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Nutrition Discussion Forum

Particulate matter loss and the polyester-bag method

Without any doubt, the polyester-bag method is a very useful and common tool when evaluating ruminal utilization of feed. The first-order kinetic model as described by Ørskov & McDonald (1979), McDonald (1981) and Dhanoa (1988) is widespread and commonly used for description of disappearance curves. The model has two drawbacks clearly identified: (a) the assumption of fraction A being totally and quickly degraded in the rumen when it is known that solubility does not necessarily mean degradability, and (b) particulate matter loss causing bias in true disappearance from the bag. I was pleased to see in the Journal a letter from Lopez *et al.* (1994) where they suggested a correction for particulate matter loss (PL) when applying the polyester-bag method. They suggested the corrected disappearance calculation as:

$$y_t^{(c)} = (y_{t(0)} - g)/(1 - g), \tag{1}$$

where $y_t^{(c)}$ denotes corrected PL, and $y_{t(0)}$ the observed disappearance;

$$g = \Delta_0/(1 - y_{t(0)}), \tag{2}$$

and Δ_0 denotes PL at zero time (for detail, see Lopez *et al.* 1994).

Correcting for PL does not affect the estimate of fractional degradation rate *c* or the lag time T, only the estimates of constants A and B (Lopez *et al.* 1994). However, the assumption made for the mathematical modelling was as PL remaining in the bag and then proportionally lost in time, '... the particulate matter loss that would still be undegraded in the bag at time *t* had there been no particulate loss from the bag.' Although I agree with the results, I must present an alternative rationale to it.

As PL is a representative sample of the bag material contents, let us assume that Δ_0 has a soluble contribution ($\Delta_0^{(a)}$) to $y_0^{(c)}$ in the same magnitude as the total sample (notation as in Lopez *et al.* 1994):

$$\Delta_0^{(a)} = \Delta_0/y_0^{(c)}, \tag{3}$$

therefore the total soluble component is made from the soluble fraction coming from the remaining bag material ($y_0^{(c1)}$) and the soluble fraction from the PL ($\Delta_0^{(a)}$). Thus, the initial incubated sample (IS) at zero time is composed of four fractions, a fraction that will remain in the bag after washout (remaining Sample (RS)) and three components of washout losses.

$$IS = RS + y_0^{(c1)} + \Delta_0^{(c)} + \Delta_0^{(a)}, \tag{4}$$

where

$$\Delta_0^{(c)} = \Delta_0 - \Delta_0^{(a)}. \tag{5}$$

As $\Delta_0^{(c)}$ and $\Delta_0^{(a)}$ represent a bias in the real disappearance due to its rapid disappearance on the first washouts (Lopez *et al.* 1994), and being a known amount of material, it therefore

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can be subtracted to $y_t^{(o)}$. This subtraction leads us to a corrected sample size, then $y_t^{(c)}$ becomes:

$$y_t^{(c)} = (y_t^{(o)} - (\Delta_0^{(c)} + \Delta_0^{(a)}))/(1 - (\Delta_0^{(c)} + \Delta_0^{(a)})). \tag{6}$$

Changing notation in the formula results as in the Lopez *et al.* (1994) letter, however the rationale differs, as now there is no account for $\Delta_0^{(c)}$ and $\Delta_0^{(a)}$. This rationale solves the problem which may arise if Δ_0 had originated from non-nutritional components (i.e. soil) as suggested by D. Hovell (personal communication). I do not suggest changing the formula from Lopez *et al.* (1994) just to clarify that their rationale does not really account for a proportional loss in time of the particulate matter. Their formula just subtracts the component $\Delta_0^{(c)} + \Delta_0^{(a)}$ and creates a corrected sample size. Changing the rationale leads to a better understanding of the process and avoids future misinterpretations.

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Particulate matter loss and the polyester-bag method – Reply by Ørskov

I am interested in the discussion on the nylon bag method. First, nobody would state that 'a' represents the soluble fractions. It is the intercept, but for many protein concentrate feeds it is very similar to the soluble fraction. For roughages it is certainly not as discussed by McDonald (1981). For this reason we have adapted to use laboratory-determined solubility for roughage which we denote A. The insoluble but fermentable fraction B is then simply $(a+b) - A$, and c, of course, is the rate constant. Here it is better to avoid short incubation times or beginning at about 8 h. The A, B and c values of roughages are used effectively to characterize roughage as it is related to voluntary feed intake (Ørskov, 1994).

However, the question is about particle loss, and this undoubtedly occurs depending on particle size of substrates and pore size of bags. Some substrates are unsuitable, e.g. bloodmeal. For plant material some correction can be made for particle loss by determining NDF loss during washing.

The simple equation

$$p = a + b(1 - e^{ct})$$

is of course the mean of a number of equations if we could accurately determine them. The question is really whether it is sufficiently accurate or whether additional complicated

maths should be involved? I think the popularity of the equation is due to its simplicity. It can be used by everybody and for most purposes accurately enough. That of course should not prevent anybody from seeking to improve it, but the objective must be kept in mind. It is a very robust tool which is an additional advantage in countries where electricity is unstable and *in vitro* systems difficult to maintain. The nylon bag technique has been, and continues to be, a very powerful tool for ruminant nutrition studies.

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Particulate matter loss and the nylon-bag method – Reply by France et al.

Substrate disappearance from nylon bags includes material that has actually been dissolved or degraded, and material that has escaped without being degraded. This latter loss is due to the interaction between pore size of the bag and particle size of the incubated sample. It occurs when the particulate matter has a smaller particle size than the pore diameter.

The loss of particulate matter from the bags can result in significant overestimation of substrate degradation by rumen microbes and their enzymes. Although it has to be accepted that disappearance and degradation do not correspond exactly, correction for particulate matter loss would reduce the difference between the two and allow ruminal degradability to be estimated more accurately.

Overestimation of substrate degradation caused by particulate matter loss is especially important during the initial stages of incubation. Particle losses occur immediately the bags are immersed in the rumen contents. Initially no degradation takes place and disappearance is due to two factors: solubility, and particle loss. Any substrate which has dissolved in the rumen contents has to be considered a component of the effective degradability. Particle loss is composed of (non-soluble) particulate matter which may or may not be degraded with time if it had remained in the bag. Overestimation of substrate degradation is due to the loss of undegraded (undegradable plus potentially degradable) particulate matter during each incubation interval, and therefore varies with time because the potentially degradable fraction of the particles lost would have been degraded to a different extent.

Using such arguments, we corrected observed cumulative substrate disappearance using the equation (see Lopez *et al.* 1994):

$$y_t^{(c)} = (y_t^{(o)} - \lambda)/(1 - \lambda), \quad (1)$$

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where

$$\lambda = \Delta_0/(1 - y_0^{(c)}). \quad (2)$$

The notation used is as follows: $y_t^{(c)}$ denotes corrected cumulative disappearance to time t, $y_t^{(o)}$ is observed disappearance, and Δ_t the particulate matter lost that would still be undegraded in the bag at time t had there been no particulate matter loss from the bag (all in units of g loss/g initially incubated). Obviously, the three entities are related:

$$y_t^{(c)} = y_t^{(o)} - \Delta_t. \quad (3)$$

Δ_0, the zero time value of Δ_t, is calculated as the difference between the washout value $y_0^{(o)}$ (observed disappearance) and the solubility of the feed $y_0^{(c)}$ measured after soaking and filtering (corrected disappearance).

The standard model for analysing substrate disappearance profiles obtained using nylon bags is the Ørskov model, a model based on simple first-order kinetics and described by Ørskov & McDonald (1979), with the lag effect incorporated as suggested by McDonald (1981) and Dhanoa (1988):

$$y_t = A + B(1 - e^{-c(t-T)}), t \geq T, \quad (4)$$

where y_t is cumulative disappearance (g loss/g initially incubated) to time t (h) and A, B, c, and T are constants. A represents the soluble fraction (assumed instantly degradable), B the degradable part of the insoluble fraction, c the fractional degradation rate (per h) and T the lag time before the commencement of degradation of B. Estimates of these parameters, used in conjunction with an estimate of ruminal rate of passage k (per h), permit evaluation of the extent of ruminal degradation E (g degraded/g ingested) by applying the formula:

$$E = A + Bce^{-kT}/(c + k). \quad (5)$$

As an alternative to correcting the data before fitting a model, the estimates of the model parameters can be corrected after fitting. With the Ørskov model, correcting for particulate matter loss does not affect the estimates of the parameters c and T, only the estimates of A and B whose corrected and uncorrected values are related by Lopez *et al.* (1994):

$$A^{(c)} = (A^{(o)} - \lambda)/(1 - \lambda), \quad (6)$$

$$B^{(c)} = B^{(o)}/(1 - \lambda). \quad (7)$$

The associated expression for correcting the extent of ruminal degradation is:

$$E^{(c)} = (E^{(o)} - \lambda)/(1 - \lambda). \quad (8)$$

It must be stressed that the use of this correction does not completely solve the problem of overestimation of degradation due to undegraded particulate matter losses from the bags. The correction is less accurate for substrates of high degradability whose undegraded particles are more readily lost, such as finely ground concentrates. With some of these concentrates, 100% disappearance is observed after as little as 24 h of incubation. In such cases the 24 h value for corrected disappearance given by Equation (1) would be unity with no possibility of subsequent correction, although some particles would have been lost and the concentrate incubated would have contained a portion of undegradable material. The correction, however, can be really useful when applied to substrates such as forages.

The determination of solubility is certainly important in obtaining estimates of actual substrate disappearance at time zero. But it is not possible from this alone to correct

cumulative disappearance at other time points and obtain an estimate for the extent of ruminal degradation corrected for any bias due to particle loss. Mathematical modelling allows a correction to be derived which significantly improves our estimates of the fraction of a feed (or feed component) degraded in the rumen. The correction is simple, its application does not require any special experimental or computing facilities, and it can be adopted for whatever model is chosen to describe the degradation profile (for a review of models see France *et al.* (1990)).

Sandoval-Castro (1997), in this Discussion Forum, suggests the expression for λ (Equation 2) be simplified, i.e.

$$\lambda = \Delta_0. \tag{9}$$

The simplification has two serious flaws. First, the observed disappearance at time zero, less the instantaneous particle loss, should equal solubility (i.e. $y_0^{(c)} = y_0^{(o)} - \Delta_0$), but this equality cannot be obtained with the simplification suggested. Second, the simplification assumes that particle loss is representative of the whole sample (including the soluble fraction).

It is clear that any soluble fraction will be lost from the bag (lost though not necessarily degraded) the instant incubation begins as a consequence (by definition) of its solubility, and not as a consequence of washout of particles. The argument that a substrate may contain soluble matter which has to be considered particle loss as it would not be degraded (e.g. soil contaminants) is, in our opinion, not valid. Probably this soluble matter is not going to be utilized (maybe not even absorbed) by the animal, but it definitely undergoes dissolution in rumen contents. The mathematical modelling used to estimate ruminal degradability in the nylon-bag technique serves to describe the degradation process, and not the further absorption and utilization of degraded substrates by the animal. On the other hand, samples are assumed to be representative of the original feed. Obviously if there is any contamination (e.g. by soil), any analytical determination (chemical composition, *in vitro* or *in situ* digestibility) is going to be inaccurate, whatever correction is used. Working with organic matter, rather than dry matter, disappearance should obviate this possible artifact.

There are other crucial errors in the analysis by Sandoval-Castro (1997):

(i) after his Equation (1), $y_t^{(c)}$ does not denote corrected particulate matter loss PL), but rather corrected total matter disappearance (g loss/g initially incubated) at time t;

(ii) in his Equation (2), $y_{t(0)}$ should read $y_0^{(c)}$;

(iii) in his Equation (4), of the three components of $y_0^{(o)}$, the fraction washed out at zero time, $y_0^{(cl)}$ is identical to $y_0^{(c)}$ used in Equations (2) and (3) because Δ_0 is equal to $y_0^{(o)} - y_0^{(c)}$.

Ørskov (1997), also in this Discussion Forum, emphasizes the utilitarian nature of his model (Equation 4). It is indeed a simple and very robust model, and the pioneering contribution of Dr Ørskov and his colleagues to the development of the nylon-bag methodology in general deserves the widest recognition. We advocate the Ørskov model as the model of the choice generally – but not universally. With forages and forage-based diets, for example, the disappearance profiles sometimes exhibit sigmoidal rather than diminishing returns behaviour (e.g. Fig. 1) and analysis based on simple first-order kinetics may not be appropriate in such cases because the underlying kinetics are more complex. Useful kinetic information is lost by fitting too simple a model. In biology, very few models are applicable universally, and in selecting a model one should adhere to Einstein's maxim that science should be simple, but not too simple. Scientists are beholden to seek simple explanations wherever possible but to avoid making them

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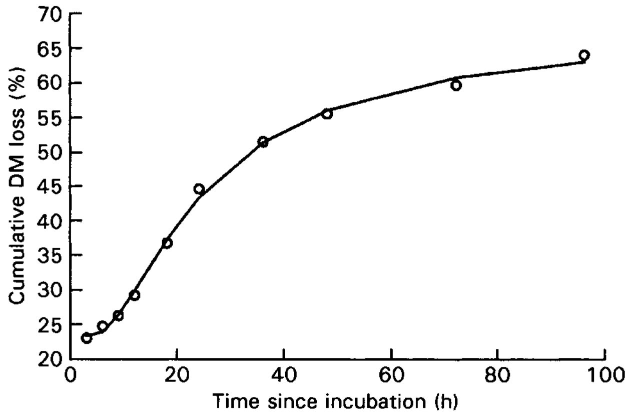

Fig. 1. Dry matter disappearance profile for a short-chopped brome hay.

simplistic. Aspects of model choice when analysing nylon-bag data are discussed by Dhanoa *et al.* (1996).

Consequently, we proposed a new model to describe degradation in the rumen (Dhanoa *et al.* 1995), which was derived by postulating that the fractional degradation rate μ (per h) varies with time according to:

$$\mu = c + d/(2\sqrt{t}), t \geq T, \tag{10}$$

where c (per h) and d (per $h^{0 \cdot 5}$) are constants. This allows for underlying kinetics which is diminishing returns or sigmoidal in type. Mathematically, the model can be considered a generalization of the Ørskov model and takes the form:

$$y_t = A + B(1 - e^{-c(t-T)-d(\sqrt{t}-\sqrt{T})}), t \geq T, \tag{11}$$

where y_t, A, B and T are defined as stated after Equation (4). When $d=0$, Equation (11) reduces to Equation (4), the Ørskov model. The extent of ruminal degradation for our model is given by the formula:

$$E = A + B(1 - kI)e^{-kT}, \tag{12}$$

where k (per h) is again the rate of passage constant and:

$$I = \int_t e^{-[(c+k)(t-T)+d(\sqrt{t}-\sqrt{T})]} dt. \tag{13}$$

The integral I, which is non-analytical and therefore has to be evaluated numerically, can be calculated using the AREA function available in most statistical software packages.

Correcting for particulate matter loss can be accomplished either before or after fitting this model. Correcting after fitting affects the parameters A and B, but not c, d and T. The corrected and uncorrected values of A, B and the extent of ruminal degradation E are once more related by Equations (6) to (8).

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Dietary nucleotides/nucleosides may have important metabolic functions but free adenine may produce adverse effects

I read with great interest the editorial comments of Grimble (1996) and the paper of López-Navarro *et al.* (1996) about essentiality of dietary nucleotides. Grimble (1996) eloquently reviewed recent literature supporting the importance of dietary nucleotides in terms of reduced survival of animals (fed on a nucleotide-free diet) after intravenous infection with *Candida albinus* or *Staphylococcus aureus* (Grimble, 1994); reduced net protein catabolism following 75 % hepatectomy during total parenteral nutrition (TPN) supplemented with a mixture of nucleosides and nucleotides (Ogoshi *et al.* 1985); reduction in first incidences of diarrhoea in Chilean children (from low socioeconomic backgrounds) by supplementing formulas with nucleotides (Brunser *et al.* 1994); and

time-dependent transient negative effects on liver structure and function associated with the lack of an adequate supply of dietary nucleotides (López-Navarro *et al.* 1996).

Apparently, no effort was made to make the nucleotide-deprived and the nucleotide-supplemented diets isonitrogenous in the study of López-Navarro *et al.* (1996). Therefore, the findings of López-Navarro *et al.* (1996) could be questioned on a scientific basis. In studies of this nature it is essential that the diets be made isonitrogenous because of the obvious relationship between protein and nucleotide metabolism.

Grimble (1996) also pointed out that serious consideration should be given to nomenclature since the exogenously administered nucleotide, nucleoside or nucleobase such as purines and pyrimidines are metabolized differently and produce different alterations in uric acid metabolism in humans and animals (Clifford *et al.* 1976; Yokozawa *et al.* 1982; Brulé *et al.* 1988, 1992).

The primary objective of this communication was to emphasize the point about the nomenclature since supplementation with some purines may produce adverse metabolic effects. In our laboratory the metabolic effects of free purines (adenine, guanine, hypoxanthine and xanthine), their nucleosides (adenosine, guanosine and inosine) and nucleotides (adenosine monophosphate, guanosine monophosphate and inosine monophosphate) were studied in rats fed on casein-based diets (20 % protein) supplemented with the free purine base, nucleoside or nucleotide (30 mmol/kg diet) for 14 d (Brulé *et al.* 1988). Addition of free adenine resulted in less weight gain than in controls, greater kidney weight, greater urine volume and higher levels of blood urea nitrogen, serum uric acid, creatinine and allantoin but lower urinary levels of allantoin, uric acid and creatinine. The adenine diet also caused nephropathy characterized by nephromegaly and deposition of crystals. A microscopic examination of the kidneys revealed deposition of crystals mainly in the lumen of convoluted tubules of the cortex. Adenine produced adverse effects only when fed in the free form and not when fed as the nucleoside or nucleotide.

Information on the levels of free adenine in foods is limited. Normally, bound and free purines in foods are determined together as total purines after heat–acid hydrolysis (Sarwar & Brulé, 1991). Free adenine (0·02–0·04 %) was, however, reported to be present in dried raw soyabean obtained from several countries (Yokozawa *et al.* 1986). Changes in the levels of total purines and release of free bases have been reported to occur during stewing and roasting of chicken meat (Young, 1982, 1983). Therefore, further studies should be carried out to assess the influence of processing on the stability of the supplemental nucleosides, nucleotides, and/or nucleobases to be added to infant formulas or TPN products.

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BMR multiples as a basis for estimating energy needs

Two recent papers from Haggarty and colleagues draw attention to potential weaknesses in the current factorial procedures for estimating energy needs based upon multiples of predicted basal metabolic rate (BMR) (Haggarty *et al.* 1994, 1997). Here I suggest a further weakness of the current system. The FAO/WHO/UNU (1985) approach adopted by the Department of Health (1991) is built on two independent assumptions: (i) that BMR can be predicted from linear functions of weight and (ii) that the energy cost of activity is determined largely by body mass. Given that the energy cost of activity and BMR are both functions of body mass, the current approach assumes that the energy expenditure in activity can be calculated as a multiple of BMR. Whilst this may appear to be a reasonable assumption in principle, its application in the derivation of energy requirements (Department of Health, 1991) leads to untenable conclusions.

Work done (measured in joules) is the product of force and distance and for most human activities is determined by body mass and the distance travelled in the horizontal and vertical planes. In the absence of differences in mechanical efficiency, e.g. as a consequence of differences in stride length or gait, the energy expended in walking a given distance is likely to be independent of age or sex and directly proportional to body mass. However, because calculations of BMR are functions of age, sex and body mass, current predictions of the energy cost of physical activity, when calculated as multiples of BMR, suggest that walking a given distance at a given speed will be more energy-expensive for men than for women of the same body mass and will decrease with age (Table 1). This seems highly improbable. A more reasonable hypothesis is that the energy cost of work will remain unchanged or will increase with age because of reduced mechanical efficiency resulting from reduced flexibility of joints. There is no *a priori* reason to expect mechanical efficiency will be greater for women than for men; indeed one might anticipate the reverse for work such as walking where a shorter stride length is likely in women compared with men of the same body mass.

In summary, this analysis suggests that the current 'multiples of BMR' approach to determining energy needs (Department of Health, 1991) is likely to introduce significant

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Table 1. *Estimated basal metabolic rate (BMR) and energy expenditure during walking for 1 h for men and women weighing 70 kg**

	Age (years)		
	18–29	30–59	60–74
Estimated BMR (MJ/d): Males	7·31	7·01	6·42
Females	6·38	5·92	5·58
Additional cost of walking (MJ): Males	0·83	0·79	0·72
Females	0·71	0·66	0·63

* Calculated from Department of Health (1991) Annex 2 for BMR and Annex 3 for additional energy expenditure during walking at 4–6 km/h for 1 h (Physical Activity Ratio = 3·7).

errors, particularly for the most physically active individuals. If it is assumed that the true absolute costs of physical activity (on which the current Dietary Reference Values are based) were determined, in the main, using young male subjects, then these discrepancies will be more apparent for older people and for women. The factorial approach to determining energy requirements has much to recommend it, not least its flexibility, but the current model appears to have introduced illogicalities in an attempt to simplify the necessary calculations.

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Skinfold thickness measurement

With reference to the Technical Note, Skinfold thicknesses: is there a need to be very precise in their location? by Durnin *et al.* (1997), we wish to make the following points.

The authors reported that one of the most common methods of assessing fatness in adults is by measuring skinfold thickness using the equations of Durnin & Womersley (1974) and Jackson & Pollock (1978). Whilst reference is made to the generalized equations of Jackson & Pollock (1978) for men (and we assume by inference the Jackson

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et al. (1980) equation for women), there is no further reference to the use or application of these equations.

It is notable therefore that the selection of sites omits any from the lower body, which are among the strongest predictors of subcutaneous adiposity. The Brussels Cadaver Study (Martin *et al.* 1985) revealed that of the six best predictors of subcutaneous adipose tissue, all but one were in the lower limb, and four of these were situated in the thigh. The suprailiac, biceps, subscapular and triceps ranked 8th, 9th, 10th and 11th respectively, with the latter not reaching significance at the $P < 0{\cdot}05$ level. The thigh and calf were also among the best predictors of hydrostatically determined body density in a group of Chinese men and women (Eston *et al.* 1995). Other independent studies have also shown that ultrasound sections of the thigh region are highly correlated with body density (Eston *et al.* 1994) and studies using dual X-ray absorptiometry (Stewart *et al.* 1997) show higher correlations with the skinfold totals which incorporated the thigh site. It is a pity that the lower limb sites were not included in the paper.

Obviously, the conclusions drawn from this study are dependent on the selection of the subcutaneous fat tissue at specific sites which, as MRI studies show, vary considerably from site to site. Of the four sites measured, the suprailiac site is one of particular concern, because the exact location varies depending on the equation used. The commonly recognized suprailiac site, such as that used by Durnin & Womersley (1974), is a horizontal fold in the mid axillary line above the iliac crest. However, the data for the generalized equations of Jackson and colleagues (1978, 1980) were determined from a diagonal fold at the anterior axillary line. Studies show that inter-tester reliability is lowest at this site (Pollock & Jackson, 1984), and that anterior sites are normally 3–6 mm lower than sites on the mid-axillary line. This was observed in the Durnin *et al.* (1997) paper, although it does not appear that the exact site used in the Jackson *et al.* (1980) studies was included in the present paper.

On a slightly separate but related issue, the authors recognize that the skinfold technique may provide the most valid estimates of fatness, but we have observed that percentage body fat estimates from the Durnin & Womersley (1974) equations are systematically higher than the Jackson equations. This is an important point when one considers the frequency with which these equations are used to estimate body fat, particularly on lean individuals and athletes. As to which equation is the more valid, from our experience of skinfold topography and the evidence of studies which have included the lower limb we are of the opinion that the equations of Jackson & Pollock (1978) provide the more valid estimate.

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Skinfold thickness measurement – reply by Durnin

Inadequate attention is often paid to the degree of precision needed for estimates of body fatness in any particular context, and excessive amounts of time and labour are spent on quite unnecessary minutiae of technology. This attitude might, of course, give rise to the erroneous impression that any old method of measurement may be satisfactory, and Stewart and Eston are rightly concerned with negating this attitude. However, it could be argued that their critical letter, far from reinforcing their viewpoint, in fact supports our own position that too much time and effort on all aspects of the methodology is not sufficiently productive. While, as responsible and careful scientists, any technique we use should be employed with maximum care, we argue in our paper (Durnin *et al.* 1997) that small variations in the measured data have little influence on our interpretation and use of the information. The criticisms of Stewart and Eston illustrate our argument. They give specific instances which obviously they regard as important but which we consider as falling into the realm of mistaken exactitude. For example, although they state that sites from the lower body are representative of a considerable proportion of the total body fat, they cite as supporting evidence for including these sites, first the Brussels Cadaver Study (Martin *et al.* 1985) which depended on measurements on cadavers: unless these were very 'fresh', they do not represent the situation in live human bodies. They also seem to suggest that, ideally, up to four skinfolds situated on the thigh would provide a significant improvement on the experimental measurements. We are at a loss to understand this argument if a wide sample of individuals is being measured. In a very large proportion of ordinary adults, with often either obese or very muscular thighs, it is not even technically possible to lift up a skinfold in order to make the appropriate measurement. The reason that we use only four skinfolds, none of which is in the lower limb (but of course two are present in the upper limb), is dependent on the classical measurements of Tanner (1953) and Edwards *et al.* (1955) on about ninety skinfolds distributed throughout the surface of the body, where the four 'standard' skinfolds appeared to represent acceptably the overall subcutaneous adipose tissue. The statements that 'the thigh and calf were among the best predictors of hydrostatically determined body density in a group of Chinese men and women' and 'that ultrasound sections of the thigh region are highly correlated with body density' or 'that

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studies using dual X-ray absorptiometry show higher correlations with skinfold totals which are incorporated in the thigh site' do little to convince us of the persuasiveness of their arguments: with all the potentially erroneous assumptions involved in using bone density or total body water or, indeed, densitometry as a very indirect method to predict fatness, who could possibly be prepared to argue the significance of comparatively small variations in the distribution of adipose tissue? The fact that some other studies using different skinfolds give data which are smaller or larger than those obtained by our four skinfolds does not necessarily prove that one of the techniques is more accurate than the other, unless there is an overpowering volume of evidence from other authors to support one of the positions, which does not, to our mind, exist. We are not too concerned that Stewart and Eston consider the equations of Jackson & Pollock (1978) to be valid; unless some unusual requirement is being sought, the difference is likely to be of little importance.

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Diet–genotype interactions: an example from tea

The interaction between diet and genotype in the development of disease is a fast emerging area in nutritional science, especially in relation to diseases in middle to late age such as cardiovascular disease, cancer and dementia. The importance of several dietary factors in the prevention of cardiovascular disease is well-established and recommendations to reduce saturated fat intake on a national level are in place (Department of Health, 1991, 1994). However, multiple genetic factors involved in the pathogenesis of coronary heart disease are important in determining individual risk within populations. Among them apolipoprotein E (ApoE) gene polymorphism is considered to be one of the most important (Castelli, 1996). Three common isoforms of the gene protein product, E2, E3, and E4, strongly affect total serum cholesterol and LDL concentrations, E4 being associated with substantially elevated levels of these substances and an increased risk of cardiovascular disease (Davignon *et al.* 1988) and Alzheimer's disease (Weisgraber & Mahley, 1996). With the availability of new genotyping methods, interactions between dietary and genetic factors at an individual level are now identifiable. As an example of this we have reanalysed our recent study of tea and cardiovascular risk factors published in this Journal, in which no effect of tea supplements on serum lipids, blood pressure, or blood clotting were found (Bingham *et al.* 1997). Having performed ApoE genotyping in

the individuals studied we have been able to reveal allele-dependent differences in levels of blood lipids and blood coagulation factors as well as differences in response during the tea-drinking period. The reanalysis results further stress the importance of a differential approach to groups of people with different genotypes in assessment of the role of dietary factors in the pathogenesis of coronary heart disease and other diseases of nutritional origin.

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