD. Understanding the factors controlling fat distribution is a subject of intensive current research; although it is widely believed to derive from multiple perturbations of the endocrine system associated with a hypersensitive hypothalamo–pituitary–adrenal axis, hyperinsulinaemia and a marked insulin resistance (Bjorntorp, 1996). Measurements of changes in body composition allow us to monitor the effect of interventions such as changes in dietary patterns, physical activity, and medical or surgical treatments. This allows some assessment of the efficacy of the intervention strategy in the short term before long-term health outcomes can be measured directly in terms of morbidity and mortality.

CONCLUSIONS : TOWARDS THE NEW MILLENNIUM

The challenge for the future is to further refine body composition methodology, both to develop simpler and more practical reference methods and new prediction techniques with improved accuracy. This is particularly important in the case of fat distribution which has received less attention to date than measures of gross composition. However, as its importance is increasingly recognized as a determinant of morbidity and mortality, more sophisticated and more practical methods are likely to be identified. There is a particular interest in the measurement of muscle mass at specific sites and in the whole-body. Measures of segmental impedance are of potential value here as a more practical alternative to imaging techniques (Organ *et al.* 1994).

In all cases accuracy and precision are vital. With the advent of sophisticated multi-compartment models it will be easier to validate absolute measurements of fat mass against a realistic *in vivo* standard and, it is hoped, ensure better cross-instrument comparability. For the measurement of changes in composition, perhaps in response to a change in diet or exercise, ill-health or treatment, precision is the key element. To measure a change of only 2 % will require 130 subjects if the precision of the method is 5 %, whereas if the precision

is better than 1% only five subjects will be required to measure this change with 90% power and 5% significance.

However the methodology is only important in so far as it allows us to make new advances in our knowledge of the physiological system. Perhaps the most significant outstanding compartment of the body is glycogen, both at a whole-body level and its distribution in liver and muscle stores. Given the central role which has been proposed for carbohydrate in the regulation of substrate disposal, *in vivo* measurements of glycogen have never been of more interest to nutritionists, yet its quantification is still elusive. The best estimates to date come from MRI spectroscopy but this is still far from being a routine clinical measure (Morris *et al.* 1994). We are also likely to see growing interest in subfractions of the gross body compartments, for example, to distinguish muscle fibre types or to identify the fatty acid composition of fat stores. Ultimately it may be taken to the point of quantifying energy stores at the level of individual cells or tissues in the form of ATP.

With access to simple but accurate techniques, the analysis of body composition at the whole-body, tissue, cellular, molecular or atomic level will become routine (Wang *et al.* 1992). Armed with these techniques we will be able to advance further our understanding of human physiology. One of the most promising areas at the present time is the use of MRI and spectroscopy, but there are currently too few biologists who understand the capabilities of the methods and too few physicists who understand the biological questions which need to be addressed. It is vital in this field that methodology and practical applications are tightly linked in order to ensure maximum progress. In this respect Dr Widdowson has played a key role in the development of body composition science by exploiting the best methods available to answer the physiological questions of the day. If we can continue in her footsteps we should see great progress into the next millennium.

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