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Measuring the Glomerular Filtration Rate in Obese Individuals without Overt Kidney Disease

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Key Words
Iohexol · Glomerular filtration rate · Creatinine · Cystatin C · Obesity

Abstract
Background: Identifying methods to accurately measure the glomerular filtration rate (GFR) in obese individuals without kidney overt kidney disease is necessary to understanding the pathophysiology and natural history of obesity-related kidney disease. ***Methods:*** Using a cross-sectional design, iohexol clearance and disposition was measured, an optimal sampling schedule was identified, and the reliability of GFR-estimating methods was described in 29 obese individuals with normal serum creatinine levels. Iohexol disposition was measured using population pharmacokinetics. The agreement with GFR-estimating equations was assessed by intraclass coefficients. ***Results:*** Mean age was 44 ± 10 years, body mass index 45 ± 10, creatinine 0.7 ± 0.2 mg/dl (62 ± 18 μmol/l) , and cystatin C 0.83 ± 0.18 mg/dl (8.3 ± 1.8 mg/l). Iohexol disposition fit a two-compartment model and 5 sampling windows were identified over a 4-hour period to optimize model accuracy and minimize blood draws. Precision was not compromised with this sampling design. Neither creatinine nor cystatin C were linearly correlated with the measured GFR though cystatin C was independent of body composition. Agreement was fair to poor between the measured GFR and GFR-estimating equations. ***Conclusion:*** This study offers a rigorous method to study obesity-related kidney disease and improve upon suboptimal GFR-estimating methods.

Introduction

The obesity public health crisis, which is expected to encompass 700 million adults by the year 2015 [1], has profound global implications for kidney disease. Aside from being a major risk factor for the development of diabetes and hypertension, the principal causes of chronic kidney disease (CKD) in the Western world, obesity may have adverse effects on kidney function independent of such intermediate disease states [2, 3].

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Unfortunately, fundamental knowledge about obesity-related kidney disease, including its prevalence, natural history, risk factors, and effective preventative strategies, is sorely lacking. Insights into these issues will require the ability to accurately measure and estimate the glomerular filtration rate (GFR), the best global indicator of kidney function [4], in obese individuals with incipient kidney disease or even normal kidney function. Iohexol, a nonionic low-osmolar contrast agent, is an ideal filtration marker for this task in that it is readily obtainable, inexpensive, nonradioactive, and does not require cumbersome urine collections. It has been used for years as a reliable GFR marker in children and adults, particularly in Europe [5–7].

There is ample reason to believe that the current GFR-estimating methods, derived primarily in lean individuals with CKD, may be less accurate in extreme states such as obesity [8]. Even reference clearance markers such as iohexol have never been formally tested in the obese population. This is a concern because the volume of distribution and/or tissue handling of clearance markers may be affected by obesity, leading to altered pharmacokinetics and renal clearance.

The project goal was to study plasma iohexol as a GFR marker in obese humans with apparently normal kidney function. In doing so, it would help establish (1) a model of iohexol disposition and an optimal iohexol sampling schedule in obese subjects that would minimize the number of blood draws yet maximize overall accuracy, and (2) the reliability of commonly used GFR-estimating methods in obesity.

Subjects and Methods

Study Population

Twenty-nine obese individuals were recruited from the Indianapolis, Ind., area between April 2004 and October 2007 as part of a larger National Institutes of Health-sponsored study (NCT00244790). The relevant institutional review boards approved the protocol and all patients gave written informed consent after reviewing a written summary of the plan. Exclusion criteria included the following: age less than 18 years, pregnant state, body mass index (BMI) less than 30, inability to give informed consent, iodine or contrast allergy, serum creatinine >1.3 mg/dl (115 μmol/l) in women and >1.5 mg/dl (133 μmol/l) in men. Each subject had up to 6 iohexol-based GFR measurements performed over a range of weeks to months.

Study Protocol

All subjects presented after an overnight fast to the Indiana University General Clinical Research Center. Subjects were encouraged to hydrate themselves prior and during the visit to avoid dehydration. Vital signs were measured and 2 peripheral intravenous lines were inserted, one for the iohexol bolus injection and one for the blood sampling. Approximately 5,000 mg iohexol (Omnipaque-300; GE Healthcare, Piscataway, N.J., USA) was injected slowly over 2 min from a preweighed syringe at time 0, followed by a 20-ml normal saline flush. The syringe was then weighed to the nearest ten-thousandths gram on the same scale used prior to the injection. The exact dose of iohexol (in grams) was calculated from the difference in syringe weights multiplied by the amount of iohexol in Omnipaque-300 (647 mg/g Omnipaque) divided by its density at room temperature (1.349). A total of 90 iohexol clearance measurements were performed in a variety of study protocols in the 29 study subjects, each on separate days. As the project evolved, the number of iohexol samplings per clearance measurement increased due to the understanding that this would increase the overall accuracy. The first 25 clearance measurements were based on 2 blood samples drawn at 120 and 240 min (subjects 1–8), the next 54 on 10 samples drawn at 15, 30, 45, 60, 90, 120, 150, 180, 210, and 240 min (subjects 8–26), and the remaining 11 on 11 samples (the previous protocol plus an extra sample drawn at 5 min) (subjects 25, 27–29). Serum was isolated and aliquots were stored at –80°C.

BMI and body surface area (BSA) were calculated [9] during each visit from measured weight and height while wearing a hospital gown and no shoes. Body composition was measured using bioelectrical impedance analysis (BIA-101Q Fluid/ Nutrition Bio-Electrical Impedance Analyzer System with Cyprus 1.0 Body Composition Analysis Software, RJL Systems, Clinton Township, Mich., USA), which has been studied in the obese population [10]. Diabetes was defined as a previous diagnosis of diabetes or a fasting plasma glucose ≥126 mg/dl (7 mmol/l) [11]. Hypertension was defined as a previous diagnosis of hypertension or 2 or more blood pressure readings of ≥140/90 mm Hg [12] on at least one visit.

Laboratory Measurements

As previously described [13], iohexol in serum was measured by capillary electrophoresis using a Model 2050 CE instrument (Beckman Instruments, Palo Alto, Calif., USA) by injecting the serum directly on untreated capillary followed by separation based on micellar electrokinetic chromatography. In this technique, iohexol in the serum is distributed between the separation buffer in the capillary and the sodium dodecyl sulfate micelle according to its distribution coefficient. It migrates based on the velocity of the micelle under the influence of the electric field. After separation from the other compounds present in the serum, iohexol is detected on the capillary by its absorption at 254 nm. The method is linear between 8 and 260 mg/l, with a relative standard deviation of peak height of 2.9%.

Cystatin C was measured using particle-enhanced immunonephelometry (Siemens/Dade Behring BNII Nephelometer, Ill., USA), and serum creatinine by the Roche/Hitachi Creatinine Plus enzymatic assay (Roche Diagnostics, Basel, Switzerland), the results of which correlate with those obtained by isotope-dilution mass spectrometry. Urine albumin and creatinine were measured using standard laboratory techniques. The GFR was predicted using the modification of diet in renal disease (MDRD) equation modified for standardized serum creatinine measurements [14], while the creatinine clearance was estimated using

the Cockcroft-Gault method [15]. Given the concern that indexing GFR by BSA may introduce bias when used in obese persons [16], we used nonindexed measurements and estimations as a rule. We removed indexing from the MDRD equation by multiplying it by BSA/1.73.

Population Pharmacokinetic Modeling and Statistics

Iohexol clearance was determined by a nonlinear mixed-effects modeling approach for population pharmacokinetic analysis using NONMEM VI (Globomax LLC, Ellicott City, Md., USA) [22, 23]. A two-compartment base model (ADVAN4 TRANS4) with a first-order conditional estimation method was found to best describe the iohexol plasma-concentration time curves. Iohexol dose, dosing time, time of blood draws, and concentrations were entered into the model for each subject, and the individual pharmacokinetic parameters, including clearance (i.e. GFR) were estimated. This approach also determines interindividual and intraindividual variability of the pharmacokinetic parameters. Additional models were evaluated based on the initial two-compartment model to include subject-specific covariates, including age, weight, lean mass and fat mass. In addition, the following categorical covariates were explored: sex, hypertension, diabetes, and the impact of interoccasion variation. Model evaluation was performed utilizing both minimization of the objective function (OBJ; –2 log likelihood) and diagnostic plots. Individual covariates were determined to be significant if they caused a decline of the OBJ by at least 3.84 ($p < 0.05$, 1 d.f. in χ^2). Continuous covariates were centered on their median value. A full model was then created with inclusion of all significant covariates. Selection between covariates that described the same biologically relevant concept (weight, lean body mass and fat body mass) was made based on that covariate which had the greatest decline in OBJ. Full model components were subsequently evaluated through stepwise backward elimination procedure, with covariates maintained in the final model which caused a change in OBJ of at least 10.83 ($p < 0.001$, 1 d.f.). Visual analysis was performed in R [17] using the Xpose4 package [18]. Individual predictions of iohexol clearance (GFR) were generated using the IPRED output from NONMEM. Determination of optimal sampling points for the GFR measurements using iohexol clearance was made with WinPOPT [19] using the simulated annealing algorithm in 1,000 simulated subjects utilizing parameter estimates from the final model produced by NONMEM. In an effort to determine an optimal blood sampling schedule within a clinically feasible study period, the latest sampling point was set to 4 h. Sampling time windows were determined using the same sample window function of WinPOPT.

Agreement of the iohexol GFR (estimated from study subjects through the prescribed population pharmacokinetic model) with the MDRD GFR and Cockcroft-Gault creatinine clearance was assessed by estimating the intraclass coefficient (ICC) and its 95% confidence interval (CI). ICCs were calculated on the statistical software program R version 2.8.0 [17] using the 'irr' package [20]. Additionally Bland-Altman plots [21] were constructed along with the Bland-Altman limits of agreement. Pearson's correlation coefficient was estimated to assess the strength of the linear relationship between GFR and physical characteristics. A significance level of 0.05 was used in all statistical tests.

Table 1. Baseline characteristics

Number of subjects	29
Age, years	44 ± 10 (22–59)
Sex, male (%)	17
Race, %	
White	66
Black	34
Height, m	1.67 ± 0.09 (1.51–1.88)
Weight, kg	124 ± 30 (74–198)
Diabetes, %	14
Hypertension, %	48
Sleep apnea, %	37
BMI	45 ± 10 (31–65)
BSA, m^2	2.27 ± 0.27 (1.70–3.04)
Fat mass, %	50.7 ± 11 (26–66)
Serum creatinine, mg/dl[1]	0.7 ± 0.2 (0.5–1.1)
Cystatin C, mg/dl[2]	0.83 ± 0.18 (0.49–1.28)
Urine albumin/creatinine, mg/g[3]	5.5 (4.0–15.5)
GFR, ml/min	121 ± 27 (78–180)
GFR, ml/min/1.73 m^2	92 ± 16 (61–130)
Estimated GFR by MDRD, ml/min	132 ± 35 (78–205)
Estimated GFR by MDRD, ml/min/1.73 m^2	101 ± 22 (57–142)
Estimated creatinine clearance by Cockcroft-Gault, ml/min	206 ± 78 (112–400)

Data shown are mean ± standard deviation (range).

[1] Conversion of serum creatinine values: 62±18 (44–97) μmol/l.

[2] Conversion of cystatin C values: 8.3±1.8 (4.9–1.28) mg/l.

[3] Data shown are median (interquartile range).

Results

Patient Characteristics

Table 1 describes at baseline the 29 study subjects, who were predominantly young-adult to middle-aged white females. The majority of subjects manifested the more severe degrees of obesity [World Health Organization classes II (n = 6) and III (n = 19)] [24]. Despite this, only a small minority had diabetes mellitus, while a larger proportion had mild hypertension and sleep apnea. Serum creatinine and cystatin C levels were well within the normal range. Albuminuria was below the microalbuminuria range for most subjects (19 with ≤10 mg/g, 7 between 10 and 30 mg/g, and 3 ≥30 mg/g). Of the 10 subjects with albuminuria of 10 mg/g or greater, only 2 had diabetes and/or hypertension. Seven individuals had GFR levels between 78 and 94 ml/min, 7 between 105 and 113 ml/min, and 15 subjects between 120 and 180 ml/min, suggesting that glomerular hyperfiltration was fairly common. Excluding subjects with

Table 2. Population model estimates for iohexol-based clearance

Equation variable	Parameter	Parameter value
θ_1	CL, l/h	7.06 ± 0.678
θ_2	V_1, l	9.87 ± 3.12
θ_3	Q, l/h	11.4 ± 3.1
θ_4	V_2, l	6.15 ± 0.876
$\theta_1 \exp^{(\theta 5 \cdot WT)}$	Influence of weight (WT) on clearance	0.00259 ± 0.00254
$\Theta_2 \cdot (1 + \theta_6 \cdot LBM)$	Influence of lean body mass (LBM) on V_1	0.0249 ± 0.01634

Values are mean ± standard deviation. CL = Drug clearance (for iohexol, renal clearance assumed); V_1 = volume of the central compartment; Q = flow between peripheral and central compartment; V_2 = volume of the peripheral compartment.

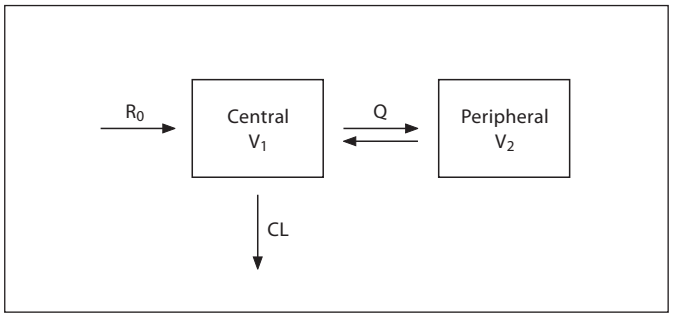

Fig. 1. Two-compartment i.v. infusion model. R_0 = Rate of drug infusion; V_1 = volume of the central compartment; CL = drug clearance (for iohexol, renal clearance assumed); Q = flow between peripheral and central compartment; V_2 = volume of the peripheral compartment.

Additional data to table 2

	Theta	Eta	Value	Standard error
Pharmacokinetic parameter				
CL	1		7.0600	0.33900
V_1	2		9.8700	1.56000
Q	3		11.400	1.55000
V_2	4		6.1500	0.43800
Weight on clearance	5		0.0026	0.00127
Lean body mass on V_1	6		0.0259	0.00817
Parameter relevance				
CL		1	0.034	0.01070
Occasion		2	0.007	0.00195
Occasion		3	0.007	0.00195
Occasion		4	0.007	0.00195
Occasion		5	0.007	0.00195
Occasion		6	0.007	0.00195
Occasion		7	0.007	0.00195
V_1		8	0.007	0.00195
Sigma (residual error)				
0.0078			–	0.00104

GFR measurements based on only 2 iohexol clearance time-point measurements did not appreciably affect these findings.

Population Pharmacokinetic Modeling and Optimal Sampling Schedule

The optimal structural pharmacokinetic model based on bolus administration of iohexol was determined by visual fit to be a two-compartment model. Parameters of the two-compartment model were clearance, volume compartment 1, volume compartment 2, and intercompartmental clearance (Q) (fig. 1). Weight and lean body mass were found to be significant positive correlates of renal clearance and volume of distribution, respectively. The final parameter estimates for the population model are presented in table 2 (for standard errors and between subject variance, see additional data to table 2). Within-subject (interoccasion) variability was 8.8%. The population model slightly underestimates iohexol concentrations at high levels. This bias was absent in the individual models, indicating an adequate fit for each individual concentration-time curve. Figure 2 shows iohexol concentrations over time as both observed by our laboratory as well as for the individual and population prediction models. Within the constraints of a clinically feasible study (i.e. over 4 h), the optimal sampling time points and sampling windows are presented in table 3. Because

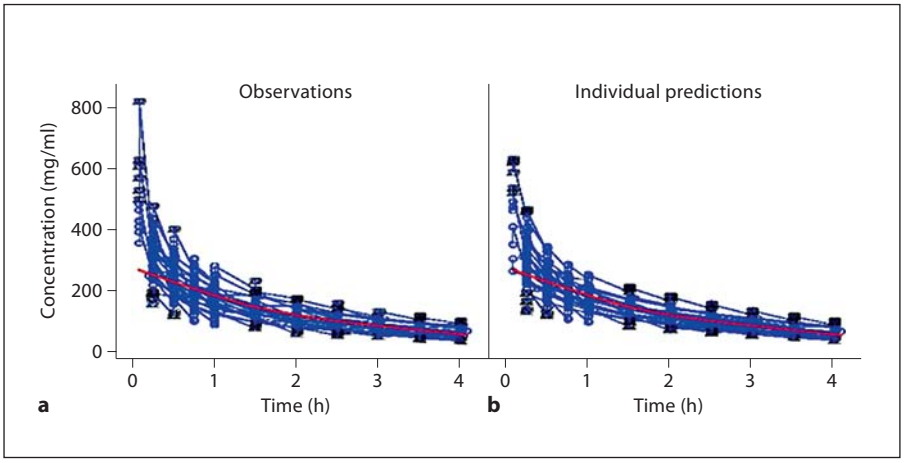

Fig. 2. Iohexol concentrations versus time as measured by our laboratory for all subjects (**a**) and predictions based on individual pharmacokinetic parameters and their weight and lean body mass (**b**).

Table 3. Optimal sampling time windows

Time point	Lower time limit	Upper time limit
1	15 s	4 min 40 s
2	5 min 3 s	17 min 52 s
3	45 min 43 s	1 h 25 min
4	2 h 10 min	3 h 4 min
5	4 h 7 min	4 h 29 min

Windows are based on the assumption that time 0 = the iohexol infusion.

the two-compartment model contains 4 parameters, at least 5 sampling points are needed, the extra one being for residual variation in the model. In table 3, 2 closely spaced initial time points are necessary to obtain early on, followed by 3 subsequent measurements. A comparison of the precision of clearance based on a reference 10-point sampling design versus the optimal sampling design derived in this study is shown in table 4. As noted, precision in both sampling designs are quite similar, suggesting that precision was not compromised using the optimal design model. In addition, the Brochner-Mortensen method (which presumes a one-compartment approach) is not as precise as our two-compartment approach (table 4).

Reliability of Standard GFR-Estimating Methods in Obesity

Comparisons of GFR by iohexol clearance, MDRD and Cockcroft-Gault equations are shown in figure 3. There was no significant linear correlation between the reference (i.e. iohexol) GFR and the inverse of either serum creatinine ($r = 0.08$, $p = 0.68$) or cystatin C ($r = 0.03$, $p = 0.88$) (fig. 4). Removing one obvious outlying data point strengthened the latter relationship, but not to statistical significance ($r = 0.20$, $p = 0.30$). As noted in figure 5, agreement of GFR with the Cockcroft-Gault-based estimated creatinine clearance was poor (ICC = –0.13, 95% CI: –0.46, 0.25) with a bias of 86 ml/min ($p < 0.001$, 95% CI: –49, 220) and Bland-Altman limits of agreement of –49 to 220. There was fair agreement between GFR and the MDRD GFR estimations (ICC = 0.51, 95% CI: 0.19, 0.74) (fig. 5), although there was a slight bias of 11.7 ml/min ($p = 0.041$) and a wide spread in the limits of agreement (–47.0, 70.3). Furthermore, 72% (41% when adjusting for BSA) and 24% of the GFR estimations by MDRD and Cockcroft-Gault, respectively, were within 30% of the true iohexol GFR. Adjusting for BSA did not improve agreement between the GFR and the 2 other measures.

In terms of physical characteristics, GFR was linearly associated with total weight ($r = 0.59$, $p < 0.001$), lean body mass ($r = 0.65$, $p < 0.001$), BMI ($r = 0.47$, $p < 0.001$), and BSA ($r = 0.60$, $p < 0.001$), with no association with

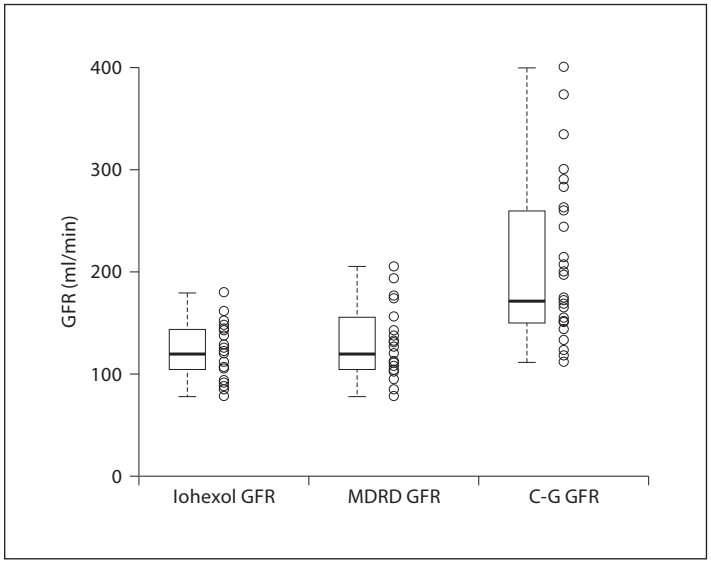

Fig. 3. Comparison of GFR boxplot and individual data by iohexol clearance, MDRD and Cockcroft-Gault (C-G) equations.

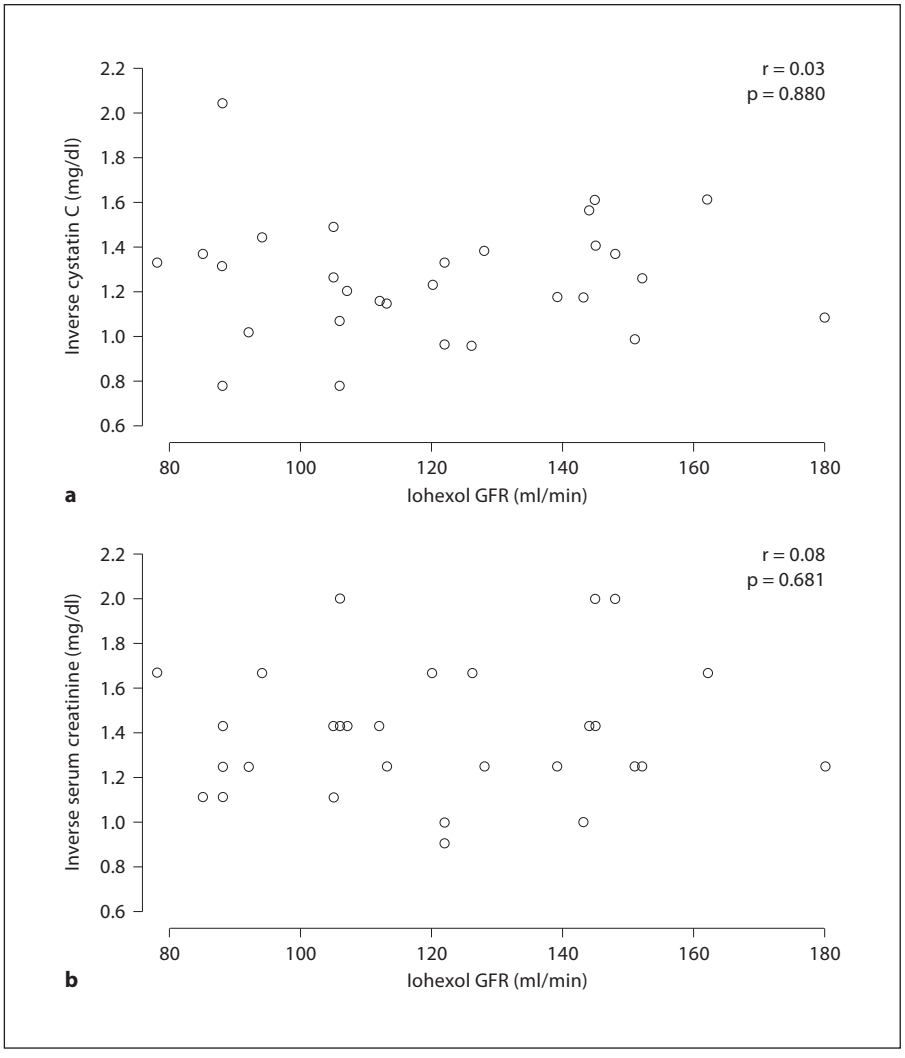

Fig. 4. a Relationship between inverse cystatin C and iohexol GFR. **b** Relationship between inverse serum creatinine and iohexol GFR.

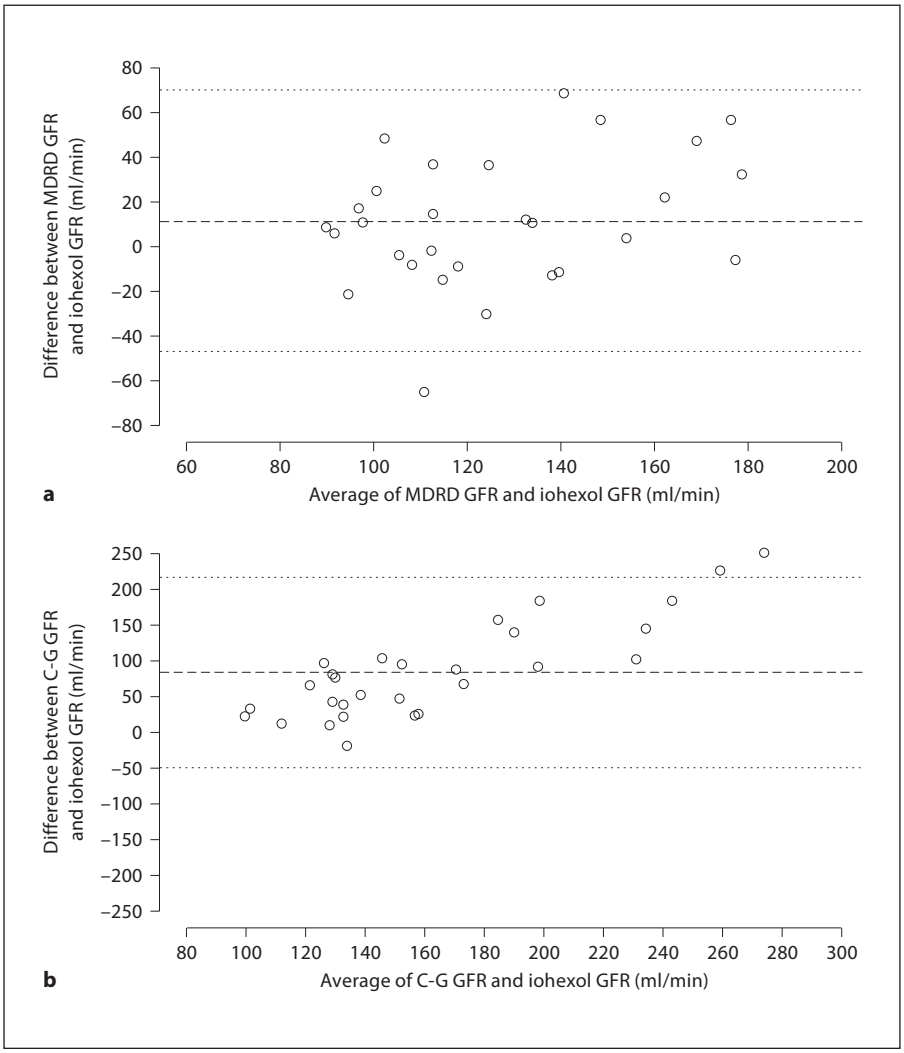

Fig. 5. a Bland-Altman plot of the difference between the MDRD-estimated GFR and the iohexol GFR. **b** Bland-Altman plot of the difference between the Cockcroft-Gault (C-G)-estimated creatinine clearance and the iohexol GFR. The dashed line represents the mean difference between the respective MDRD or Cockcroft-Gault results and the iohexol GFR. Dotted lines represent the upper and lower limits of agreement (95% CI).

Table 4. Comparison of sampling point design

Parameter	Value	Reference sampling design (10 sampling points)		Optimal sampling design (5 sampling points)		Brochner-Mortensen approach (5 sampling points)	
		SE	precision (%)[1]	SE	precision (%)[1]	SE	precision (%)[1]
CL, l/h	7.06	0.68	9	0.88	12.4	0.93	13.1%
V_1, l	9.87	3.12	32	3.74	37.9	4.12	41.7%
Q, l/h	11.4	3.1	27	3.72	32.6	NA	NA
V_2, l	6.15	0.88	14	1.144	18.5	NA	NA

CL = Drug clearance (for iohexol, renal clearance assumed); V_1 = volume of the central compartment; Q = flow between peripheral and central compartment; V_2 = volume of the peripheral compartment; SE = standard error.

[1] Precision = SE/value (%).

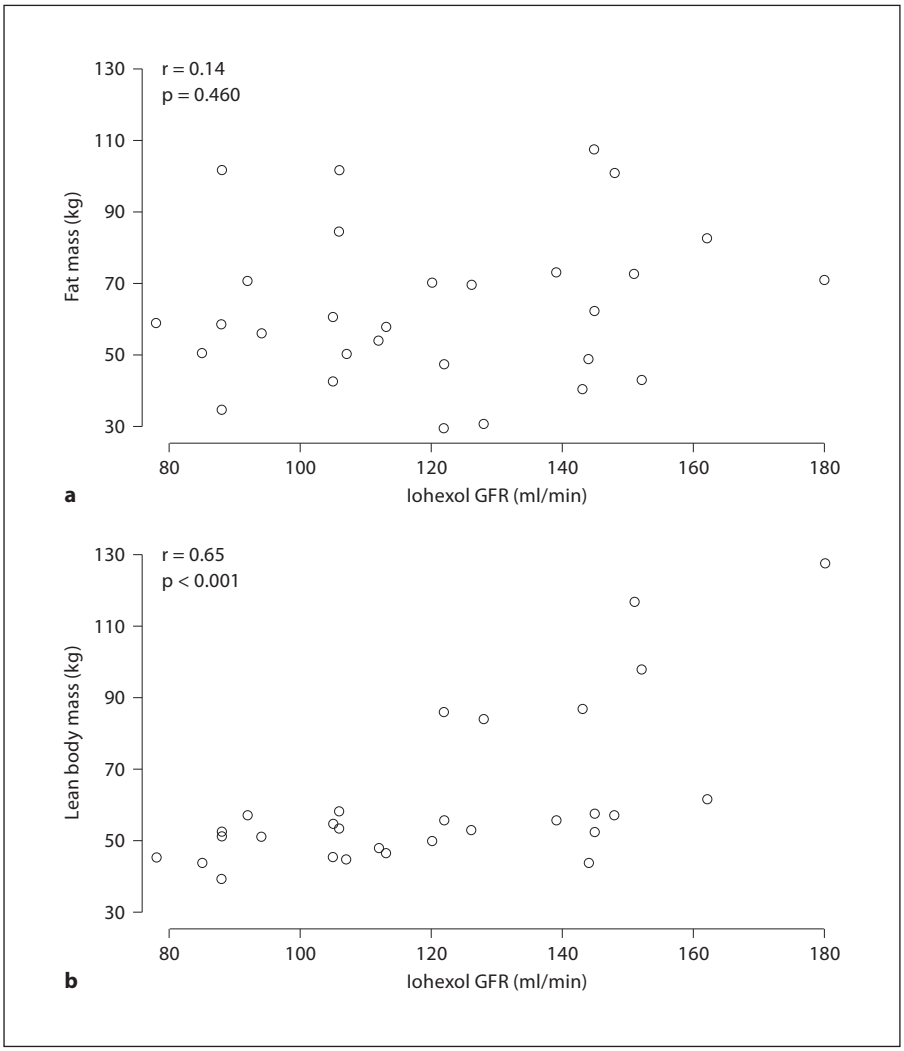

Fig. 6. a Relationship between fat mass and iohexol GFR. **b** Relationship between lean body mass and iohexol GFR.

height (r = 0.35, p = 0.067) or fat mass (r = 0.14, p = 0.46) (fig. 6). Serum creatinine was inversely related to fat mass (r = –0.54, p < 0.002) and positively related to height (r = 0.43, p = 0.021), while no association was noted with lean body mass (r = –0.34, p = 0.076) or total weight (r = –0.14, p = 0.457). In contrast, cystatin C was not significantly associated with any anthropometric or body composition variables.

Discussion

Recent years have noted a convergence between the growing obesity problem and the emerging recognition of the existence of obesity-related kidney disease [2, 3, 25, 26]. These challenges require a reassessment of traditional GFR measurements and estimation methods, which were generally developed in lean populations with overt CKD [15, 27]. To this end, we decided to study very obese individuals with apparently normal kidney function, since it is in this at-risk subgroup that incipient CKD will need to be identified and treated early. In doing so, we used population pharmacokinetics, a fairly novel method that has revolutionized the field of drug research and development [28].

Population pharmacokinetics offered to us a number of advantages over traditional pharmacokinetic methods, including the ability to (1) utilize data from all study visits, including those where sparse sampling for GFR measurement was performed (in this case, the 26 visits with 2 sampling points), (2) identify sources of variability within and between subjects (weight and lean body mass

identified here as covariates) and (3) establish the simplest and most accurate iohexol sampling schedule in a particular population that could then be applied to clinical and research settings [29–31]. Only a handful of studies have thus far employed population pharmacokinetics to study GFR [32–35], with this being the first in obese individuals.

While iohexol is, for a variety of previously described reasons [36], an excellent GFR marker, it has never been studied specifically in obese adults, whose physiology and body composition may affect iohexol handling. We found that iohexol pharmacokinetics in obesity follows a two-compartment model, which confirms the existence of a 'peripheral' compartment into which iohexol is distributed (in addition to being excreted by the kidney). While this may not necessarily be surprising, it is a useful fact to establish for modeling purposes. In addition, the theoretical total volume of distribution, which reflects the relationship between serum iohexol concentrations and total body iohexol, was very similar to that previously observed in a group of healthy young men [37], suggesting that iohexol does not significantly distribute into the fat compartment.

The identification of an iohexol-based 5-point sampling schedule with windows of time during which the sampling should occur offers a convenient, flexible, and accurate method with which to measure GFR in obese subjects without overt CKD. We expect to use it in future studies involving similar populations. Schwartz et al. [36] derived a similar 4-point sampling schedule in a pediatric CKD population, though theirs was derived empirically rather than through pharmacokinetic modeling. Their sampling times included at least 2 points each on the early (fast) and later elimination curves (slow), as did ours, allowing for more accurate measurement of each of the individual slopes.

The characteristics of our obese cohort were similar to what others have found. While glomerular hyperfiltration was common, micro- or macroalbuminuria was noted in only a minority of patients [38–41]. That total weight and lean body mass (and not fat mass) were the 2 factors influencing GFR modeling supports the premise that the metabolic load is an important influence on GFR. This issue becomes relevant when addressing the ongoing controversy over indexing GFR by BSA or other physiological parameters [16]. Indexing by BSA is performed in order to fairly compare renal hemodynamics between people of varying body sizes. In the setting of obesity, however, body surface area is disproportionately affected by fat mass, which in our cohort did not significantly affect GFR. Therefore, indexing by BSA in obese individuals artificially lowers the GFR and masks any ongoing glomerular hyperfiltration. This is why we focused primarily on nonindexed measurements.

We found the endogenous filtration markers creatinine and cystatin C to be poorly correlated with GFR throughout the narrow ranges we studied. Though an ongoing controversy exists as to whether cystatin C is influenced by body composition [42–45], the lack of association between the two in this study suggests that it may in fact be a superior filtration marker under certain circumstances because it is less prone to being influenced by body composition. Correlations between GFR and estimating equations were superior to individual filtration markers though the Bland-Altman plots revealed at best modest agreement, suggesting that improved methods of GFR estimation are needed in this population. Of note, we found the MDRD equation overestimated GFR, which conflicts with previous observations [8, 46]. This could be related to differences in the creatinine ranges studied, the prevalence of hyperfiltration, and/or the creatinine assays used.

Our study has a number of limitations. The cohort included mostly women with serum creatinine levels within a narrow range and was of modest size. This may have limited our statistical power and ability to describe important relationships. Whether our findings are applicable to men remains to be seen, though sex differences in iohexol pharmacokinetics have not been reported [6, 47]. Having studied only obese individuals, we cannot comment on whether the optimal sampling time points would have been different for lean individuals. A recent report found that GFR measured over longer durations (i.e. up to 10 h) may improve precision and accuracy [48]. However, that report included individuals with advanced kidney disease (i.e. estimated GFR of 32 ml/min/1.73 m^2 and true median GFR of 48 ml/min). That study population would be expected to have much slower plasma clearance and therefore require measurements over longer periods than the one profiled in this report. Therefore, our results may not necessarily apply to obese subjects with grossly abnormal serum creatinine levels. Finally, we chose not to measure iohexol urinary clearance as a comparison method because of the well documented difficulties in ensuring urinary collections are complete [36].

In the coming years, the ability to accurately measure GFR in obese individuals without overt CKD is likely to be increasingly important in both the clinical and research arenas. Furthermore, given the limited reliability

of current GFR-estimating strategies in obese subjects that we and others have described, efforts should be made to identify more accurate methods of estimation. Our study offers a simple and accurate iohexol-based protocol with which to initiate such endeavors.

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