Clinical Kidney Journal, 2021, vol. 14, no. 1, 181–188

doi: 10.1093/ckj/sfz167
Advance Access Publication Date: 26 November 2019
Original Article

ORIGINAL ARTICLE

Measurement of renal functional response using iohexol clearance—a study of different outpatient procedures

Bjørn Steinar Lillås [1,2], Camilla Tøndel[2,3], Anna Gjerde[1,2] and Bjørn Egil Vikse[1,2]

[1]Department of Medicine, Haugesund Hospital, Haugesund, Norway, [2]Department of Clinical Medicine, University of Bergen, Bergen, Norway and [3]Department of Pediatrics, Haukeland University Hospital, Bergen, Norway

Correspondence to: Bjørn Steinar Lillås; E-mail: bjorn.lillas@uib.no

ABSTRACT

Background. Glomerular filtration rate (GFR) increases after a heavy protein load; an increase termed renal functional response (RFR). Decreased RFR could be a marker of early kidney damage, but published methods are cumbersome in the outpatient setting. The present study investigates the use of iohexol clearance to measure RFR in outpatients using both one- and two-sample methods.

Methods. Fourteen healthy volunteers with a mean ± SD age of 42 ± 12 years were included (six males and eight females). GFR was measured using plasma iohexol clearance with one- and two-sample methodologies. Four measurements in each individual were performed: one baseline test and three protein loading tests containing 80 g protein (commercially available protein supplementations from Myo Nutrition and Proteinfabrikken and 350 g chicken breast). RFR was calculated as percentage increase in GFR from the baseline test.

Results. Mean RFR was 11.4 ± 5.4% and 12.1 ± 6.4% using one- and two-sample methods, respectively. The three different protein loads resulted in similar mean RFR but there was considerable intra-individual variability. One- and two-sample methods for measurement of RFR showed similar results with near-identical means, but there was some intra-individual variation that was similar for different protein loads. The overall 95% limit of agreement between one- and two-sample methods for calculating RFR was −8.7 to 7.3.

Conclusions. RFR can be investigated using plasma iohexol clearance in an outpatient setting. Protocols using commercially available protein supplementation showed a mean RFR of about 12%. One- and two-sample methods for measuring RFR yield similar results.

Received: 6.6.2019; Editorial decision: 21.10.2019

Keywords: GFR, iohexol clearance, outpatient, renal functional reserve, renal functional response, renal reserve capacity, renal stress test

INTRODUCTION

Kidney function is assessed by estimation of glomerular filtration rate (GFR) for most clinical circumstances. In patients with relatively preserved GFR in whom a precise knowledge of GFR is necessary, such as in the evaluation of kidney donors and before the use of certain chemotherapies, measurement of GFR with iohexol or iothalamate is however recommended [1, 2]. When glomeruli are lost, hyperfiltration in the remaining glomeruli may maintain a near-normal GFR [3, 4] and measuring GFR may thus still be an imprecise measure of functional renal mass. In the early 1980s, Bosch *et al.* [5] showed that a heavy protein meal induced an acute increase in GFR. The difference between maximum GFR and baseline GFR was termed renal functional reserve, and it was hypothesized that a reduction in functional kidney mass would exhaust this reserve capacity before reduction of GFR. The concept was, however, criticized as studies showed that even patients with reduced GFR had preserved renal reserve capacity [6, 7]. In recent years, the concept has, however, received renewed interest [8, 9], and in a recent review, De Moor *et al.* [10] suggested replacing the term renal functional reserve with renal functional response (RFR). This proposes that the GFR response after a protein load does not necessarily represent a renal reserve, but the ability of single nephron hyperfiltration, and that an absent response might imply that single nephron hyperfiltration has already taken place [11, 12].

Stimulation of GFR to estimate RFR has been done using different methods in different studies. The original method was by a heavy protein meal with beef [13–15], but chicken breast has also been used [16, 17]. Adaption of protocols with the use of milk-based protein supplementation [15, 18, 19] and intravenous amino acid infusion [20–22] has also been tested. In a renal stress test, the most commonly used method for measuring GFR has been urinary creatinine clearance [5, 13, 15], but tubular secretion of creatinine may result in overestimation of GFR [10, 23], and difficulties with urinary sampling may complicate matters further. Protocols using the clearance of inulin or iothalamate have, therefore, been used [12, 24–26], but these methods are cumbersome and not readily available in the outpatient setting.

In the present study, we aimed to develop and test a protocol for measurement of RFR using single injection of iohexol clearance that is easy and feasible in the outpatient setting. We tested whether a one-sample iohexol clearance method yielded similar results as a two-sample iohexol clearance. We hypothesized that RFR could be tested using this protocol and aimed to select one test for use in future clinical studies.

MATERIALS AND METHODS

Study design

This is an experimental, controlled, prospective, crossover study, where clinical examination was performed from November 2017 to April 2018.

Participants

Fifteen healthy volunteers were included in the study. Exclusion criteria were previously known hypertension, kidney disease, diabetes and cardiovascular disease, as well as the use of regular medication for any other purpose. One participant withdrew after baseline measurement due to pregnancy and her results are not shown. All participants provided a urine sample that was dipstick negative for protein and blood.

Overview of study

The study involved four separate test days, all separated by at least 1 week to ensure complete washout of iohexol. Day 1 included baseline measurement of GFR and Days 2–4 included measurements of GFR after different protein loads. Participants followed an *ad libitum* protein diet on days before and after GFR tests. During days of GFR measurements, they were advised to eat a light breakfast and lunch and refrain from food rich in animal and milk protein—this was verified with self-reported lists of food intake. Iohexol was injected between 07:49 am and 10:47 am, with a mean intra-individual variation between the test days of 1 h and 6 min, and a maximum of 2 h and 1 min. All participants completed all test days except one participant (Participant 2) who, due to taste, could not drink Protein powder 2. We suspected erroneous baseline GFR in another participant (Participant 14); this measurement was repeated, and the last measurement was used for analyses.

Baseline GFR measurement

Participant height and weight were measured before the baseline GFR test. Weight was measured at all test days and used for the respective calculation of iohexol GFR. The mean weight was used for statistical analyses. Blood pressure was measured seated once per test day, and the lowest mean arterial pressure was used for statistical analyses. An intravenous catheter was inserted in the cubital vein of the dominant arm to be used for the injection of iohexol. We injected 5 mL Omnipaque® 300 mg I/mL (GE Health care, Oslo, Norway; equals 647 mg iohexol/mL) over 2 min, followed by flushing with 10 mL of normal saline. The syringe and packaging were weighed before and after the injection, with an accuracy of 0.01 g. Omnipaque 300 mg I/mL weighs 1.35 g/mL and provides 647 mg iohexol/mL. The participants were observed for 30 min for adverse reactions. Blood samples were taken from the opposite arm, either via an indwelling intravenous catheter or via venipunctures. Samples were taken at 2 and 4 h, except for baseline measurement of Participants 1–3, who had samples taken at 2 and 5 h.

GFR measurement after protein stimulation

For test days 2–4, a protein meal was served to be ingested within 30 min after the injection of iohexol. Otherwise, the method was exactly as for the baseline GFR measurement. We used a fixed protein dose for all participants providing 80 g of protein regardless of the participant's weight. Three different protein meals were tested: Protein powder 1: 'TriWhey' from Myo Nutrition (Melhus, Norway), 100 g of powder mixed with 3.75 dL of water; Protein powder 2: '100%Whey' from Proteinfabrikken (Sandefjord, Norway), 111 g of powder mixed with 6.5 dL of water; and cooked chicken breast measured to 350 g before preparation. Both powders were dosed and mixed with water according to the manufacturers' labelling, and

protein contents specified by the manufacturer were used for calculation of amounts. The complete composition of the protein powders is available in Supplementary data, Table S1.

Iohexol analysis and calculations

Blood samples were allowed to stand for a minimum of 30 min and a maximum of 2 h and were then centrifuged at 2200 r.c.f. (relative centrifugal force) for 15 min at 20°C, and kept overnight at 4°C before a 4-h shipment to Haukeland University Hospital. After shipping, the samples were stored at −20°C until analysis. Serum concentrations of iohexol were determined by high-performance liquid chromatography using previously published methods [27].

GFR was calculated both using the two-sample formula according to Jødal–Brøchner-Mortensen [using samples taken at 2 and 4 h or 2 and 5 h (baseline GFR for Participants 1–3)] [28] and one-sample according to Jacobsson [4 or 5 h (baseline GFR for Participants 1–3)] [29].

Jødal–Brøchner-Mortensen:

$$Cl_1 = \frac{Q_0}{AUC_{slow}} = \frac{Q_0}{c_1/b_1} \quad Cl = \frac{Cl_1}{1 + f * Cl_1}$$

where Q_0 is injected iohexol, c_1 slope, b_1 intercept, Cl_1 clearance in slow component only, the factor $f = 0.0032 * BSA^{-1.3}$ and BSA is body surface area.

Jacobsson:

$$Cl = \frac{1}{\frac{t}{V'} + 0.0016} \times \ln\left(\frac{Q_0}{V' \times C(t)}\right)$$

$V' = V/m$, where $m = 0.991 – 0.00122 \times Cl$.

V = volume of distribution and equals $166 \times$ weight $+ 2490$ for males and $95 \times$ weight $+ 6170$ for females. C is the concentration of iohexol at time t.

RFR was calculated as the difference between stimulated GFR and baseline GFR divided by baseline GFR—reported in percent.

Statistics

All statistical analyses were performed using SPSS Statistics version 24 (IBM Corp., Armonk, NY, USA) and Stata/SE 15.1 for Windows (Stata Corp LLC, College Station, TX, USA). Data were tested for normality using the Shapiro–Wilk test and also evaluated with normal q–q plot. Values are given as mean ± SD for normally distributed data and median (minimum and maximum) for non-normally distributed data. Differences between stimulated GFR and baseline GFR were tested using paired sample t-test. RFR for all protein loads and sample methods was compared using one-way analysis of variance. Correlation between one- and two-sample methods was tested with Pearson's correlation coefficient (r). Concordance was tested with Lin's concordance coefficient [30]. Linear regression statistics was used to investigate associations between baseline characteristics and RFR; these analyses are described in the Results section. Significance level for all tests was set to 0.05.

Ethics

The study was approved by the regional ethics committee (REK2017/927) and was conducted in accordance with the Helsinki Declaration, and all participants gave informed consent before inclusion in the study.

Table 1. Characteristics of participants

	Total ($n = 14$)	Males ($n = 6$)	Females ($n = 8$)
Age, years	42 ± 12	42 ± 13	41 ± 12
Height, cm	173 ± 9	180 ± 7	167 ± 7
Mean weight, kg	74 ± 11	83 ± 9	67 ± 7
BMI, kg/m^2	24.7 ± 2.7	25.8 ± 2.6	23.9 ± 2.7
BSA, m^2	1.87 ± 0.19	2.03 ± 0.13	1.75 ± 0.11
Systolic blood pressure, mmHg	123 ± 16	134 ± 13	115 ± 12
Diastolic blood pressure, mmHg	64 ± 14	75 ± 8	56 ± 11

BMI: body mass index; BSA: body surface area. All variables are given as mean ± SD.

RESULTS

Data from 14 participants, 6 males and 8 females aged 25–64 years (mean 42 ± 12), were included in the study. Summary characteristics of the participants are presented in Table 1 and individual values are presented in Table 2. Three men (50%) but no women had systolic blood pressure >140 mmHg. No participants had diastolic blood pressure >90 mmHg nor body mass index >30 kg/m^2. Self-reported birth weight was 3525 ± 655 g. Three participants had birth weight <3000 g and one of these <2900 g.

Mean baseline GFR was 104 ± 15 mL/min using the one-sample method and 104 ± 16 mL/min using the two-sample method. GFR after stimulation with Protein powder 1 was measured to 115 ± 16 and 116 ± 18 mL/min using the one- and two-sample methods, respectively; the corresponding RFR was calculated as 10.9 ± 5.4% using the one-sample method and 11.7 ± 6.2% using the two-sample method (Table 2). Similarly, for Protein powder 2, mean RFR was 13.5 ± 5.0% using the one-sample method and 13.7 ± 6.0% using the two-sample method (Table 3), and for chicken breast the respective values were 9.9 ± 5.6% and 11.1 ± 7.1% (Table 4). Regardless of the sampling method, all protein loads yielded an RFR that was significantly different from zero ($P < 0.001$). There were no significant differences between mean RFR using different methods. Individual responses are presented in Tables 2–4. Figure 1 shows that both sampling methods and all three protein loads yielded positive values for RFR for virtually all participants.

RFR estimation using Protein powder 1 had a mean difference of −0.8 ± 3.1 between one- and two-sample methods, giving a 95% limit of agreement of −6.8 to 5.3. Three participants had a difference in RFR of >3 percentage points between sampling methods and two participants had a relative difference of >30% (Table 2). Only one participant had both an absolute difference >3 percentage points and a relative difference >30% between sampling methods. For Protein powder 2, the mean difference was −0.2 ± 4.2 and for chicken breast −1.2 ± 5.0. Protein powder 2 had 5 (out of 13) participants with a difference in RFR >3 percentage points and a relative difference >30% between sampling methods (Table 3), while chicken breast had 4 (out of 14) (Table 4).

We analysed all pairs of one- versus two-sample methods combined, regardless of the method of protein loading. The correlation coefficient (Pearson's r) between one- and two-sample methods was 0.772, while Lin's concordance correlation coefficient (ρ_c) was 0.756. The 95% limit of agreement for all RFR pairs was −8.7 to 7.3 (Table 5). Table 5 also presents the percentage of

Table 2. RFR one-sample versus two-sample using Protein powder 1

					Baseline GFR		RFR		RFR difference	
Participant	Gender	Age group (years)	BMI (kg/m^2)	Blood pressure (mmHg)	One-sample (mL/min)	Two-sample (mL/min)	One-sample (%)	Two-sample (%)	Absolute (percentage points)	Relative (%)
1	Male	30–50	22.8	127/77	120	120	7.8	9.9	−2.0	−20.4
2	Male	30–50	29.8	142/86	118	123	11.6	4.3	7.3[a]	169.3[b]
3	Female	30–50	26.4	112/48	110	115	17.3	20.1	−2.8	−13.8
4	Male	>50	26.3	126/64	88	89	20.4	18.2	2.2	12.1
5	Male	<30	27.3	150/82	138	141	9.0	11.5	−2.5	−21.7
6	Female	>50	23.0	112/71	77	79	9.9	11.9	−2.0	−16.8
7	Female	<30	27.7	137/60	109	104	4.4	1.9	2.5	126.9[b]
8	Male	>50	23.4	145/73	100	95	2.5	4.6	−2.1	−46.4[b]
9	Female	<30	25.6	112/63	101	94	9.7	12.8	−3.1[a]	−24.0
10	Female	>50	23.0	113/34	90	91	13.7	18.5	−4.8[a]	−26.1
11	Female	30–50	25.0	101/54	97	98	9.7	11.7	−2.0	−17.4
12	Female	30–50	20.9	104/55	102	99	19.8	21.7	−1.9	−8.6
13	Female	30–50	19.9	125/61	98	100	11.7	10.3	1.4	13.5
14	Male	30–50	25.2	116/68	110	110	5.5	6.1	−0.6	−10.0
Mean ± SD					104.2 ± 15.06	104.3 ± 16.17	10.93 ± 5.40	11.68 ± 6.18		

BMI: body mass index. Participant number with corresponding gender, age group, BMI, blood pressure and baseline GFR is the same for Tables 2–4. RFR is the percentage increase from baseline GFR—one- or two-sample methods, respectively.
[a]Absolute difference >3 percentage points.
[b]Relative difference >30%.

Table 3. RFR one-sample versus two-sample using Protein powder 2

		RFR		RFR difference	
Participant	Baseline GFR Two-sample (mL/min)	One-sample (%)	Two-sample (%)	Absolute (percentage points)	Relative (%)
1	120	20.8	20.8	0.0	0.1
2	123	N/A	N/A	N/A	N/A
3	115	16.1	11.3	4.7[a]	41.8[b]
4	89	20.5	17.6	2.9	16.6
5	141	8.8	9.0	−0.2	−1.7
6	79	13.3	12.9	0.4	3.2
7	104	13.2	16.3	−3.1[a]	−18.8
8	95	3.9	8.2	−4.3[a]	−52.3[b]
9	94	17.7	24.3	−6.6[a]	−27.1
10	91	6.9	5.4	1.5	27.4
11	98	11.9	8.8	3.1[a]	34.9[b]
12	99	14.6	22.5	−7.8[a]	−34.8[b]
13	100	11.5	10.8	0.7	6.6
14	110	16.5	10.5	6.0[a]	57.0[b]
Mean ± SD	104.3 ± 16.17	13.52 ± 5.02	13.71 ± 5.99		

BMI: body mass index. Participant numbers are the same as in Table 2. Gender, age group, BMI and baseline one-sample GFR are not repeated in this table.
[a]Absolute difference >3 percentage points.
[b]Relative difference >30%.

samples with agreement between the two methods for the different protein loads. Overall, no significant differences were seen, but Protein powder 1 seemed better in the most relevant analyses of percentage with one-sample RFR within ±30% of two-sample RFR, and RFR difference <3 percentage points. The absolute difference and the 95% limit of agreement for the individual protein loads are shown in Figure 2.

We analysed whether RFR was associated with the patient characteristics described in Table 1 using univariate and multivariate linear regression statistics. Average RFR using all six methods was used as the dependent variable. In the univariate statistics, we found a non-significant relation between systolic blood pressure and RFR ($P = 0.056$). No other factors were associated with RFR (all $P > 0.15$). In the multivariate statistics, we adjusted for gender and age and tested one baseline characteristic in each analysis. In these analyses, none of the baseline characteristics was associated with RFR (all $P > 0.2$).

DISCUSSION

This is, to our knowledge, the first study to use plasma clearance of iohexol after a single injection to measure RFR. Using three different 80-g protein loads, we found a mean RFR of 11.4 ± 5.4% and 12.1 ± 6.4% using the one- and two-sample

Table 4. RFR one-sample versus two-sample using chicken breast

Participant	Baseline GFR Two-sample (mL/min)	RFR		RFR difference	
		One-sample (%)	Two-sample (%)	Absolute (percentage points)	Relative (%)
1	120	6.7	9.0	−2.3	−25.9
2	123	4.1	0.0	4.1[a]	12 935.7[b]
3	115	12.9	11.0	1.9	17.4
4	89	7.3	9.3	−2.0	−21.4
5	141	13.1	17.1	−3.9[a]	−23.1
6	79	21.4	21.2	0.1	0.7
7	104	14.1	26.9	−12.9[a]	−47.8[b]
8	95	7.2	8.1	−0.8	−10.3
9	94	10.9	13.4	−2.5	−18.7
10	91	8.9	9.5	−0.6	−6.2
11	98	12.4	10.1	2.3	23.1
12	99	−0.1	8.9	−9.0[a]	−101.5[b]
13	100	3.6	0.8	2.8	361.7[b]
14	110	15.3	9.6	5.7[a]	59.2[b]
Mean ± SD	104.3 ± 16.17	9.85 ± 5.57	11.07 ± 7.07		

BMI: body mass index. Participant numbers are the same as in Table 2. Gender, age group, BMI and baseline one-sample GFR are not repeated in this table.
[a]Absolute difference >3 percentage points.
[b]Relative difference >30%.

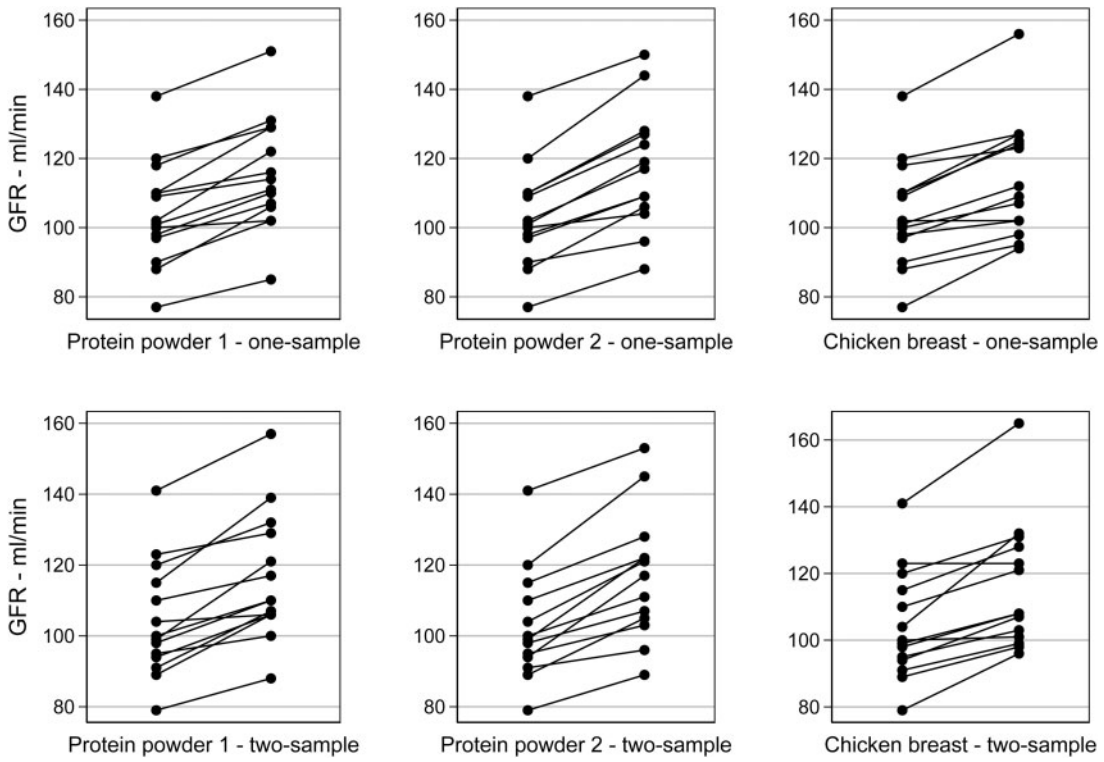

FIGURE 1: Baseline and stimulated GFR for all protein loads showing an individual increase. For all graphs, left column shows baseline GFR using one- or two-sample methods and right column shows corresponding stimulated GFR.

methods, respectively. Our study shows that plasma clearance after a single injection of iohexol can measure an acute increase in GFR after a protein load, and together with a baseline GFR this can give an estimate of RFR. Calculation of RFR using one- versus two-sample method and different protein loads yielded similar results.

A renal stress test measures GFR before and after a protein load. We used plasma clearance after a single injection of iohexol to measure GFR. This method is well described, and both one- and two-sample methods are in broad clinical use and yielded results similar to a multisample regime [31]. A major advantage is its safety profile, and the fact that it is non-radioactive [32]. In addition, GFR measured by iohexol has low day-to-day variation [33]. This is important as our method requires two test days. After the ingestion of the protein load, a temporary increase in GFR occurs. However, the onset of this

increase, the duration and magnitude varies in different studies [5, 15, 24, 34] and most likely depend on baseline GFR, the presence of renal disease and the size of the protein load. In addition, obesity could postpone this increase [35]. As safety protocol requires observation of the participant for 30 min after the injection of iohexol, it was deemed practical for the participants to ingest the protein load during this time. A maximum time limit of 30 min was chosen to resemble the original protocol of Bosch *et al.* [5]. Although other timing protocols could have been used, we believe that our method represents a good compromise between different options. We chose chicken meat as protein load due to easier availability in our hospital compared with beef, and chicken meat has shown comparable results to beef [36]. The use of milk-based protein powders has previously been questioned because of failure to increase GFR [18], but in our study, both powders yielded comparable results to chicken meat. The practical advantage of protein powders over meat is that it can be prepared very easily in the same room and by the same personnel as conducting the test itself. Studies on GFR increase after vegetable proteins are conflicting [37, 38] and vegetable proteins were not tested in our study. We used a fixed 80-g protein load similar to the Bosch protocol [13] and found no association between weight and RFR. Other studies have used various weight-adjusted protein loads demonstrating a dose–response relationship [15, 24]. We do not know why this relationship was not seen in our study, but a higher dose of protein load could be tested in future studies.

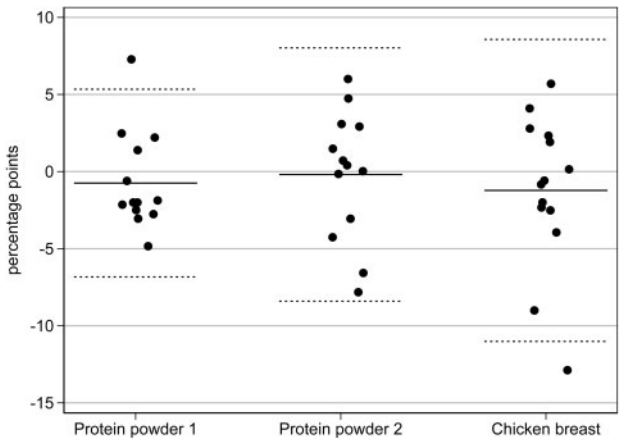

FIGURE 2: Bland–Altman plot showing agreement between one- and two-sample methods for the three protein loads. Solid line is a mean difference (absolute bias) between one- and two-sample methods. Dashed line is the 95% limit of agreement.

The RFR found in our study is lower than seen in studies using creatinine clearance as the method of measuring GFR [13, 15]. We believe this is because tubular secretion of creatinine leads to overestimation of GFR [10, 39], possibly more so after protein stimulation than before. Previous studies usually report peak RFR that is not available in our method. This may also explain our results being lower. Rodenbach *et al.* [39] reported both mean and peak GFR when comparing cimetidine-inhibited creatinine clearance to continuous iohexol clearance. They found a mean RFR that was similar to ours for both methods. At the same time, they showed a peak RFR that is only slightly lower than found in previous studies using creatinine clearance. This suggests that our results are comparable to previous results. And as the timing of the peak GFR is not uniform, a method averaging the GFR increase may yet be preferable [34].

We found no significant bias between the one- and two-sample methods. The overall agreement between the two methods may seem weak; however, the sample size is very small. This could explain the large limit of agreement that for some participants even extends the size of the RFR itself. We believe that the two-sample method most closely resembles a multi-sample regimen and suggests this as the reference method for single injection of iohexol RFR. With no significant bias, the one-sample method is probably an acceptable alternative.

The strength of our study is that it is a crossover study where all but one participant finished all tests. It was an easy-to-perform protocol requiring few blood samples and therefore fewer resources. We have used a method for measuring GFR that is in broad use, avoids the use of radioactive compounds and avoids sampling errors from using urinary clearance [1]. The limitations are a small sample of only young and middle-aged healthy participants. We do not know if these results are valid also for older patients or patients with renal disease. The lack of fasting means that especially the baseline GFR may be overestimated, yielding a falsely low RFR. We did not measure a 24-h urine collection (where urea might reflect protein consumption) nor the hydration state, both of which could influence the results. With two test days required, equal baseline GFR is assumed. This means it could be difficult to discern random day-to-day variation in GFR from measured RFR or absent RFR.

Table 5. Correlation between one- and two-sample methods

	Protein powder 1	Protein powder 2	Chicken breast	All
No. of samples	14	13	14	41
Correlation coefficient Pearson's *r*	0.843	0.789	0.736	0.772
Lin's concordance correlation coefficient—ρ_c	0.823	0.784	0.690	0.756
Difference (mean ± SD)	−0.8 ± 3.4	−0.1 ± 3.9	−1.3 ± 5.1	−0.7 ± 4.1
The 95% limit of agreement	−7.4 to 5.9	−7.4 to 7.5	−11.3 to 8.8	−8.7 to 7.3
P10 (%)	14.3	30.8	14.3	19.5
P30 (%)	78.6	61.5	64.3	68.3
P50 (%)	85.7	84.6	71.4	80.5
RFR difference <3 percentage point (%)	78.6	46.2	64.3	63.4
RFR difference <5 percentage point (%)	92.9	76.9	78.6	82.9
Percentage with RFR difference ≤3 percentage point or <30% difference	92.9	61.5	71.4	75.6

The last column summarizes all three tests for the different participants. The overall 95% limit of agreement is the mean difference ± 1.96 SD of the difference. P10, 30 and 50 are the percentage with one-sample RFR within ± 10, 30 and 50%, respectively, of two-sample RFR.

In conclusion, measuring RFR using plasma clearance after a single injection of iohexol is feasible with an easy protocol that can be used in the outpatient setting. Both chicken meat and commercially available milk-based protein powders can be used. One- and two-sample methods gave comparable results, but a larger study is needed to verify the agreement. Comparing the method to other methods measuring RFR is recommended, as well as validating the method for renal disease patients and other age groups. We also recommend comparing RFR with other aspects of kidney function, including magnetic resonance imaging for both structural and functional measurements as well as kidney biopsies.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

ACKNOWLEDGEMENTS

We wish to thank Lillian Skumnsnes and Monika Sobota-Curylo for their participation in planning, organizing and conducting the experiments.

FUNDING

This work was funded by a cooperation between Western Norway Regional Health Authority and Helse-Fonna HF. Project number in Western Norway Regional Health Authority (912186).

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Delanaye P, Melsom T, Ebert N *et al.* Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 2: why to measure glomerular filtration rate with iohexol? *Clin Kidney J* 2016; 9: 700–704
2. Gaspari F, Guerini E, Perico N *et al.* Glomerular filtration rate determined from a single plasma sample after intravenous iohexol injection: is it reliable? *J Am Soc Nephrol* 1996; 7: 2689–2693
3. Ronco C, Bellomo R, Kellum J. Understanding renal functional reserve. *Intensive Care Med* 2017; 43: 917–920
4. Thomas DM, Coles GA, Williams JD. What does the renal reserve mean? *Kidney Int* 1994; 45: 411–416
5. Bosch JP, Saccaggi A, Lauer A *et al.* Renal functional reserve in humans. Effect of protein intake on glomerular filtration rate. *Am J Med* 1983; 75: 943–950
6. Zuccala A, Gaggi R, Zucchelli A *et al.* Renal functional reserve in patients with a reduced number of functioning glomeruli. *Clin Nephrol* 1989; 32: 229–234
7. Krishna GG, Kapoor SC. Preservation of renal reserve in chronic renal disease. *Am J Kidney Dis* 1991; 17: 18–24
8. Sharma A, Mucino MJ, Ronco C. Renal functional reserve and renal recovery after acute kidney injury. *Nephron Clin Pract* 2014; 127: 94–100
9. Ronco C, Chawla LS. Glomerular and tubular kidney stress test: new tools for a deeper evaluation of kidney function. *Nephron* 2016; 134: 191–194
10. De Moor B, Vanwalleghem JF, Swennen Q *et al.* Haemodynamic or metabolic stimulation tests to reveal the renal functional response: requiem or revival? *Clin Kidney J* 2018; 11: 623–654
11. ter Wee PM, Rosman JB, van der Geest S *et al.* Renal hemodynamics during separate and combined infusion of amino acids and dopamine. *Kidney Int* 1986; 29: 870–874
12. Messchendorp AL, van Londen M, Taylor JM *et al.* Kidney function reserve capacity in early and later stage autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol* 2018; 13: 1680–1692
13. Bosch JP, Lauer A, Glabman S. Short-term protein loading in assessment of patients with renal disease. *Am J Med* 1984; 77: 873–879
14. Ronco C, Brendolan A, Bragantini L *et al.* Renal functional reserve in pregnancy. *Nephrol Dial Transplant* 1988; 3: 157–161
15. Sharma A, Zaragoza JJ, Villa G *et al.* Optimizing a kidney stress test to evaluate renal functional reserve. *Clin Nephrol* 2016; 86: 18–26
16. Hellerstein S, Hunter JL, Warady BA. Creatinine excretion rates for evaluation of kidney function in children. *Pediatr Nephrol* 1988; 2: 419–424
17. Loo CS, Zaki M, Sulaiman AB *et al.* Acute protein loading in the assessment of renal reserve. *Med J Malaysia* 1994; 49: 36–43
18. Jones MG, Lee K, Swaminathan R. The effect of dietary protein on glomerular filtration rate in normal subjects. *Clin Nephrol* 1987; 27: 71–75
19. Mansy H, Patel D, Tapson JS *et al.* Four methods to recruit renal functional reserve. *Nephrol Dial Transplant* 1987; 2: 228–232
20. Losito A, Fortunati F, Zampi I *et al.* Impaired renal functional reserve and albuminuria in essential hypertension. *Br Med J (Clin Res Ed)* 1988; 296: 1562–1564
21. Rondeau E, Paillard F, Peraldi M-N *et al.* Role of the renin-angiotensin system on the renal functional reserve in renal transplant recipients. *Kidney Int* 1993; 44: 165–172
22. Pluvio C, de Pascale E, Giordano M *et al.* Correlation between glomerular morphology and renal haemodynamic response to amino-acid administration in patients with IgA nephropathy. *Nephrol Dial Transplant* 1996; 11: 2421–2425
23. van Acker BA, Koomen GC, Koopman MG *et al.* Creatinine clearance during cimetidine administration for measurement of glomerular filtration rate. *Lancet* 1992; 340: 1326–1329
24. Rodriguez-Iturbe B, Herrera J, Garcia R. Relationship between glomerular filtration rate and renal blood flow at different levels of protein-induced hyperfiltration in man. *Clin Sci (Lond)* 1988; 74: 11–15
25. Fliser D, Zeier M, Nowack R *et al.* Renal functional reserve in healthy elderly subjects. *J Am Soc Nephrol* 1993; 3: 1371–1377
26. ter Wee PM, van Ballegooie E, Rosman JB *et al.* Renal reserve filtration capacity in patients with type 1 (insulin-dependent) diabetes mellitus. *Nephrol Dial Transplant* 1987; 2: 504–509
27. Tondel C, Bolann B, Salvador CL *et al.* Iohexol plasma clearance in children: validation of multiple formulas and two-point sampling times. *Pediatr Nephrol* 2017; 32: 311–320
28. Jødal L, Brøchner-Mortensen J. Reassessment of a classical single injection 51Cr-EDTA clearance method for determination of renal function in children and adults. Part I: analytically correct relationship between total and one-pool clearance. *Scand J Clin Lab Invest* 2009; 69: 305–313

29. Jacobsson L. A method for the calculation of renal clearance based on a single plasma sample. *Clin Physiol* 1983; 3: 297–305
30. Lin LI. A concordance correlation coefficient to evaluate reproducibility. *Biometrics* 1989; 45: 255–268
31. Delanaye P, Ebert N, Melsom T *et al*. Iohexol plasma clearance for measuring glomerular filtration rate in clinical practice and research: a review. Part 1: how to measure glomerular filtration rate with iohexol? *Clin Kidney J* 2016; 9: 682–699
32. Gaspari F, Thakar S, Carrara F *et al*. Safety of iohexol administration to measure glomerular filtration rate in different patient populations: a 25-year experience. *Nephron* 2018; 140: 1–8
33. Gaspari F, Perico N, Matalone M *et al*. Precision of plasma clearance of iohexol for estimation of GFR in patients with renal disease. *J Am Soc Nephrol* 1998; 9: 310–313
34. De Santo NG, Anastasio P, Cirillo M *et al*. Sequential analysis of variation in glomerular filtration rate to calculate the haemodynamic response to a meat meal. *Nephrol Dial Transplant* 1995; 10: 1629–1636
35. Anastasio P, Viggiano D, Zacchia M *et al*. Delay in renal hemodynamic response to a meat meal in severe obesity. *Nephron* 2017; 136: 151–157
36. Simon AH, Lima PR, Almerinda M *et al*. Renal haemodynamic responses to a chicken or beef meal in normal individuals. *Nephrol Dial Transplant* 1998; 13: 2261–2264
37. Kontessis P, Jones S, Dodds R *et al*. Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. *Kidney Int* 1990; 38: 136–144
38. Deibert P, Lutz L, Konig D *et al*. Acute effect of a soy protein-rich meal-replacement application on renal parameters in patients with the metabolic syndrome. *Asia Pac J Clin Nutr* 2011; 20: 527–534
39. Rodenbach KE, Fuhrman DY, Maier PS *et al*. Renal response to a protein load in healthy young adults as determined by iohexol infusion clearance, cimetidine-inhibited creatinine clearance, and cystatin c estimated glomerular filtration rate. *J Ren Nutr* 2017; 27: 275–281