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In Focus

Measured glomerular filtration rate is the goal, but how to measure it?

Henry N. Hulter[1] and Reto Krapf[2]

[1]Department of Medicine, University of California San Francisco, San Francisco, CA, USA and [2]Department of Internal Medicine, University of Basel, Klinik St Anna, Lucerne, Switzerland

Correspondence and offprint requests to: Henry N. Hulter; E-mail: hulter@cgl.ucsf.edu

Accurate and precisely measured GFR values are clinically necessary for renal function-specific subsets of the CKD population [1] and also serve as primary end points in clinical trials investigating treatment effects on the rate of GFR loss [2, 3]. Precise GFR slope differences therefore have the potential to provide an approval pathway for new drugs for prevention of CKD progression with reasonable sample size. Numerous studies have found from both cross-sectional and longitudinal data that serum creatinine-based measurements of estimated GFR (eGFR) exhibit excessive inaccuracy leading to recommendations for the use of measured GFR (mGFR), particularly when measuring the rate of progression of CKD [4, 5].

The choice of mGFR method for achievement of the desired accuracy and precision is not straightforward. Measured GFR can, in general, be performed either as a urinary clearance during constant infusion of a GFR marker (e.g. inulin, iothalamate or iohexol) or as a plasma clearance performed during either constant infusion (assuming constant urinary excretion and GFR) or as a single injection with calculation of GFR based on injected dose and an estimated AUC (area under the curve, assuming a constant GFR over many hours). In both adult [6, 7] and pediatric [8] CKD patients, the precision of mGFR has been reported to be better with plasma clearance methods due to the observed high variation in GFR marker urinary excretion rate, attributed to variable bladder emptying with incomplete sampling. However, the urinary clearance method when used with a bladder emptying correction based on a constant infusion of ortho-iodohippurate (OIH, hippuran, 100% renal clearance without metabolism) provides superior values for intra-test and inter-test precision (1.9 and 2.9%) and is supported by the accuracy (lack of bias versus simultaneous inulin urinary clearance) of iothalamate as a GFR solute [9] as well as superior precision of the slope of GFR decline (SEM <1 mL/min/year) for use in longitudinal studies necessary for drug approval and for clinical management/treatment of renal risk factors in individual patients [10].

A substantial problem with the use of the best urinary clearance methods for GFR in clinical and multi-center research settings, despite their robust qualifications as a 'gold standard' [10], is the use of radioactive solutes for constant infusion urinary clearance which includes radiolabeled OIH for correcting mGFR values for variable bladder emptying. Unfortunately, no validated non-radioactive bladder emptying-corrected GFR data have been reported, with para aminohippurate rendered unsuitable due to its metabolism. Bladder emptying variance is likely of greater magnitude in the enlarging elderly subset of the CKD population. Accordingly, there would be great clinical and research value in achieving a validated non-radioactive version of the Apperloo *et al.* mGFR method [10] using non-radioactive infusions of iothalamate or iohexol with non-radioactive OIH that might demonstrably approach that method's reported precision both for individual GFR measurements and GFR slopes.

Given the unmet need for non-radioactive validated mGFR methods that minimize or eliminate bladder emptying variance, there is an expected widespread interest in plasma clearance methods that obviate urine collection. Single injection plasma clearance mGFR calculations depend on true GFR remaining constant during the hours of measurement of GFR marker decay in plasma specimens and, while this has been observed in some reports over 2–3 h intervals [6, 7], there is abundant evidence for large diurnal variation in true GFR in both normal subjects and CKD patients as exemplified by a rising inulin clearance and falling plasma inulin level during late morning and afternoon during constant inulin infusion and rigorous control of protein intake (Figure 1) [11, 12]. In the current issue of NDT, Ebert *et al.* [13] provide a potential time- and convenience-improving method to estimate mGFR

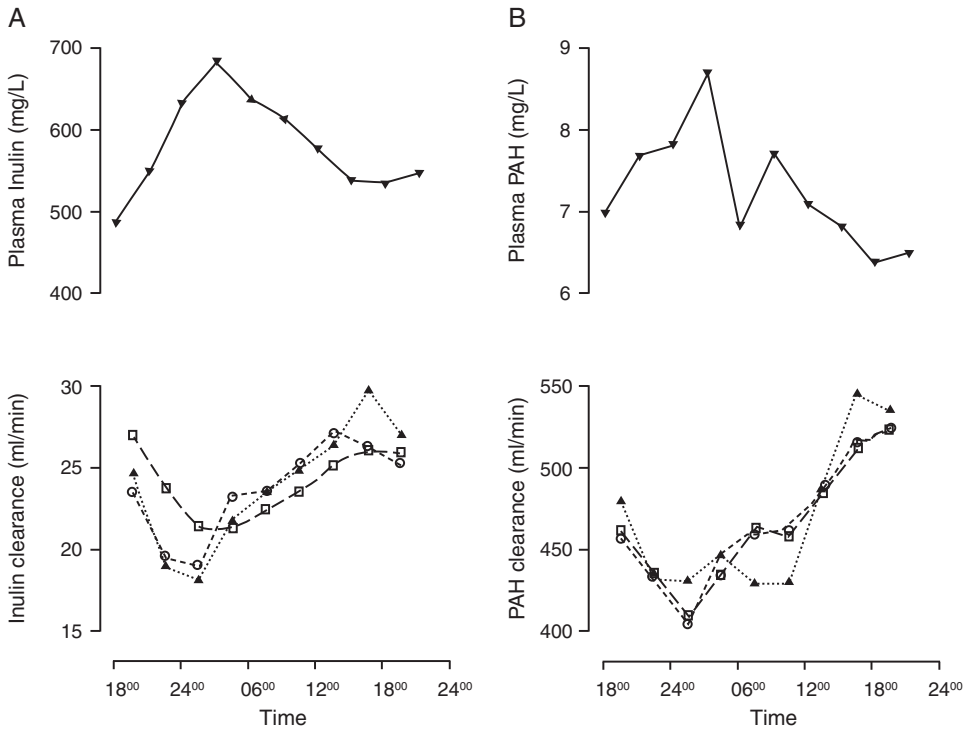

FIGURE 1: Time course of plasma inulin and inulin clearance (**A**) and of plasma para aminohippurate (PAH) concentration and PAH clearance (**B**) in a representative CKD patient. Symbols refer to differing methods of calculation: square, 24-h constant infusion method with clearance = infusion rate/plasma concentration; triangle, urinary clearance = urinary excretion rate/plasma concentration; circle, modified constant infusion method with adjustment for accumulating solute when plasma concentration is changing. Reproduced from Van Acker *et al.* [11].

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(mGFR = IV dose of iohexol/$AUC_{0\ to\ inf}$) as the plasma clearance of iohexol after a single IV injection. The authors have derived an estimating equation to empirically adjust mGFR values calculated from plasma iohexol levels obtained over a 5-h sampling period to a value designed to approximate a hypothetically more accurate value based on 24-h blood sampling. If future studies can provide validation of this equation to a 'gold standard' mGFR method (e.g. Apperloo *et al.* [10]), then this equation could, in theory, simplify mGFR measurements.

Both Ebert *et al.* [13] and prior studies [14, 15] of mGFR based on single injection iothalamate or iohexol plasma clearances have found that a shorter duration of plasma level measurements (2–9 h) leads to large and clinically meaningful overestimations of mGFR as compared to that derived from a longer sampling interval (10–24 h). As their basis for choosing the primacy of a 24-h iohexol plasma clearance sampling period, Ebert *et al.* [13] have cited a comparison of 4, 6 and 24-h plasma clearance sampling periods undertaken with validation versus simultaneous (first 6-h post-IV iohexol) urinary clearance measurements in 343 renal transplant patients with a mean iohexol urinary mGFR of 49 mL/min/1.73 m^2 [15]. These allograft results demonstrated a substantial 'overestimation' of plasma clearance mGFR, as compared to a 5-h urinary mGFR (using a falling post-injection plasma iohexol concentration) for both the 4-h (+27.2%) and 6-h (+20.6%) plasma clearance sampling periods, but found that a 24-h sampling period reduced the 'overestimation' to a more modest value (+6.8%) and concluded that a 24-h sampling period is necessary for accuracy in subjects with reduced mGFR. Stolz *et al.* [15] did not consider diurnal or meal-related GFR effects on mGFR in explaining their 'overestimation' of mGFR in their 4- and 6-h (early AUC) versus 24-h (long duration) AUC as compared to their early 5-h urinary clearance. Stolz *et al.* [15] also did not consider the reported finding that a non-steady state (falling) plasma GFR marker level causes the urinary mGFR to falsely overestimate values found in a steady state mGFR, a reported methodological confounder of mGFR accuracy in the MDRD study [16]. This error is due to the delay in glomerular marker transit time from glomerulus to bladder urine during rapid fall in venous plasma GFR marker concentration after IV or even SQ injection during a timed urinary clearance period and is accompanied by an additional error due to generation of an arteriovenous gradient (arterial level > mixed venous blood sample) created by the rapid and un-replaced renal loss of GFR marker from filtered plasma [16–18].

Ebert *et al.* [13] are correct in pointing out that normal diurnal variation in mGFR, as accompanied by parallel diurnal changes in renal plasma flow, may be a factor in explaining the 5-h plasma clearance value exceeding the corresponding 24-h value. Although not as pronounced as in an animal model (hummingbird [19]), there is a large and consistent diurnal increase in mGFR during the late morning and afternoon in normal and CKD human subjects [11, 12, 20], CKD patients [11, 21] and in renal allografts [22], and it remains evident even when diet protein intake is carefully controlled and administered steadily every 3-h [11, 12, 22]. Protein intake, *per se*, also causes large acute increases in mGFR [23, 24]. Both the diurnal and diet protein effects on mGFR are independent of systemic blood pressure [11, 12, 24].

Accordingly, when applied to the data of Ebert *et al.* [13], with a reported average iohexol injection time of ∼9:30 am and the 5-h sample time at ∼2:30 pm, it might be predicted (Figure 1) that the reported late morning and afternoon rise in GFR, plus the potential for random protein meals, will increase true GFR in that time interval such that the 5-h procedure may not be 'overestimating' mGFR, but simply reflect expected physiology. Similarly, acceptance of the 24-h iohexol plasma GFR values obligates one to accept both intrinsic variations in diurnal (afternoon/evening) increases in true GFR as well as diet protein-induced variations in GFR. While a 24-h mGFR by single injection plasma clearance may have value in reflecting 'real world' conditions, it has the major disadvantage of introducing a loss of accuracy and precision that is gained by shorter measurements under standardized physiological conditions. That is, it is important to decide whether the goal is to measure GFR under conditions designed to increase variance such as long (>2–4 h) or varying time of day clearance periods and with potentially random diet protein effects or to pursue the goal to remove as much of the protein and diurnal variation as possible. This has been approximated previously by starting a urinary clearance procedure 2-h following a fixed low protein breakfast followed by fasting, with clearance periods commencing 2-h post-meal, with clearance periods not exceeding 2-h and occurring at a fixed time of day [10]. The future addition of a validated non-radioactive method to minimize bladder-emptying variance during urinary clearance periods might be an optimal solution for both clinical and clinical research needs and thereby avoid the need for single injection mGFR measurements that are subject to large diurnal and protein intake GFR contributions.

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CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part.

(See related article by Ebert *et al.* Iohexol plasma clearance measurement in older adults with chronic kidney disease—sampling time matters. *Nephrol Dial Transplant* 2015; 30: 1307–1314.)

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