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Measurement of albumin synthesis in the pig by using L-[$^2H_5$]phenylalanine as a stable isotope tracer

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Summary

Two experiments with growing pigs were performed to measure the albumin synthesis rate using [$^2H_5$]phenylalanine as a stable isotope tracer. The isotope was infused together with unlabeled phenylalanine at massive doses of 45 and 125 mg/kg body weight in order to "flood" all possible albumin precursor pools as uniformly as possible in Experiments 1 and 2, respectively. Blood samples were collected frequently up to 90 min after the infusion and the time courses of isotopic enrichment of the albumin-precursor pool (assumed to be represented by the plasma-free phenylalanine enrichment) and of albumin were followed. The enrichment of the precursor pool decreased significantly more steeply in Experiment 1 than in Experiment 2 as indicated by the isotopic enrichment 60 min after the infusion compared to the extrapolated enrichment at zero, suggesting that the "flooding" of the precursor pool was more efficient in the latter experiment. Time dependent isotope incorporation into albumin was shown to be linear in both experiments despite the obviously poorer flooding of the precursor pool in the first study. Although no significant differences were detected for the estimated fractional albumin synthesis rates between both studies (16-30 % per day of the intravascular albumin mass) it is recommended to use the higher dose of 125 mg phenylalanine/kg body weight to ensure a sufficiently high and uniform labeling of all possible precursor pools, which is a crucial precondition for an adequate estimation of protein synthesis rates according to the applied so-called flooding dose technique.

Keywords: Albumin synthesis, pig, stable isotope

Zusammenfassung

Messung der Albuminsynthese des Schweins unter Verwendung von L-[$^2H_5$]Phenylalanin als Stabilisotopen-Marker

Es wurden zwei Experimente mit Mastschweinen durchgeführt, um die Syntheserate von Albumin mittels des Stabilisotop-Markers [$^2H_5$]Phenylalanin zu messen. Das Isotop wurde zusammen mit unmarkiertem Phenylalanin in massiven Dosen von 45 (Exp. 1) und 125 (Exp. 2) mg/kg Körpergewicht infundiert, um alle möglichen Albumin-Precursor-Pools so einheitlich wie möglich zu markieren und zu „fluten". Es wurden mehrere Blutproben bis zu 90 Minuten nach der Infusion entnommen, um den Zeitverlauf der Isotopen-Anreicherung des Albumin-Precursor-Pools (hierbei wurde angenommen, dass dieser durch die Anreicherung des freien Phenylalanins im Plasma repräsentiert würde) und von Albumin verfolgen zu können. Im Experiment 1 sank die Anreicherung des Albumin-Precursor-Pools signifikant steiler als in Experiment 2, wenn die Isotopen-Anreicherung 60 min nach der Infusion mit der extrapolierten Anreicherung zum Zeitpunkt null ins Verhältnis gesetzt wurde. Daher kann angenommen werden, dass das „Fluten" des Albumin-Precursor-Pools im letzten Experiment effizienter war. Die zeitabhängige Isotopen-Inkorporation in das Albumin war, trotz der offensichtlich weniger effizienten „Flutung" des Albumin-Precursor-Pools im ersten Experiment, in beiden Experimenten linear. Obwohl keine signifikanten Unterschiede der geschätzten fraktionellen Syntheserate (16 - 30 % pro Tag der intravaskulären Abumin-Masse) zwischen den beiden Studien nachgewiesen werden konnte, wird empfohlen, die höhere Dosis von 125 mg Phenylalanin/kg Körpergewicht zu verwenden, um eine ausreichend hohe und gleichmäßige Markierung aller möglichen Albumin-Precursor-Pools als eine entscheidende Vorbedingung zur adäquaten Schätzung der Protein-Syntheseraten entsprechend der angewendeten so genannten „flooding dose"-Technik, zu gewährleisten.

Schlüsselwörter: Albumin Synthese, Schwein, Stabil-Isotop

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1 Introduction

The plasma albumin concentration does not necessarily reflect its synthesis by the liver as it is just the net balance between albumin synthesis, degradation and transcapillary escape and might be further influenced by changes in plasma volume (Ballmer et al., 1990). Hence, a direct measurement of albumin synthesis by using isotope techniques is helpful in interpreting many of the nutritional, physiological and pathological conditions of this tissue. There are two main techniques, referred as to "constant infusion method" and "flooding method," using either radioactively or stable isotope labeled amino acids for measurement of *in vivo* protein synthesis (Garlick et al., 1994). Both methods rely on an achievement of a constant plateau of the specific activity or isotopic enrichment of the precursor pool. Disadvantages of the constant infusion method, which requires an approximately 6 h constant infusion of the tracer amino acid, were discussed by Garlick et al. (1994). These mainly include a need for the maintenance of a steady state over this measurement period and a non-uniform labeling of plasma-free, intracellular-free and tRNA-bound amino acids. Instead, the flooding method is based on the infusion of a large dose of the tracer amino acid as a bolus which raises the concentration of that amino acid in all free pools many times over whereby the mentioned possible precursor pools are labeled more uniformly within a short period of time. This enables a simplified kinetic evaluation of the incorporation of the label into newly synthesized proteins such as albumin.

In humans, albumin synthesis was measured with this flooding method by using stable isotopes. The doses which were used to flood the free pools ranged between 43 and 57 mg/kg body weight and the corresponding fractional synthesis rates of albumin (FSR) varied between approximately 3 and 10 % of the intravascular albumin pool per day depending on the experimental factors examined (Hunter et al., 1995, 2001; Ballmer et al., 1990, 1995a, b, 1996; McNurlan et al., 1996; Slater et al., 1995). In pigs, the FSR of albumin was frequently measured by the constant infusion method only and values ranged between approximately 10 and 40 % (Jahoor et al., 1994, 1999; Mackenzie et al., 2003) which would generally suggest a higher level in the growing pig.

The aim of the present study was to adopt the flooding dose technique - with L-[$^2H_5$]phenylalanine as the tracer amino acid which was measured by gas chromatography mass spectrometry (GC-MS) - for the measurement of albumin synthesis in pigs. For this purpose, two experiments were carried out. In the first experiment, the flooding dose of the tracer amino acid phenylalanine was chosen according to the cited human studies, whereas in the second experiment the dose was increased to account for the obviously higher FSR of albumin in the pig. By comparing the results of both studies it should be evaluated whether a flooding of the free pool was achieved or not, which is crucial for the estimated fractional albumin synthesis rates.

2 Material and Methods

2.1 Procedures

2.2.1 Experiment 1

Three female Polish Large White x (Pietrain x Duroc) cross-bred pigs (39 kg ± 3 kg, pig 1-3) were used. They were surgically equipped with a permanent polyvinyl chloride catheter which was placed in the jugular vein, subsequently tunnelled subcutaneously and dorsally exteriorized. Pigs were kept in individual balance cages after the operation during the following procedures. On the measurement day, a large dose of a mixture of L-phenylalanine (P 8324, Sigma-Chemie, Deisenhofen, Germany) and L-[$^2H_5$]phenylalanine (DLM 1258, Promochem, Wesel, Germany) in physiological saline (104 mmol/L, 11 atom percent excess, MPE) was infused intravenously (45 mg phenylalanine per kg body weight) after finishing the morning meal. Infusion was finished within 3 min. Blood samples were taken at 2, 5, 10, 15, 20, 30, 50, 70 and 90 min after infusion, prepared for plasma and kept frozen until prepared for further analyses.

2.1.2 Experiment 2

For this experiment, four castrated male pigs, crossbred German Landrace x Pietrain, were used (38 kg ± 3 kg, pig 4-7). In principle, pigs were treated as described for Experiment 1, with the exception that the infused dose of phenylalanine was increased to 125 mg/kg body weight (150 mmol/L). The enrichment of the infusion solution was also increased to 29 MPE to improve the reliability of measurements of the very low isotopic enrichment in the albumin fraction. Moreover, sampling times differed from Experiment 1 in that blood samples were collected only 30, 40, 50, 60 and 90 min after infusion for pig 6, and 30, 45 and 60 min after infusion for Pigs 4, 5 and 7.

2.2 Analyses

The principle steps in plasma preparation for the measurement of isotopic enrichment in albumin and in plasma-free phenylalanine, which was assumed to be the indicator of the precursor pool, are depicted in Figure 1.

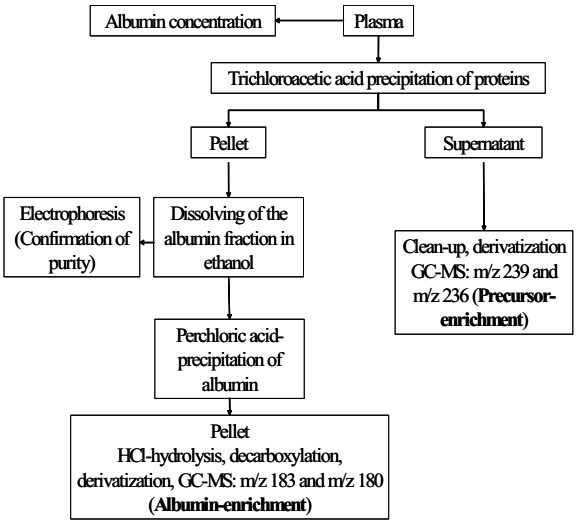

Figure 1:
Flow chart of plasma preparation for measurement of albumin synthesis rates

2.2.1 Isolation of albumin from plasma and preparation of albumin-bound phenylalanine for GC-MS analysis

Isolation of albumin from plasma is based on its solubility in ethanol and was performed according to the principles outlined by Korner and Debro (1956). Briefly, 10 ml of ice-cold trichloroacetic acid (TCA, 12 %) and 3 glass beads were added to 1 ml of plasma, thoroughly vortex mixed and left on ice for 10 min. The sample was then mixed again and centrifuged at 4 °C and 3500 rpm for 20 min. The supernatant was collected and kept frozen until further prepared for GC-MS-analysis of enrichment of plasma-free phenylalanine (see Section 2.2.3). The pellet was washed with 10 ml of TCA (12 %) and centrifuged at 4 °C and 4000 rpm for 20 min. The supernatant was discarded and the pellet was homogenized to a creamy paste to which 10 ml of absolute ethanol were added. The mixture was left at room temperature with one intermediate vortex mix. The sample was then centrifuged at room temperature and 4000 rpm for 20 min. Three ml of the albumin-containing supernatant were collected and kept frozen for electrophoresis whereas the remaining 7 ml were evaporated to dryness at 40 °C using a gentle stream of nitrogen. The dry residue was re-dissolved in 7 ml of 0.3n sodium hydroxide (NaOH) and incubated in a water bath at 37 °C for 20 min. Two ml of ice-cold perchloric acid (PCA, 20 %) were added and mixed with the sample, and left on ice for 5 min. The mixture was centrifuged at room temperature and 3500 rpm for 20 min. The supernatant was discarded and the pellet was washed twice by using 6 ml of ice-cold PCA (2 %) and centrifugation as described above. The final pellet was soaked with 0.5 ml of 0.3n NaOH and subsequently hydrolyzed with 6 ml of 4n hydrochloric acid (HCl) at 110 °C in the drying oven for 18 h. Three ml of the hydrolysate were taken to dryness at 60 °C in the drying oven, washed with 5 ml of doubly distilled water and dried again. The residue was taken up into 1 ml of citrate buffer (pH = 6.3) and transferred to an Eppendorf vial (2 ml). Further preparation steps for formation of the tertiary-butyldimethylsilyl (t-BDMS) derivates of phenylethylamine from phenylalanine of the hydrolysate which was finally measured by GC-MS are described in detail by Dänicke et al. (2001).

2.2.2 Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 10 %) was performed to confirm the purity of the albumin fraction isolated from plasma (see Section 2.2.1). A wide molecular weight marker (M4038, Sigma-Chemie, Deisenhofen, Germany) and porcine albumin (A1173, Sigma-Chemie, Deisenhofen, Germany) were used as standards. SDS-PAGE was performed by using a Mini-Protean® electrophoresis cell (Bio-Rad Laboratories, München, Germany) according to the Laemmli buffer system as described by the manufacturer.

2.2.3 Preparation of plasma-free phenylalanine for GC-MS-analysis

The supernatant collected from the TCA-precipitation of the plasma (see Section 2.2.1), containing the free amino acids, was thawed and loaded onto 1.2 ml of AG-50W-X8 cation exchange resin (H^+ form, 100 - 200 mesh, Bio-Rad Laboratories, München, Germany) and washed with 4 ml of distilled water. Amino acids were eluted with 2 ml of 2 M NH_4OH followed by 1 ml of distilled water. The eluate was dried under a stream of nitrogen at 40°C and then derivatised to yield the t-BDMS-phenylalanine derivative according to Calder and Smith (1988).

2.2.4 GC-MS of t-BDMS-phenylethylamine (albumin enrichment) and t-BDMS-phenylalanine (free pool enrichment)

Both derivatives were measured at the same GC-MS instrument which consisted of the autosampler A200S (CE Instruments) the GC 8000 (Fisons Instruments) and the quadrupole mass spectrometer MD 800 (Fisons Instruments).

The system was calibrated for measurement of low enrichment of albumin-bound phenylalanine in the range between 0.01 and 1.0 MPE. The conversion of phenylalanine to phenylethylamine prior to derivatisation was shown to improve the sensitivity of GC-MS for measurement of very low enrichment of newly synthesized proteins (Calder et al., 1992; Slater et al., 1995; Dänicke et al., 2001). The peak areas at m/z 183 and m/z 180 of samples and standards were recorded in the selected ion

recording mode under electron ionization conditions and subsequently used for isotope ratio analysis. The relationships between the calculated MPE of phenylalanine standards (0.01 - 1 MPE) and the ratio between the peak areas at m/z 183 and m/z 180 were strongly linear ($r^2 \sim 0.99$). Further details of measurement and of running the instruments are given by Dänicke et al. (2001).

The measurement of the enrichment of the plasma-free phenylalanine did not require such a pre-derivatisation step since its substantially higher enrichment compared to that of the albumin-bound phenylalanine enables an easier sample treatment which provides reliable isotope ratio monitoring. Ratios between the peak areas at m/z 239 and m/z 234 were used for calculation of MPE of plasma-free phenylalanine according the equation by Campbell (1974). GC and MS-conditions were similar to that described by Slater et al. (1995). At our instrument, the relationships between the calculated MPE of phenylalanine standards (1-30 MPE) and the ratio between the peak areas at m/z 239 and m/z 234 were strongly linear ($r^2 \sim 0.99$).

2.2.5 Albumin concentration

Albumin concentration was determined by the bromocresol green method in all plasma samples.

2.3 Calculations and statistics

The fractional albumin synthesis rate (FSR) was calculated from the ratio between the time-related linear increase in albumin enrichment and the corresponding area under the time-MPE curve of the precursor (plasma-free phenylalanine) according to Ballmer et al. (1990):

$$FSR = \frac{MPE_{albumin}(t_2) - MPE_{albumin}(t_1)}{A} \cdot 100 \qquad (1)$$

where FSR = fractional albumin synthesis rate (%/d), i.e., the percentage of intravascular albumin mass which is daily newly synthesized; $MPE_{albumin}$ (t_1) and $MPE_{albumin}$ (t_2) = enrichment of albumin-bound phenylalanine at times t_1 and t_2 (min), respectively; A = area under the precursor-enrichment curve (MPE · min).

$MPE_{albumin}$ (t_1) and $MPE_{albumin}$ (t_2) were estimated from the linear regressions of time after the infusion on albumin-bound phenylalanine enrichment. Two linear regression models were used in evaluating the data from Experiments 1 and 2 owing to the fact that the label appears in the blood after a certain time lag which is known as the albumin secretion time (e.g., Ballmer et al., 1990), i.e., the time required for synthesis and processing of albumin in the liver and its subsequent release in the blood circulation, and due to different plasma collection times.

Experiment 1:

$$MPE_{albumin} = (t_s \cdot b + a) \cdot (t \leq t_s) + (a + b \cdot t) \cdot (t > t_s) \qquad (2)$$

where $MPE_{albumin}$ = albumin-bound phenylalanine MPE; t = time (min); t_s = albumin secretion time (min); b = slope (MPE/min), a = intercept on ordinate (MPE).

Experiment 2:

$$MPE_{albumin} = a + b \cdot t \qquad (3)$$

where abbreviations as in equation 2. The albumin secretion time (t_s) is estimated from the extrapolated intercept on the x-axis when $MPE_{albumin} = 0$ as:

$$t_s = -\frac{a}{b} \qquad (4)$$

The knowledge of t_s is essential for a correct assignment of the area under the precursor-enrichment curve (A) which was calculated on the basis of the linear regression of time after infusion on the plasma-free phenylalanine MPE, corrected for t_s as estimated by equations (2) and (4), respectively:

$$MPE_{free} = a + b \cdot t \qquad (5)$$

where MPE_{free} = plasma-free phenylalanine MPE; t = time (min); b = slope (MPE/min), a = intercept on ordinate (MPE). The regression coefficients were used to estimate the MPE_{free} at times t_1 and t_2 as a precondition for estimation of "A "which is necessary in calculation of FSR according to equation (1):

$$A = \frac{(MPE_{free}(t_2 - t_s) + MPE_{free}(t_1 - t_s)) \cdot Ät}{2} \qquad (6)$$

where abbreviations as in equations (1) and (5).

The absolute synthesis rates of albumin (ASR) were estimated on the basis of the FSR multiplied with the intravascular albumin mass, which, in turn, was calculated from the measured albumin concentration in plasma multiplied with the estimated plasma volume:

$$ASR = VP \cdot AC \cdot FSR/100 \qquad (7)$$

where ASR = fractional absolute albumin synthesis rate (g/d); VP = plasma volume (l) = 1.06 + 0.037 · body weight (kg) (Yang and Lin, 1997) and AC = plasma albumin concentration (g/l).

Results of both experiments were compared by a t-test. All statistics were carried out using the Satistica for the Windows™ operating system (StatSoft, 1984).

3 Results and discussion

To the authors' knowledge, there is no report in the literature on the measurement of albumin synthesis in the pig by the flooding technique. Thus, experimental protocol and dose of the tracer amino acid for the first experiment were adopted from human studies (Ballmer et al., 1990, 1996; Slater et al., 1995). In these studies, the fall in the isotopic enrichment of the plasma-free amino acid during the period of 60 min after infusing the isotope amounted to approximately 67, 77 and 79 % when subjects were dosed with 57 mg leucine, 56 mg phenylalanine or 43 mg phenylalanine, respectively, per kg body weight. Although a comparable dose of 45 mg phenylalanine per kg was used in the present pig study (Experiment 1), the isotopic enrichment of plasma-free phenylalanine decreased to 46 % of the initial value after the same time period (Table 1, Figure 2). The increase in the infusion dose to 125 mg/kg body weight in Experiment 2 resulted in a significantly higher proportion of the isotopic enrichment of the precursor pool of 86 % after 60 min compared to the extrapolated enrichment at time zero (Table 1, Figure 3). Thus, the flooding of the precursor pool, which means a rapid, massive and uniform elevation of the tracer amino acid in all possible protein synthesis precursor pools, such as plasma-free, intra- and intercellular-free and tRNA-bound amino acids, was more successful in the second experiment. However, the validity of the assumption that the enrichment of plasma-free phenylalanine can be viewed as an indicator for the enrichment of liver-free phenylalanine, which is the ultimate precursor for albumin synthesis, can not be entirely deduced from the changes of enrichment in plasma-free phenylalanine. To substantiate this assumption it would be necessary to follow the time-course of enrichment in liver-free phenylalanine, which would require a time-related comparative slaughter of a number of pigs to obtain liver samples. Such an approach was tested by Bregendahl et al. (2004) who injected pigs intraperitoneally with 250 mg [$^2H_5$]phenylalanine per kg body weight. The enrichment of the liver-free phenylalanine decreased by approximately 0, 9, 6, 17 and 6 % relative to the isotopic enrichment of plasma-free phenylalanine when measured after 15, 30, 45, 60 and 75 min, respectively. Dänicke et al. (2005) reported this proportion to be 28 % when a similar protocol was used as in Experiment 2 of the present study. Since plasma-free enrichments were corrected for the albumin secretion time (t_s) in the present study, these enrichment values would

Table 1:
Experimental details and summary of results from Experiments 1 and 2

	Experiment		Probability	PSEM
	1	2		
Sex	female	male		
n	3	4		
Phenylalanine enrichment of the injection solution (MPE)	11	28		
Phenylalanine dose (mg/kg body weight)	45	125		
Live weight (kg)	39.0	37.5	0.470	1.4
Plasma-free phenylalanine MPE				
Linear slope (MPE/min)	-0.061	-0.054	0.762	0.015
Intercept (MPE)	6.7	22.1	<0.001	1.0
Ratio between MPE after 60 min and MPE at zero (%)	46.0	86.1	0.001	4.2
Area under the curve (MPE·min)	178	635	<0.001	25
Albumin-bound phenylalanine MPE				
Linear slope (MPE/min)	0.0007	0.0035	0.004	0.0004
Intercept (MPE)	-0.009	-0.099	0.002	0.011
Albumin secretion time (min)	13	28	0.005	2
Fractional albumin synthesis rate (%/d)	23.0	24.2	0.776	2.8
Albumin concentration (g/l)	20.4	32.5	0.006	1.8
Plasma volume (l)[1]	2.50	2.45	0.470	0.05
Intravascular albumin mass (g)	51.0	79.4	0.005	4.2
Absolute albumin synthesis (g/d)	11.8	19.2	0.076	2.3
Absolute albumin synthesis (mg/kg body weight per d)	306	508	0.057	58
Absolute albumin synthesis (% of total body protein synthesis)[2]	2.6	4.3	0.057	0.5

Abbreviations: MPE - molar % excess, PSEM - pooled standard error of means
[1] Plasma volume (l) =1.06 + 0.037·body weight (kg) (Yang et al., 1997)
[2] Assumed data: Empty body protein content = 17 %, Fractional whole body protein synthesis = 7 %/d (Simon, 1989)

correspond to a time window of approximately 0 to 30 min after infusion, where liver-free phenylalanine enrichment closely parallels that of plasma-free phenylalanine.

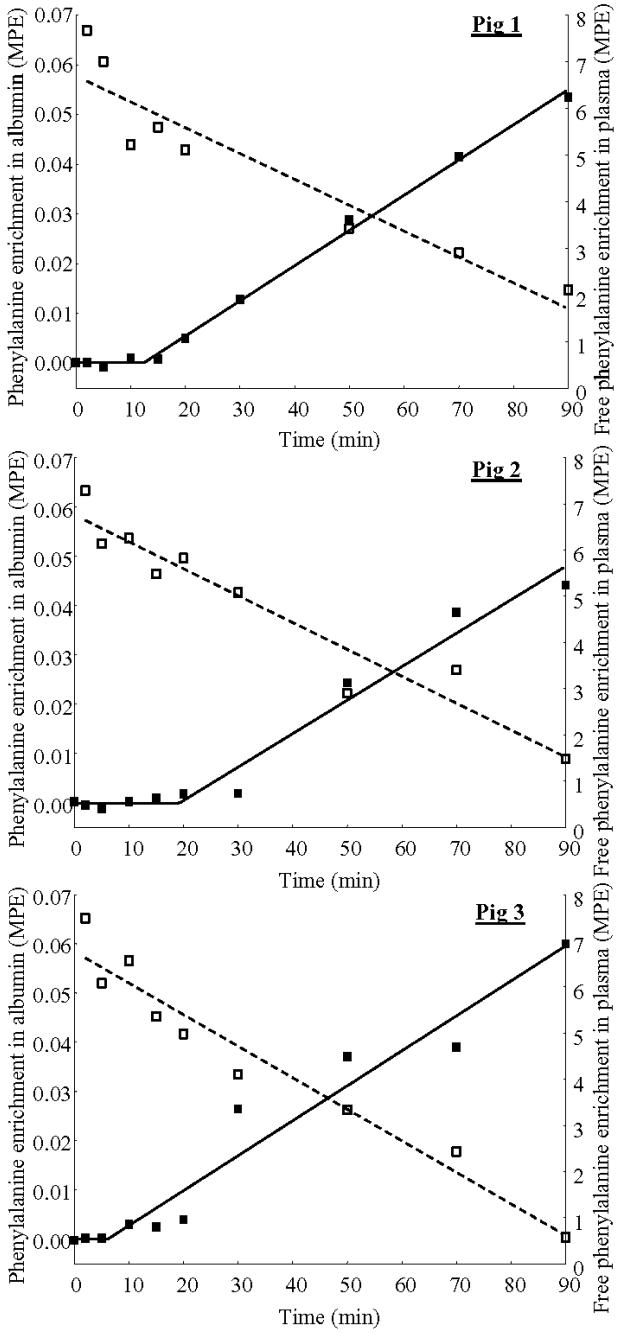

Figure 2:
Time course of molar % excess (MPE) in albumin-bound and plasma-free phenylalanine of individual pigs of experiment 1.
MPE in albumin-bound phenylalanine = $(t_S \cdot b + a) \cdot (t \le t_S) + (a + b \cdot t) \cdot (t > t_S)$, where t = time (min), t_S = secretion time of albumin (min), a = intercept on ordinate and b = slope of MPE in albumin-bound phenylalanine.
MPE in plasma-free phenylalanine = a - b · t, where t = time (min), a = intercept on ordinate, b = slope of MPE in plasma-free phenylalanine.

Thus, plasma-free phenylalanine enrichment can be viewed as a reliable indicator for albumin synthesis.

The purity of the albumin which is isolated from plasma is essential for a reliable measurement of its enrichment since contamination of this fraction with the highly enriched plasma-free phenylalanine or with other proteins from the TCA-precipitable plasma fraction differing in enrichment might result in isotopic adulterations. The contamination with highly enriched plasma-free phenylalanine was avoided by repeated washings of the pellet during sample preparation (Section 2.2.1). It was clearly demonstrated by Slater et al. (1995) that free phenylalanine is completely removed from the albumin fraction after the third wash. In the present study, the albumin pellet was also washed three times. Together with the two initial washes of the TCA-precipitable fraction (total plasma proteins) a contamination of the albumin fraction with the

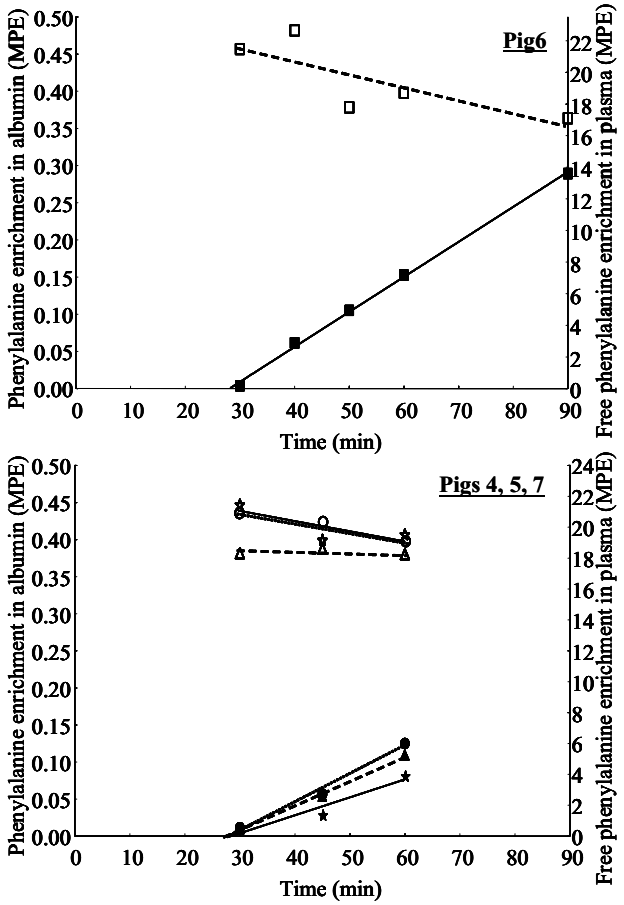

Figure 3:
Time course of molar % excess (MPE) in albumin-bound and plasma-free phenylalanine of individual pigs of experiment 2.
MPE in albumin-bound phenylalanine = a + b · t, where t = time (min), a = intercept on ordinate, b = slope of MPE in albumin- bound phenylalanine, ts = secretion time of albumin (min) = -a/b.
MPE in plasma-free phenylalanine = a - b · t, where t = time (min), a = intercept on ordinate, b = slope of MPE in plasma-free phenylalanine.

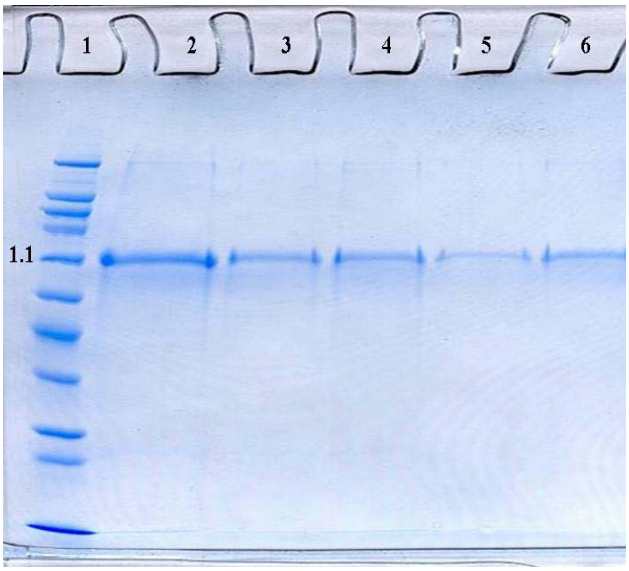

Figure 4:
Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS 10%) of isolated albumin from pig 2 (Experiment 1) compared with a molecular weight standard and with porcine albumin standard (1 – molecular weight standard, 1.1 – bovine serum albumin (MW: 66000), 2 - Albumin-pig 2 (dilution 1), 3 - Albumin-pig 2 (dilution 2), 4 - Albumin-pig 2 (dilution 3), 5 – Porcine albumin standard (MW: 68000, 1 µg), 6 – Porcine albumin standard (2 µg).

free phenylalanine can be excluded. A possible contamination of the albumin fraction with other proteins was checked by electrophoresis (Figure 4). No contamination of the isolated albumin fraction (Lanes 2 - 4) with other proteins could be detected.

The isotope incorporation was found to be strongly linear in both experiments from the time when the label appeared in the plasma (Figures 2 and 3), despite the fact that flooding in the first experiment was marginal. The slopes of the linear part of the regressions were significantly different due to the fact that the infusion solution used in Experiment 1 was substantially less enriched than in Experiment 2 (Table 1). The higher enrichment in Experiment 2 was chosen to induce a higher enrichment in the albumin to facilitate the GC-MS-measurements. The lower albumin enrichments in Experiment 1, especially shortly after the infusion, were close to the sensitivity of the GC-MS instrument used, and might have contributed to some variation (see Pig 3, Figure 2). The albumin FSRs finally calculated were not significantly different between both experiments despite the substantial differences in the experimental protocols, supporting the view that flooding of the precursor pool was successful even in Experiment 1. Albumin FSRs varied between 20 and 27 %/d in Experiment 1, and between 16 and 30 %/d in Experiment 2, respectively, and fit well into the range of 10 and 40 %/d as estimated by the continuous infusion technique (Jahoor et al., 1994, 1999; Mackenzie et al., 2003).

Based on the experiences with Pigs 1, 2 and 3 (Experiment 1, 45 mg phenylalanine per kg body weight), and with Pig 6 (Experiment 2, 125 mg phenylalanine per kg body weight) it became obvious that it would not be necessary to analyze blood samples so frequently. Only three measurement points are necessary within the range of the linear increase in albumin enrichment. It is also sufficient to analyze the corresponding plasma-free phenylalanine enrichment within this time slot, as its decrease is linear over up to 90 min (Figures 2 and 3). A further advantage of such a procedure is that the analysis of very low enrichment in albumin at natural abundance, typically occurring at times close to or lower than the albumin secretion time can be avoided. It is not necessary to estimate this secretion time with a broken-line regression (Equation 2) but simply by extrapolating the linear increase in albumin enrichment to zero enrichment (Equation 4).

To the authors' knowledge this is the first report on albumin secretion times of pigs which varied between 6 and 28 min. Reported mean secretion times were 15 min for rats (Peters, 1962) and varied between 27 and 37 min in humans (Ballmer et al., 1990; Hunter et al., 1995; Slater et al., 1995). In the present study, the albumin secretion time was significantly shorter in Experiment 1 (Table 1, 6 - 19 min) than in Experiment 2 (27 - 28 min), but this difference was not caused by the different approaches which were used to estimate this time (Equation 2 vs. 4), as the intercept on the x-axis was exclusively determined by the linear slope in the isotopic enrichment in both experiments. A biological reason for the shorter albumin secretion time can not be given at present, but it has to be considered that animals of both experiments differed not only in sex but also in their genetic background. These aspects need also to be taken into consideration when interpreting the significantly lower albumin concentration of the female pigs used in Experiment 1 (Table 1). Albumin con-

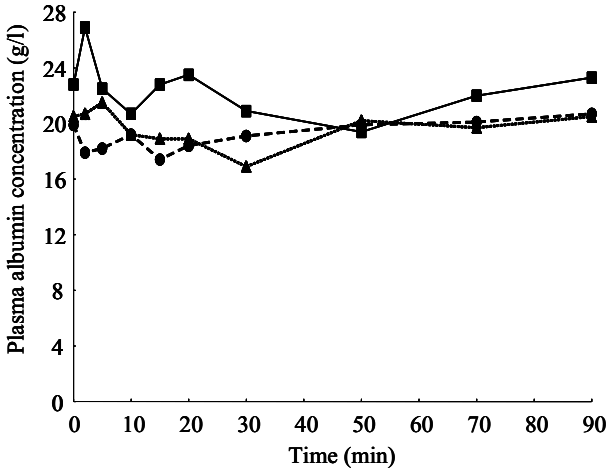

Figure 5:
Time course of plasma albumin concentration of individual pigs of experiment 1.

centrations were not altered during the experimental period due to the procedure and manipulations (Figure 5, Experiment 1) and estimation of absolute albumin synthesis rates (ASR) were therefore based on mean albumin concentrations.

As albumin FSRs were not significantly different between both experiments, the significantly lower albumin ASRs estimated for Experiment 1 according to the equation (7) were the result of the lower plasma-albumin concentration (Table 1). Albumin ASRs varied between 236 and 405 mg/kg body weight in Experiment 1, and between 347 and 624 mg/kg body weight in Experiment 2, respectively. Respective values for humans ranged between 120 and 230 mg/kg body weight (Ballmer et al., 1990) and those for pigs between 60 and 600 mg/kg body weight, depending on experimental factors examined (Jahoor et al., 1999; Mackenzie et al., 2003). Thus, the values obtained in the present study by using the flooding dose technique gave values which corresponded well to those obtained by the constant infusion technique.

Another interesting parameter is the proportion of the albumin synthesis to the whole body protein synthesis of the pig. This was estimated based on the assumption that whole pigs of a given body weight contain approximately 170 g protein/kg body weight, which is synthesized daily at a rate of 7 % (Simon 1989). The so-estimated albumin synthesis proportions of whole body protein synthesis varied between 2 and 5 %. As protein synthesis is a high-energy consuming process, physiologically or nutritionally induced alterations in albumin synthesis rates could have some impact on the whole body protein turnover and energy metabolism.

4 Conclusions

The flooding dose technique using [$^2H_5$]phenylalanine as the stable isotope tracer amino acid is suitable for measuring the albumin synthesis of the pig. A dose of at least 125 mg phenylalanine/kg body weight is recommended to ensure that flooding of all possible albumin synthesis precursor pools occurs. The enrichment of the infusion solution should be at least 11 molar % excess, but preferentially higher to ensure a good performance of the GC-MS. Biologically, further experiments should examine the effects of breed, age, sex and varying nutritional conditions on albumin synthesis of the pig more specifically.

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