

Estimation of protein and amino acid metabolism using [¹⁵N] and [¹⁴C] labeled tracer amino acids

**K. Krawielitzki¹, F. Kreienbring¹, Ruthild Schadereit², M. Gabel¹
and V.V.A.M. Schreurs³**

¹ *Institute for Ecological Compatible Animal Husbandry
Justus-von-Liebig-Weg 8, 18051 Rostock, Germany*

² *Research Institute for the Biology of Farm Animals
Department of Nutritional Physiology "Oskar Kellner"
18059 Rostock, Germany*

³ *Department of Human and Animal Physiology, Agricultural University
Haarweg 10, N-6709 PJ Wageningen,
The Netherlands*

(Received 5 March 1996; accepted 9 July 1996)

ABSTRACT

The possibilities of estimating parameters for whole body protein metabolism (synthesis and breakdown) by the help of the end product method and a compartment model using ¹⁵N- and/or ¹⁴C labeled amino acids (AA) as tracers are demonstrated. First, the fundamentals of the method, the choice of tracer AA and the determinable parameters are described. The influence of endogenous and exogenous factors on protein synthesis and breakdown is demonstrated using selected results of tracer experiments on rats. Differences relating to animal species and the effect of age, body weight, protein intake, β -agonist administration and training, are shown in detail.

The main conclusions are:

- the tracer method in combination with modeling provides parameters of protein metabolism on intact animals;
- the estimated data are relative values and they reflect the main processes of protein and/or AA metabolism with sufficient accuracy;
- the method makes it possible to assess the influence of different endogenous and exogenous factors on the protein synthesis rate.

KEY WORDS: amino acid, protein, metabolism model, isotope tracer

INTRODUCTION

The understanding of protein synthesis, breakdown, and of the mechanisms involved in these processes enables optimizing protein deposition and minimizing N pollution due to animal production and provides the opportunity to control protein metabolism with optimal efficiency. Today, the tracer technique in combination with compartment models is often used in animal and human nutrition. It enables the complex use of experimentally estimated tracer data and the calculation of such important parameters of protein metabolism as N flux, protein synthesis and breakdown, which either cannot, or only with great difficulty, be estimated using other methods.

In contrast to other methods for determining protein synthesis and breakdown, for instance the precursor method or the flooding method, the so-called end product method, is non invasive and can be used on intact organisms. Therefore, it is also suitable for larger animals, and even for human beings. Furthermore, it allows the determination of protein synthesis to be repeated on the same animal or individual and to check the time course of this process during growth. Other parameters can be estimated simultaneously, for instance energy metabolism. These are the most important advantages of this isotope or tracer method.

THEORETICAL FUNDAMENTALS

Protein metabolism is a dynamic balance between protein synthesis and breakdown. In steady state situations both are equal and there is no protein deposition (Figure 1). This is normal for adults. For growth, synthesis must be higher than breakdown, and the greater the difference, the greater the protein deposition.

There are 5 possibilities allowing protein deposition to increase:

1. synthesis is increased, and breakdown remains constant
2. breakdown is decreased, and synthesis remains constant
3. both are enhanced, but synthesis to a higher degree
4. both are decreased, but synthesis to a smaller degree
5. synthesis is increased, and breakdown is decreased (optimal case).

What we can estimate directly is always only the deposition rate, but we do not know how it was realized.

The aims of our research are:

- to check which of these 5 possibilities is true for different situations
- to approximate the mechanisms of these processes, and
- to find possibilities of influencing protein metabolism.

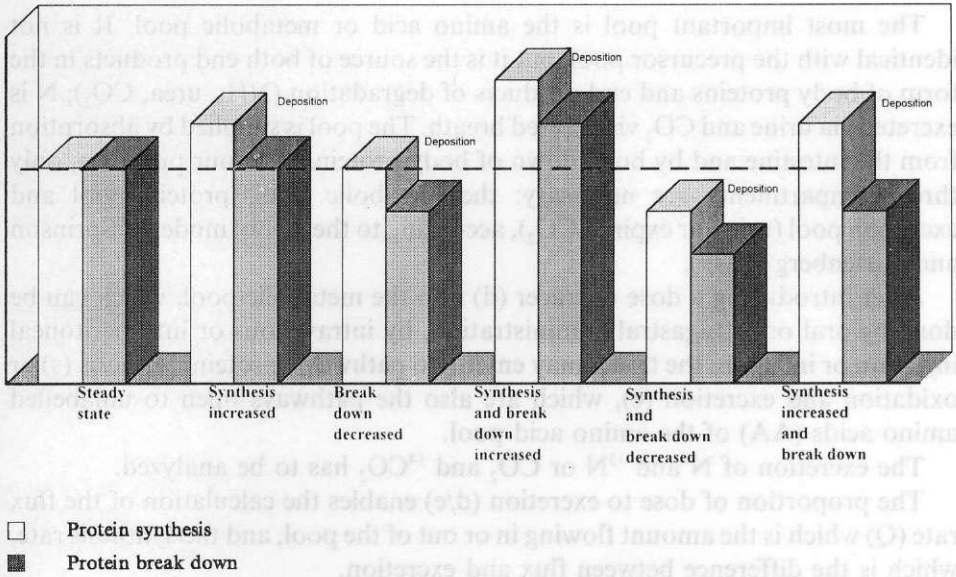


Figure 1. Possibilities for increasing of protein deposition

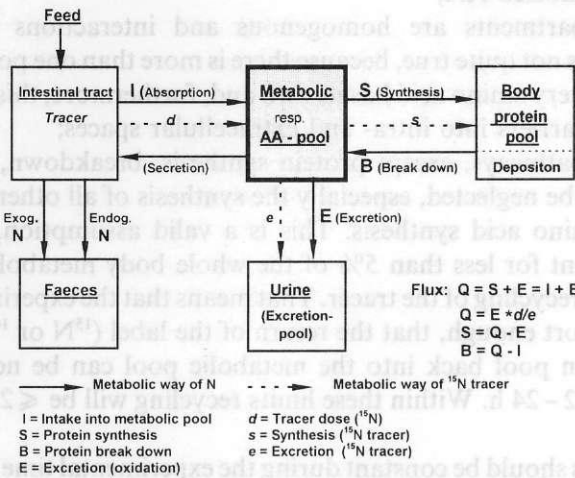


Figure 2. Compartment model for calculation of the synthesis rate

Protein synthesis and breakdown can only be estimated indirectly by the help of isotope or tracer methods and by use of models. Figure 2 shows the compartment model of protein metabolism used here, with its different pools.

The most important pool is the amino acid or metabolic pool. It is not identical with the precursor pool, but it is the source of both end products in the form of body proteins and end products of degradation (NH_3 , urea, CO_2); N is excreted via urine and CO_2 via expired breath. The pool is supplied by absorption from the intestine and by breakdown of body proteins. For our purposes, only three compartments are necessary: the metabolic pool, protein pool and excretion pool (urine or expired CO_2), according to the 3 pool model of Sprinson and Rittenberg (1949).

After introducing a dose of tracer (d) into the metabolic pool, which can be done by oral or intragastral administration, by intravenous or intraperitoneal injection or infusion, the tracer may enter two pathways: protein synthesis (s) or oxidation and excretion (e), which are also the pathways open to unlabelled amino acids (AA) of the amino acid pool.

The excretion of N and ^{15}N or CO_2 and $^{14}\text{CO}_2$ has to be analyzed.

The proportion of dose to excretion (d/e) enables the calculation of the flux rate (Q) which is the amount flowing in or out of the pool, and the synthesis rate, which is the difference between flux and excretion.

This model like others, also includes some assumptions:

1. the metabolism of the tracer amino acid is identical to that of the amino acids in the pool. This means that there are no isotope effects between ^{14}N and ^{15}N or ^{12}C and ^{14}C labeled AA;

2. the compartments are homogenous and interactions are first-order processes. This is not quite true, because there is more than one pool for all amino acids. Rather, every amino acid has its own and, furthermore, this pool is divided by membrane barriers into intra- and extracellular spaces;

3. all other pathways, except protein synthesis, breakdown, oxidation and excretion, must be neglected, especially the synthesis of all other N compounds and denovo amino acid synthesis. This is a valid assumption, because these processes account for less than 5% of the whole body metabolism;

4. there is no recycling of the tracer. That means that the experimental time has to be chosen short enough, that the return of the label (^{15}N or ^{14}C labeled AA) from the protein pool back into the metabolic pool can be neglected; this is possible up to 12–24 h. Within these limits recycling will be $\leq 2\%$ of the tracer dose;

5. parameters should be constant during the experimental time. In reality, they oscillate during the day and the obtained value is an average.

CHOICE OF THE TRACER AA

$[^{15}\text{N}]$ glycine. More than 50 % of the published tracer experiments are carried out using $[^{15}\text{N}]$ glycine (Faust et al., 1983). ^{15}N of this amino acid will be

transported by transamination and by other chemical transformations very quickly to other amino acids, and therefore it represents more or less the N metabolism of the whole spectrum of AA. But on the other hand, glycine is also the precursor for many other metabolites like purines, nucleic acids, bile acids and others. These pathways cannot be neglected without consequences.

[¹⁵N]lysine does not take part in transamination processes. The ¹⁵N of this tracer represents, therefore, in nearly perfect form, lysine N metabolism which of course does not necessarily have to be identical with that of the whole body N. Especially if lysine is the limiting amino acid in feed, this choice will surely be flawed.

[¹⁵N]leucine is in an intermediate position between both of these amino acids. [¹⁵N]leucine is the second most commonly used tracer amino acid after [¹⁵N]glycine.

Labeling amino acids with ¹⁴C eliminates the problem of transamination. But the question how exactly the metabolism of the chosen tracer amino acid reflects the metabolism of the whole body N, is of course relevant (Krawielitzki et al., 1986).

We can conclude:

- ¹⁵N from only one labeled amino acid is not equally distributed to or incorporated in all amino acids, thus it only imperfectly reflects whole body N metabolism;
- excess dietary amino acids are catabolized in a greater proportion than the limiting amino acid, and the tracer will be excreted predominantly in urine (¹⁵N) or in breath (¹⁴C);
- protein synthesis and amino acid breakdown do not have identical precursor pools and, because of this, tracer amino acids (sometimes) have different probabilities of incorporation into protein and being used in catabolism.

To minimize these problems, we consider a mixture of all (or nearly all) amino acids labeled by ¹⁵N, adapted to the amino acid composition of the feed protein or the body protein of the experimental animals to be the best tracer. Of course, this is much more expensive than the use of only one ¹⁵N labeled AA (Krawielitzki et al., 1989).

However, cost sometimes imposes compromises, thus the use of a single labeled amino acid often chosen. For the same reason, most of the experiments are carried out with rats as model animals.

Despite all of the limitations and although all of the assumptions can be realized only approximately, under standardized conditions this method creates good opportunities to estimate the metabolic parameters of protein metabolism in the whole body and reflects the main pathways (protein synthesis, breakdown, oxidation and excretion) with sufficient accuracy.

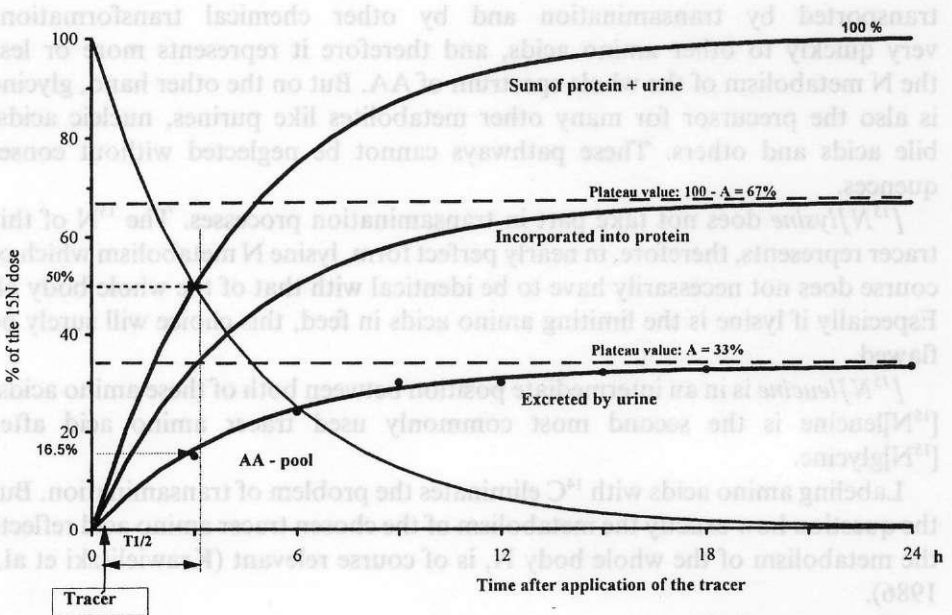


Figure 3. Course of tracer in different pools after a single ^{15}N dose

Figure 3 demonstrates tracer distribution after application of a single dose, and it is in principle the same whether an ^{15}N or ^{14}C labeled AA is used.

After administration of the tracer, its level in the metabolic pool decreases exponentially against zero. The tracer is transported into two pools, the protein pool and excretion pool (urine in the case of ^{15}N , expired CO_2 in the case of ^{14}C). Other pathways will be neglected. Both of these curves increase exponentially and reach a plateau 12–24 h after administration of the ^{15}N label, but in the case of a ^{14}C label, 4 h after application. From the curves of Figure 3 one can calculate:

- how much of the AA is incorporated into protein (here 67%);
- how much is oxidized and excreted (here 33%);
- the half life of this process (here 3 h).

Further determinable parameters are:

- pool sizes
- metabolic rates
- reutilization
- turnover time and half lives of the pools.

But there exists a fundamental difference in the fate of the label of AA ^{15}N and AA ^{14}C which shall be demonstrated in Figure 4.

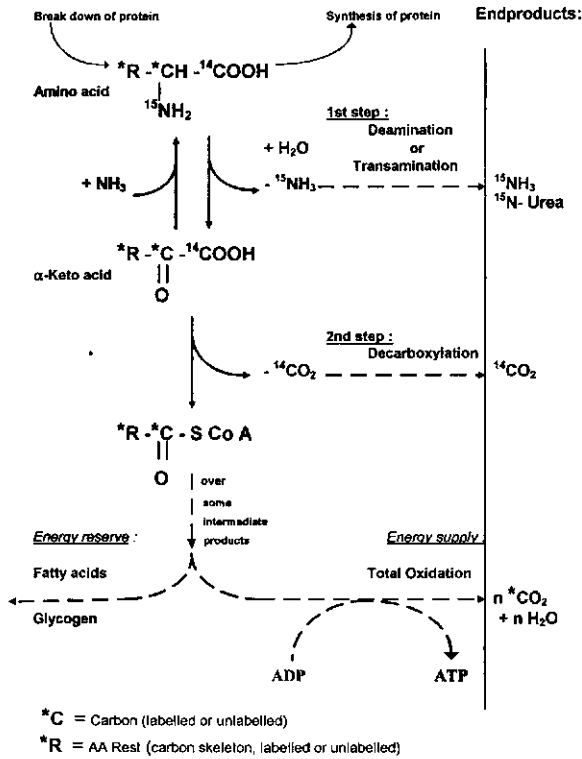


Figure 4. Break-down mechanism of amino acids

The first step of degradation for an amino acid is deamination. During this process, the acid loses its ^{15}N amino group. The $^{15}NH_2$ -group will be transaminated to another amino acid or, through some intermediates, will be excreted in the form of ^{15}N urea or $^{15}NH_3$ into the urine as end products of degradation. The deamination process is reversible, but the reamination product is no longer labeled by ^{15}N . Both amino acids, the original ^{15}N labeled and the rebuilt unlabelled one, will be incorporated into protein, and this can lead to underestimation of protein synthesis using ^{15}N labeled AA.

The second step of degradation is decarboxylation and this process is not reversible. If the carboxyl group (C1) is labeled, then the ^{14}C will be excreted by expired CO_2 and can be estimated by a breath test. Because this label is not eliminated during deamination and reamination of the AA, it gives better results for protein synthesis.

For the unlabelled rest of the AA, the carbon skeleton, two metabolic pathways exist:

- it can be transformed into fatty acids or to glycogen and then stored in the body as an energy reserve,
- it can enter the citric acid cycle and be oxidized to the end products, CO_2 and H_2O by forming ATP (energy supply).

If this amino acid residue is also ^{14}C -labeled, the end product $^{14}\text{CO}_2$ is labeled, too. The difference between $^{14}\text{CO}_2$ coming from decarboxylation and $^{14}\text{CO}_2$ coming from the carbon skeleton represents the energy reserve (Schreurs et al., 1991, 1994).

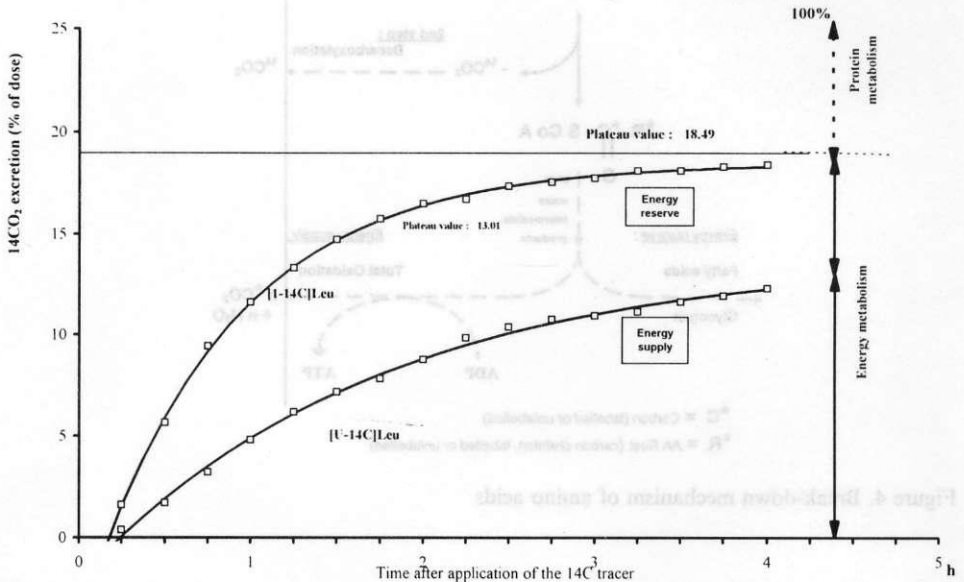


Figure 5. Course of the $^{14}\text{CO}_2$ -excretion

Figure 5 shows the estimated $^{14}\text{CO}_2$ excretion curves

of $[1-^{14}\text{C}]$ leucine = carboxyl labeled AA

of $[U-^{14}\text{C}]$ leucine = universally labeled AA.

In the example presented here, decarboxylation reaches an end value of 18.5%

total oxidation reaches an end value of 13.0%

energy reserve $(18.5 - 13.0) = 5.5\%$

and incorporation into protein $(100 - 18.5) = 81.5\%$ of the whole leucine intake.

Both protein synthesis and breakdown are influenced by endogenous factors (such as species, age or live weight, breed and sex of the animals) and, more importantly, by exogenous factors (protein and energy intake, protein quality, training effects and promoters, for instance anabolics or hormones).

INFLUENCE OF ENDOGENOUS AND EXOGENOUS FACTORS ON PROTEIN SYNTHESIS

Table 1 illustrates protein synthesis in different species.

The following conclusions can be drawn from these figures:

- the greater the animal,
- the greater the protein synthesis [g/d],
- the longer the half life of protein ($T^{1/2}$),
- the smaller the Fractional Synthesis Rate, (FSR) [%/d].

Protein synthesis compared to metabolic body weight seems to be relatively constant, 15–25 g protein/d x kg^{0.75}.

Table 2 shows the influence of age and live weight demonstrated on rats, summarized from different experiments (Millward, 1978; Krawielitzki et al., 1991).

TABLE 1

Protein synthesis of different species (Adults)

Species	Live weight kg	Protein synthesis			Half life $t^{1/2}$ [d]	Authors
		g/d	g/kg ^{0.75} /d	%/d		
Mouse	0.02	0.77	14.5	17.4	4.0	Garlick and Marshall (1972)
	0.04	1.78	19.5	19.6	3.5	Waterlow et al. (1978)
Rat	0.51	10.5	17.4	9.6	7.5	Millward et al. (1976)
	0.55	14.1	22.1	12.2	5.7	„
Rabbit	3.6	65	24.8	7.8	8.9	Nicholas et al. (1977)
Dog	10.2	123	21.5	5.7	12.2	Everett and Sparrow (1980)
Sheep	67	356	15.9	2.7	25.7	Bryant and Smith (1980)
Man	77	434	16.7	2.9	24.0	James et al. (1976)
Cattle	628	2350	18.7	1.9	36.7	Lobley et al. (1980)

TABLE 2

Influence of age resp. live weight (Albino rats)

Age d	Live weight g	Synthesis rate FSR, %/d	Break-down rate FBR, %/d	Growth rate FGR, %/d	Efficiency of protein synthesis, %
23	37 ± 1	28.6	22.5	6.1	21.3
46	70 ± 5	16.1	13.1	3.0	18.6
65	116 ± 8	11.5	9.8	1.7	14.8
130	223 ± 32	5.3	4.6	0.7	13.2
330	511 ± 84	4.2	4.1	0.1	2.4
700	ca. 400	2.9	2.9	0	0

TABLE 3

Influence of protein intake (Albino rats, 100 g LW)

Protein level % protein	Protein synthesis rate		N deposition rate		Efficiency of deposition %
	mgN/d	%/d	mgN/d	%/d	
0	192	6.6	-44	-1.5	-
6	216	7.3	13	0.4	6
10	205	8.5	138	5.7	67
17	281	10.4	184	6.8	65
26	306	12.0	183	7.2	60

The data show that both synthesis and breakdown decrease with age or live weight, but synthesis more than break-down. At the age of about 300 days both become equal, deposition approaches zero and growth stops.

Of greatest influence is of course the protein intake. Table 3 shows our results from different experiments with rats (Krawielitzki et al., 1987, 1991).

With increasing N intake, nitrogen deposition and protein synthesis rise, and even during no protein nutrition, remarkably high protein synthesis occurs, amounting to about 6.6% of body protein per day. This enables the organism to keep the protein content of vital organs constant for a longer time, at the expense of other organs such as muscles.

The next table (Table 4) presents the effect of administering clenbuterol (an β -agonist) or clenbuterol + propanolol (a combination of β -agonist + β -antagonist) on the protein metabolism of female rats (Reichel et al., 1993). In the case of clenbuterol, protein synthesis and breakdown decline, but synthesis to a smaller degree which causes a gross increase of growth. The combination of clenbuterol and propanolol leads to intermediate metabolic rates between the control and the test animals receiving only clenbuterol.

TABLE 4

Influence of β -agonist resp. β -agonist + β -antagonist (Female rats, LW 150g)

	Protein synthesis rate		Break-down rate		Deposition rate	
	mgN/d	%/d	mgN/d	%/d	mgN/d	%/d
Control group (normal diet)	370	8.1	296	6.5	74	1.6
Test group (diet + clenbuterol)	255	5.6	155	3.4	100	2.2
Test group (diet + clenbuterol + + propanolol)	281	6.1	193	4.2	88	1.9

TABLE 5

Effect of training on metabolic fate of glycine-C and N (Experiment with rats)

	Control group		Trained group	
	C, %	N, %	C, %	N, %
21% protein				
Protein metabolism	77.2	80.1	78.6	78.4
Energy metabolism	22.8	19.9	21.4	21.6
from this:				
for reserve (fat, glycogen)	9.0		6.6	
for supply (oxidation and excretion)	13.8		14.8	
7.5% protein				
Protein metabolism	80.6	95.0	84.0	93.6
Energy metabolism	19.4		16.0	
from this:				
for reserve (fat, glycogen)	9.6		5.0	
for supply (oxidation and excretion)	9.8	5.0	11.0	6.4

The effect of training is demonstrated in Table 5 (Weijs et al., 1993; Schreurs et al., 1994). The data indicate no or only marginal effect of training on protein metabolism. At a high protein level (21%) the utilization of carbon and nitrogen of glycine are equal in the control and trained groups (77–80%). At the lower level (7.5% protein) the proportion for protein synthesis is higher as compared with the high protein level. N is metabolized more effectively for protein synthesis than C, which is caused by the high reutilisation of N. Differences between trained and untrained rats exist only in terms of energy reserve, which, in trained animals, is only 73 or 52% of that in controls. In principle, the fate of leucine-N and leucine-C leads to analogous findings.

CONCLUSIONS

The tracer method in combination with modeling allows calculation of the parameters of protein metabolism on intact animals, which are inaccessible or only accessible with difficulty by using other methods. The use of ¹⁵N and ¹⁴C or ¹³C as tracers are not competing methods – they complement one another.

The estimated data are relative values and they reflect the main processes of protein and amino acid metabolism with sufficient accuracy. It depends on the choice of the tracer whether it reflects the whole body protein metabolism or that of a specific amino acid.

The method enables estimation of the influence of different endogenous and exogenous factors on protein synthesis.

Comparability of values demands standardized experimental conditions.

REFERENCES

- Bryant B.T.W., Smith R.W., 1980. Protein Metabolism, In: M. Florkin, A. Neuberger, L.L.M. Van Deeken (Editors). *Comprehensive biochemistry 19B, Part 1*. Amsterdam-Oxford-New York, Elsevier Science Publication Company
- Evertt A.W., Sparrow M.P., 1980. Protein Metabolism, In: M. Florkin, A. Neuberger, L.L.M. Van Deeken (Editors). *Comprehensive biochemistry 19B, Part 1*. Amsterdam-Oxford-New York, Elsevier Science Publication Company
- Faust H., Czarnetzki H.-D., Hartig W., 1983. Untersuchungen mit ^{15}N zum Ganzkörperstoffwechsel von Glycin-N beim Menschen. *ZFI Mitteilungen, Berichte des Zentralinstitutes für Isotopen- und Strahlenforschung, Leipzig, Nr. 77*, 49-61
- Garlick P.J., Marshall J., 1972. A technique for measuring brain protein synthesis. *J. Neurochem.* 19, 577-583
- James W.P.T., Garlick P.J., Sender P.M., Waterlow J.C., 1976. Studies of amino acid and protein metabolism in normal man with $[\text{U-}^{14}\text{C}]$ -tyrosine. *Clin. Sci. Mol. Med.* 50, 525-532
- Krawielitzki K., Schadereit R., Herrmann U., 1986. Die Eignung verschiedener ^{15}N - Verbindungen als Tracer für Körpermarkierung und für Untersuchungen des Protein- und Aminosäuremetabolismus. *Isotopenpraxis* 22, 262-269
- Krawielitzki K., Schadereit R., Wünsche J., 1987. Kinetische Parameter des Proteinstoffwechsels der Ratte bei proteinreicher Ernährung. *Arch. Anim. Nutr.* 37, 1041-1053
- Krawielitzki K., Schadereit R., Klein M., Hoffmann L., Wünsche J., Schiemann R., 1991. Bestimmung von kinetischen Parametern des Proteinmetabolismus bei wachsenden Ratten in Verbindung mit Energieumsatzmessungen. 1. Mitt. Bestimmung von kinetischen Parametern des Proteinmetabolismus. *Arch. Anim. Nutr.* 41, 583-600
- Krawielitzki K., Schadereit R., Wünsche J., 1989. Methodische Empfehlungen zur Bestimmung von Gesamtkörper-Proteinsyntheseraten im Tracerversuch- Übersichtsarbeit. *Arch. Anim. Nutr.* 39, 799-811
- Lobley G.E., Milne V., Lovie J.M., Reeds P.J., Pennie K., 1980. Whole body and tissue protein synthesis in cattle. *Brit. J. Nutr.* 43, 491-501
- Millward D.J., Garlick P.J., Nnanyelugo D.O., Waterlow J.C., 1976. The relative importance of muscle protein synthesis and break-down in the regulation of muscle mass. *Biochem. J.* 156, 185-188
- Millward D.J., 1978. The regulation of muscle protein turnover in growth and development. *Biochem. Soc. Transaction*, London 6, 494-499
- Nicholas G.A., Lobley G.E., Harris C.J., 1977. Use of constant infusion technique for measuring rates of protein synthesis in New Zealand White rabbit. *Brit. J. Nutr.* 38, 1-17
- Reichel K., Rehfeldt C., Weikard R., Schadereit R., Krawielitzki K., 1993. Einfluß eines β -Agonisten und einer β -Agonist / β -Antagonist -Kombination auf Muskelwachstum, Körperzusammensetzung sowie Proteinstoffwechsel bei Ratten. *Arch. Anim. Nutr.* 42, 221-225
- Schreurs V.V.A.M., Krawielitzki K., Boekholt H.A., Koopmanschap R.E., Wejls P.J.M., 1991. Recovery of metabolic endproducts from ^{14}C - and ^{15}N leucin. A comparison at different levels of protein intake. *Proceedings of 6th International Symposium on Protein Metabolism and Nutrition*, Herning (Denmark), EAAP- Publication No. 99, 139-141
- Schreurs V.V.A.M., Krawielitzki K., Koopmanschap R.E., 1994. A study on the metabolic fate of C and N from tracer amino acids. *Stable Isotopes in Nutrition and Metabolic Research. 2nd World Conference, Rotterdam, Abstract*, p. 39
- Sprinson D.B., Rittenberg D., 1949. The rate of interaction of the amino acids of the diet with the tissue proteins. *J. Biol. Chem.* 180, 715-726

- Waterlow J.C., Garlick P.C., Millward D.C., 1978. Protein turnover in mammalian tissues and in the whole body. Amsterdam-New York-Oxford, North Holl. Publication Company
- Weijs P.J.M., Schreurs V.V.A.M., Helmes M.H.B., Bujko J., Koopmanschap R.E., Boekholt H.A., Keller J., 1993. Effects of training and chronic protein restrictions on metabolic leucin utilisation after exercise and feeding. In: P.J.M. Weijs (Editor). Dietary protein, physiologic condition and metabolic amino acid utilization. - Prom. A, Landbouwniversiteit Wageningen (Netherland), pp. 71-80

STRESZCZENIE

Wyznaczenie parametrów metabolizmu białka i aminokwasów przy użyciu aminokwasów znakowanych ^{15}N i ^{14}C

Przedstawiono możliwość oznaczania parametrów metabolizmu (syntezy i rozpadu) białka w organizmie przy zastosowaniu metody opartej na określeniu produktów końcowych metabolizmu i modelu przedziałów, używając aminokwasów znakowanych ^{15}N lub ^{14}C . Opisano założenia metody, wybór znakowanego aminokwasu i oznaczanych parametrów. Omówiono wpływ czynników endo- i egzogennych na syntezę i rozkład białka na przykładzie wyników doświadczeń przeprowadzonych na szczurach z zastosowaniem znakowanych pierwiastków. Przedstawiono różnice w parametrach w zależności od gatunku zwierząt, wieku, masy ciała, ilości pobranego białka, zastosowania związków β -agonistycznych i powstających produktów ubocznych. Stwierdzono, że przy stosowaniu w badaniach pierwiastków znakowanych oraz proponowanego modelu można oznaczyć parametry metabolizmu białka na zwierzętach, bez konieczności stosowania zabiegów chirurgicznych. Otrzymane wartości parametrów mają wartość względną i odzwierciedlają główne procesy przemian białkowych lub aminokwasów z zadowalającą dokładnością. Metoda umożliwia oszacowanie wpływu różnych endo- i egzogennych czynników na tempo syntezy białka.