Determination of urea kinetics by isotope dilution with $[^{13}\text{C}]$urea and gas chromatography–isotope ratio mass spectrometry (GC–IRMS) analysis

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1. Stable urea isotopes can be used to study urea kinetics in humans. The use of stable urea isotopes for studying urea kinetic parameters in humans on a large scale is hampered by the high costs of the labelled material. We devised a urea dilution for measurement of the distribution volume, production rate and clearance of urea in healthy subjects and renal failure patients using the inexpensive single labelled $[^{13}\text{C}]$urea isotope with subsequent analysis by headspace chromatography–isotope ratio MS (GC–IRMS) of the $[^{13}\text{C}]$urea enrichment.

2. The method involves measurement of the molar percentage excess of $[^{13}\text{C}]$urea in plasma samples taken over a 4 h period after an intravenous bolus injection of $[^{13}\text{C}]$urea. During the sample processing procedure, the plasma samples together with calibration samples containing a known molar percentage excess of $[^{13}\text{C}]$urea are acidified with phosphoric acid to remove endogenous CO$_2$, and are subsequently incubated with urease to convert the urea present in the plasma samples into CO$_2$. The $^{13}\text{C}$ enrichment of the generated CO$_2$ is analysed by means of GC–IRMS. This method allows measurement of the molar percentage excess of $[^{13}\text{C}]$urea to an accuracy of 0.02%.

3. Reproducibility studies showed that the sample processing procedure (within-run coefficient of variation (CV) <2.8% and between-run CV <8.8%) and the GC–IRMS analysis (within-day CV <1.3% and between-day CV <1.3%) could be repeated with good reproducibility.

4. In clinical urea kinetic studies in a healthy subject and in a renal failure patient without residual renal function, reproducible values of the distribution volume, production rate and clearance of urea were determined using minimal amounts of $[^{13}\text{C}]$urea (25–50 mg).

5. Because only low $[^{12}\text{C}]$urea enrichments are needed in this urea dilution method using GC–IRMS analysis, the costs of urea kinetic studies are reduced considerably, especially in patients with renal failure.

INTRODUCTION

To assess the quality of the haemodialysis treatment of patients with chronic renal failure, kinetic modelling of urea is widely used. Clinically, the most relevant results from urea kinetic modelling are $K/V$ ('dose of dialysis') and protein catabolic rate (a measure of protein intake under stable conditions). An essential parameter to determine the protein catabolic rate is the urea distribution volume (UDV) [1]. The UDV, which is assumed to equal total body water [2], comprises multiple compartments of the human body. The size of the UDV can be determined by kinetic modelling of urea during the haemodialysis treatment. However, it is difficult to obtain an accurate measurement of the UDV by single-pool urea kinetic modelling due to compartmentalization of urea. Therefore, complex multiple-pool urea kinetic models have been proposed for studying urea kinetics during haemodialysis [3].

The reference method for the determination of the UDV is the dilution of exogenous urea. Natural urea and various urea isotopes have been used in these dilution studies [4–8]. In a previous paper we have described a urea dilution method using the triple labelled $[^{13}\text{C},^{15}\text{N}_2]$ urea isotope and gas chromatography–MS (GC–MS) analysis for study-

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Abbreviations: CV, coefficient of variation; GC–IRMS, gas chromatography–isotope ratio MS; MPE, molar percentage excess; UDV, urea distribution volume.
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ing urea kinetics in normal humans [9]. In patients with chronic renal failure, however, the use of the dilution method using the \[^{13}\text{C}^{15}\text{N}_2\] urea isotope is limited, since more of the costly labelled material is needed. The blood urea concentration in these ureamic patients is about 4–6-fold higher than that in healthy subjects. Consequently, considerably more \[^{13}\text{C}^{15}\text{N}_2\] urea is required to achieve detectable enrichments. To reduce the costs of urea isotope dilution studies in uremic patients, it is worthwhile investigating dilution methods that require less expensive urea isotopes and low isotope enrichments.

In this paper we describe a urea dilution method using the inexpensive single labelled \[^{13}\text{C}\]urea isotope and headspace gas chromatography-isotope ratio MS (GC–IRMS) analysis for determining \[^{13}\text{C}\]urea enrichments. We have succeeded in devising a urea dilution method in which minimal amounts of the \[^{13}\text{C}\]urea isotope suffice to determine urea kinetic parameters in normal subjects and in ureamic patients.

**MATERIALS AND METHODS**

**Chemicals**

\[^{13}\text{C}\]Urea (99% \(^{13}\text{C}\)) was obtained from Campro Scientific, Veenendaal, The Netherlands, and 85% phosphoric acid (\(\text{H}_3\text{PO}_4\)) and amylalcohol from E. Merck Nederland B.V., Amsterdam, the Netherlands. Urease (cat. nr. U 4002, 80.000 units/g) was purchased from Sigma Chemical Company, St Louis, MO, U.S.A. and stored at 0–5°C until use.

**Standard solutions**

\[^{13}\text{C}\]Urea. A stock solution of \[^{13}\text{C}\]urea was prepared by dissolving approximately 50 mg of \[^{13}\text{C}\]urea in 100 ml of distilled water and this solution was then diluted 50-fold. Aliquots (2.5 ml) of this stock solution were stored frozen at \(-20°C\). Each time GC–IRMS analyses were performed, a 10-fold (healthy person) or 5-fold diluted (renal failure patient) working solution was made from the stored aliquots using 0.9% \(\text{NaCl}\) as diluent.

Urease. A stock solution of urease containing approximately 3000 units/ml was prepared by dissolving 0.26 g of urease in 3.5 ml of \(\text{H}_2\text{O}\) and 3.5 ml of glycerol, and was stored at 4–6°C. This solution was stable for 6 months. Each time GC–IRMS analyses were performed a working solution (30 units/l) was made by diluting the stock solution 100-fold with a 10 g/l EDTA,\(\text{Na}_2\) buffer solution (pH 6.9). The EDTA,\(\text{Na}_2\) buffer solution was stable for 6 months.

**Headspace GC–IRMS**

A Delta-S GC–IRMS combination (Finnigan MAT, Bremen, Germany), equipped with a Varian 3400 GC, was used to determine \(^{13}\text{C}_2\text{O}_2/^{12}\text{C}_2\text{O}_2\) ratios in the gas mixture present in the Vacutainer tubes after conversion of the \[^{13}\text{C}\]urea/\[^{12}\text{C}\]urea mixtures by means of urease (see analytical procedures below). Gas mixtures were injected on a 25 m Poraplot Q capillary column (Chrompack, Middelburg, The Netherlands), internal diameter 0.32 mm, with helium as the carrier gas. The injection temperature was 120°C, oven temperature 70°C, split ratio 1:10. \(^{13}\text{CO}_2/^{12}\text{CO}_2\) ratios were determined as delta (\(\delta\)) value against the international PDB-standard carbon dioxide [10]. An increase in \(\delta\) value of 1000 corresponds to an increase of 1% in the \(^{13}\text{CO}_2/^{12}\text{CO}_2\) ratio [\(=\) molar percentage excess (MPE) of \(^{13}\text{CO}_2\)].

**Calibration lines**

Calibration lines were constructed from calibration mixtures containing known \[^{13}\text{C}\]urea/\[^{12}\text{C}\]urea ratios (= MPE of \[^{13}\text{C}\]urea). For this purpose a pool of plasma was prepared from EDTA plasma samples obtained from healthy volunteers. The urea concentration (4.6 mmol/l) was determined by means of a Kodak Ektachem 500 analyser (Eastman Kodak Company, Rochester, NY, U.S.A.). Aliquots of this pooled plasma were stored frozen at \(-20°C\) until use. To prepare the calibration mixtures, aliquots of pooled plasma (0.5 ml) were pipetted into unstoppered 10 ml Vacutaine® tubes (Becton Dickinson Vacutainer Systems Europe, France). To each tube 0, 125, 250, 375 or 500 \(\mu\)l of \[^{13}\text{C}\]urea working solution was added. Each series of calibration mixtures covered a range of 0–0.35 MPE of \[^{13}\text{C}\]urea. Reproducibility tests were performed using these calibration mixtures.

In the clinical urea kinetic studies, a calibration line was constructed for each subject individually from plasma samples (0.5 ml) drawn from the studied subject immediately before administration of labelled urea. To cover the range up to 0.30 MPE of \[^{13}\text{C}\]urea, the 10-fold diluted \[^{13}\text{C}\]urea solution (15.89 \(\mu\)mol/l) was used for the healthy volunteer and the 5-fold diluted \[^{13}\text{C}\]urea solution (31.78 \(\mu\)mol/l) for the renal failure patient.

**Analytical procedures**

Sample processing for GC–IRMS analysis. Appropriate amounts of \(\text{H}_2\text{O}\) were added to the calibration mixtures and to the plasma samples (0.5 ml) collected during the urea kinetic studies in order to bring all solutions to the same volume (2.0 ml). Calibration mixtures and patient plasma samples were then processed in one run using the same procedure.

The samples were placed in aluminium holders in a dry block heater, set at 37°C. To facilitate the removal of \(\text{CO}_2\) originating from \(\text{HCO}_3^-\) present in the mixtures, the samples were acidified to pH 2.0–3.0 by adding 25 \(\mu\)l of 1 mol/l \(\text{H}_3\text{PO}_4\) and
mixed. A vigorous stream of \( \text{N}_2 \) gas was passed from the bottom through the contents of each tube. To prevent foaming, 125 \( \mu \)l of amylalcohol was added to all samples. After 30 min the endogenous CO\(_2\) present in the samples was maximally expelled and the pH was about 6.5. The tubes were then closed rapidly with the rubber stoppers supplied, taking care no air was enclosed. Then 1 ml of urease working solution (30 units/l, pH 6.9) was added by injection through the stopper and the contents of the tubes was again thoroughly mixed. During an incubation period of 15 min at 37°C the urea in the solution was converted into CO\(_2\) and NH\(_3\). To liberate the generated CO\(_2\) from the solution, the pH was adjusted to less than 2 by adding 75 \( \mu \)l of 1 mol/l H\(_3\)PO\(_4\) through the stopper. The stoppered tubes were then rotated for 30 min on an orbital shaker. The gas mixtures remained stable for at least 2 weeks at 2-6°C.

**GC-IRMS analysis.** Analysis of the \( ^{13}\text{C} \) enrichment of the generated CO\(_2\) was performed within 2 weeks after sample processing. Samples (100 \( \mu \)l) of the gas mixture present in the Vacutainer\textsuperscript{®} tubes were injected manually into the GC–IRMS combination by means of a gas-tight syringe. Samples were injected in order of increasing enrichment to minimize memory effects.

**Analysis of plasma urea concentration.** Urea concentrations of the separate plasma samples from the urea kinetic studies were determined by means of a Kodak Ektachem 500 analyser and converted into plasma water concentration by dividing the measured values by 0.93 [11].

**Reproducibility studies**

**Sample processing procedure.** The within-run reproducibility of the sample processing procedure was determined by measurement of the \( \delta \) values of eight series of calibration mixtures that were prepared, processed and analysed in one run on the same day. The between-run reproducibility was determined by measuring the \( \delta \) values of eight series of calibration mixtures that were prepared, processed and analysed on separate days over a period of 1 year. Reproducibility of the sample processing procedure was expressed as the coefficient of variation (CV = mean/SD) of the \( \delta \) values of the eight calibration series. In addition, the slope of the calibration line was determined and the within-run and between-run reproducibility of the slope was calculated.

**GC–IRMS analysis procedure.** Reproducibility of the GC–IRMS analysis itself was determined according to the following procedure: eight series of calibration mixtures were prepared and processed in one run on the same day. The calibration series were analysed in random order. To determine the within-day reproducibility of the GC–IRMS analysis procedure, the \( \delta \) values of the eight calibration mixtures were measured three times on the same day. The CV values of each series of the eight calibration mixtures were calculated and averaged to obtain the mean within-day reproducibility. To determine the between-day reproducibility, the \( \delta \) values of the calibration mixtures were measured again on three separate days within 1 week. Again the corresponding CV values were calculated and averaged to obtain the mean between-day reproducibility of the GC–IRMS analysis procedure.

**Clinical urea kinetic study.** To evaluate the repeatability of the urea kinetic parameters in man, four urea kinetic studies were performed in a healthy volunteer within 4 months.

**Clinical urea kinetic studies**

The clinical studies were carried out after approval of the Medical Ethics Committee of the University of Groningen and the informed consent of participants was obtained.

**Subjects.** Urea kinetics were determined in two male subjects: one healthy volunteer (age, 57 years; height, 182 cm; body weight, 86 kg) and one stable renal failure patient without residual renal function on thrice weekly haemodialysis (age, 38 years; height, 177 cm; body weight, 71.5 kg). The patient with renal failure was studied during an interdialytic period.

**Urea kinetics.** Both subjects were studied in the morning after a 12 h overnight fast, and remained fasting during the course of the study. The subjects received an intravenous injection of \([^{13}\text{C}]\text{urea} \) dissolved in 24 ml of 0.9% NaCl solution in 45 s through a heparin lock. The healthy volunteer received a dose of 24 mg ( = 0.39 mmol) and the renal failure patient 48 mg ( = 0.79 mmol) of \([^{13}\text{C}]\text{urea} \). The solution was prepared in and dispensed from 30 ml syringes under sterile conditions on the day of the study. Blood samples were drawn in 4 ml Vacutainer\textsuperscript{®} tubes containing lithium-heparin through a heparin lock in the opposite arm just before the injection (\( t = 0 \)) and at 2, 5, 10, 15 and 30 min, and every 30 min until 4 h after injection. Plasma was separated from the red blood cells and aliquots of 0.5 ml were pipetted into sterile Vacutainer\textsuperscript{®} tubes without additive and stored at \(-20°C\) until GC–IRMS analysis. In addition, separate 400 \( \mu \)l aliquots were pipetted and stored for the analysis of plasma urea concentration.

**Calculations and statistical analysis**

**Calibration lines.** Regression parameters of the calibration lines, relating measured \( \delta \) values to known MPEs of \([^{13}\text{C}]\text{urea} \), were determined using least-squares linear-regression analysis.

**Clinical urea kinetic studies.** In the clinical urea kinetic studies the \([^{13}\text{C}]\text{urea} \) enrichments of the plasma samples of each subject were calculated...
using the slope and intercept of the calibration lines that were prepared from their own plasma samples obtained before the injection of $[^{13}\text{C}]$urea. In the calculations of the urea kinetic parameters it is assumed that the kinetic handling of the labelled $[^{13}\text{C}]$urea and unlabelled $[^{12}\text{C}]$ urea is equal. After the injected labelled urea is completely distributed within the body, the change over time of the natural logarithmic value of the ratio labelled/unlabelled urea follows a straight line with a negative slope. If UDV, the production rate of urea and the plasma urea concentration are assumed to be constant, this change is defined by eqn. (1): 

$$\ln R_t = \ln R_0 - t \cdot k_p$$  \hspace{1cm} (1)$$

in which $R_t$ represents the ratio labelled/unlabelled urea at time $t$, $R_0$ is the ratio at $t = 0$, and $k_p$ is the production constant of urea (the slope of the regression line of the natural logarithmic values of $R$ plotted against time) [5]. The UDV can be calculated from the intercept of the extrapolated regression line, representing the apparent ratio $R_0$ at $t = 0$, according to eqn. (2): 

$$\text{UDV} = \text{amount of } [^{13}\text{C}]\text{urea injected}/(R_0 \cdot U)$$  \hspace{1cm} (2)$$

The production rate of urea ($G_U$) is calculated from UDV, $k_p$ and the plasma urea concentration ($U$) according to eqn. (3) [5]: 

$$G_U = k_p \times \text{UDV} \cdot U$$  \hspace{1cm} (3)$$

To determine the clearance (CL) of the $[^{13}\text{C}]$urea isotope from the body, $[^{13}\text{C}]$urea plasma water concentrations were calculated by multiplying the $[^{13}\text{C}]$urea/$[^{12}\text{C}]$urea ratios by the measured plasma water urea concentration in each sample. Plotting the logarithmic $[^{13}\text{C}]$urea concentrations against time gives a straight declining line whose slope represents the elimination constant ($k_e$). CL is calculated from eqn. (4) and the half-life time ($t_{1/2}$) from eqn. (5): 

$$\text{CL} = k_e \times \text{UDV}$$  \hspace{1cm} (4)$$

$$t_{1/2} = 0.693/k_e$$  \hspace{1cm} (5)$$

Statistics. Results are shown as means±SD. Reproducibility is expressed as CV. Regression analysis was performed using least-squares linear-regression analysis.

RESULTS

Calibration lines

We found an excellent linear correlation ($r = 0.99999$) between the MPEs of $[^{13}\text{C}]$urea and the measured $\delta$ values, with a recovery of 92%. Consequently, the slope of the calibration line was lower (914.7±16 $\delta$ per MPE $[^{13}\text{C}]$urea) than the expected slope (1000 $\delta$ per MPE $[^{13}\text{C}]$urea) (Fig. 1). The $\delta$ values of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ gas mixtures were measured at the peak heights of about 0.3 V and $\delta$ values that corresponded to as little as 0.02 MPE of $[^{13}\text{C}]$urea could be measured.

Reproducibility studies

The within-run reproducibility (CV 0.6–2.8%) of the sample processing procedure was good and the between-run reproducibility (CV 1.8–8.8%) was acceptable (Table 1). The within-day (CV 0.4–1.3%) and between-day (CV 0.4–1.3%) reproducibility of the GC–IRMS analysis was even better. The variability of the processing procedure and GC–IRMS analysis tended to be somewhat higher in the samples containing low $[^{13}\text{C}]$urea enrichments.

The variability of the processing and analytical procedures had only a small effect on the reproducibility of the slope of the calibration curves that were prepared, processed and analysed on the same day (within-run CV 0.6%) and on different days within one year (between-run CV 1.8%).

Clinical urea kinetic studies

Administration of the $[^{13}\text{C}]$urea solution was well tolerated. Figure 2 (upper panel) shows the decay curves of the natural logarithmic values of the MPE of $[^{13}\text{C}]$urea in the volunteer and renal failure patient. The change in the MPE of $[^{13}\text{C}]$urea over time can be described by a two-compartment model. The $[^{13}\text{C}]$urea isotope was injected into the central compartment and was diluted over the central and peripheral compartment in about 90 min. After 90 min, the change in the logarithmic MPE of $[^{13}\text{C}]$urea values followed a straight line. The apparent ratio ($R_0$) at $t = 0$ was determined from the intercept of the extrapolated regression line of

![Fig. 1. Calibration line: $\delta$ values measured by GC–IRMS versus MPE of $[^{13}\text{C}]$urea](image-url)
Table 1. Reproducibility of the δ values determined from calibration mixtures containing known MPE of [13C]urea. For experimental details see the reproducibility studies section under Material and methods. The mean δ values of calibration mixtures with five different MPE of [13C]urea values and the corresponding CVs are shown.

<table>
<thead>
<tr>
<th>MPE of [13C]urea (%)</th>
<th>Sample processing</th>
<th>GC–IRMS analysis</th>
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<tr>
<td></td>
<td>Within-run</td>
<td>Between-run</td>
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<tr>
<td></td>
<td>δ</td>
<td>CV (%)</td>
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<td>50.822</td>
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<td>0.2409</td>
<td>202.79</td>
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<tr>
<td>0.3213</td>
<td>278.9</td>
<td>0.6</td>
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The MPE of [13C]urea values after 90 min. Then UDV was calculated according to eqn. (2). The UDV divided by the body weight of the healthy volunteer was higher (65% of body weight) than that of the renal failure patient (52% of body weight) (Table 2). The reproducibility of the repeated UDV measurements was acceptable with a CV value of 3.5%.

The production constant of urea ($k_p$) was determined from the slope of the regression line of the MPE of [13C]urea values after 90 min and $G_U$ was calculated according to eqn. (3). The production rate of urea was about the same for both subjects, but varied considerably between the repeated measurements in the healthy volunteer.

The decay curves of the natural logarithmic values of the [13C]urea plasma water concentrations in the volunteer and renal failure patient are shown in Fig. 2 (lower panel). The elimination constant ($k_e$) of urea was determined from the slope of the regression line of [13C]urea concentration values after 90 min. The $k_e$ of urea was about 6-fold higher in the healthy volunteer ($0.0674 \text{ h}^{-1}$) than in the renal failure patient ($0.0120 \text{ h}^{-1}$). Calculated urea clearance in the healthy volunteer was also higher than in the renal failure patient. However, [13C]urea clearance in the renal failure patient was still 7.4 ml/min, despite the fact that the patient had no residual renal function.

**DISCUSSION**

We have described a urea dilution technique using the stable urea isotope [13C]urea and headspace GC–IRMS analysis for measuring kinetic parameters of urea in healthy subjects and chronic haemodialysis patients. The use of the relatively inexpensive [13C]urea isotope in the dilution method described in this paper reduces the high costs that are associated with the previously reported urea isotope dilution methods [6–9].

We have shown that there is an excellent linear correlation between the MPE of [13C]urea and the δ values measured by GC–IRMS and that this correlation could be reproduced accurately. Of interest is the measured δ value for a given MPE of [13C]urea in the calibration mixtures (Fig. 1). Since an increase of 1δ is defined to be equivalent to 0.001
MPE, it was expected that an increase of 1 MPE of \[^{13}\text{C}\]urea would have led to an increase of 1000\(^\delta\). An increase of 1 MPE \[^{13}\text{C}\]urea corresponds to an increase of 1000\(^\delta\) if the following conditions are met: (1) the \[^{13}\text{C}\]urea isotope is 100% pure; (2) the endogenous CO\(_2\) is completely expelled from the samples during the sample processing procedure; (3) the breakdown of \[^{13}\text{C}\]urea and \[^{12}\text{C}\]urea by urease is quantitatively equal; (4) the GC-IRMS is correctly calibrated; and (5) the determination of the natural plasma urea concentration is precise and accurate. In practice, however, the \(\delta\) values of the calibration mixtures prepared from pooled plasma were lower than the expected values, resulting in a calibration line with a slope of about 9156 per MPE of \[^{13}\text{C}\]urea. The recovery of 92% probably demonstrates that not all the conditions required for obtaining the maximal \(\delta\) values were fulfilled.

Two of the conditions mentioned are most likely responsible for the lower \(\delta\) values. One reason may be an incorrect determination of the plasma urea concentration by the Kodak analyser. The urea concentration of the pooled plasma was determined only once. The recovery of the calibration mixtures would have been 100% if the true urea concentration of the pooled plasma was only 8% lower than the urea concentration determined by the analyser.

The main reason, however, is probably the presence of residual endogenous CO\(_2\) that is not completely expelled from the samples. Since the \[^{13}\text{C}\]enrichment of endogenous CO\(_2\) is zero, residual CO\(_2\) will lower the \[^{13}\text{C}\]enrichment of the CO\(_2\) derived from the \[^{13}\text{C}\]urea in the enriched plasma samples. Bennett et al. [12] used a similar procedure to convert urea into CO\(_2\) in a study on the incorporation of \[^{13}\text{C}\]bicarbonate into urea \textit{in vivo} [12]. The authors described a sample processing procedure in which a larger quantity of H\(_3\)PO\(_4\) is used to liberate natural plasma CO\(_2\). To improve the \(\delta\) values we also used a larger quantity of H\(_3\)PO\(_4\) and increased the duration of CO\(_2\) removal, but these alterations in the sample processing procedure did not have any beneficial effects. If a larger quantity of H\(_3\)PO\(_4\) is used, the solution has to be adjusted to pH 7.0 using an alkaline solution. Bennett et al. [12] used NaOH for this adjustment, but this may increase the risk of contaminating the CO\(_2\)-free solution with natural CO\(_2\) that may be dissolved in the NaOH solution, since CO\(_2\) is easily trapped by this alkaline solution. We only added approximately twice the required number of hydrogen ions to liberate all endogenous CO\(_2\). In this way, no additional NaOH was needed, minimizing contamination with CO\(_2\). Because we were not able to improve the recovery of the \[^{13}\text{C}\]urea enrichments in the calibration mixtures, the endogenous CO\(_2\) was probably expelled maximally. This does not mean that endogenous CO\(_2\) was expelled completely. Because the endogenous plasma CO\(_2\) and urea concentrations can differ significantly between subjects, and especially between healthy persons and renal failure patients, we decided to prepare a calibration line for each individual separately from plasma samples drawn from the studied subject just before \[^{13}\text{C}\]urea injection. By using individual calibration lines, differences in the experimental conditions between the calibration mixtures and plasma samples were minimized, including the effect of residual endogenous CO\(_2\).

After intravenous injection, \[^{13}\text{C}\]urea was completely distributed over the UDV in approximately 90 min in both the healthy subject and renal failure patient (Fig. 2). Although it cannot be concluded from this small clinical study that the duration of the equilibration period is the same in healthy subjects and patients with chronic renal failure, our results are in accordance with the study by Pearson et al. [8], who concluded that end-stage renal failure patients do not have two-compartment characteristics that are substantially different from normal subjects.

In the calculation of UDV of subjects with normal renal function an error may be made due to excess renal excretion of the \[^{13}\text{C}\]urea isotope from the intravascular compartment during the period of equilibration between plasma and the urea space [6, 13]. We estimated this excess loss of \[^{13}\text{C}\]urea during the 90 min equilibration phase from the data obtained in the healthy subject. Firstly, the average \[^{13}\text{C}\]urea plasma concentration of each sampling period up to 90 min was calculated by logarithmic averaging. Corresponding average \[^{13}\text{C}\]urea concentrations were calculated from the extrapolated regression line shown in Fig. 2. These \[^{13}\text{C}\]urea concentrations represent the plasma concentrations if equilibration of \[^{13}\text{C}\]urea had been instantaneous. Then, the amount of \[^{13}\text{C}\]urea excreted during the 90 min equilibration period was calculated using the
actual and extrapolated $^{13}$C-urea concentrations, assuming a total $^{13}$C-urea clearance as calculated from eqn. (4). By comparing both amounts, the excess $^{13}$C-urea loss during the equilibration period was estimated to be 14–33%. When compared with the injected total amount of $^{13}$C-urea this excess loss was only 1.4–3.1% of the injected dose. Walser and Bodenbos [13] calculated a larger range (0–20%) of excess urea loss in normal subjects. In a study in four normal subjects by Varcoe et al. [6] excess loss of urea isotope was less than 2%. Thus the error that is made in the calculation of UDV due to excess loss of $^{13}$C-urea during the equilibration period appears to be very small.

The values of UDV that were measured in the two subjects studied are comparable with the values found in other urea dilution studies using intravenously injected urea isotopes [5–9]. Watson et al. [14] analysed multiple reports on dilution measurements of total body water, which is assumed to approximately equal the UDV, and showed that total body water can vary widely from 38 to 73% of body weight between individual males. We have previously determined UDV by urea kinetic modelling during haemodialysis in the renal failure patient [15]. By relating the total amount of urea excreted into the dialysate to the decrease in plasma urea concentration during the dialysis UDV can be calculated. UDV determined by urea kinetic modelling during haemodialysis was 35.3 litres (50% of body weight), whereas it was 37.3 litres (52% of body weight) by $^{13}$C-urea dilution method. This suggests that, at least in this patient, the $^{13}$C-urea dilution method and urea kinetic modelling during haemodialysis yield comparable results.

The production rate of urea ($\dot{G}_U$) was comparable with the production rates reported in the literature and was about the same for the healthy subject and the renal failure patient [5, 7]. The urea production rate varied considerably among the repeated studies in the healthy subject, although the subject was studied after an overnight fast and remained fasting during the studies. Since we did not prescribe a particular protein diet to the volunteer, the variability in $\dot{G}_U$ may originate from differences in protein intake during the days before the studies. When a subject switches from a normal diet to a low-protein diet it takes several days until a new equilibrium with a decreased level of nitrogen excretion is established, indicating that adaptation of the urea production rate to changes in protein intake is not instantaneous [16].

Not surprisingly, the clearance of urea in the healthy volunteer was much higher than that in the renal failure patient. Although the patient had no residual renal function urea clearance was 7.4 ml/min. Probably, the extrarenal $^{13}$C-urea clearance in renal failure patients originates from hydrolysis of urea in the gastro-intestinal tract by urease-containing bacteria [13]. The measured urea clearance in our patient was somewhat lower than the clearance values in patients with end-stage renal failure that have been reported by Pearson et al. [8]. Those authors found urea clearance values between 13.8 and 43.6 ml/min. However, some of these patients had various degrees of residual renal function.

Our principal interest was to develop a urea dilution method that is as precise as the earlier described method using $^{13}$C-$^{15}$N$_2$-urea [9] but less costly. Since the price of single labelled $^{13}$C-urea isotope is only one fifth of that of $^{13}$C-$^{15}$N$_2$-urea, and since we were able to reduce the required amount of labelled material 4-fold due to the low enrichments required in GC–IRMS analysis, the total cost of the labelled material was reduced by a factor of 20.

In summary, we have described a urea dilution technique using the stable urea isotope $^{13}$C-urea and headspace GC–IRMS analysis for measuring urea kinetic in healthy human subjects and patients without residual renal function. Very low enrichments of $^{13}$C-urea were sufficient to measure the distribution volume, production rate and clearance of urea in a healthy subject and a patient with chronic renal failure with good reproducibility. The low cost of this $^{13}$C-urea dilution method provides the possibility of studying urea kinetics on a larger scale, especially in patients with end-stage renal failure.

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