An assessment of the methods available to determine nutritional equilibrium in patients with chronic renal failure

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Assessment of status and nitrogen balance

The nutritional assessment of patients with chronic renal failure includes an assessment of body fat, fat free mass (subdivided into somatic and visceral protein) [1] and dietary intake. Figure 1 shows some of the methods that can be used to assess fat free mass and dietary intake. A few are applicable to everyday clinical practice whereas others should be seen only as research tools. Methods of dietary assessment include precise weighing, a weighed inventory, a dietary history and dietary recall [2,3]. In clinical practice a 3 day dietary history is commonly used but lacks the accuracy necessary for clinical research. Most such studies use a weighed inventory. However, with increasing complexity of dietary assessment there is a danger that patients will change their normal dietary practice to facilitate the assessment. Another method that is commonly used is to measure the urea production rate and then using a variety of regression equations derived from other studies to estimate total nitrogen output. It is then assumed that the patient is in neutral nitrogen balance (i.e. what is going in equals what is coming out) and the dietary protein intake is therefore the product of the total nitrogen output and 6.25 (assuming that on average 16% of protein is nitrogen). A major problem associated with this is that most patients are not in balance at any one time. To overcome this one needs to undertake nitrogen balance whereby patients are admitted to a metabolic ward and placed either on a diet with the same caloric and nitrogen content as their usual diet (isocaloric, isonitrogenous) or an experimental diet if this the aim of the study [4,5]. All urine and faeces are collected and the nitrogen content (UN and FN) measured directly. The nitrogen intake (IN) is calculated by the direct measurement of nitrogen in an aliquot from a homogenate of a parallel total daily dietary intake. In CRF it is necessary to take into account any changes that may occur in the body urea nitrogen pool (BUN × Vd) during the period of assessment with daily measurements of BUN. Using linear regression the change in the pool (ΔN) can then be estimated and used to calculate nitrogen balance (BN) where:

\[ B_N = I_N - U_N - F_N - \Delta N \]

To undertake accurate nitrogen balance studies requires meticulous attention to detail [6]. It is not a tool that can be used in routine clinical practice and there are now only a few clinical research centres world wide capable of undertaking it. If the aim of the study is to assess the effect of a dietary intervention it is necessary to allow a period of equilibration of up to one week before starting to measure nitrogen balance.

The concept of protein turnover

Nitrogen balance ignores the fact that there is a turnover of protein within the body which is approximately five times greater than the daily protein intake [7]. For example, protein turnover in an individual consuming a 60 g protein diet is of the order of 300 g per day. It is the daily balance between whole body protein degradation (PD) and whole body protein synthesis (PS) that dictates whether an individual is in neutral nitrogen balance, positive nitrogen balance (anabolic) or negative nitrogen balance (catabolic).

Measurement of protein turnover

Using tracer techniques it is possible to directly measure both whole body and specific tissue protein turn-
over. It was Rudolf Schoenheimer who in 1939 first described the use of leucine isotopes in the study of protein metabolism in vivo in humans [8]. To undertake such studies requires a model of protein metabolism which can be used to calculate PD and PS. In the model shown in Figure 2 the flux of an amino acid (Q) is equivalent to dietary intake (I) plus the input from protein breakdown (B) and the input from de novo synthesis (N). Flux is also equivalent to incorporation of amino acid into body protein (S), plus oxidation (C) and other forms of metabolism (M). Therefore,

\[ Q = I + B + N = S + M + C \]

This model can be simplified further by choosing an appropriate amino acid as a tracer. For instance, use of an essential amino acid allows the component of flux due to de novo synthesis (N) to be ignored. Likewise, choice of an amino acid that is oxidized by only one pathway and has no other metabolic fate results in further simplification of the model such that the equation for flux becomes

\[ Q = B + I = S + C \]

The most frequently used amino acid is leucine, a branched chain amino acid with only one pathway of catabolism. It is predominantly metabolized in muscle and provides by transamination an intracellular marker of leucine carbon, \( \alpha \)-ketoisocaproic acid (\( \alpha \)-KIC). Human experiments now usually use leucine labelled with \( ^{13} \)C. The initial step in the metabolism of \( \text{L-}[1-{^{13}} \text{C}] \)leucine is the reversible transamination reaction catalysed by branched chain amino acid aminotransferase (BCAAT) to \( \alpha \)-[\( ^{13} \)C-KIC]. The plasma isotopic enrichment of this is measured in preference to \( \text{L-}[1-{^{13}} \text{C}] \)leucine because it has been shown to better reflect intracellular leucine enrichment. Following this step, \( [1-{^{13}} \text{C}] \)KIC undergoes irreversible oxidative decarboxylation by branched-chain keto acid dehydrogenase (BCKAD) to isovaleryl-CoA with loss of \( ^{13} \)C as \( ^{13} \)CO\(_2\). This can be measured in

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**Fig. 2.** Generalized model of protein turnover.

**Fig. 3.** Nitrogen balance using classical (measured) and isotopic methods in CRF patients and normal controls on both a low protein (LP) and high protein (HP) diet. (Reproduced with the permission of the Journal of the American Society of Nephrology.)
expired air using isotope ratio mass spectrometry (IRMS). L-[1-13C]leucine is usually administered as a primed constant intravenous infusion; a priming dose of NaH13CO3 is also given and this enables isotopic equilibrium to be reached in as short a time as possible. Typically a 4–5 h infusion will be undertaken. Samples of expired air and plasma are taken before the start of the infusion and at 15 min intervals during the last hour of the study. The plasma samples are derivatized to a product of KIC which can be analysed for 13C enrichment by gas chromatography mass spectrometry. The leucine flux is calculated from the L-[1-13C]leucine infusion rate and the [1-13C]KIC enrichment at isotopic equilibrium. Leucine oxidation rate is calculated from the 13CO2 expiration rate and the [1-13C]KIC enrichment at isotopic equilibrium. This involves measuring CO2 production rate (VCO2) which is now easily done using open-circuit indirect calorimetry with a ventilated hood system. If studies are done fasting the aforementioned equation is simplified to

\[ Q = B = S + C \]

Because C is leucine oxidation S can be solved. Studies in the fed state can be solved provided that I is quantified with regard to the leucine intake. If subjects are studied both fasting and fed then it is possible to calculate a balance between leucine disappearance into body protein (S) and leucine appearance from body protein (I) in both states. This leucine balance can be converted to a nitrogen equivalent, assuming that body protein contains (by weight) 8% leucine and 16% nitrogen. Using both this technique and classical nitrogen balance Goodship et al. were able to compare the two in CRF patients and control subjects consuming diets containing both 0.6 (LP) and 1.0 (HP) g kg\(^{-1}\) day\(^{-1}\) protein [9]. The absolute results were not the same but the results for the difference between the two dietary protein levels were identical. This is shown in Figure 3. The discrepancy between the results obtained using the two methods may be explained by the experimental design: the subjects were fed continuously for 6 h to obtain the isotopic results, while the normal intermittent feeding pattern over 5 days was used to measure ‘classical’ nitrogen balance.

Assumptions made when measuring protein turnover

Besides measuring whole body amino acid kinetics, stable isotopes can also be used to measure flux across a tissue bed such as forearm muscle [10] and the fractional synthesis rate of individual proteins such as albumin. However, the use of these methods with a constant primed infusion of tracer make several assumptions [11], including the following.

(i) A steady state is present.
(ii) The dose of tracer will not have any effect on metabolism of tracee (i.e. is a true tracer). This may particularly be a problem with the flooding dose method [12] whereby a large bolus of (3–4 g) of labelled and unlabelled amino acid is give to rapidly flood all the free amino acid pools to a common value which can be determined by sampling the plasma pool.
(iii) The labelled and unlabelled amino acids are handled identically.
(iv) No significant recycling of isotope should take place.
(v) The enrichment measured (in plasma) should be representative of that at the site of protein synthesis.

Despite these assumptions the use of stable isotopes in nutritional research is now a well established technique that has significant advantages over the ‘classical’ methods of nutritional assessment such as nitrogen balance.

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References