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Isolation of plasma albumin by ethanol extraction is inappropriate for isotope ratio measurements during the acute phase response

Rita Jacobs[a,1], Hans Demmelmair[b,1], Peter Rittler[a], Josef Kellermann[c], Berthold Koletzko[b], Maximiliane Krick[a], Karl-Walter Jauch[a], Wolfgang H. Hartl[a,*]

[a] *Department of Surgery, Klinikum Grosshadern, Ludwig-Maximilian University, Marchioninistr. 15, D-81377 Munich, Germany*
[b] *Department of Pediatrics, Dr.v. Haunersches, Kinderspital, Ludwig-Maximilian University, Munich, Germany*
[c] *Analytical Protein Chemistry Group, Max-Planck Institute of Biochemistry, Martinsried, Germany*

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Abstract

Isolation of high-purity albumin from plasma is essential to study albumin kinetics in vivo with tracer techniques. Because of its simplicity ethanol extraction has been repeatedly used for albumin purification. However, it cannot be excluded that this single-step procedure completely prohibits contamination by other proteins, especially those known to be produced at an accelerated rate during the acute phase response. In the present study, we wanted to examine the reliability of ethanol extraction in different clinical conditions and to study the effects of potential impurities on albumin enrichment during stable isotope tracer studies. SDS-PAGE revealed a contaminating protein band at about 25,000 Da in healthy subjects and postoperative patients during the acute phase response, but not in critically ill patients. According to densitometry about 8% of proteins after ethanol extraction were contaminants. To examine potential contaminant effects on tracer enrichment 1-[^{13}C]-leucine was given to healthy subjects and postoperative patients. Blood samples were taken after various amounts of time, and albumin enrichments (tracer/tracee ratios) were determined from isotope ratios obtained by mass spectrometry. Irrespective of the magnitude of tracer enrichment, postoperative tracer/tracee ratios were significantly higher (on average +10%) in samples exclusively analysed by ethanol extraction than in samples which had undergone additional electrophoretic purification. No significant effect of the contaminant was seen in healthy subjects. N-terminal protein sequencing revealed contaminants to mainly consist of apolipoprotein A-1. Its physiology and pathophysiology may sufficiently explain its variable effects of albumin enrichment. Our findings suggest that exclusive ethanol extraction is inappropriate for albumin isolation in tracer studies performed during the acute phase response. Ethanol extraction may also not be advisable in all other situations known to be associated with a rise in apolipoprotein A-1 turnover.

Keywords: Albumin; Ethanol extraction; Mass spectrometry; Acute phase response

1. Introduction

Isolation of highly pure albumin from plasma is a prerequisite for tracer studies designed to examine albumin turnover in vivo. For scientific purposes, several methods for albumin isolation have been examined in the past. Traditional methods like cold ethanol purification according to Cohn [1] work via fractionation but cannot produce sufficiently pure albumin [2,3]. Alternatively, single-step chromatographic methods have been employed [2,4], but these also cannot guarantee the desired high degree of purity [2,3]. Other procedures such as multi-step chromatography [3,5] or automated FPLC [6] will produce the desired purity, but are either laborious and time costly [3,5], or technically demanding [6].

Because of its simplicity dissolution of albumin in acidified ethanol [7] has become popular for albumin isolation in biological tracer experiments. This method has repeat-

* Corresponding author. Tel.: +49 89 7095 3510; fax: +49 89 700 4418.
E-mail address: whartl@gch.med.uni-muenchen.de (W.H. Hartl).
[1] Both authors contributed equally.

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edly been claimed to yield sufficiently pure albumin [7–13]. However, because of the nature of this single step procedure this view has been questioned in the past [6,14,15]. Furthermore, if impurity exists indeed after ethanol extraction, no one has so far studied the relevance of this phenomenon for the accuracy of enrichment measurements. It was the aim of the present study to examine purity of albumin after ethanol extraction from samples obtained in different clinical conditions and to analyse interfering effects of potential contaminants on albumin enrichment during tracer application.

2. Experimental

2.1. Subjects

Studies were performed in six healthy volunteers, 10 postoperative patients and five critically ill patients. Postoperative patients had had limited colorectal cancer and had undergone curative, elective abdominal surgery. These patients were studied during the acute phase response after surgery, had an uneventful postoperative course and were free from signs of organ malfunction and local or systemic infection. No patient (before surgery) had a history of previous weight loss or clinical and laboratory signs of malnutrition or metabolic diseases. Critically ill patients were severely septic and had been in the intensive care unit since more than 10 days. Except in critically ill patients, informed consent was obtained from all subjects after the experimental protocol had been explained in detail. In critically ill patients an informed consent was not required since neither blood samples were taken for the purpose of the study, nor did we perform experimental measures of any kind. Blood analysis was exclusively done in left-overs from blood samples drawn for routine blood gas analysis. The study was approved by the local institutional review board (protocol #134/97).

2.2. Experimental protocol

After 10 p.m., all subjects/volunteers remained postabsorptive, except for consumption of mineral water. On the next day, heparinized venous or arterial blood was obtained from each subject. Then, all volunteers consumed an oral bolus (4 mg/kg) of 1-[^{13}C]-leucine (Tracer Technologies, Sommerville, Massachusetts, USA, 99.3 at.% enrichment) at 7 a.m. Subsequent blood samples were taken at 2, 4, 5, 6 and 8 h after tracer application. In seven postoperative patients a primed-constant intravenous infusion of 1-[^{13}C]-leucine was started at 7 a.m. The isotope infusion rate was 0.16 μmol/kg min (prime 9.6 μmol/kg) and lasted 3, 6 or 10 h. Arterialized blood samples were taken at the end of isotope infusion. In all subjects who received 1-[^{13}C]-leucine, an additional blood sample was collected before isotope administration to determine the background enrichment of protein-bound leucine.

2.3. Procedure

2.3.1. Ethanol extraction

Ethanol extraction was performed according to Korner [7] and Slater and Preston [13]. Five hundred microliters of 10% trichloroacetic acid (TCA) at 4 °C were added to 500 μl serum. The protein fraction was separated by precipitation. Samples were centrifuged at 11,000 rpm for 10 min (Hettich Mikroliter, Hettich Zentrifugen, Tuttlingen, Germany). The supernatant was removed and the pellet was re suspended in 500 μl absolute ethanol. The ethanolic albumin solution was centrifuged again at 11,000 rpm for 10 min. The ethanolic supernatant and 5 ml of distilled water were transferred to an ultrafiltration device (50,000 molecular mass cut-off Centriplus ultrafiltration cone; Centriplus YM-50 Millipore Corporation Bedford, Massachusetts, USA) placed above an universal bottle. Samples were centrifuged at 5000 rpm for 30 min or until the fluid had been completely cleared by the filter. The filtrate was discarded. Sediments attached to the filter were resuspended by adding 1 ml of distilled water to the inverted back side of the filter and by spinning the samples at 1500 rpm for 15 min (Hettich Roto Silenta/RPm, Hettich Zentrifugen, Tuttlingen, Germany). Samples were than divided into several aliquots for further analyses (analytical electrophoresis only, hydrolysation/leucine derivatization/mass spectrometry only, preparative electrophoresis with subsequent hydrolysation/leucine derivatization/mass spectrometry).

2.3.2. Analytical electrophoresis

An aliquot of the supernatant was used to monitor albumin purity or identification, using a 10% sodium dodecyl sulphate polyacrylamide vertical slab gel electrophoresis (SDS-PAGE) and visualization by Coomassie staining. Electrophoretically obtained bands of samples were compared with bands of commercially obtained human albumin and molecular weight markers (rainbow molecular weight marker RPN 800 and 756, respectively, Amersham Pharmacia Biotech, Uppsala, Sweden). Electrophoresis was performed using a vertical slab gel apparatus (PerfectBlue^{TM} double gel system Twin S, PEQLAB Biotechnologie GmbH, Erlangen, Germany). The 12.06 ml resolving gel was prepared using 4.4 ml of solution containing 30% acrylamide, 0.8% bisacrylamide and 69.2% H_2O. To this solution we added 120 μl of 10% sodium dodecyl sulfate (SDS), 3 ml of 1.5 M Tris/HCl buffer at pH 8.8, 4.4 ml H_2O, freshly prepared 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 80 μl) and freshly prepared 10% ammonium peroxodisulfate (60 μl). The stacking gel was prepared using 1.26 ml of the above acrylamide solution, 75 μl of 10% SDS, 1.95 ml of 0.5 M Tris/HCl buffer at pH 6.8, 2.55 ml H_2O, freshly prepared 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 108 μl) and freshly prepared 10% ammonium peroxodisulfate (54 μl). The electrode buffer contained 192 mM glycin, 25 mM Tris and 10 g SDS in 1 l H_2O, and the sample buffer 20 μg bromophenolic blue, 0.1 M Tris/HCl at pH 6.5, 20 g glycerol and 4 g SDS in 100 ml

H_2O. The resolving gel solution was poured between the glass plates and covered with distilled water. Polymerisation was completed after 30 min. Then, water was removed and the stacking gel was added. The length of the resolving gel was 6.5 cm and the length of the stacking gel 3.5 cm. Wells on the slab gel were prepared by a 10-tooth well former allowing for 10 different samples to be analyzed on one slab gel. No airspace was left between the well former and the acrylamide solution. After completion of the polymerization the well former was removed. To add an adequate amount of albumin, albumin concentration in the sample was determined photometrically and the samples were diluted down to a final concentration of 0.5 g/l. Fifty microliters of sample were combined with 50 μl of sample buffer, and 35 μl of this mixture were applied to each well of the gel. Electrophoresis was then performed at a constant current of 50 mA (electrophoresis power supply, EPS 1001, Amersham Pharmacia Biotech, Uppsala, Sweden) for about 1.5 h until bromophenol blue had passed the slab gel. After electrophoresis the gel was cut into two pieces thereby removing the stacking gel. For staining the remaining gel was incubated with 0.1% Coomassie® Brilliant Blue R-250, 50% H_2O, 40% ethanol and 10% acetic acid. After shaking for 12 h (Celloshaker Variospeed, Biotec-Fischer, Munich, Germany) at 60 rpm/min, protein bands were made visible by removing the extra staining by adding 50% H_2O, 40% ethanol and 10% acetic acid.

2.3.3. Densitometry

After analytical electrophoresis the protein bands were quantified by densitometry (Elscript 3, Hirschmann Geratebau, Unterhaching, Germany) with integration of the curve.

2.3.4. Preparative electrophoresis

The resolving gel, the electrode buffer and the sample buffer were prepared as described for analytical electrophoresis. The stacking gel was prepared using 0.84 ml of the above acrylamide solution (Section 2.3.2), 50 μl of 10% SDS, 1.3 ml of 0.5 M Tris/HCl buffer at pH 6.8, 1.7 ml H_2O, freshly prepared 10% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED, 36 μl,) and freshly prepared 10% ammonium peroxodisulfate (72 μl). For eluting buffer we used a sodium phosphate buffer 0,05 M pH 7.2 (sodiumdihydrogenphosphate, pH adjusted with sodium hydroxide). The resolving gel solution was poured into a cylinder and covered with distilled water. Polymerisation was completed after 30 min. Then, water was removed and the stacking gel was added and covered with distilled water. After completion of the polymerization, the cylinder with the gel was inserted into the electrophoresis apparatus (Bio-Rad Model 491 Prep-Cell, Bio-Rad Laboratories, Hercules, California, USA). The length of the resolving gel was 3 cm and the length of the stacking gel 1 cm. Eluting buffer and electrode buffer were added into the corresponding chambers. Seven hundred and fifty microliters of sample were added to 750 μl of sample buffer, and by using a tube, the mixture was directly transferred on the gel. The speed of the eluting buffer was set at 1 ml/min and electrophoresis was initially performed at a constant current of 40 mA (electrophoresis power supply, EPS 1001, Amersham Pharmacia Biotech, Uppsala, Sweden) for about 2–2.5 h until bromophenolic blue had passed the gel. Subsequently, electrophoresis was continued at a constant current of 80 mA for 2.5–3 h. During this period 100 consecutive samples of 1.5 ml each were collected by a fraction collector (Bio-Rad Model 2110 Fraction collector, Bio-Rad Laboratories Hercules, California, USA). Every fifth sample was analyzed by an analytical electrophoresis (see above) to detect the sample fraction containing the albumin (on average samples ranging from #45/50 to #100). Samples containing albumin were combined and concentrated by filtration through a Centriplus filter (Centriplus ultrafiltration cone Centriplus YM-50, Millipore Corporation, Bedford, Massachusetts, USA) with an exclusion pore size of 50,000 Da. Albumin attached to the filter was resuspended by adding 1 ml of distilled water to the inverted back side of the filter and by spinning the samples at 2000 rpm for 15 min.

2.3.5. Mass spectrometry

Methods and data analysis were discussed in detail previously [16]. Samples were dried under vacuum with a speed vac (Savant Instruments, Farmingdale, NY, USA). The protein was hydrolyzed under nitrogen at 110 °C for 36 h in 6 N HCl. For capillary gas chromatography and combustion isotope ratio mass spectrometry analysis, amino acids from proteins (on average 7–8 ng) were then converted to the *N*-acetyl-propyl (NAP) ester. NAP-amino acid derivatives were analyzed in a capillary gas chromatography and combustion isotope ratio mass spectrometry system (GC-IRMS) that consisted of a Hewlett-Packard 5890 Series II gas chromatograph interfaced to a mass spectrometer Delta S (Finnigan MAT, Bremen, Germany). In an individual sample, all measurements were done in duplicate.

For biomedical studies isotopic enrichment needs to be expressed as the tracer/tracee ratio (*z*). GC-IRMS analysis only yields $\delta^{13}C$ values which can be converted into isotopic ratios ($^{13}C/^{12}C$) of the entire NAP-1-[^{13}C]-leucine molecule by the formula

$$^{13}C/^{12}C = \left(\frac{\delta^{13}C}{1000 + 1}\right) \times 0.011$$

where 0.011 represents $^{13}C/^{12}C$ in the Pee Dee Belemnate limestone (PDB) standard for GC-IRMS. However, a correction is necessary. In contrast to the actual 1-[^{13}C]-leucine molecule, in NAP-1-[^{13}C]-leucine one of a total of 11 carbon atoms is labelled. Because of the nature of GC-IRMS analysis, enrichment is determined by measuring the ratio of ^{13}C to ^{12}C in the CO_2 resulting from the combustion of the total derivatized NAP-1-[^{13}C]-leucine molecule, yielding

$$Z_{\text{NAP–leucine}} = (^{13}C/^{12}C)_{SA} - (^{13}C/^{12}C)_{BK}$$

$(^{13}C/^{12}C)_{SA}$ represents the ratio of ^{13}C to ^{12}C in a sample and $(^{13}C/^{12}C)_{BK}$ is the naturally occurring background ratio.

Multiplication of $Z_{NAP–leucine}$ with a correction factor that takes into account dilution of the label at position 1 by carbon atoms at position 2–11 will yield the tracer/tracee ratio for 1-[^{13}C]-leucine ($Z_{leucine}$) in the sample [17]. As we have previously shown [16] the correction factor equals 10.02 and $Z_{leucine}$ can be calculated by the formula

$$Z_{\text{leucine}} = \frac{10.02 Z_{\text{NAP–leucine}}}{1 + [1 - 10.02] Z_{\text{NAP–leucine}}}$$

2.3.6. Protein sequencing

The purified contaminant was desalted on a ProSorb column (Applied Biosystems, Foster City, CA, USA) and N-terminally sequenced on a pulsed liquid phase sequencer Procise cLC (Applied Biosystems, Foster City, CA, USA) according to the manufacturers instructions [18].

2.4. Statistics

Data are expressed as mean ± SEM. δ^{13}C values and tracer/tracee ratios in samples with or without electrophoretic purification were compared by the paired *t*-test. Correlations were analysed by Pearson's product moment correlation. A *p* value of less than 0.05 was taken as indicating a significant difference.

3. Results and discussion

Albumin has well-established and important functions in health. There is evidence, that its kinetic and dynamic properties are significantly altered by surgical stress, chronic renal failure but also by nutritional status and type of diet [19]. However, findings are not always consistent, and especially rates of albumin synthesis were found to vary during the acute phase response after surgical trauma. Thus, the cytokine-mediated reduction of albumin synthetic capacity described in vitro [20–22] does not correspond to in vivo findings showing an accelerated albumin turnover [11,23–25]. A portion of these discrepancies may originate from imprecise analytical methods.

Stable isotopic tracers are an established investigational tool for the in vivo investigation of albumin synthesis [12]. For appropriate measurement of tracer incorporation isolation of pure albumin from plasma is essential. Because of its simplicity, ethanol extraction has been widely used for this purpose and has originally been claimed to yield pure albumin [7]. Our results clearly indicate this assumption not to be the case in healthy subjects and in patients during the acute phase response after surgical trauma. Furthermore, exclusive use of ethanol extraction with the latter patients will result in a significant overestimation of tracer enrichments in albumin (on average +10%). As can be seen on the SDS-PAGE in Fig. 1 (healthy subjects) and 2 (postoperative patients), albumin extracted by ethanol is not pure but is accompanied by a contaminating protein. This contamination was found in

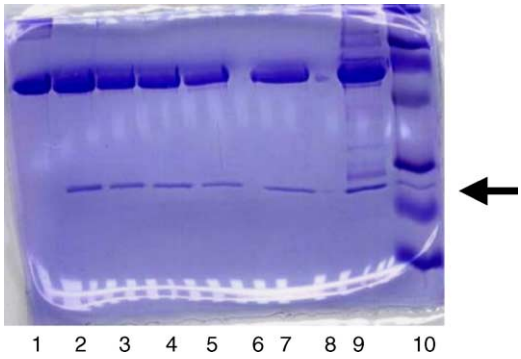


Fig. 1. SDS-PAGE of albumin from five healthy control subjects. Albumin was separated exclusively by ethanol extraction. Lane 1: albumin (control); lane 2–5, 7: samples after ethanol extraction; lane 9: untreated sample; lane 10: molecular weight marker; lane 6 and 8: empty. The arrow indicates the contaminant.

all postoperative patients and healthy subjects and could only be removed by subsequent preparative electrophoresis. Comparison with standard molecular weight markers revealed this contaminating compound to possess a molecular weight of approximately 25,000 Da. Protein sequencing identified apolipoprotein A-1 (apoA-1) as major source of the contamination. ApoA-1 is synthesised as proapo A-1 in the liver and gut, secreted into the plasma and transformed into mature apoA-1 by a proteolytic cleavage over a period of 10–24 h. ApoA-1 associates with lipids to form plasma high density lipoproteins (HDL) [26,27].

The findings in healthy subjects correspond to results from two other studies where exclusive ethanol extraction was also not capable of yielding pure albumin [14,15]. In contrast, the group of Garlick and co-workers reported several times ethanol extraction to yield pure albumin in healthy volunteers [8,9,12] or in subjects during laparoscopic surgery [10]. Purity was verified either by matrix-assisted laser desorption time of flight mass spectrometry [10] or by SDS-PAGE and silver-staining, and pictures of corresponding gels were published twice [8,9]. Similar findings in healthy subjects were also published by Slater and Preston [13] who used SDS-PAGE and Coomassie-staining for verification of purity after ethanol extraction (Fig. 2).

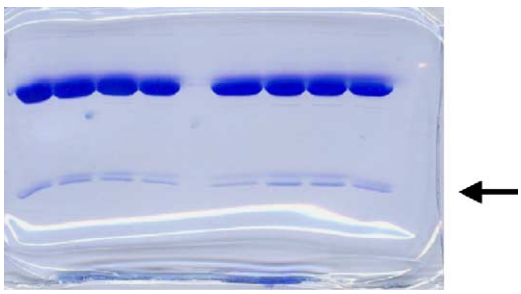


Fig. 2. SDS-PAGE of albumin from eight postoperative patients. Albumin was separated exclusively by ethanol extraction. All lanes represent samples after ethanol extraction. The arrow indicates the contaminant.

In contrast to healthy and post-surgical subjects, we and others [11] could not identify relevant impurities in albumin isolated from critically ill patients by ethanol extraction. The latter authors [11] compared ethanol extraction with anion exchange chromatography (FPLC) which has been shown to result in a more than 99% purity of the isolated albumin [6]. Albumin was isolated in parallel by both procedures in the same patients and the authors found identical bands according to SDS-PAGE [11].

In an attempt to improve the purity and to eliminate apoA-1 from samples in healthy and postoperative subjects we examined several modifications of the procedure for ethanol extraction. The following modifications were tested:

(a) Use of EDTA, heparinized or citrate plasma instead of serum.
(b) Reduction of the sample volume from 500 to 250 μl in order to avoid an eventual clogging of the filter.
(c) Use of ethanolic instead of aqueous TCA.
(d) Reduction of the TCA volume (200 μl instead of 750 μl).
(e) Two subsequent TCA precipitation steps instead of one.
(f) Ultracentrifugation at 4 °C.
(g) Washing of the samples with acid buffer instead of distilled water.
(h) Washing of the samples with SDS and mercaptoethanol instead of distilled water. Thereby it was hoped to separate albumin from the contaminating protein(s) by breaking up disulfide bridges during the filtration step.

None of the above modifications was capable of eliminating apoA-1 according to the respective SDS-PAGE which still revealed an additional band. These results suggest that apoA-1 must be tightly bound to albumin and is resistant to simple alterations of the described procedure for ethanol extraction. It is, however, unclear why some [8,9,12,13] but not all [15] previous authors missed apoA-1 in their SDS-PAGE of samples from healthy subjects. Presumably, variations in the SDS-PAGE procedure itself may explain the discrepancies. Thus, Slater et al. [13] used an SDS-PAGE according to Maguire [28] of which the staining procedure included only 0.025% Coomassie Blue, one fourth of the concentration used by us. Furthermore, it is clear that variations in the amount of albumin used for analytical electrophoresis will have an effect on staining. In postoperative patients on average 8% of the isolated proteins were not albumin (as determined by densitometry), and this percentage was even lower in healthy subjects. Garlick and co-workers used less than 1.4 μg albumin per well for analytical electrophoresis [8]. These albumin amounts are markedly smaller than those used by us (about 9 μg albumin per well), and they may eventually contain too little apoA-1 to be detected in healthy subjects even by silver staining [8,9,12]. A similar mechanism may also explain the negative findings from specific mass spectrometry analysis [10]. In the latter study, samples were taken intraoperatively at a time when hepatic protein synthesis is markedly depressed [29], and when fluid administration and blood loss will globally reduce levels of plasma proteins. Thereby concentrations of apoA-1 will fall to very low values and this phenomenon may have possibly prevented their detection after ethanol extraction.

In ICU patients with multiple organ failure similar mechanisms may have prevented the identification of apoA-1 after exclusive ethanol extraction. In critical illness very low levels of apoA-1 have been described resulting from the combined effects of impaired hepatic lipoprotein synthesis, accelerated apoA-1 catabolism and losses into the interstitial space [30–32]. Thus, apoA-1 concentrations may have been also too low to interfere significantly with ethanol extraction.

The question remains whether apoA-1 contamination is relevant for tracer studies aiming at the measurement of albumin synthesis. Our results indicate a relevant confounding effect in postoperative patients. In healthy volunteers, this effect seems to be of minor importance. In our healthy subjects $\delta^{13}C$ values in plasma albumin measured by GC-IRMS at various times after 1-[^{13}C]-leucine administration did not depend on the analytical method. Corresponding average values (-7.46 ± 3.60 with electrophoretic purification, and -7.37 ± 3.48 without purification) were comparable. There was also an excellent correlation between the $\delta^{13}C$ values obtained by the different methods emphasizing the approximate identity of albumin enrichment and enrichment of apoA-1 (Fig. 3). These findings are in line with published normal rates of fractional albumin and apoA-1 synthesis being fairly similar in healthy subjects [33,34].

Plasma from postoperative subjects yielded different results. There, $\delta^{13}C$ values in plasma albumin varied depending on the analytical procedure. Corresponding average values were significantly higher with exclusive ethanol extraction than with ethanol extraction and subsequent electrophoretic purification (-5.54 ± 3.42, $p < 0.01$ versus -8.19 ± 3.06).

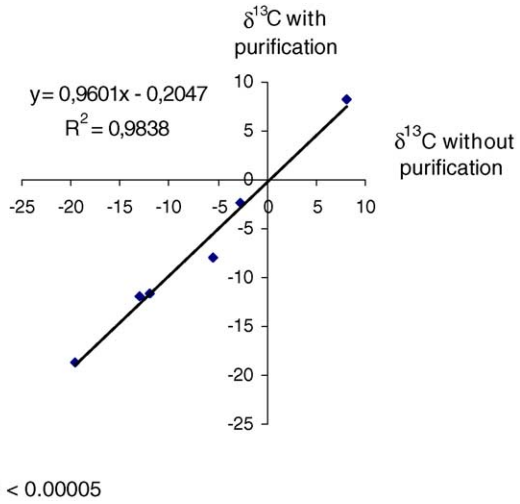
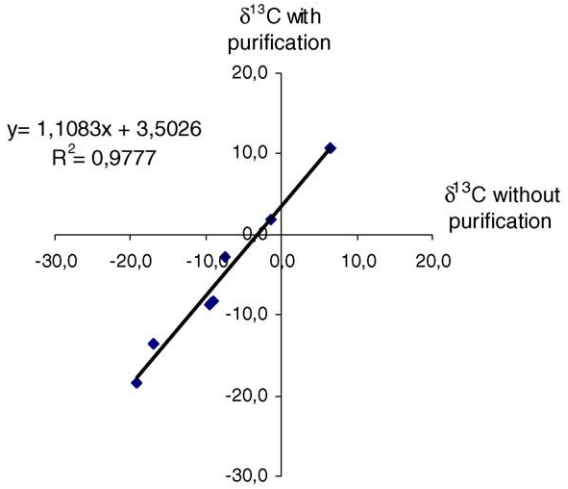
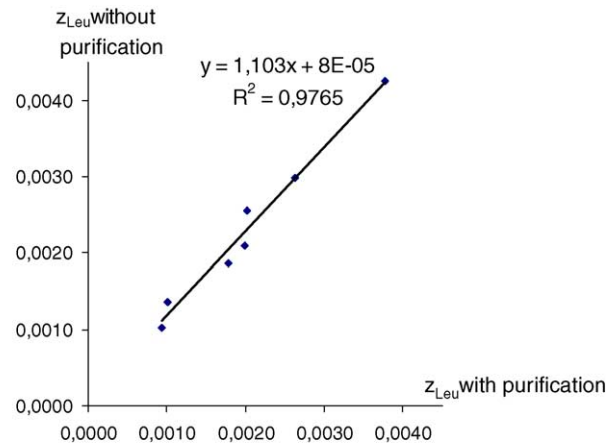


Fig. 3. Correlation between $\delta^{13}C$ values in plasma albumin from healthy volunteers. 1-[^{13}C]-leucine was given orally and blood samples were taken after various amounts of time. $\delta^{13}C$ values were measured by GC-IRMS in the same sample after ethanol extraction with or without subsequent electrophoretic purification.



p < 0.00005

Fig. 4. Correlation between $\delta^{13}C$ values in plasma albumin from postoperative patients. 1-[^{13}C]-leucine was infused over various amounts of time and $\delta^{13}C$ values were measured by GC-IRMS in the same blood sample after ethanol extraction with or without subsequent electrophoretic purification.



p < 0.00005

Fig. 5. Correlation between tracer/tracee ratios (Z_{leu}) for 1-[^{13}C]-leucine from plasma albumin in postoperative patients. 1-[^{13}C]-leucine was infused over various amounts of time and Z_{leu} was calculated from $\delta^{13}C$ values determined in background blood samples and blood samples taken after tracer infusion. $\delta^{13}C$ values were obtained by GC-IRMS in the same sample after ethanol extraction with or without subsequent electrophoretic purification.

Furthermore, a highly significant linear correlation was found between the values obtained by different methods. $\delta^{13}C$ values from impure samples were about 1.11 times higher than those from purified samples (Fig. 4). This ratio remained almost the same when background enrichments and isotope dilution 1-[^{13}C]-NAP-leucine were taken into account. Thus, tracer/tracee ratios also varied according to the analytical procedure (Table 1) and were significantly higher in impure than in pure samples (0.0023 ± 0.0004, $p < 0.01$ versus 0.0020 ± 0.0003). Correspondingly, also a highly significant correlation between corresponding tracer/traceee ratios was found, and the ratios from impure samples were on average 1.1 times higher than those from pure samples (Fig. 5). In individual samples, this difference was as high as 40% (Table 1).

Table 1
Tracer/tracee ratios (Z_{leu}) for 1-[^{13}C]-leucine from plasma albumin in postoperative patients

Sample number	Duration (h) of tracer infusion	Z_{leu} (without purification)	Z_{leu} (with purification)
1	3	0.0010	0.0009
2	6	0.0014	0.0010
3	6	0.0019	0.0018
4	6	0.0021	0.0020
5	10	0.0026	0.0020
6	10	0.0030	0.0026
7	10	0.0043	0.0038

1-[^{13}C]-leucine was infused over various amounts of time and Z_{leu} was calculated from $\delta^{13}C$ values determined in background blood samples and blood samples taken after tracer infusion. $\delta^{13}C$ values were obtained by GC-IRMS in the same sample after ethanol extraction with or without subsequent electrophoretic purification.

Therefore, it is clear that in postoperative patients enrichment of apoA-1 must be considerably higher than the corresponding albumin enrichment. Most likely these differences in enrichment also reflect differences in synthesis or turnover, implying that the postoperative rate of apoA-1 synthesis should be faster than that of albumin and also faster than the apoA-1 synthetic rate seen in healthy subjects. This theory is in line with physiology and pathophysiology of apoA-1 metabolism. Because of perioperative fluid and blood losses, a temporary minor decline of apoA-1 concentration can be observed after surgery [35,36]. In an attempt to compensate for apoA-1 losses, apoA-1 synthesis rises significantly [37–39], possibly mediated by cytokines, steroids or colloid osmotic pressure [40,37].

Our results suggest that exclusive ethanol extraction is inappropriate to isolate albumin in human tracer studies. In postoperative patients the resulting error may potentially be large. Even if analysis of samples from postabsorptive healthy subjects seems to be less affected, use of ethanol extraction is still not advisable since various dietary, hormonal and medicamentous manipulations are known to alter apoA-1 kinetics.

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