Journal of Chromatography, 617 (1993) 197–203
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6899

Determination of taurine metabolism by measurement of ^{15}N-enriched taurine in cat urine by gas chromatography–mass spectrometry

Andreas A. Stämpfli, Olivier Ballèvre and Laurent B. Fay*

Friskies Research, P.O. Box 44, 1000 Lausanne 26 (Switzerland)

(First received February 18th, 1993; revised manuscript received April 26th, 1993)

ABSTRACT

To understand the biological function of taurine, a study of taurine kinetics in the cat was undertaken. This paper describes a method developed for the accurate determination of ^{15}N-taurine enrichment in cat urine by gas chromatography–mass spectrometry. ^{15}N-Taurine was given to six animals as an oral bolus dose of 20 mg/kg body weight, and the urine was pooled on a daily basis. The hydrolysed or non-hydrolysed urine samples (for total and free taurine, respectively) were directly derivatized without further purification. The N-pentafluorobenzoyl di-*n*-butyl amide derivative obtained was analysed, and the fragment [M − (di-*n*-butyl amide)]$^+$, carrier of the labelled nitrogen atom, was selectively recorded at *m/z* 302 (^{14}N-taurine) and *m/z* 303 (^{15}N-taurine). Calibration curves prepared in hydrolysed and non-hydrolysed urine samples spiked with ^{15}N-taurine gave similar slopes to the calibration curve prepared in water. The average coefficient of variation observed for the mole percent excess in the non-hydrolysed samples was 1.22% (n = 92) and for the hydrolysed urine 1.00% (n = 98). There was no significant difference between free and total taurine enrichment. The half-life of taurine in cat body was found to be 29.3 ± 2.9 h and 35.0 ± 1.4 h for free and total taurine, respectively (non-significant). The taurine body pool, calculated by extrapolation of the curve to zero time, had a value of 137 ± 22 ng/kg and 157 ± 11 mg/kg for free and total taurine, respectively.

INTRODUCTION

Taurine (2-aminoethanesulphonic acid) is one of the most abundant amino acids in animal tissues [1] and is described as an essential amino acid for human and other mammalian species [2]. Apart from its conjugation with bile acids, its biological functions are not yet fully understood. In the cat, taurine deficiency has been associated with retinopathy and retinal degradation [2,3], and more recently with dilated cardiomyopathy [4], and therefore taurine is supplemented to cat foods in order to meet the daily need. Studies of taurine kinetics have been investigated in different species, including humans [5], rats [6] and mice [7] using radioactive tracers, or in monkeys [8] and cats [9] using stable isotopes. In order to study the physiological basis of the taurine requirement in cats, we studied taurine kinetics with a bolus dose of ^{15}N-taurine and measured taurine enrichment in urine.

Although gas chromatography–isotope ratio mass spectrometry (GC–IRMS) is the method of choice for measurements of low levels of isotopic enrichment, conventional gas chromatography–mass spectrometry (GC–MS) is frequently used, especially in the case of amino acid studies [10–12]. For taurine, the main difficulty is to form a volatile derivative of the sulphonic function. Di-

* Corresponding author.

0378-4347/93/$06.00

methylaminomethylene methyl ester [8,10] and trimethylsilyl ether [13] derivatives have been used, but these derivatives did not show sufficient reproducibility for measuring isotopic enrichment. To overcome this problem, an alternative approach was used consisting of analyses of ^{14}N-taurine and ^{15}N-taurine by fast atom bombardment mass spectrometry after purification by ion-exchange chromatography [9]. This method was satisfactory, but was almost impossible to automate. Because of the number of analyses necessary for taurine kinetics measurements, an easily automated GC–MS method was developed. This paper presents the results obtained with the N-pentafluorobenzoyl di-*n*-butyl amide derivative described by Kataoka *et al.* [14].

EXPERIMENTAL

Chemicals

Taurine was obtained from Fluka (Buchs, Switzerland) and ^{15}N-taurine was prepared with an isotopic purity greater than 98% according to the method described by Philippossian *et al.* [15]. Standard working solutions were prepared in deionized water and stored at 4°C. Pentafluorobenzoyl chloride (PFB-Cl) was purchased from Fluka, tetrabutylammonium hydrogen sulphate ($TBA^+HSO_4^-$) and thionyl chloride from Aldrich (Buchs, Switzerland), and di-*n*-butylamine (DBA) from Sigma (Buchs, Switzerland). All other chemicals were of analytical grade.

Instrumentation

The equipment used was a Hewlett-Packard 5890 gas chromatograph (HP, Geneva, Switzerland) with an HP-7673 autosampler, a Finnigan MAT-8430 double-focusing mass spectrometer, and an SS300 data system (Finnigan, Bremen, Germany). The GC–MS conditions were as follows: DB-5 J&W fused-silica capillary column (30 m × 0.32 mm I.D., film thickness 0.25 μm); carrier gas, helium at 69 kPa; splitless injection at 250°C; oven temperature programme: 60°C (1 min), increased by 30°C/min to 200°C and then by 10°C/min to 300°C (2 min); interface temperature, 250°C. The mass spectrometer was operated under electron ionization conditions with an electron energy of 70 eV and an ion source temperature of 180°C. The ions at *m/z* 301.9910 (^{14}N-taurine) and 302.9881 (^{15}N-taurine) were monitored by selected-ion monitoring at a resolution power of 3500.

Sample preparation

Urine samples (1.0 ml) were hydrolysed by adding 1 ml of 12 *M* hydrochloric acid. The sealed tubes were kept for 24 h at 130°C. After evaporation to dryness under vacuum, the residue was resuspended in 1 ml of water, ready for derivatization.

Calibration curves were prepared by spiking various amounts of ^{15}N-taurine in a urine sample (hydrolysed or not) containing a known amount of ^{14}N-taurine.

Derivatization

The preparation of the N-pentafluorobenzoyl di-*n*-butylamine derivative was slightly modified from the procedure described by Kataoka *et al.* [14]. Briefly, an aliquot of 100 μl of urine (hydrolysed or not) was diluted in 900 μl of water. After the addition of 100 μl of 0.5 *M* sodium hydroxide, 100 μl of PFB-Cl were added and the mixture was shaken for 5 min at room temperature. The pH was adjusted to 1–2 with 300 μl of 0.5 *M* hydrochloric acid, and the mixture was washed with 3 ml of diethyl ether. Then 100 μl of $TBA^+HSO_4^-$ (10% in methanol) were added, and the ion-pair compounds formed were extracted into 3 ml of methylene chloride. The organic layer was dried with anhydrous ammonium sulphate and evaporated at 60°C under a stream of nitrogen. A 200-μl volume of thionyl chloride was added to the residue, and the mixture was heated at 80°C for 15 min. Excess thionyl chloride was removed at 60°C under nitrogen, and 200 μl of DBA were added. The mixture was allowed to stand for 5 min at room temperature. After acidification with 1 ml of 20% orthophosphoric acid, the compounds were extracted into 4 ml of pentane. This solvent was then evaporated to dryness, and the residue redissolved in 100 μl of ethyl acetate.

Animal studies

Six adult European cats were used for the taurine kinetics studies. The animals were fed once a day on a commercial canned diet containing an adequate amount of taurine to prevent any deficiency symptoms. ^{15}N-Taurine was given as an oral bolus dose of 20 mg/kg of body weight. Total urine was collected for the following 5 days. Urine was pooled on a daily basis, acidified with 2 *M* HCl, and stored at −20°C until analysis. Taurine kinetics were calculated using an open one-pool model, assuming a rapid equilibrium of the dose within the body. Each urine sample was assumed to reflect the mean taurine enrichment throughout the collection period. For each cat, the kinetics were calculated by using the linear regression of the natural logarithm of the enrichment *versus* time (mid-time of the collection period). In some animals, miction did not occur every day, and therefore we used the mid-time of two days as reference time. The pool size and the half-life were determined using single pool kinetic equations [16].

RESULTS

Identification of the taurine derivatives

The mass spectra of the derivatives of ^{14}N-taurine and ^{15}N-taurine obtained after electron impact (EI) at 70 eV are presented in Fig. 1. The EI mass spectrum of ^{14}N-taurine (Fig. 1, top) was characterized by the absence of the molecular ion at *m/z* 430, two fragments at *m/z* 302 ([M − (di-*n*-butylamine)]$^+$) and *m/z* 238 ([M − SO_2-di-*n*-butylamine]$^+$), and a base peak at *m/z* 195 corresponding to the pentafluorobenzoyl group. The molecular mass of 430 of the derivative was confirmed by the ion [M + NH_4]$^+$ at *m/z* 448 after positive chemical ionization with ammonia as reagent gas.

The spectrum of ^{15}N-taurine showed similar fragmentation, but compared with ^{14}N-taurine, the two ions at *m/z* 302 and 238 were shifted by the presence of the ^{15}N atom to *m/z* 303 and 239, respectively.

For the quantitation of ^{15}N-taurine enrichment in urine, the isotopic fragment ions [M − di-*n*-butylamine)]$^+$, carriers of the nitrogen atom, were selectively recorded at *m/z* 301.9910 (^{14}N-taurine) and 302.9881 (^{15}N-taurine). A resolution power of 3500 was found to be a good compromise between selectivity, needed for the analysis of non-purified urine samples, and sensitivity, rapidly lost when high resolution is employed.

Analysis of urinary ^{14}N- and ^{15}N-taurine

A typical GC–MS analysis of taurine in cat urine is displayed in Fig. 2, and shows the good chromatographic properties of the N-pentafluorobenzoyl-di-N-butylamide derivatives on a non-polar column. The same GC profile was obtained from both crude urine and hydrolysed samples. The amount of injected taurine was *ca.* 2 pmol for each GC run.

To measure the ^{15}N-taurine enrichment, the ratio between the ^{15}N- and the ^{14}N-taurine before and after urine hydrolysis was determined. With the double-focusing Finnigan MAT 8430 mass spectrometer, the measurement of the isotopic ratio *m/z* 303/302 did not change significantly when the amount of injected taurine was increased (Fig. 3). Therefore no correction was made and the mole percent excess (MPE) [17] of the unknown samples was obtained directly from the calibration curves.

The calibration curves were established for non-hydrolysed urine (free taurine) and hydrolysed urine samples (total taurine). For each calibration point, the enriched ratio *m/z* 303/302 was plotted against the theoretical MPE [16]. The linearity of the measurements was demonstrated across the range of the expected enrichments for the urinary samples, with a correlation coefficient better than 0.996. These calibration curves have similar slopes to the curve obtained with standard compounds spiked in pure water. It seems that no urinary compound present interfered with the measured ^{15}N- and the ^{14}N-taurine.

The accuracy and precision of the ^{15}N-taurine enrichment determination are given in Table I. The results indicate that the method is able to detect a minimum enrichment lower than 0.5% in both non-hydrolysed and hydrolysed urine,

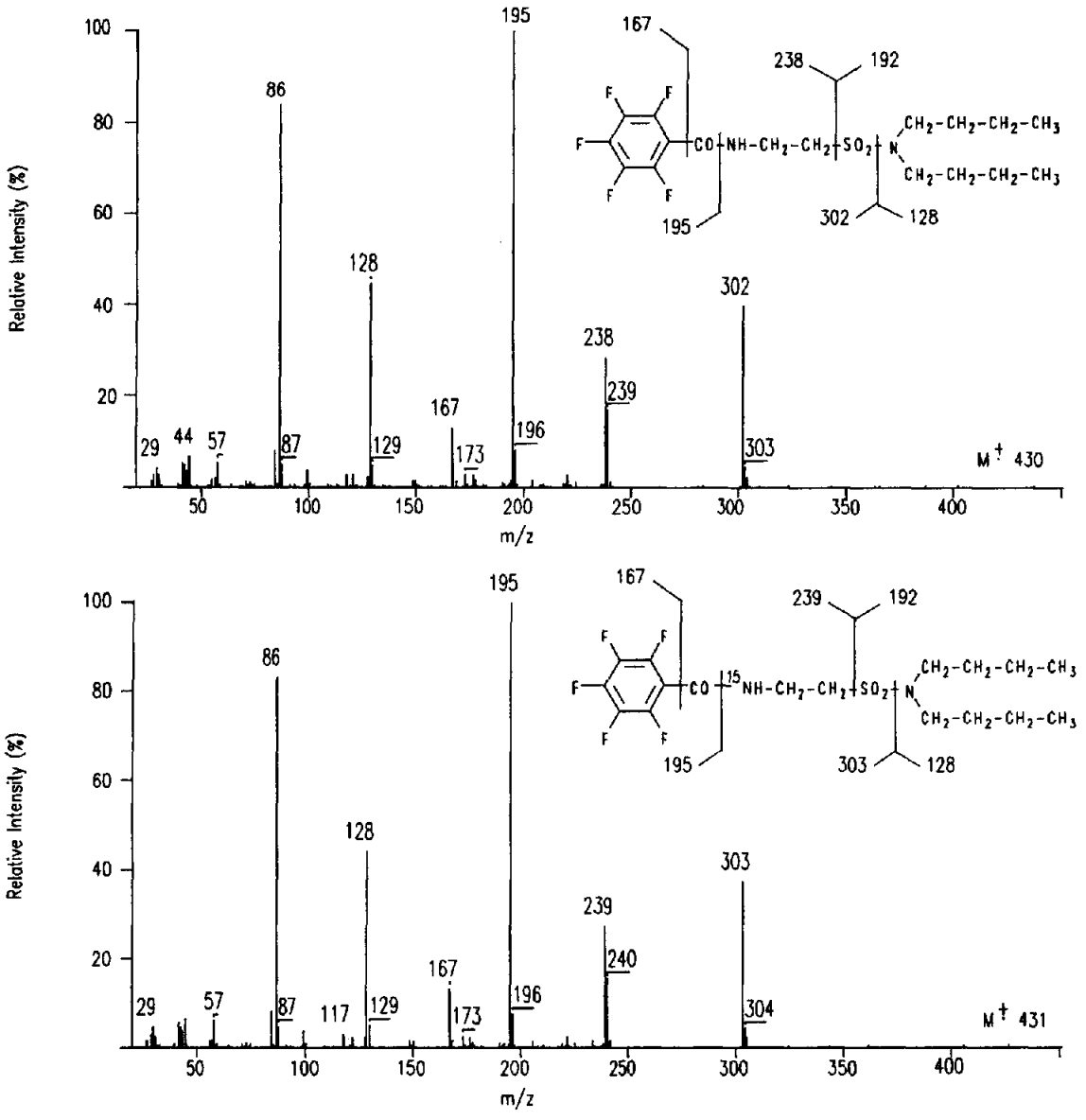

Fig. 1. Electron impact mass spectrum of the N-pentafluorobenzoyl di-*n*-butylamide derivatives of ^{14}N- and ^{15}N-taurine.

which was adequate for measurements in the biological samples in this study.

Fig. 4 displays a typical curve of MPE *versus* time in a semilogarithmic scale for six cats (mean ± S.D.). The average coefficient of variation (C.V.) observed for the MPE measured in duplicate in non-hydrolysed urine was 1.22% (n = 92) and for hydrolysed urine 1.00% (n = 98). The evolution of taurine enrichment after the bolus dose describes an exponential decay curve for both free and total taurine. There was no significant difference between free and total taurine enrichment. The half-life of taurine in the cat body was 29.3 ± 2.9 h and 35.0 ± 1.4 h for free and total taurine, respectively (difference non significant). The taurine body pool, calculated by extrapolation of the curve to zero time, had a value of 137 ± 22 mg/kg and 157 ± 11 mg/kg for free and total taurine, respectively.

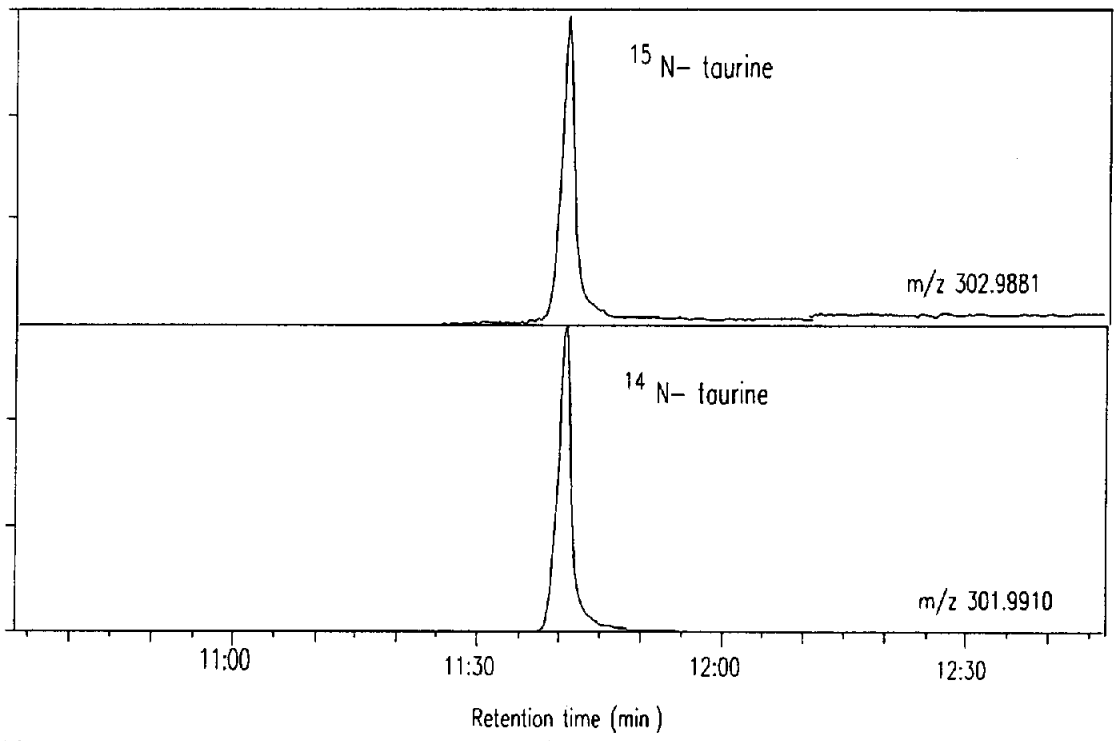

Fig. 2. GC–MS trace obtained after selective detection of ^{14}N- and ^{15}N-taurine in cat urine.

DISCUSSION

Taurine deficiency is an important problem in cat nutrition, and the regulation of taurine metabolism is not fully understood. The addition of non-radioactive isotope-labelled taurine to the

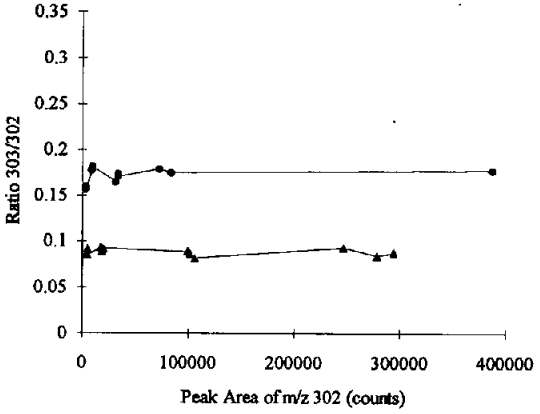

Fig. 3. Variation of the measured ratio 303/302 *versus* the injected amount of the sample (peak area of the ion *m/z* 302) for two different ^{15}N-taurine enrichments: (●) MPE = 7.1; (▲) MPE = 0.0.

TABLE I

ACCURACY AND PRECISION OF THE GC–MS ANALYSIS

Free and total taurine were enriched with increasing amounts of ^{15}N-taurine in non-hydrolysed and hydrolysed urine, respectively. Each MPE value is the mean of three determinations.

Theoretical MPE	Measured MPE	Standard deviation
Non-hydrolysed urine		
0.00	0.01	0.003
0.51	0.58	0.004
1.02	1.03	0.001
3.90	4.27	0.009
7.60	7.17	0.002
14.10	12.16	0.727
Hydrolysed urine		
0.00	0.02	0.000
0.34	0.36	0.003
1.36	1.81	0.029
5.24	4.51	0.048
9.96	8.57	0.106
18.13	16.29	0.233

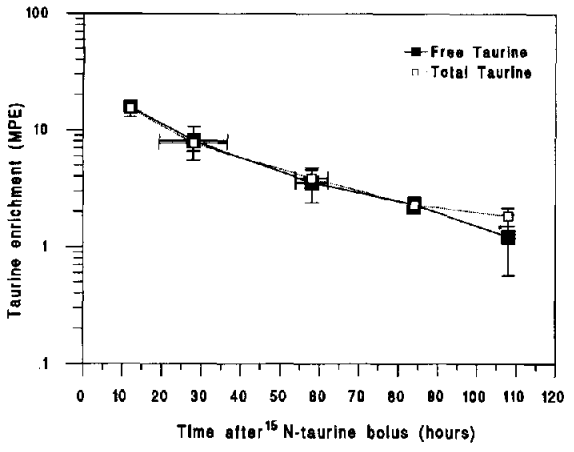

Fig. 4. Semi-logarithmic evolution of the ^{15}N-taurine enrichment in cat urine after a bolus dose of ^{15}N-taurine. The taurine enrichment was determined in non-hydrolysed (free taurine) and in hydrolysed (total taurine) urine samples. Each point corresponds to mean ± S.D. (n = 6 animals).

diet of animals, together with urine collection, opens the way to a better understanding of the regulation of different pathways of taurine metabolism (absorption, synthesis, degradation and excretion) in a non-invasive way. This contrasts with previous studies using radioactive tracers (*e.g.* ref. 5) or requiring the inhalation of ^{18}O gas through a mask [10], both methods that are difficult to apply to cats.

The present study required the development of a mass spectrometric technique adapted to a precise measurement of taurine enrichment in numerous biological samples. The use of a convenient GC–MS methodology offered a good alternative in terms of rapidity, sensitivity and selectivity. The previously reported difficulties encountered during derivatization and analysis [9] were solved by using the N-pentafluorobenzoyl di-*n*-butyl amide derivative described by Kataoka *et al.* [14].

The results obtained for spiked non-hydrolysed and hydrolysed urine samples (Table I) show that the measurement of ^{15}N-taurine enrichment is accurate, precise and reproducible. It allows a fast determination of enrichment in urine samples and can easily be applied to other biological samples, such as plasma, whole blood and tissues, because a complete analysis required only 50 nmol.

The analysis of urine samples is suitable for taurine kinetics because the half-life of taurine is longer than a day in the cat. Therefore, any imprecision in the determination of the time-related evolution of enrichment in daily pooled urine samples is minimized. Thus the different pathways of taurine metabolism can be determined accurately from taurine pool size and rate constant, using a stochastic analysis of the open one-pool model [18].

Because the enrichments of free and bound taurine were similar, the half-life and kinetics data were also similar using both non-hydrolysed and hydrolysed samples of urine. This illustrates that the bound taurine pool is in equilibrium with the free taurine pool. In these urine samples, 85% of urinary taurine was in the free form [19]. This is related to the fact that taurine intake was in excess of the requirements for cats [20], which is not always achieved on a diet of unsupplemented commercial petfood. The comparison between free and bound taurine kinetics using both sample enrichment determinations (free and total) should help in the understanding of taurine metabolism in cats and optimize the taurine content in cat foods.

ACKNOWLEDGEMENTS

This study is a part of a joint project on taurine metabolism in cats with Prof. R.M. Batt and Dr. K. Johnston (Royal Veterinary College, London, UK) and Friskies Research. The authors are grateful to Dr. D.M. Bier (St. Louis, MO, USA) for helpful discussions, Dr. I. Horman for review of the manuscript and S. Marti for technical assistance.

REFERENCES

1 J. G. Jacobsen and L. H. Smith Jr., *Physiol. Rev.*, 48 (1968) 424–511.
2 K. C. Hayes, *Nutr. Res. Rev.*, 1 (1988) 99–113.
3 K. C. Hayes, R. E. Carey and S. Y. Schmidt, *Science*, 88 (1975) 949–951 .
4 P. D. Pion, M. D. Kittleson, Q. R. Rogers and J. G. Morris, *Science*, 237 (1987) 764–768.

5 J. A.. Sturman, G. W. Hepner, A. F. Hofmann and P. J. Thomas, *J. Nutr.*, 105 (1975) 1206–1214.
6 R. J. Huxtable, *J. Nutr.*, 111 (1981) 1275–1286.
7 R. J. Huxtable and S. E. Lippincott, *J. Nutr.*, 112 (1982) 1003–1010.
8 Y. Matsubara, Y. Y. Lin, J. A. Sturman, G. E. Gaull, L. M. Marks and C. S. Irving, *Life Sci.*, 36 (1985) 1933–1940.
9 A. A. Stämpfli, O. Ballèvre and L. Fay, *Rapid Commun. Mass Spectrom.*, 6 (1992) 547–549.
10 C. S. Irving and P. D. Klein, *Anal. Biochem.*, 107 (1980) 251–259.
11 O. Ballèvre, J. L. Prugnaud, M. L. Houlier and M. Arnal, *Anal. Biochem.*, 193 (1991) 212–219.
12 A. G. Calder, S. E. Anderson, I. Grant, M. A. McNurlan and P. J. Garlick, *Rapid Commun. Mass Spectrom.*, 6 (1992) 421–424.
13 O. Stokke and P. Helland, *J. Chromatogr.*, 146 (1978) 132–136.
14 H. Kataoka, N. Ohnishi and M. Makita, *J. Chromatogr.*, 339 (1985) 370–374.
15 G. Philippossian, D. H. Welti, R. Fumeaux, U. Richli and K. Anantharaman, *J. Labelled Compd. Radiopharm.*, 27 (1989) 1267–1273.
16 R. R. Wolfe, in *Tracers in Metabolic Research: Radioisotope and Stable Isotope/Mass Spectrometry Methods*, Alan R. Liss, New York, 1984, pp. 189–206.
17 J. Rosenblatt, D. Chinkes, M. Wolfe and R. R. Wolfe, *Am. J. Physiol.*, 263 (1992) E584–E596.
18 O. Ballèvre, C. Piguet, A. Stämpfli, G. L. Czarnecki and K. Acheson, *Proc. Nutr. Soc.*, (1993) in press.
19 O. Ballèvre, unpublished results.
20 E. N. Glass, J. Odle and D. H. Baker, *J. Nutr.*, 122 (1992) 1135–1142.