Physiol. Res. 42: 257–260, 1993

Distribution of Radioactivity of Intraperitoneally Administered ^{14}C-leucine and ^{14}C-alanine in Tissues of Suckling Lambs

S.I. VOVK, S.V. BRODIN, M. MAROUNEK[1], V.G. JANOVIČ

Institute of Animal Physiology and Biochemistry, Ukrainian Agricultural Academy, Lvov, Ukraine and [1]Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Prague, Czech Republic

Received March 3, 1993
Accepted May 3, 1993

Summary

^{14}C-labelled leucine and alanine were administered intraperitoneally to suckling male lambs aged 30. The distribution of radioactivity was investigated in the liver, heart, quadriceps femoris muscle, subcutaneous adipose tissue, skin and expired air. Most of the radioactivity (per 1 g of dry tissue) was found in proteins of liver, followed by proteins of the heart, skin, muscle and adipose tissue. The radioactivity found in lipids and glycogen was much lower, with the exception of high radioactivity of leucine-derived lipids of the adipose tissue. Incorporation of alanine into proteins was lower than that of leucine. On the other hand, more alanine was oxidized to CO_2 than leucine.

Key words

Amino acids – Tissue metabolism – Ruminants

Introduction

Ruminants convert feed nitrogen into protein with a rather low efficiency in comparison with monogastric animals. Only about 17 percent of the initial nitrogen intake can be converted into protein available for growth of a ruminant animal fed at its approximate protein requirement (Preston 1970). Similar conversion was reported by Lobley (1992). Corresponding values of nitrogen retention in pigs are higher : 50-60 % in young animals and cca 25 % in the last period of fattening (Oslage and Fliegel 1965). The inefficiency of nitrogen utilization in ruminants is caused by peculiarities of ruminant digestion and also by the metabolic processes in ruminant tissues. A better understanding of intermediary metabolism of amino acids is necessary to increase protein deposition in productive animals. Amino acids absorbed from the intestinal tract are used for the synthesis of proteins, gluconeogenesis, or they may be oxidized to obtain energy. A key role in intermediary metabolism of nitrogen in ruminants is played by amino acids with branched chains (BCAA), which integrate the metabolism of peripheral tissues with the metabolic and oxidative functions of the liver. The BCAA (leucine, isoleucine, valine) are not precursors of other small biologically active molecules. Therefore, they are either used for proteosynthesis or catabolized (Papet *et al.* 1992). The synthesis of proteins is their main metabolic pathway. There are certain differences in BCAA metabolism between fasted and *ad libitum* fed ruminants (Ballard *et al.* 1976, Pell *et al.* 1986, Hammond *et al.* 1987) and between preruminant and adult animals (Bergen *et al.* 1988). Unlike most of the essential amino acids, BCAA are degraded in the hindquarters of ruminants rather than in the liver. In preruminant lambs, the skeletal muscle appears to be the principal site of BCAA catabolism (Papet *et al.* 1988), whereas the adipose tissue in adult sheep is more important than other tissues (Bergen *et al.* 1988). The catabolism of BCAA involves deamination and decarboxylation to form acyl-CoA compounds. These CoA derivatives can be further oxidized in reactions

similar to those by which fatty acyl-CoA are degraded (Papet *et al.* 1992).

Leucine metabolism has been studied more extensively than metabolism of other BCAA. Available studies in ruminants investigated leucine absorption, plasma flux, incorporation into proteins and oxidation (Schaefer *et al.* 1986, Hammond *et al.* 1987, Van Veen *et al.* 1987, Krishnamurti and Janssens 1988), activity of leucine catabolizing enzymes (Papet *et al.* 1988), and effect of the physiological state and diet on leucine metabolism in different tissues (Nissen and Ostaszewski 1985, Pell *et al.* 1986, Papet *et al.* 1988, Bergen *et al.* 1988).

The purpose of this study was to expand data on the metabolism of amino acids in ruminants. In this paper we describe the distribution of radioactivity in tissues of preruminant lambs after an intraperitoneal administration of ^{14}C-leucine and ^{14}C-alanine. The radioactivity of $^{14}CO_2$ in expired air was also measured.

Methods

Six suckling Précoce male lambs, 30 days of age, were kept in individual pens with their dams. Their live weights were 5.8-6.4 kg. Ewes were offered a diet consisting of concentrate (0.3 kg), beet (2 kg), meadow hay (1 kg) and barley straw *ad libitum*. Three lambs were given 3145 kBq of L-[-^{14}C] leucine per 1 kg of live weight and three lambs received the same amount of L-[U-^{14}C] alanine. Radioactive amino acids were administered intraperitoneally, three hours after the morning feed. Lambs were then placed into respiration chambers for 5 hours to collect expired $^{14}CO_2$. Carbon dioxide was trapped in 20% (w/v) NaOH. Animals were then killed and samples of liver, skin, subcutaneous adipose tissue, heart and quadriceps femoris muscle were excised. Samples were immediately frozen in liquid nitrogen, freeze-dried and pulverized. Pulverized tissues were extracted with chloroform/methanol (2 : 1) according to Folch *et al.* (1957). Extracts were evaporated at 60 °C under vacuum and lipids were dissolved in a commercial scintillation fluid. Glycogen and proteins were extracted from lipid-free samples of tissues with 30 % KOH (100 °C/h). Glycogen was precipitated by alcohol and washed after standing overnight in the cold. Pure glycogen was dissolved in water and mixed with a scintillation fluid. Protein was precipitated with 20 % TCA washed with TCA solution and dissolved in 5 M KOH (80 °C/2 h). The alkaline solution was neutralized with 5 M acetic acid, dried and mixed with a scintillation fluid. The radioactivity of samples was measured in a LKB liquid-scintillation counter.

L-[U-^{14}C] leucine (8.9 GBq/mmol) and L-[U-^{14}C] alanine (4.4 GBq/mmol) were purchased from the Institute for Research, Production and Applications of Radioisotopes (Prague). The scintillation fluid SLD-41 and aqueous-compatible scintillation fluid SLT-41 were purchased from Spolana (Neratovice).

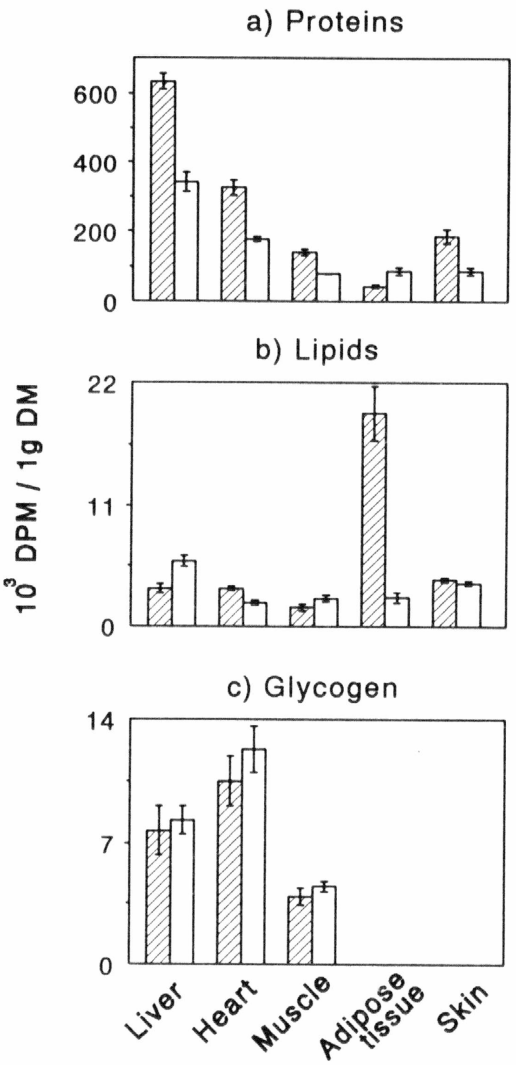

Fig. 1
Radioactivity of proteins, lipids and glycogen in tissues of lambs, 5 h after the intraperitoneal administration of ^{14}C-leucine or ^{14}C-alanine. Vertical bars represent standard deviations. DM - dry matter

Results

Most of radioactivity, intraperitoneally administered to suckling lambs in the form of ^{14}C-leucine and ^{14}C-alanine, was recovered in proteins in all the tissues examined (Fig. 1). Liver proteins exhibited the highest radioactivity (on a dry substance basis) followed in descending order by the heart, skin, quadriceps femoris muscle and adipose tissue. The radioactivity found in lipids was 18 to 97-fold lower than the radioactivity recovered in proteins. The

exception to this was the high radioactivity of leucine-derived lipids of the adipose tissue. The radioactivity found in glycogen was 14 to 82-fold lower than the radioactivity recovered in proteins. The highest radioactivity was found in glycogen of the heart tissue. Oxidation of leucine to CO_2 was only 29.6 % of that of alanine (Table 1). Values given in Table 1 would be approximately five times higher if related to dry body weight of a lamb.

Table 1
Radioactivity of $^{14}CO_2$ found in expired air of lambs during a 5-hour-interval after intraperitoneal administration of ^{14}C-leucine or ^{14}C-alanine

Substrate	Radioactivity (10^6 dpm per kg of body weight)
L-[U-^{14}C] leucine	8.4 ± 0.4
L-[U-^{14}C] alanine	28.4 ± 2.8

Means ±S.D. (n=3)

Discussion

It was estimated that skeletal muscles, adipose tissue and the liver represent 37.0, 6.5 and 2.7 % of total body weight of preruminant lambs, respectively (Papet *et al.* 1988). In view of these assessments, skeletal muscles, but not the liver, appear to be the principal site of leucine and alanine incorporation into proteins. Incorporation of alanine into proteins was about one half of that of leucine, with the exception of the adipose tissue. However, the content of protein in adipose tissue is low, a few percent only. The skin in growing animals can account for a large fraction of protein synthesis. Incorporation of leucine and alanine into proteins of skin suckling lambs was higher than the incorporation into proteins of the quadriceps femoris muscle.

Incorporation of radioactivity of amino acids into the lipid fraction was generally low, except for the incorporation of leucine carbon into lipids of the adipose tissue. It is known that leucine is catabolized to acetoacetate and acetyl-CoA (Greenberg 1961). Both compounds are potential substrates for lipogenesis. Adipose tissue thus appears metabolically capable of using leucine (but not alanine) as substrate for lipogenesis.

Incorporation of radioactivity of leucine and alanine into glycogen was somewhat higher than incorporation into lipids. It is difficult, however, to assess the extent of gluconeogenesis from our data, as only a part of glucose is used for the synthesis of glycogen, the radioactivity of which was measured. The involvement of leucine and alanine in gluconeogenesis in suckling lambs was probably low.

Oxidation of leucine was relatively low in comparison with its incorporation into proteins. This is in agreement with the findings of Pell *et al.* (1986) and Krishnamurti and Janssens (1988) who found that leucine oxidation in adult sheep accounted for 15 and 10.7 % of plasma leucine flux, respectively.

In conclusion, the data presented in this paper demonstrate that incorporation of leucine and alanine into tissue proteins was the principal reaction involved in intermediary metabolism of both amino acids in suckling lambs. Suckling lambs did not differ from adult sheep in this respect (Pell *et al.* 1986). Incorporation of alanine into proteins was lower than that of leucine. On the other hand, more alanine was oxidized to CO_2 than leucine. The uptake of leucine carbon by the adipose tissue and its incorporation into lipids was considerably higher than the uptake of alanine.

References

BALLARD F.J., FILSELL O.H.,JARRETT I.G.: Amino acid uptake and output by the sheep hind limb. *Metabolism* **25**: 415–418, 1976

BERGEN W.G., BUSBOOM J.R., MERKEL R.A.: Leucine degradation in sheep. *Br. J. Nutr.* **59**: 323–333, 1988.

FOLCH J., LEES M., STANLEY G.H.S.: A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509, 1957.

GREENBERG D.M.: Carbon catabolism of amino acids. In: *Metabolic Pathways,* Vol. II. GREENBERG D.M. (ed.), Academic Press, London and New York, 1961, pp. 79–172.

HAMMOND A.C., HUNTINGTON G.B., REYNOLDS P.J., TYRRELL H.F., EISEMANN J.H.: Absorption, plasma flux and oxidation of L-leucine in heifers at two levels of intake. *J. Anim. Sci.* **64**: 420–425, 1987.

KRISHNAMURTI C.R., JANSSENS S.M.: Determination of leucine metabolism and protein turnover in sheep, using gas-liquid chromatography mass spectrophotometry. *Br. J. Nutr.* **59**: 155–164, 1988.

LOBLEY G.E.: Control of the metabolic fate of amino acids in ruminants: A review. *J. Anim. Sci.* **70**: 3264–3275, 1992.

NISSEN S., OSTASZEWSKI P.: Effects of supplemental dietary energy on leucine metabolism in sheep. *Br. J. Nutr.* **54**: 705–712, 1985.

OSLAGE H.J., FLIEGEL H.: Nitrogen and energy metabolism of growing-fattening pigs with an approximately maximal feed intake. In: *Energy Metabolism,* BLAXTER K.L.(ed.), Academic Press, London and New York, 1965, pp. 297–306.

PAPET I., BREUILLE D., GLOMOT F., ARNAL M.: Nutritional and metabolic effects of dietary leucine excess in preruminant lamb. *J. Nutr.* **118:** 450–455, 1988.

PAPET I., GRIZARD J., BONIN D., ARNAL M.: Regulation of branched chain amino acid metabolism in ruminants. *Diabetes Metab.* **18:** 122–128, 1992.

PELL J.M., CALDARONE E.M., BERGMAN E.N.: Leucine and α-ketoisocaproate metabolism and interconversions in fed and fasted sheep. *Metabolism* **35:** 1005–1016,1986.

PRESTON R.L.: 10th Annual Ruminant Nutrition Conference : Amino Acids in Ruminant Nutrition. Introductory remarks. *Fed. Proc.* **29:** 33–34, 1970.

SCHAEFER A.L., DAVIS S.R.,HUGHSON G.A.: Estimation of tissue protein synthesis in sheep during sustained elevation of plasma leucine concentration by intravenous infusion. *Br. J. Nutr.* **56:** 281–288, 1986.

VAN VEEN L.C.P., TENG C., HAY W.W., MESCHIA G., BATTWAGLIA F.C.: Leucine disposal and oxidation rates in the fetal lamb. *Metabolism* **36:** 48–53, 1987.

Reprint Requests
M. Marounek, Institute of Animal Physiology and Genetics, Czech Academy of Sciences, 104 00 Prague 10, Uhříněves, Czech Republic.