

Monitoring the menstrual cycle using urinary oestrone glucuronide: the relationship between excretion rate and concentration

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Abstract – Oestrone glucuronide (E1G) is monitored during the menstrual cycle because it reflects the follicular production of oestradiol (E2), the rate of which increases as the follicles grow. Despite several recent assertions to the contrary, the urinary E1G concentration is an inadequate indicator of E2 because it is confounded by variations in the rate of urine production. We show that (a) the serum E2 concentration is proportional to the E1G excretion rate, which is the product of the urinary concentration of E1G and the urine production rate, (b) that it is almost as important to measure the urine production rate as it is to measure the urinary E1G concentration itself, and (c) that the distribution of urine production rate is positively skewed, from which it is inferred that extreme values are more likely than would be expected were the distribution normal. If urinary E1G is to be used to monitor the menstrual cycle it is essential that the concentration is corrected for fluctuations in urine production rate: the E1G excretion rate is reliable, urinary E1G concentration is not

Keywords – analyte, concentration, excretion rate, menstrual cycle, oestrone glucuronide, urine

1. Introduction

The menstrual cycle usually lasts for about a month, during the first (follicular) phase of which a follicle grows until, towards the middle of the cycle, an ovum is released (ovulation) and then the remnants of the follicle develop into a corpus luteum during the luteal phase. It is widely accepted that ultrasound is the most reliable means of estimating follicle size, but it suffers from at least two obvious difficulties: it is rarely practicable every day and, even if it is possible, it can be challenging to follow individual follicles reliably [2]. However, among the several hormones involved, oestradiol (E2) is secreted by the growing follicle and both E2 and progesterone (P) are secreted by the corpus luteum. Together, these hormones or their metabolites provide a means of monitoring the cycle [3-5], although other methods have also been employed [6].

In the first phase of the cycle the rate of E2 secretion increases with follicle size [7], so serum E2 concentration is used to monitor follicular growth and physiological status. However, E2 is rarely measured every day because daily blood sampling may not be acceptable to many women and the laboratory assays of serum E2 measurements are costly. Consequently, a simple, cost-effective, non-invasive technique is needed that can be carried out at home, every day by a woman without assistance. This inevitably requires that appropriate analytes are assayed in a medium other than blood, such as saliva or urine. While there are reports of measurements in saliva [8, 9], urine is generally the alternative medium of choice. The most useful urinary analytes for monitoring

womens' fertility are luteinising hormone (LH), follicle-stimulating hormone (FSH), oestrone 3-glucuronide (E1G) and pregnanediol glucuronide (PdG) [4]. The latter two analytes (E1G and PdG) are metabolites of the E2 and progesterone, respectively, that emanate directly from the ovary [4]. One well-established and validated tool for assaying E1G and PdG in urine is the Ovarian Monitor [10-14], but a variety of lateral flow assay systems are also in development [15].

Measurements of serum E2 concentration are used to monitor follicle development [16, 17], although the serum concentration represents the balance between the rate of E2 secretion and its excretion or enzymatic conversion to other species. Measurements of urinary E1G are also used to monitor follicle development, but the concentration in urine is the result of the interaction between the changing rate of E1G excretion and the fluctuating rate of urine production [18]. The consequence of this interaction is that urinary analyte concentration estimates must be corrected for the rate of urine production, as has long been appreciated [1]. Frequently, urine production rate correction is approximated by normalising the concentration to the urinary creatinine concentration [19] or to the urine specific gravity [20]. Despite this, attempts have been made recently to avoid actually correcting for the rate of urine production. For example, Miro *et al.* [21] proposed that deviations from the smoothed, logarithmically transformed urinary PdG concentration could be used to 'correct' the estimated concentrations of urinary E1G and LH. Interestingly, they did not demonstrate the success of this approach by comparing it

with data corrected for the rate of urine production. They seemed to have no appreciation of the implication of comparing ‘concentrations’ derived in this way and seemed unaware of the information lost by their treatment of the PdG data [22]. Others have not corrected for the urine production rate at all [23-33] and, in at least some reports, physiological significance has been attached to particular urinary E1G and PdG concentrations [24, 27, 33].

Two issues arise from these observations. First, that while there is widespread appreciation that correction for the rate of urine production is necessary, it need not translate into implementation from which we infer that it is not actually understood. Second, that uncorrected measurements of analyte concentration can be surprisingly effective. Here, we address these issues using a simple mathematical approach. This analysis is written in terms of E1G, but it also applies to other urinary analytes.

2. Materials and methods

2.1. Data

Urine samples were collected according to the protocol employed in a United Nations Development Programme/World Health Organisation (WHO)/World Bank Special Programme of Research, Development and Research Training in Human Reproduction Study #90905 on the Ovarian Monitor [11-14]. Participants collected urine samples either overnight or during the day, but the collection period in each case was at least three hours. Urinary E1G and PdG were determined using the Ovarian Monitor [10-14]. Ethical permission was obtained from Massey University’s ethics committee and written informed consent was obtained from each participant.

2.2. Analysis

The E2 secreted by a follicle is rapidly converted into a variety of compounds, including oestrone (E1), oestriol (E3) and several more soluble conjugates made by glucuronidation or sulphonation in the liver. These various species are themselves interconvertible, further complicating the dynamics. Of the many possible urinary markers of ovarian E2 production, oestrone 3-glucuronide (E1G) is usually taken to be the most suitable [34] because it is rapidly excreted and present in reasonable quantities in urine. Moreover, there is good correlation between the excretion rate of E1G in urine and the concentration of E2 in serum [35-40]. There is also a linear relationship between the concentrations of E2 and E1G in both plasma [41] and serum [42, 43], although the values are not necessarily comparable because analyte concentrations in plasma and serum usually differ and need not be well correlated [44, 45]. We infer from these data that it is reasonable to assume that serum [E1G]/[E2] is approximately constant.

The transfer of E1G from serum to urine is reflected by the renal clearance rate for E1G, which depends on the rates of glomerular filtration, tubular secretion and tubular reabsorption [46]. As a first approximation, these rates are proportional to the serum E1G concentration (s), so the rate can be written as $(k_{GF} + k_{TS} - k_{TR})s$, where k_{GF} , k_{TS} and

k_{TR} are rate constants for glomerular filtration, tubular secretion and tubular reabsorption, respectively [47]. However, the relatively large concentration gradient between the urine and the serum leads us (largely for completeness) to consider the possibility of some flow of E1G from the urine to the serum across the urinary bladder epithelium [48-51]. Given this, the rate of transfer of E1G between serum and urine is

$$a) \quad \frac{dn}{dt} = (k_{GF} + k_{TS} - k_{TR})s - k_d u = k_n s - k_d u, \quad (1)$$

where n is the number of moles of E1G in the urine, $k_n = k_{GF} + k_{TS} - k_{TR}$ and k_d is the rate constant for any flow from the urine to the serum.

The concentration of E1G in urine depends on both the rate of transfer from the serum (1) and the rate of urine production. By definition, the concentration of the conjugate in the urine (u in units of mol L⁻¹) depends on both the quantity in the urine (n in mol) and the volume (V in L)

$$b) \quad u(t) = \frac{n(t)}{V(t)}. \quad (2)$$

Differentiating (2) with respect to time (t) yields

$$b) \quad \frac{du}{dt} = \frac{1}{V} \frac{dn}{dt} - \frac{n}{V^2} \frac{dV}{dt} = \frac{1}{V} \left(\frac{dn}{dt} - u \frac{dV}{dt} \right), \quad (3)$$

where dn/dt is the rate of transfer of conjugate into the urine and dV/dt is the rate of urine production.

An expression for s can be obtained by substituting (3) into the rate equation (1) for the transfer of the conjugate from the serum to the urine

$$c) \quad k_n s - k_d u = V \frac{du}{dt} + u \frac{dV}{dt} \quad (4)$$

and solving (4) for s

$$d) \quad s = \frac{1}{k_n} \left(k_d u + V \frac{du}{dt} + u \frac{dV}{dt} \right). \quad (5)$$

From (5) it can be inferred that (i) u is not equivalent to s and (ii) the relationship between s and u depends not only on the excretion rates (Vdu/dt and udV/dt), but also on the rate constants (k_n and k_d), so the correlation may well vary between women. Clearly, u alone is not an adequate proxy for s because it ignores the other terms on the right of (5) which do not cancel out (3). Taking (4) and (5) together, it is clear that if $k_d = 0$, so that there is no diffusion of conjugate from the urine to the serum, then (1) becomes $dn/dt = k_n s$ and only the terms in the two excretion rates remain.

3. Results and discussion

3.1. The excretion rates

In (3), both Vdu/dt and udV/dt are excretion rates, but what is usually used [11-14] is based on the measured concentration (u) and the urine production rate (dV/dt). So what is Vdu/dt and how do the two excretion rates compare?

The fundamental data are based on discrete samples and are the void volume (V), which may or may not leave urine in the bladder (the post-void residual volume (r)), the time elapsed since the last void (Δt), and the concentration (u). From these the rate of urine production ($dV/dt \approx V/\Delta t$),

also known as the ‘urine volume’, and an excretion rate ($udV/dt \approx uV/\Delta t$) are estimated [11-14].

The two excretion rates are not equal ($Vdu/dt \neq udV/dt$, Appendix 1) unless the urinary analyte concentration of one void is the same as that of the previous void ($u_i = u_{i-1}$) or there is no post-void residual volume ($r = 0$). It would be unreasonable to assume that u does not change, but it is not possible to measure r routinely, as the post-void residual volume is usually measured using transurethral catheterisation or ultrasound [52]. The post-void residual volume may be negligible ($r < 5$ mL) [53], rather larger ($r \approx 40$ mL) [54] or much larger ($r > 400$ mL) in individuals with voiding dysfunction [55], but should not exceed 50 mL in asymptomatic women [56]. As the normal void volume (V) range is 250-600 mL [57] and may be even larger, if $r < 50$ mL it is a relatively small proportion of V and it might be pragmatic to assume that $r \approx 0$. This is the approach recommended by Brown [3], but he emphasises that at least three hours should have elapsed since the last void ($\Delta t \geq 3$ h) to ensure that V is likely to be large and even this is potentially problematic if the rate of urine production is small (Figure 1, and see Fortin *et al.* [58] for similar data). This approach has at least two consequences. First, it is equivalent to assuming that $Vdu/dt = udV/dt$ (Appendix 1) and, second, it has the effect of smoothing the data if $r > 0$ (Appendix 2). Assuming that the two excretion rates are equal to udV/dt (and can each be approximated by $uV/\Delta t$) almost always (unless $r = 0$) leads to an over-estimate of dn/dt (3). Given this assumption, (5) becomes

$$e) \quad s \approx \frac{1}{k_n} \left(k_d u + 2u \frac{V}{\Delta t} \right), \quad (6)$$

and the significance of the two terms on the right ($k_d u$ and $2uV/\Delta t$) depends on their relative magnitude ($k_d \Delta t / 2V$). To assess this we require estimates of the parameters.

3.2. Parameter estimates

Estimation of k_n . The value of k_n is given by the renal clearance of the solute. Brown *et al.* [59] reported renal clearances for E1, E2, E3 and creatinine as 0.717 L h⁻¹, 0.528 L h⁻¹, 12.330 L h⁻¹ and 7.878 L h⁻¹, respectively. The first two of these values are slightly higher than those given by Wright *et al.* [60] for E1 and E2. For other steroids, glucuronidation increases renal clearance about 10-fold [61, 62], so we assume that $k_n \approx 10$ L h⁻¹ for E1G.

Estimation of k_d . To estimate k_d we observe that the rate constant is the product of the permeability (P) of the bladder epithelium and the bladder surface area (A). There are relatively few reports of measurements of P for steroids and steroid conjugates. Faassen *et al.* [63] reported values for estradiol and progesterone of 1.7×10^{-5} cm s⁻¹ and 2.4×10^{-5} cm s⁻¹, respectively, which compare well with the value of 6.6×10^{-5} cm s⁻¹ for hydrocortisone reported by Xiang and Anderson [64]. We know of no measurements of P for steroid glucuronides, but it is well established that $\log_{10}(P)$ is positively correlated with $\log_{10}(K)$, where K is the partition coefficient of the solute [64, 65]. The $\log_{10}(K_{\text{octanol/water}})$ values for oestradiol, oestrone and progesterone are 4.01, 3.13 and 3.87 [66], respectively. Since glucuronidation makes a steroid more water soluble, the glucuronide will have a lower

$\log_{10}(K_{\text{octanol/water}})$ than the unconjugated steroid (for example, the theoretical (XLogP3) estimate of the partition coefficient for E1G is 1.1) and, therefore, a smaller $\log_{10}(P)$. Given this, we take $P < 5 \times 10^{-5}$ cm s⁻¹ for E1G, which is a generous upper limit. The surface area of the bladder (A) of an adult woman is about 250 cm² [67], so $k_d = PA \approx 250 \times 5 \times 10^{-5}$ cm³ s⁻¹ \times 3600 s h⁻¹ = 45 mL h⁻¹. We emphasise that this estimate of k_d is probably close to the upper limit: if $dn/dt > 0$, as is the case for E1G, then $k_d/k_n < s/u$ and, given the data in Table 1, $u/s \approx 100$ and $k_d < 0.01k_n = 100$ mL h⁻¹.

Values of u and dV/dt . It is clear from Figure 1A that dV/dt is approximately lognormally distributed, which, because it is positively skewed, means that extreme values are more likely than would be the case were the distribution Gaussian. Even for the small sample size shown, the range of dV/dt is considerable and varies between subjects (Figure 1B). While this is the case even with a small number of urine samples, given the distribution it is likely that the range would tend to

Table 1. Literature values for serum E2 and E1G and urinary E1G concentrations (\pm SD).

	Menstrual cycle phase	
	Follicular	Luteal
Serum E2 ¹ (pg mL ⁻¹ , $n = 19$)	38.40 \pm 20.40	103.66 \pm 73.27
Serum E1G ¹ (pg mL ⁻¹ , $n = 19$)	54.19 \pm 24.71	141.12 \pm 75.40
Urinary E1G ² (ng mL ⁻¹)	5-50	10
Serum E1G:E2 (mol/mol, $n = 19$)	0.9 \pm 0.5	0.8 \pm 0.5
Urinary E1G:serum E1G (mol/mol)	90-920	70

¹ Taken from Table 3 of Caron *et al.* [42]. Xu *et al.* [71] reported comparable values ($n = 2$) for serum E2.

² Approximate values estimated from Figure 2 of Miro *et al.* [21].

increase with the sample size [1, 58, 68-70]. The mean dV/dt of the samples included in Figure 1 is about 49 mL h⁻¹, but the highest value in Figure 1 is more than 190 mL h⁻¹, almost four times the mean, and Fortin *et al.* [58] report several such values, including one of 437 mL h⁻¹, in a study of the variability of the rates of urine production obtained from 15 subjects in the course of one day. We take $dV/dt = 49$ mL h⁻¹, which is the mean value of the data shown in Figure 1. Miro *et al.* [21] show daily measurements of uncorrected urinary E1G concentration (u) for one menstrual cycle. The values are about 5 ng mL⁻¹ throughout the early follicular phase, rise to about 50 ng mL⁻¹ at the E1G peak day and are about 10 ng mL⁻¹ in the luteal phase. MacGregor *et al.* [29] show similar uncorrected data, averaged over 120 cycles, in which the early follicular phase, E1G peak day and luteal phase concentrations are about 10, 40 and 30 ng mL⁻¹, respectively. Johnson *et al.* [30] provide ‘normal’ ranges for urinary E1G that are consistent with these. We take $u = 5$ ng mL⁻¹ and $u = 10$ ng mL⁻¹ as estimates of the uncorrected urinary E1G concentrations in the early follicular and luteal phases of the cycle, respectively. These values are, inevitably, highly susceptible to variation [30], but provide some indication of the order of magnitude of u and its variation.

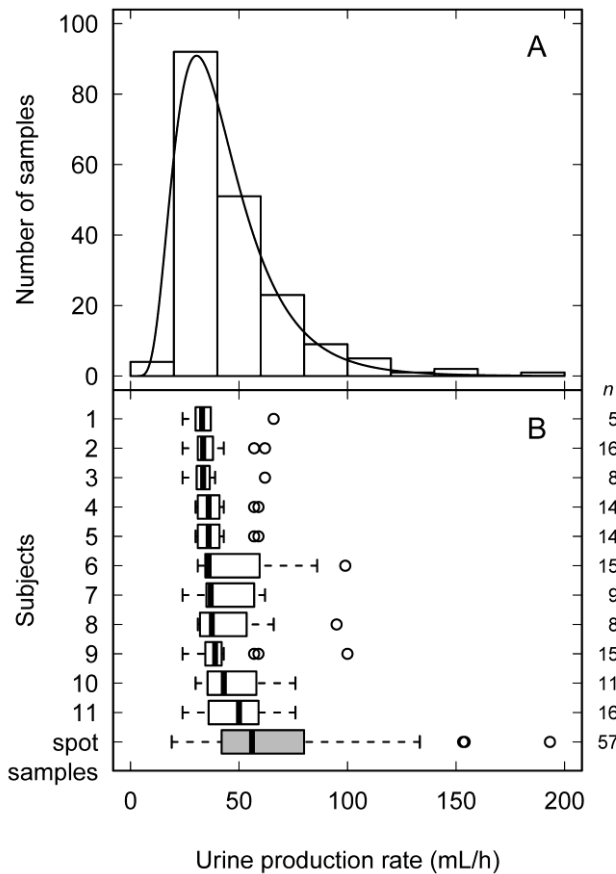


Figure 1. Distribution of the urine production rate ($n = 188$ samples from 68 different women). In (A) the values ranged from 12 mL h^{-1} to 193 mL h^{-1} (interquartile range: $34\text{-}59 \text{ mL h}^{-1}$; mean ($\pm \text{SEM}$) = $49 \pm 2 \text{ mL h}^{-1}$; median = 39 mL h^{-1}) and the smooth curve is a lognormal distribution fitted to the data by maximum likelihood. In (B) the same data are plotted for those subjects from whom several samples were obtained (white bars) and for a number of subjects from each of whom provided a single sample (grey bar). The numbers on the right hand side of (B) are the corresponding sample sizes (n).

3.3. Relative significance of u and udV/dt

Two tests can be applied using (6) and the estimated parameters. First, we determine whether values of the right order of magnitude are generated, and, second, we consider the relative significance of u and dV/dt .

Substituting the parameter estimates ($k_n = 10 \text{ L h}^{-1}$, $k_d = 45 \text{ mL h}^{-1}$, $dV/dt = 49 \text{ mL h}^{-1}$ and $u = 5 \text{ ng mL}^{-1}$) in (6) yields an estimate for the upper limit of the serum E1G concentration

$$\begin{aligned} f) \quad s &\leq \frac{1}{k_n} \left(k_d u + 2u \frac{V}{\Delta t} \right) \\ &= \frac{45 + (2 \times 49)}{10000} \times 5 \text{ ng mL}^{-1} = 71.5 \text{ pg mL}^{-1} \end{aligned}$$

g) in the early follicular phase and about 140 pg mL^{-1} in the luteal phase. These estimates correspond reasonably to those reported by Caron *et al.* [42] and Xu *et al.* [71] for the serum E1G concentration (Table 1). This provides weak confirmation that the parameter estimates we have used are at least of the right order of magnitude relative to one another.

The relative significance of the two terms of (6) is independent of u and is $k_d \Delta t / 2V = 45/98 \approx 0.5$. However, while $V/\Delta t$ might vary from about 25 mL h^{-1} to almost 200

mL h^{-1} (Figure 1), k_d may well be smaller than 45 mL h^{-1} , so we can conclude with some confidence that $k_d \Delta t / 2V < 1$. While this implies that u alone is less significant than udV/dt in the estimation of s , it should be considered another way. The right hand side of (6) is

$$f = \frac{1}{k_n} \left(k_d + 2 \frac{V}{\Delta t} \right) u$$

and the sensitivity of f to changes in u and $V/\Delta t$ are

$$\frac{\partial \ln f}{\partial \ln u} = 1 \quad \text{and} \quad \frac{\partial \ln f}{\partial \ln (V/\Delta t)} = \frac{2(V/\Delta t)}{\left(k_d + 2 \frac{V}{\Delta t} \right)},$$

respectively. What this means for the former is that a 1% change in u would cause a 1% change in f , as would be expected. Using the same parameter estimates, the latter varies from 0.68 to 0.82 as $V/\Delta t$ ranges from 25 mL h^{-1} to 200 mL h^{-1} (Figure 1), which implies that f is very nearly as sensitive to changes in $V/\Delta t$ (a 1% change causes a 0.68-0.82% change in f) as it is to changes in u , and if a smaller value of k_d is more appropriate, then this sensitivity would be enhanced (and would equal 1 if $k_d = 0$). We infer from this that it is very nearly as important to correct for changes in dV/dt as it is to measure u in the first place if accurate estimates of s are to be obtained.

3.4. A representative cycle

Despite the impression conveyed by some recent reports [21, 28-31], the importance of correcting for the rate of urine production has long been accepted [1]. The failure to correct for the rate of urine production seems to arise from an erroneous perception that it is difficult to estimate the urine production rate. We illustrate some of the relevant issues using data obtained from a single menstrual cycle (Figure 2). First, the dynamics of the E1G excretion rate (Figure 2A) and the concentration (Figure 2B) are quite different, even when u is high, as it is around the E1G peak day, or the rate of urine production is low or constant, as it happens to be around the E1G peak day or in the last few days of this cycle (Figure 2C). Second, critical parameters such as the day on which the fertile period starts and the E1G peak day [12] can be misidentified using u alone. In this case the E1G peak day is either one day earlier or three days later (days 14 or 18, Figure 2B) than would be the case using udV/dt (day 15, Figure 2A). A naïve view might take the later of the two peaks in E1G concentration as the correct peak day, since it reaches the greater concentration and a peak in the PdG concentration follows this E1G peak. It should be noted that the baseline concentration of PdG exceeds $5 \mu\text{mol L}^{-1}$ from day 13 (Figure 2B). This would add to the confusion because it has been suggested [72] that a PdG concentration greater than $5 \mu\text{mol L}^{-1}$ is proof of ovulation, but this occurs in the follicular phase of this cycle (Figure 2B). The beginning of the fertile window would be judged to start on what is actually the first E1G concentration peak day (day 14, Figure 2B), with potentially disastrous consequences since the excretion rate E1G peak day is the next day. Third, the rate of urine production changes unpredictably (Figure 2C), but with an approximately lognormal distribution (Figure 1A), so the range of intra- and inter-subject variation can be considerable. If the

menstrual cycle profiles are to be accurately represented, the urine production rate profile, which is impossible to anticipate, must be taken into account.

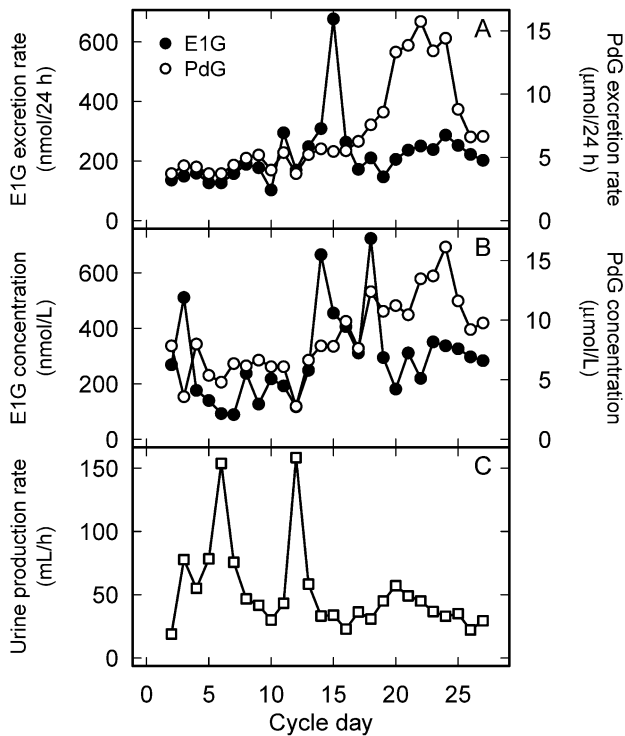


Figure 2. Variation of excretion rate (A) and concentration (B) of E1G (●) and PdG (○), and the urine production rate (C) during a single menstrual cycle. The E1G and PdG excretion rates were estimated using the Ovarian Monitor [75].

However, the analysis we have shown yields more insight. For example, combining (3) and the approximation in (A1.4) yields

$$\frac{dn}{dt} \leq 2u \frac{dV}{dt}$$

or

$$\log(u) \geq -\log(2) + \log\left(\frac{dn}{dt}\right) - \log\left(\frac{dV}{dt}\right), \quad (7)$$

from which we infer that (a) an upper limit to the rate of transfer between serum and urine (dn/dt) can be estimated and (b) u is inversely proportional to dV/dt . Both of these inferences are substantiated by the data shown in Figure 2, as is illustrated by Figure 3. Firstly, it is clear that u declines as dV/dt increases, although the relationship is relatively noisy, presumably because of variation in dn/dt (Figure 3). Second, despite the noise in the data, the relationship is bounded from above by a line with unit slope, and, indeed, it appears that these data are similarly bounded from below (Figure 3). From these bounds, it can be estimated from (7) that the rate of E1G transfer from serum to urine (dn/dt) is less than about 66 ng h^{-1} and appears to be greater than about 18 ng h^{-1} for these three cycles (Figure 3). Similar data have been reported for other urinary analytes [73] and the analysis we have described also applies to them.

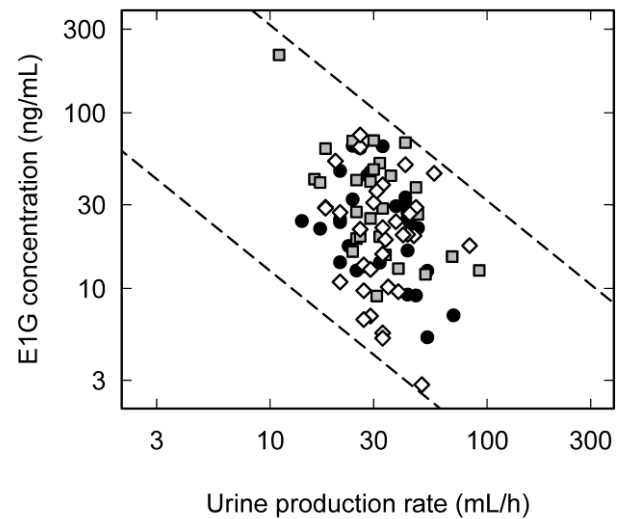


Figure 3. The relationship between urinary E1G concentration and the urine production rate. The lines have unit slope and represent the bounds of the data (only the upper bound is predicted by (7)) and the different symbols (●, ■, ◇) represent measurements from three menstrual cycles from a single woman.

The general inverse relationship between u and dV/dt illustrated in Figure 3, and predicted by (7), explains some of the success of those who have elected not to correct for urine production rate [28-30] and, perhaps, of the smoothing approach of Miro *et al.* [21]. The tendency for the concentration to decline as the urine production rate increases would, taken to an extreme, lead to $dn/dt = \text{constant}$ and, therefore, that the excretion rate $udV/dt = \text{constant}$. If this happened to be true, then correction for the rate of urine production would not be necessary, but neither of these possibilities is substantiated by the data. Instead, it is clear from Figures 2A and 3 that the excretion rate varies considerably and the values of dn/dt appear to vary by a factor of about 3.7 (Figure 3).

• Finally, this analysis naturally applies to other analytes, such as creatinine, and so it can be used to consider the assumptions underlying creatinine correction of urinary analyte concentrations. There is a substantial literature concerning the use of creatinine in the estimation of glomerular filtration rate and the assessment of renal function. This is predicated on the assumptions that (a) tubular secretion and reabsorption of creatinine are negligible ($k_{TS} = k_{TR} = 0$) so that $k_n = k_{GF}$, (b) there is no transfer across the bladder epithelium ($k_d = 0$) and (c) the rate of creatinine production is constant, so that any changes in the serum creatinine concentration (s_c) are indicative of changes in kidney function. Each of these assumptions has been questioned [74], but if it is assumed that they are justified, (5) for creatinine becomes

$$s_c = \frac{1}{k_{GF}} \left(V \frac{du_c}{dt} + u_c \frac{dV}{dt} \right),$$

where u_c is the urinary creatinine concentration. Since s_c is assumed to be constant for a healthy individual, and using (A1.4),

$$2u_c \frac{V}{\Delta t} \geq V \frac{du_c}{dt} + u_c \frac{dV}{dt} = k_{GF} s_c = \text{constant}, \quad (8)$$

which means that

$$\frac{V}{\Delta t} \geq \frac{k_{GF} S_c}{2u_c} = \frac{\text{constant}}{u_c}.$$

In other words, dividing the concentration of a urinary analyte by the urinary creatinine concentration is roughly equivalent to correcting for the urine production rate. However, in this case several additional assumptions have been made that are not necessary when the rate of urine production is used directly. Among these is the implication of (8) that the rate of creatinine transfer (dn_c/dt) into the urine is constant (3), which is certainly not the case for E1G (Figure 3) or calcium [73], and may not be the case for creatinine either [74].

4. Conclusions

If the point of monitoring urinary E1G is to provide an indication of the serum E2 concentration, and hence ovarian function [4], rather than having to measure it directly, then it is insufficient simply to report the concentration of E1G in a urine sample. The reason for this is that the concentration reflects both the rate of E1G accumulation in the bladder and the rate of urine production. We have shown that it is almost as important to correct for the rate of urine production as it is to determine the concentration of E1G in the first place.

5. Appendix 1. The two excretion rates

As the data are usually acquired only once a day, the derivatives have to be approximated discretely, so, using a subscript i to denote void i , the excretion rates are

$$\bullet \quad V \frac{du}{dt} \approx V_i \frac{u_i - \frac{u_{i-1} r_{i-1}}{V_i}}{\Delta t_i} = \frac{u_i V_i}{\Delta t_i} \left(1 - \frac{r_{i-1}}{V_i} \frac{u_{i-1}}{u_i} \right) \quad (\text{A1.1})$$

and

$$u \frac{dV}{dt} \approx u_i \frac{V_i - r_{i-1}}{\Delta t_i} = \frac{u_i V_i}{\Delta t_i} \left(1 - \frac{r_{i-1}}{V_i} \right) \quad (\text{A1.2})$$

where r_{i-1} is the post-void residual volume present in the bladder at the start of the accumulation of V_i . It is also useful to observe that

$$0 \leq \frac{r_{i-1}}{V_i} < 1 \quad \text{and} \quad 0 \leq \frac{u_{i-1}}{u_i} < M,$$

where M is a finite number determined by the physiology of the subject. Two things are immediately apparent from these expressions:

- i. $Vdu/dt \neq udV/dt$ unless (a) $u_i = u_{i-1}$ or (b) $r_{i-1} = 0$, and
- ii. both rates depend on r_{i-1} , but Vdu/dt also depends on u_{i-1} .

The corollary of this is that the estimation of udV/dt as $uV/\Delta t$ is equivalent to presuming that $r = 0$ and, therefore, that $udV/dt = Vdu/dt$. The error arising from this assumption is given by the difference between the two rates

$$V \frac{du}{dt} - u \frac{dV}{dt} \approx u_i V_i \frac{1 - \frac{r_{i-1}}{V_i} \frac{u_{i-1}}{u_i}}{\Delta t_i} - u_i V_i \frac{1 - \frac{r_{i-1}}{V_i}}{\Delta t_i} \quad (\text{A1.3})$$

$$= \frac{u_i V_i}{\Delta t_i} \frac{r_{i-1}}{V_i} \left(1 - \frac{u_{i-1}}{u_i} \right)$$

which varies in sign as u changes (it is positive if $u_{i-1} < u_i$, negative if $u_{i-1} > u_i$ and, as indicated above, zero if $u_{i-1} = u_i$ or $r_{i-1} = 0$). Given this, and the fact that (5) depends on the sum of the two excretion rates,

$$V \frac{du}{dt} + u \frac{dV}{dt} \approx u_i V_i \frac{1 - \frac{r_{i-1}}{V_i} \frac{u_{i-1}}{u_i}}{\Delta t_i} + u_i V_i \frac{1 - \frac{r_{i-1}}{V_i}}{\Delta t_i} \quad (\text{A1.4})$$

$$= \frac{u_i V_i}{\Delta t_i} \left[2 - \frac{r_{i-1}}{V_i} \left(1 + \frac{u_{i-1}}{u_i} \right) \right] \leq 2 \frac{u_i V_i}{\Delta t_i}$$

This means that assuming that the two excretion rates are equal to udV/dt almost always (that is unless $r = 0$) leads to an over-estimate of dn/dt (3). This is consistent with the fact that du/dt can be positive or negative (Figure 2B), but $dV/dt \geq 0$ (A1.2). In practice, it seems impossible to avoid this because that would require an estimate of r , but it is possible to minimise its effect as recommended by Brown [3]. In practice, perhaps, the most significant effect of a relatively small r is the smoothing of the urinary analyte excretion rate profile (Appendix 2).

6. Appendix 2. Smoothing effect of non-zero post-void residual volumes

For a sequence of n consecutive urine samples $i = 1, 2, \dots, k, \dots, n$ with analyte concentration u_i , void volume V_i and elapsed time Δt_i , equation (A1.1) indicates that u_i depends on u_{i-1} . Writing (A1.1) for the k th sample of Vdu/dt as

$$J_k = \frac{u_k V_k}{\Delta t_k} \left(1 - \frac{r_{k-1}}{V_k} \frac{u_{k-1}}{u_k} \right) \quad (\text{A2.1})$$

and rearranging yields

$$u_k = \frac{J_k \Delta t_k + r_{k-1} u_{k-1}}{V_k}. \quad (\text{A2.2})$$

A general expression for the n th sample can be obtained by iteratively substituting (A2.1) into itself

$$u_n = u_0 \prod_{k=1}^n \frac{r_{k-1}}{V_k} + \frac{1}{\prod_{k=1}^n V_k} \sum_{k=1}^n \left(J_k \Delta t_k \prod_{j=1}^k V_{j-1} \prod_{j=k}^{n-1} r_j \right) \quad (\text{A2.3})$$

where $V_0 = 1$ and u_0 and r_0 are the concentration and the post-void residual volume of the void immediately prior to the first sample. Substituting (A2.3) into (A2.1) yields

$$J_n = \frac{u_n V_n}{\Delta t_n} \left(1 - \frac{r_{n-1}}{V_n} \frac{u_{n-1}}{u_n} \right) = \frac{u_n V_n}{\Delta t_n} \left(1 - \frac{r_{n-1}}{u_n \prod_{k=1}^n V_k} \sum_{k=1}^{n-1} \left(J_k \Delta t_k \prod_{j=1}^k V_{j-1} \prod_{j=k}^{n-1} r_j \right) \right), \quad (\text{A2.4})$$

which is a variably weighted function of the earlier J_k s if $r_k > 0$. Moreover, since $r_k/V_k < 1$, declines rapidly with increasing n , so (A2.4) can be approximated as

$$J_n \approx \frac{u_n V_n}{\Delta t_n} \left(1 - \frac{r_{n-1}}{u_n \prod_{k=1}^n V_k} \sum_{k=1}^{n-1} \left(J_k \Delta t_k \prod_{j=1}^k V_{j-1} \prod_{j=k}^{n-1} r_j \right) \right), \quad (\text{A2.5})$$

so J_n is a variably weighted function of the earlier J_k s,

which is essentially a form of smoothing. This just describes the inevitable effect of a sequence of non-zero post-void residual volumes.

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