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Pharmacology

Bch 204 Assignment

Nutrition

**Question**

1. WHAT DO YOU UNDERSTAND BY THE TERM ''BIOLOGICAL VALUE OF PROTEINS"

2. LIST AND EXPLAIN THE VARIOUS METHODS OF ASSESSMENT OF PROTEIN QUALITY.

1. Biological Value Of Protein

**Biological value** (**BV**) is a measure of the proportion of absorbed [protein](https://en.m.wikipedia.org/wiki/Protein) from a food which becomes incorporated into the proteins of the organism's body. It captures how readily the digested protein can be used in [protein synthesis](https://en.m.wikipedia.org/wiki/Protein_biosynthesis) in the [cells](https://en.m.wikipedia.org/wiki/Cell_(biology)) of the organism. Proteins are the major source of [nitrogen](https://en.m.wikipedia.org/wiki/Nitrogen) in food. Biological Value assumes protein is the only source of nitrogen and measures the proportion of this nitrogen absorbed by the body which is then excreted. The remainder must have been incorporated into the proteins of the organisms body. A [ratio](https://en.m.wikipedia.org/wiki/Ratio) of nitrogen incorporated into the body over nitrogen absorbed gives a measure of protein "usability" – the Biological Value

Unlike some measures of protein usability, biological value does not take into account how readily the protein can be [digested](https://en.m.wikipedia.org/wiki/Digestion) and absorbed (largely by the [small intestine](https://en.m.wikipedia.org/wiki/Small_intestine)). This is reflected in the experimental methods used to determine Biological Value.

Biological Value uses two similar scales:

1. The true percentage utilization (usually shown with a percent symbol).
2. The percentage utilization relative to a readily utilizable protein source, often [egg](https://en.m.wikipedia.org/wiki/Egg_(food)) (usually shown as unit less).

These two values will be similar but not identical.

The Biological Value of a food varies greatly, and depends on a wide variety of factors. In particular the Biological Value, value of a food varies depending on its preparation and the recent diet of the organism. This makes reliable determination of Biological Value difficult and of limited use — fasting prior to testing is universally required in order to ascertain reliable figures.

Biological Value is commonly used in nutrition science in many [mammalian organisms](https://en.m.wikipedia.org/wiki/Mammals), and is a relevant measure in humans. It is a popular guideline in [body building](https://en.m.wikipedia.org/wiki/Bodybuilding) in protein choice.

For accurate determination of Biological value:

1. the test organism must only consume the protein or mixture of proteins of interest (the test diet).
2. the test diet must contain no non-protein sources of nitrogen.
3. the test diet must be of suitable content and quantity to avoid use of the protein primarily as an energy source.

These conditions mean the tests are typically carried out over the course of over one week with strict diet control. Fasting prior to testing helps produce consistency between subjects (it removes recent diet as a variable).

There are two scales on which Biological value is measured; percentage utilization and relative utilization. By convention percentage Biological value a percent sign (%) suffix and relative Biological Value has no unit.

### Percentage utilization

Biological value is determined based on this formula.

BV = ( *Nr* / *Na* ) \* 100

Where:

*Na* = nitrogen absorbed in proteins on the test diet

*Nr* = nitrogen incorporated into the body on the test diet

However direct measurement of *Nr* is essentially impossible. It will typically be measured indirectly from nitrogen excretion in [urine](https://en.m.wikipedia.org/wiki/Urine). [Faecal](https://en.m.wikipedia.org/wiki/Faeces) excretion of nitrogen must also be taken into account - this part of the ingested protein is not absorbed by the body and so not included in the calculation of Biological value. Estimate is used of the amount of the urinary and faecal nitrogen excretion not coming from ingested nitrogen. This may be done by substituting a protein-free diet and observing nitrogen excretion in urine or faeces, but the accuracy of this method of estimation of the amount of nitrogen excretion not coming from ingested nitrogen on a protein-containing diet has been questioned.

BV = ( ( *Ni* - *Ne(f)* - *Ne(u)* ) / (*Ni* - *Ne(f)*) ) \* 100

Where:

*Ni* = nitrogen intake in proteins on the test diet

*Ne(f)* = (nitrogen excreted in faeces whilst on the test diet) - (nitrogen excreted in faeces not from ingested nitrogen)

*Ne(u)* = (nitrogen excreted in urine whilst on the test diet) - (nitrogen excreted in urine not from ingested nitrogen)

Note:

*Nr* = *Ni* - *Ne(f)* - *Ne(u)*

*Na* = *Ni* - *Ne(f)*

This can take any value from 0 to 100, though reported Biological value could be out of this range if the estimates of nitrogen excretion from non-ingested sources are inaccurate, such as could happen if the endogenous secretion changes with protein intake. A Biological value of 100% indicates complete utilization of a dietary protein, i.e. 100% of the protein ingested and absorbed is incorporated into proteins into the body. The value of 100% is an absolute maximum, no more than 100% of the protein ingested can be utilized (in the equation above *Ne(u)* and *Ne(f)* cannot go negative, setting 100% as the maximum biological value).

### Relative utilization

Due to experimental limitations Biological value is often measured *relative* to an easily utilizable protein. Normally [egg](https://en.m.wikipedia.org/wiki/Egg_(food)) protein is assumed to be the most readily utilizable protein and given a Biological value of 100. For example:

Two tests of BV are carried out on the same person; one with the test protein source and one with a reference protein (egg protein).

relative BV = ( *BV(test)* / *BV(egg)* ) \* 100

Where:

*BV(test)* = percentage BV of the test diet for that individual

*BV(egg)* = percentage BV of the reference (egg) diet for that individual

This is not restricted to values of less than 100. The percentage BV of egg protein is only 93.7% which allows other proteins with true percentage BV between 93.7% and 100% to take a relative BV of over 100. For example, [whey protein](https://en.m.wikipedia.org/wiki/Whey_protein) takes a relative BV of 104, while its percentage BV is under 100%.

The principal advantage of measuring BV relative to another protein diet is accuracy; it helps account for some of the metabolic variability between individuals. In a simplistic sense the egg diet is testing the maximum efficiency the individual can take up protein, the BV is then provided as a percentage taking this as the maximum.

### Conversion

Providing it is known which protein measurements were made relative to it is simple to convert from relative BV to percentage BV:

*BV(relative)* = ( *BV(percentage)* / *BV(reference)* ) \* 100

*BV(percentage)* = ( *BV(relative)* / 100 ) \* *BV(reference)*

Where:

*BV(relative)* = relative BV of the test protein

*BV(reference)* = percentage BV of reference protein (typically egg: 93.7%).

*BV(percentage)* = percentage BV of the test protein

While this conversion is simple it is not strictly valid due to the differences between the experimental methods. It is, however, suitable for use as a guideline.

**2.Methods of assessment of protein quality**

1. Biological Value (BV)
2. Net Protein Utilization (NPU)
3. Amino Acid Score
4. Critique
5. Protein Efficiency Ratio (PER)
6. Net Protein Ration (NPR)
7. Relative Nutritive Value (RNV).
8. Nitrogen Balance Index
9. Tissue Regeneration
10. Microbiological Assays
11. Biological Value (BV)

Biological value, as defined by Thomas (4) and Mitchell (5,6) has long been

considered the method of choice for estimating the nutritive value of proteins. It has

been defined as the "percentage of absorbed nitrogen retained in the body" and a

complete evaluation of the dietary protein includes measurement of the Biological

Value and the Digestibility. These values are obtained by measuring the fecal and

urinary nitrogen when the test protein is fed and correcting for the amounts

excreted when a nitrogen-free diet is fed. True digestibility is defined as the

percentage of food nitrogen absorbed from the gut

and Biological Value as,

BV = I – ( F – F0) – (U - U0) × 100

. I – ( F – F0)

where

I = Nitrogen intake of test protein

F = Fecal nitrogen

Fo = Fecal nitrogen on nitrogen-free diet (Metabolic N)

U = Urinary nitrogen

Uo = Urinary nitrogen on nitrogen-free diet (Endogenous N)

In practice Mitchell (6) found that the endogenous N was very similar to that

obtained when a small amount of very high quality protein was fed and preferred to

feed limited amounts of egg protein rather than a nitrogen-free diet in order to

prevent severe weight loss. The basic assumption made in the measurement of

Biological Value is that the endogenous N and metabolic N are constant values and

can be legitimately subtracted from the test values as shown in the equation. There

is limited information to suggest that this may not always be true. For example, the

excretion of urinary nitrogen in rats and dogs on a nitrogen-free diet may be

lowered substantially by the administration of methionine (7,8) yielding a Biological

Value of methionine alone much above 100%. This may not happen in man (9) but

has not been thoroughly studied. Also, Mitchell et al. (10) found the Biological Value

of gelatin to be 20%, i.e., 20% as satisfactory as the best quality proteins. Since

animals will not survive on gelatin alone, this must be an overestimate of the real

nutritive value. The discrepancy here appears to be similar to that observed by

Bender (11) in NPU values for diets that provided low intakes of most of the

essential amino acids.

The overall nutritive value of a protein (Net Protein Value) should be obtained from

the Mitchell method as Biological Value x Digestibility and this should be identical

with NPU as defined below.

1. Net Protein Utilization (NPU)

Like Biological Value, NPU estimates nitrogen retention but in this case by

determining the difference between the body nitrogen content of animals fed no

protein and those fed a test protein. This value divided by the amount of protein

consumed is the NPU which is defined as the "percentage of the dietary protein

retained". Miller (12) proposed a procedure which involved replicate groups of 4

weanling rats housed in group cages which were fed either the "protein-free" or the

"test" diet for 10 days. These conditions were chosen empirically and the particular

merits of these conditions remain to be demonstrated. Since in young animals there

is a high correlation between body nitrogen and body water content (13-16), the

substitution of body water measurements for body nitrogen measurements has

been widely used. Indeed, measurement of body water may be more accurate than

measurement of body nitrogen because sampling errors are eliminated; also, it is

much more convenient and less expensive.

Since both NPU and BV are based upon estimates of "retained nitrogen", they

should measure the same thing except that in the calculation of NPU the

denominator is the total protein eaten whereas in the calculation of BV it is the

amount absorbed. BV would be expected to be higher than NPU by the amount of

nitrogen lost owing to lack of digestibility (lack of absorption). In weanling rats, it is

possible that total carcass analysis is a more accurate measure of "retained

nitrogen" that can be obtained from nitrogen balance measurements although this

has not been proven. It is certainly less tedious. Nitrogen balance measurements

must be used in large animals and in studies on man.

1. Amino Acid Score

Block and Mitchell (17) originally proposed that since all amino acids must be

present at the site of protein synthesis in adequate amounts if protein synthesis is

to proceed, a comparable deficit of any amino acid would limit protein synthesis to

the same degree. Thus, they suggested that if the composition of an "ideal protein"

was known, i.e., a protein which contained every essential amino acid in sufficient

amounts to meet requirements without any excess, then it should be possible to

compute the nutritive value of a protein by calculating the deficit of each essential

amino acid in the test protein from the amount in the "ideal protein". The "most

limiting amino acid", the one in greatest deficit, would presumably determine the

nutritive value.

1. Critique

As has been stated, the use of estimates of protein quality to calculate the amount

of protein needed to meet requirements when different diets are consumed requires

that the estimate of quality vary in some known fashion, preferably in linear fashion,

from zero to 100% utilization. Actually, when Block and Mitchell (17) first proposed

the use of Amino Acid Scores (Fig. 1), they found that Biological Value did not

follow the predicted relationship with Amino Acid Score. Rather, the regression line

relating BV and Amino Acid Score indicated that proteins completely lacking an

essential amino acid and which would therefore have an Amino Acid Score of zero

would apparently yield a BV of approximately 25% This would mean that the

requirement could be met with such proteins if they were fed at a level providing

four times the estimated minimal protein requirement. This presumably cannot be

true since it would imply that the protein needs could be met without a supply of all

of the essential amino acids.

This apparent discrepancy between theoretical predictions and experimental data

has been largely ignored. Indeed, the FAO Committee of 1955 simply assumed that

the relationship must fit theoretical expectations. Figure 2 is taken from that

publication. Obviously with the scatter of the data available on BVs and

uncertainties as to the amino acid composition of the proteins actually tested for

BV, the true relationship was difficult to ascertain. However, it now seems quite

clear that the relationship proposed by Block and Mitchell is, in fact, substantially

correct. The values presented in Table 1 are plotted in Fig. 3 to show the

relationship between BV and Amino Acid Score. The regression line calculated

indicates that a protein of zero score would be predicted to have a BV of 40%. If BV

is to be accepted as the true measure of protein quality, then proteins of zero score

should be capable of meeting protein needs if they are fed in amounts 2½ times

greater than that required with egg protein.

Comparison of NPU and Amino Acid Score values taken from Table 1 shows

essentially the same relationship (Fig. 4) although with somewhat less deviation

from expectation. According to this plot, a protein of zero score yields an NPU of

approximately 25%. Thus, if NPU be accepted as the true measure of protein

quality, protein needs can be met by feeding proteins of zero score at 4 times the

minimal requirement.

The weakness of collecting values from a widely scattered literature in which the

accuracy of neither the biological determination nor the amino acid analysis is

known is, of course, recognized. However, this does not negate the clear fact that

Amino Acid Score does not measure the same thing as NPU and BV.

It can be pointed out, of course, that when one is concerned with diets in which

protein quality is reasonably high - NPU, BV or Amino Acid Score above 60 or 70,

for example - the error in the correction will be relatively small regardless of which

measure of protein quality is used. However, it is with diets of poor quality that

correction is of real practical importance and for these the significance of the

various measures of protein quality is in doubt.

The reasons for the discrepancy between theoretical prediction and experimental

fact are now beginning to become clear. In essence the results deny the supposed

fact that equivalent deficiencies of any essential amino acid will produce the same

limitation on protein synthesis. Whether measures of BV or NPU reflect Amino Acid

Score depends upon which of the essential amino acids is limiting although there is

still disagreement on the details of the relationship. It is clear that proteins limiting in

lysine yield much higher BVs and NPUs than would be predicted by the Amino Acid

Score. Thus Bender (11) concluded that a lysine-free diet will yield an NPU of

approximately 40 and Said and Hegsted (18) reached similar conclusions. Values

for proteins limiting in lysine are most divergent from theoretical predictions and

there is disagreement as to how far values for proteins limiting in other essential

amino acids deviate. However, protein scores of zero rarely yield NPUs or BVs of

zero. Since many of the natural proteins with low NPUs or BVs which have been

studied are limiting in lysine, it is to be expected that the relationship such as shown

in Figs. 1, 3, and 4 is probably influenced largely by such proteins.

As previously mentioned, the basic assumption underlying the thesis that Amino

Acid Score and BV or NPU ought to measure the same thing is that protein

synthesis should be limited to an equivalent degree by a comparable degree of

deficiency of any essential amino acid and that protein synthesis should cease if the

diet is devoid of any essential amino acid. Thus, a diet of zero score is expected to

be equivalent to a protein-free diet. Since diets devoid of various essential amino

acids do not produce comparable losses in body protein, and only in some

instances are the losses comparable to those obtained with a nitrogen-free diet, this

thesis is no longer entirely tenable. One can only assume that the body has varying

degrees of ability to conserve different essential amino acids when they are in short

supply. When body tissues are broken down during catabolism, certain of the amino

acids are efficiently conserved and thus supplement the supply of amino acids from

dietary sources. According to the results obtained by Said and Hegsted (18) with

the adult rat, lysine is the most efficiently conserved of all essential amino acids and

this is supported by considerable information in the literature. They found threonine,

isoleucine, and total sulfur amino acids to be least efficiently conserved although

this is not in entire agreement with Bender. Information on nitrogen balance in adult

women (19, 20) supports the contention that the adult human being responds, at

least in general terms, in a manner similar to the adult rat.

These departures from the theory upon which protein metabolism has been based

for many years raise many questions for which adequate answers are not available.

If the body has varying ability to conserve specific essential amino acids and the

mechanisms controlling this are unknown, there is a question as to whether a

general "ideal amino acid pattern" can be defined. The data accumulating with

animals and with human subjects (22, 23) indicate that the amino acid requirements

probably vary depending upon the protein status of the subject. They also point to

substantial differences in the pattern of amino acids required for maintenance and

for growth. With regard to growth, it should be emphasized that accretion of new

body protein does require essential amino acids over and above the maintenance

requirement. The results thus point toward a difference in the "ideal" pattern for

growth and for maintenance. As might be expected in view of the above discussion

indicating that lysine is rather efficiently conserved, the lysine requirement for the

growth of the young rat appears to be substantially higher (relative to several other

essential amino acids) than for maintenance. The conclusion to be drawn from this

in terms of human nutrition is not very clear, however. The data available upon the

amino acid requirements of human beings of different ages have generally been

interpreted to mean that the relative proportions of essential amino acids required at

different ages are rather similar, although it cannot be proven that they are the

same (24). It must be emphasized that even in relatively young children the rate of

growth compared to body size is very slow compared to the rates of growth of

young rats and many other species. Thus, the major proportion of the dietary

protein which is required is utilized for the maintenance of tissues already formed

rather than for the formation of new tissue proteins. The question must, therefore,

be raised as to whether estimates of protein quality based upon rapidly growing

young rats are an adequate estimate of the quality of proteins for human beings,

even for rather young infants and children.

Mitchell (17, 25) concluded that the Biological Values obtained with various species

(rats, dogs, pigs, and man) follow approximately the same relationship when

compared to amino acid composition. Mitchell (26) believed that failure of much of

the data obtained with man to correlate well with Amino Acid Score was probably

due to "imperfections in technique, quite understandable in a field of research beset

with so many difficulties". However, the combined data (25) from different species

plotted against Amino Acid Score yielded a regression similar to that obtained by

Block and Mitchell (17). Thus, the departure from theory appears not to be due to

the fact that most of the data in the literature have been obtained with rats. Rather,

it appears to be a general phenomenon in several species. Mitchell specified that

BV must be measured at or below the maintenance requirement, and thus these

conclusions do not necessarily bear upon the appropriateness of BVs for infants

and children or, indeed, for other species when they are fed sufficient protein to

allow for growth.

An additional technical point with regard to the determination of NPU and BV should

be made. If the nitrogen retained is designated Y and the nitrogen eaten or

absorbed is designated X, then the ratio Y:X which is NPU or BV is the slope of the

regression line relating Y to X. Obviously, if NPU or BV are constant and

characteristic of the protein being studied, the slope of the regression line is

constant which is to say that there is a linear relationship between Y and X. It has

been tacitly assumed, but little investigated, that this relationship is generally true

for all proteins. As shown in Fig. 6, some proteins such as lactalbumin do

approximately fulfil expectation. However, with most proteins and to varying

degrees, the situation is more like that shown for gluten in the same figure.

Extension of the linear portion of the regression line would indicate that animals fed

no gluten should lose approximately 12 g of body water whereas, in fact, animals

fed no protein lost approximately 25 g of body water. The true line must

approximate that shown by the dashed curved line at the lower right hand portion of

the figure, although it is difficult to define the curve exactly.

As has been indicated in the discussion above, proteins limiting in lysine (12,18,27)

are apparently most deviant from expectation. The reason for the curvature in the

line must be that whereas at high levels of gluten intake in Fig. 6 lysine is the

limiting factor, at some low level of intake either total nitrogen or some other

essential amino acid becomes limiting. In any event, the major point which must be

recognized is that NPU or BV as usually determined is not a constant or

characteristic of the protein.

In the scheme developed by Miller and Payne (28,29) to combine protein quality

and amount of protein into a single value, called NDpCals %, they assumed first

that NPU measured at low levels of intake would yield a value equivalent to the

Amino Acid Score. It is apparent that this is far from true especially for proteins of

rather poor quality. They also assumed that NPU measured at low intakes was

constant but that NPU fell progressively at levels above the maintenance

requirement. This also is an erroneous assumption as is indicated above. Indeed,

variations in NPU measured with young rats as the intake is increased are primarily

due to the nature of the response shown in Fig. 6, line B, rather than decreased

efficiency of utilization at higher levels of intake as they assumed. Thus, attractive

as this concept appeared to be originally, it does not adequately reflect the

response of animals to proteins of differing value fed at various levels of intake. It

should also be pointed out that since the protein and amino acid needs of young

rats are dominated by the requirements for growth, the application of such formulas

to human diets is of very doubtful validity.

1. Protein Efficiency Ratio (PER)

As has been indicated, qualitative differences in protein quality can be

demonstrated by many methods. Protein Efficiency Ratio (PER) has been the

method most widely used because of its simplicity. Osborne, Mendel and Ferry (30)

observed that young rats fed certain proteins gained little weight and ate little

protein whereas those which were fed better quality proteins gained more weight

and consumed more protein. In an attempt to compensate for the difference in food

intake, they calculated the gain in weight per gram of protein eaten and this has

been called PER. It is known that the PER for any protein is dependent upon the

amount of protein incorporated in the test diet. Standardized conditions have

therefore been proposed (31). These include the use of 10 weanling rats per test

group, diets containing 9.09% protein (N × 6.25), a test period of 4 weeks' duration,

and that each experiment include a group which receives standardized casein. The

PER is calculated as the average total weight gain divided by the average grams of

protein consumed. Since PER in various laboratories was not constant for the same

protein, it was recommended that a corrected value be calculated using an

assumed PER of the standardized casein of 2.50 (Corrected PER = 2.50 ×

PER/PER of reference casein).

1. Net Protein Ration (NPR)

A major criticism of the PER has been that it does not take into account the protein

required for maintenance since only gain in weight is used in the calculation.

Bender and Doell (36) suggested that this criticism could be avoided by the

inclusion in each test of a group of animals fed a protein-free diet. Net Protein Ratio

(NPR) was then calculated as the overall difference in gain (gain in weight of the

test group plus loss in weight of the protein-free group) divided by the protein eaten.

It is apparent that if body composition is constant, this procedure is identical to NPU

except that it is expressed in arbitrary units which are less useful than the

percentage of protein utilized. The weaknesses are, of course, identical with those

discussed under NPU.

1. Relative Nutritive Value (RNV)

Hegsted et al. (34, 37, 38, 39) proposed a slope-ratio assay using rats in which the

slope of the regression line relating body protein (or body water) of a standard

protein (egg protein or lactalbumin) assumed to have maximal nutritive value was

compared to that of the test protein. The tacit assumption made in the

measurement of NPU or BV that these values are independent of the level of

protein fed is thus tested in this procedure. As in the calculation of NPU and BV the

original assumption was made that the regression line should bisect the Y axis at

the point defined by the group fed the protein-free diet. As has already been

discussed above, this often and perhaps, usually, does not happen. The regression

lines above the maintenance level of intake are, however, linear over a substantial

range of intakes with young growing rats (40) contrary to the conclusions of Miller

and Payne (28). In young growing rats where maintenance requirements are

relatively small compared to the growth requirements, this method is probably the

most logically defensible of the assays available as an estimate of the protein

quality for growth. The important question remains as to whether estimates of

protein quality for growth in young rats are adequate estimates of quality for man

including those of the young infant. Presumably, many proteins will be more

efficiently utilized in human beings than they are for young growing rats.

1. Nitrogen Balance Index

Allison and Anderson (41) showed, as has been discussed above, that Biological

Value is the slope of the regression line relating nitrogen balance and nitrogen

intake and suggested that this might have certain advantages in practice over the

usual method of determining BV. The concept of this index is rather similar to

Relative Nutritive Value discussed above. Since it is becoming increasingly clear

that nitrogen retention is not linearly related to nitrogen intake in the region of intake

below maintenance, the validity of this index requires confirmation.

1. Tissue Regeneration

A variety of techniques involving the recovery of weight or of specific tissues after

protein depletion have been proposed (42, 43, 44, 45). The specific merits of such

assays as opposed to weight gain of young rats, for example, remain to be

demonstrated.

1. Microbiological Assays

Many micro-organisms require the essential amino acids required by monogastric

animals. If it were possible to find organisms which required not only the same

pattern of amino acids but in the same relative amounts, their growth response

when supplied with limited amounts of various proteins or protein hydrolysates

would provide a simple and efficient assay of nutritive value. Considerable effort

has been directed toward this (46, 47, 48, 49) and it is clear that the responses of

some organisms resemble those observed with some of the rat assays described.

The difficulties are clear, however, since the limitations in the animal assays mean

that they provide an inadequate base for comparison with assays of this kind.

Plasma Amino Acids

As has been indicated in another section of this report, changes in plasma amino

acid levels after the feeding of various proteins can under certain conditions yield

estimates of the nutritional quality. It may be noted, however, that the range of each

of the amino acids in the plasma in normal animals is relatively large. This variability

imposes serious limitations upon the quantitative interpretation of any changes in

the levels observed. Thus, while it may be possible to identify the limiting amino

acid in certain proteins by this technique, the likelihood that good quantitative

assays for nutritional quality can be developed using plasma amino acid levels is

not promising.