Simultaneous Extraction of Urea and Glucose by Reverse Iontophoresis *in Vivo*

Anke Sieg,[1,2] Richard H.Guy,[1,3] and M. Begoña Delgado-Charro[1,3,4]

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Purpose. Reverse iontophoresis extracts glucose across the skin in the GlucoWatch Biographer, a device to monitor glycemia in diabetes. However, the device must first be calibrated with an invasive "finger-stick" and this has been perceived as a disadvantage. Here, urea, a neutral "internal standard" is extracted simultaneously in an attempt to render the technique completely non-invasive.

Methods. In a 5-h experiment in human volunteers, reverse iontophoretic fluxes of glucose and urea (J_{glu} and J_{urea}, respectively) were measured periodically and correlated with the corresponding blood levels. In the case of glucose, a finger-tip blood sample was taken at the beginning of each collection interval; for urea, three blood samples were assayed: one before, one during, and one at the end of iontophoresis.

Results. The ratio J_{glu}/J_{urea} divided by the ratio of the systemic concentrations (C_{glu}/C_{urea}) yielded an extraction coefficient (K) that could be compared between subjects. Though $J_{glucose}$ tracked C_{glu} faithfully when the volunteers were challenged with an oral glucose load, J_{urea} remained quite stable reflecting the fact that C_{urea} did not change appreciably during the experiment. However, whereas the variability (expressed as the coefficient of variation) in the normalized extraction flux of urea (J_{urea}/C_{urea}) was on the order of 25%, that for glucose was greater (>45%), with the result that the values of K (0.45 ± 0.25) were less constant than anticipated.

Conclusions. Although urea performed quite reasonably as an internal standard, in that its extraction flux and systemic concentration both remained quite constant, the normalized, transdermal, iontophoretic flux of glucose showed interindividual variability due to mechanisms that were not entirely governed by electrotransport. That is, despite good qualitative tracking to blood levels, there appear to be other (biochemical, metabolic, contamination?) factors that impact upon the quantitative results obtained.

KEY WORDS: electroosmosis; iontophoresis; non-invasive glucose monitoring; skin; transdermal.

INTRODUCTION

The GlucoWatch Biographer uses reverse iontophoresis to non-invasively extract glucose across the skin (1). It allows glycemia in diabetics to be monitored over the course of a day. The iontophoretic extraction is based on the application of a low electric current, which drives the migration of ions across the skin. This biologic membrane, under physiologic conditions, is negatively charged and, as a result, permselective to cations. The preferential passage of counter-ions across the skin induces electroosmosis, a convective solvent flow, which carries small neutral molecules such as glucose toward the skin surface. The electroosmotic flux of a solute (J_{EO}) is proportional to the potential gradient established by the electric field ($-d\Phi/dx$) and the subdermal concentration of the analyte C_i (2):

$$J_{EO} = L_{VE} \cdot \frac{-d\Phi}{dx} \cdot C_i \tag{1}$$

where the electroosmotic flow coefficient L_{VE} describes the direction and the magnitude of the volume flow. In addition to convective flow, passive diffusion may additionally contribute to the total transport across the skin. For a neutral species, the total flux J_i ($nmol \cdot h^{-1}$) can therefore be expressed as the sum of the electroosmotic (J_{EO}) and passive diffusion contributions (J_p):

$$J_i = J_{EO} + A \cdot J_p \tag{2}$$

where A (cm^2) is the surface area of the skin across which transport is occurring. Note that J_p is typically expressed, for example, in units of $nmol \cdot cm^{-2} \cdot h^{-1}$ and the total amount transported across a membrane by this mechanism depends directly on the available diffusion area (A). Further, it is generally accepted that J_p will be a function of the size of the diffusing molecule (3). In contrast, at constant current, J_{EO} is governed by the charge on, and hence the permselectivity of, the skin and appears to be area-independent (4). Additionally, J_{EO} is insensitive, or at least much less sensitive, to the size of the convected species (2). The magnitude of convective flow may be an important factor behind the variability in iontophoretic fluxes of neutral solutes.

Such inter- (and even intra-) individual differences mean that glucose monitoring with the GlucoWatch requires calibration against a conventional blood sample at each use. The invasive calibration procedure (a classic "finger-stick" to obtain a blood sample) has been perceived as a disadvantage, despite the fact that the device provides highly valuable information about the blood glucose profile over time and allows hypoglycaemia to be anticipated (5).

The goal of the work presented here is to avoid the conventional calibration step via the use of an "internal standard." This approach involves the extraction and subsequent analysis of two species simultaneously, the analyte of interest (glucose) whose concentration varies as a function of time, and the internal standard (IS), an endogenous molecule of known and essentially fixed physiologic levels (6). If the iontophoretic transport of glucose is independent of that of the other, then their iontophoretic extraction fluxes (J_{glu} and J_{IS}, respectively) should obey the following relationship:

$$\frac{J_{glu}}{J_{IS}} = K \cdot \frac{C_{glu}}{C_{IS}} \tag{3}$$

where C_{glu} and C_{IS} are the blood concentrations of the two substances, and K is a proportionality constant (or extraction coefficient), which may be defined by the combination and rearrangement of Equations 2 and 3:

[1] School of Pharmacy, University of Geneva, 1211 Geneva 4, Switzerland.
[2] Procter & Gamble, Rusham Park Technical Centre, Egham, Surrey, TW20 9NW, United Kingdom.
[3] Department of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY, United Kingdom.
[4] To whom correspondence should be addressed. (e-mail: B.Delgado-Charro@bath.ac.uk or r.h.guy@bath.ac.uk)

$$K = \frac{J_{glu}}{J_{IS}} \cdot \frac{C_{IS}}{C_{glu}} = \frac{(J_{EO,glu} + A \cdot J_{p,glu})}{(J_{EO,IS} + A \cdot J_{p,IS})} \cdot \frac{C_{IS}}{C_{glu}} \quad (4)$$

Initial work investigated this idea *in vitro* and *in vivo* using the sodium ion as the internal standard (6,7). Though encouraging results were obtained *in vitro*, significant inter- and intra-individual variation in K was observed for a subset of the study population *in vivo*. This variability was due to fluctuations in the extraction efficiency of glucose; the Na^+ electrotransport remained remarkably constant as hypothesized. Further *in vitro* studies suggested that a small neutral molecule extracted by the same mechanism as glucose would better reflect modified electroosmotic transport (Sieg *et al.,* submitted), and urea was identified as a potentially interesting internal standard candidate for *in vivo* evaluation. This article presents the results from a preliminary evaluation of this idea.

MATERIALS AND METHODS

Study Population

Nondiabetic subjects (age range: 25 to 40 years; 1 male, 5 females), with no history of skin disease participated in the study. Informed consent was obtained, the study protocol having been approved by the internal review board of the University of Geneva in accord with the principles outlined in the Declaration of Helsinki. Five subjects participated on a single occasion, while 1 volunteer participated twice.

Chemicals

Tris base (α,α,α-Tris-(hydroxymethyl)-methylamine), sodium chloride, D-glucose, hydrochloric acid, sodium hydroxide, diacetylmonoxime, thiosemicarbazid, sulfuric acid, and iron (III) chloride were purchased from Sigma-Aldrich (St. Quentin Fallavier, France) and were at least analytical grade. Deionized water (resistivity > 18.2 Mohm/cm^2) was used to prepare all solutions.

Iontophoresis

Two cylindrical glass cells (diameter 1.6 cm, extraction surface 2 cm^2), separated by a distance of 7 cm, were fixed with foam tape (3M Health Care, St. Paul, MN, USA) on the subject's ventral forearm. The anodal chamber was filled with 1.2 ml of 10 mM Tris-buffer at pH 8.5 containing 100 mM NaCl; the cathodal chamber contained the same volume of 10 mM Tris buffer alone. The slightly alkaline pH was used to maximize electroosmotic flow, a strategy already adopted in the GlucoWatch (8). Custom-made Ag/AgCl electrodes were inserted into the solutions and fixed 3–4 mm above the skin surface to ensure that no physical contact occurred. Direct current (I = 0.6 mA, current density = 0.3 mA/cm^2) was passed for a total of 5 h and was controlled by a Phoresor II Auto (Iomed, Salt Lake City, UT, USA), an FDA-approved, constant current, iontophoretic power supply. Every 15 min post-initiation of the current, the entire cathodal solution was collected and replaced by 1.2 ml of fresh buffer. The samples were immediately frozen until analysis.

After 2.5 h of iontophoresis, the subjects ingested 75 g of glucose dissolved in 300 ml of water (Glucosum monohydricum Ph.Eur., Hänserler AG, Herisau, Switzerland), so as to provoke a significant change in blood sugar. From this point onward, glycemia was measured before each subsequent 15-min collection interval using a conventional blood glucose monitor (Glucotrend 2, Roche Diagnostics, Mannheim, Germany). Blood urea levels were determined in 32 μl of capillary blood from the finger-tip using a Reflotron Benchtop Analyser (Roche Diagnostics, Mannheim, Germany). Three spot measurements were made: immediately before starting iontophoresis, during the experiment at the time of the first blood glucose measurement, and directly after termination of iontophoresis.

Analytical Chemistry

Glucose was assayed by high-performance anion chromatography with pulsed amperometric detection on a gold electrode using an ion chromatograph (Dionex 600 system, Dionex, Sunnyvale, CA, USA) (7). Urea was determined by a colorimetric reaction using diacetylmonoxime reagent (9,10). This method had a linear response over the concentration range from 5 to 70 μM; only citrulline provided a significant interference. The colored complex was allowed to form at room temperature, and absorption was measured at a wavelength of 520 nm (Perkin Elmer 554 Spectrophotometer, Perkin Elmer, Norwalk, CT, USA).

Data Analysis and Statistics

Iontophoretic fluxes were calculated from the amounts extracted in each collection period and plotted at the mid-time point of each interval; blood glucose concentrations were the values at the actual time of measurement. Blood urea levels for each sampling interval were estimated by linear extrapolation between the three spot measurements; for each volunteer, the regression ($r^2 \geq 0.97$) indicated that this approximation was reasonable. Typically, urea concentrations declined slightly over the 5-h experiment (0.25 ± 0.05 mM/h).

Normalized fluxes were determined by dividing the iontophoretic flux values of glucose and urea by the corresponding blood concentrations at each interval. Statistical differences were assessed by ANOVA, followed by a Newman-Keuls multiple comparison test, using GraphPad Prism 3.02 software (San Diego, CA, USA). Individual values of K were determined by linear regression of the extracted flux ratio vs. the ratio of the corresponding blood levels, for which significance was tested by ANOVA. Slopes of the regression line were then compared by analysis of covariance as described in Ref. 11. Unless otherwise stated, data are expressed as mean ± SD.

RESULTS AND DISCUSSION

Local Effects of Iontophoresis

All subjects experienced a mild tingling sensation when the current was applied. The sensation was typically asymmetric, being more noticeable at the anode than at the cathode. Generally, the sensation diminished with time of current application and lasted no longer than 30 min. Iontophoresis caused the skin beneath the electrode chambers to become slightly erythematous, an effect which disappeared within 24 h of current termination. In addition, a few, small, punctuate lesions remained after the redness disappeared; these marks persisted for several days. These completely reversible effects

are similar to those that have been reported in the literature (12), and do not appear to impair skin barrier function.

Glucose Extraction

The iontophoretic transport of glucose, an uncharged, polar molecule, occurs by electroosmosis and is directly proportional to the subdermal concentration (2). However, a "warm-up" period is necessary to establish a pseudo-steady electroosmotic flow and to empty the glucose reservoir from the skin. The latter is due, at least in part, to local metabolism and is not reflective of glucose levels in the blood. The recommended "warm-up" period for the GlucoWatch G2 is 2 h, a period similarly adopted in this study. Figure 1 shows the reverse iontophoretic extraction profiles of glucose for 3 volunteers. Though the fluxes accurately tracked the systemic glucose concentration, the extraction efficiency varied between subjects. Table I illustrates this point and shows, for each experiment, the average extraction fluxes of glucose normalized by the corresponding blood concentrations (i.e., the values of J_{glu}/C_{glu} which, in turn, are equal to the apparent electroosmotic flow rates). Although the intra-individual variability was modest (the mean coefficient of variation was 21%), there were considerable differences between individuals: for the seven experiments performed, $J_{glu}/C_{glu} = 8.5 \pm 3.2$ $\mu l \cdot h^{-1}$ (CV = 38%). While it is tempting, when considering the J_{glu}/C_{glu} data in Table I, to suggest that two subpopulations might exist, there are insufficient data from this study to justify the premise. However, if the results are compared to the earlier *in vivo* investigation (7), then this trend is supported more clearly. Previously, it was found that, in 12 experiments, $J_{glu}/C_{glu} = 7.1 \pm 4.7$ $\mu l \cdot h^{-1}$, a result not significantly different from that found here. Again, intra-individual differences, with one exception, showed CVs of no more than 20%. But more striking is the observation shown in Fig. 2, wherein the combined data for normalized glucose flux from the earlier work and from this study appear to separate into a "high-extraction" group ($J_{glu}/C_{glu} > 8$ $\mu l \cdot h^{-1}$), and a group for which extraction is less (and, sometimes, much less) efficient.

Urea Extraction

Reverse iontophoresis has been used to monitor blood urea in patients with renal insufficiency (13), and a linear correlation between the iontophoretically extracted analyte and the corresponding blood levels was obtained. Extraction was performed, however, for only 5 min, during which time the samples almost certainly included significant levels of endogenous urea from the skin (14). This is supported by the normalized fluxes reported, which ranged from 190 to 610 $\mu l \cdot h^{-1} \cdot cm^{-2} \cdot mA^{-1}$, that is 15–20 times higher than those observed in the study described here (17.4 ± 4.7 $\mu l \cdot h^{-1} \cdot cm^{-2} \cdot mA^{-1}$; CV = 27%).

Typical reverse iontophoretic urea fluxes are shown in the lower panels of Fig. 1(A–C). After the initially very high levels obtained, which were outside the calibration range of the assay (and therefore not shown), fluxes stabilized after ~60 min and generally followed the slight decrease in urea blood levels that occurred during the 5-h experiment. As for glucose, the small, neutral urea is principally extracted by electroosmosis. Passive diffusion, nevertheless, has been shown to contribute significantly to urea electrotransport

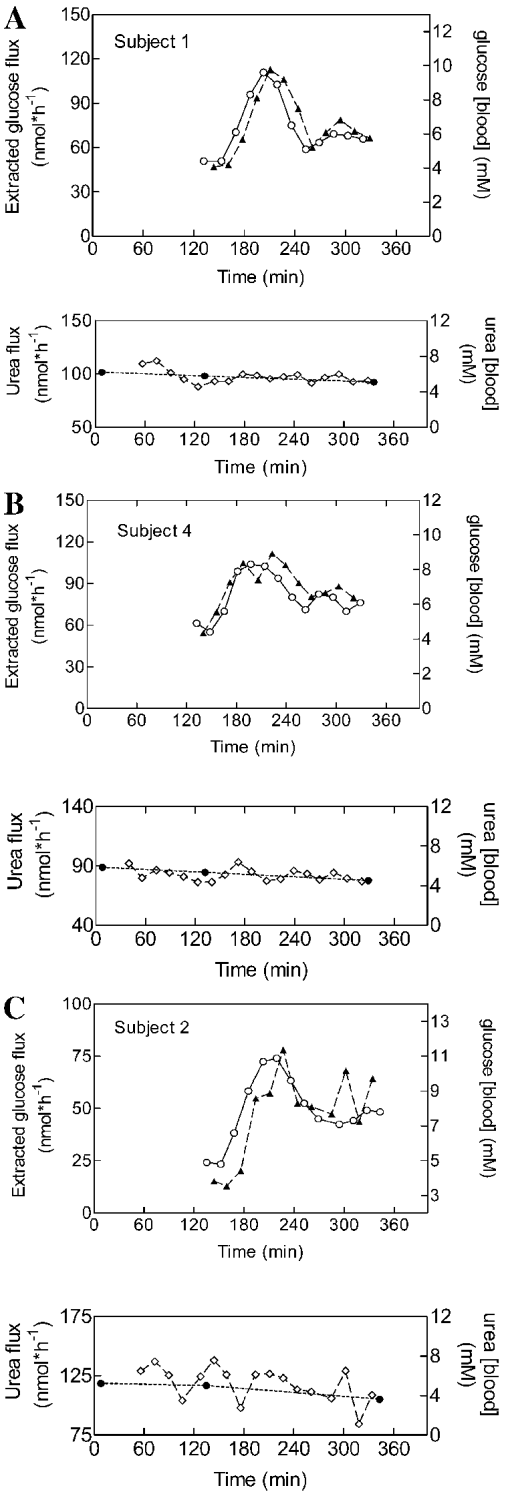

Fig. 1. Glucose (upper panels) and urea (lower panels) extraction profiles in 3 subjects. -○- blood glucose (mM), -▲- glucose extraction flux ($nmol \cdot h^{-1}$), -●- blood urea (mM), -●- urea extraction flux ($nmol \cdot h^{-1}$)

(Sieg *et al.,* submitted), meaning that J_{urea}/C_{urea} is greater than J_{glu}/C_{glu}, and that K should be less than 1 (see Eq. 4). Though the normalized urea fluxes (20.7 ± 5.6 $\mu l \cdot h^{-1}$) also showed some inter-individual variation (Table I), the coefficient of variation (27%) was reasonable.

Table I. Iontophoretic Glucose and Urea Extraction Fluxes Normalized to the Corresponding Blood Levels[a]

Subject	Blood glucose range (mM)	Blood urea range (mM)	Normalized glucose flux ($\mu l \cdot h^{-1}$)	Normalized urea flux ($\mu l \cdot h^{-1}$)	K ± SD	r^2
1	4.4–9.6	5.0–6.3	11.8 ± 0.9	17.6 ± 0.8	0.67 ± 0.04[c]	0.97
2	4.8–10.9	3.6–5.3	5.8 ± 2.1[b]	26.1 ± 3.6[b]	0.34 ± 0.05[d]	0.79
3	4.6–10.3	3.8–5.4	11.1 ± 3.1	16.5 ± 2.2	0.86 ± 0.11[c]	0.88
4	4.4–8.3	4.4–5.8	13.6 ± 1.5	16.5 ± 1.1	0.63 ± 0.09[c]	0.83
5	2.6–10.9	5.3–7.1	5.8 ± 1.5[b]	15.9 ± 2.1	0.44 ± 0.05[d]	0.89
6	3.7–7.6	5.0–6.2	4.6 ± 1.0[b]	28.5 ± 2.5[b]	0.16 ± 0.04[d]	0.62
3B	4.7–9.3	3.8–5.0	6.5 ± 1.1[b]	24.0 ± 2.7[b]	0.29 ± 0.07[d]	0.70

[a] The extraction constant K was determined from linear regression as shown in Fig. 2, and the goodness of fit is expressed with r^2.
[b] Significantly different from the fluxes of subjects 1–3 ($p < 0.001$).
[c,d] Slopes are not significantly different from one another.

The apparent "bimodal" behavior with respect to glucose electrotransport was reinforced when the urea and glucose data were combined and analyzed by Eq. 3. Figure 3 plots the ratio J_{glu}/J_{urea} against the corresponding ratio of the blood concentrations, and suggests quite persuasively that the subject population is divided into two subsets. Statistically, three of the seven regression slopes (i.e., the values of K given in Table I) were significantly different from the other four (0.72 ± 0.12 vs. 0.31 ± 0.12). Inspection of Table I reveals that this divergence originates primarily from the glucose data, rather than from those for urea, the CV for the normalized glucose fluxes being nearly twice that for urea. The effect is exacerbated by the fact that there is a weak inverse relationship between the measured values of J_{urea}/C_{urea} and J_{glu}/C_{glu}.

Mechanism

Mechanistically, it was anticipated that the normalized glucose and urea fluxes would be closely correlated given that both molecules are moved across the skin by the same principal mechanism, i.e., electroosmosis. The higher passive permeation of urea was expected to result in a value of K less than 1 (Sieg *et al.,* submitted) but, given the relatively stable values of J_{urea}/C_{urea}, this cannot account for the divergent extraction coefficients observed. Equally, it seems unlikely that the explanation lies in the potential interference of citrulline in the urea assay. Although this substance is present in the epidermis as a part of the skin's "natural moisturizing factor" (15,16), this reservoir will have been substantially, if not completely, depleted by the 2 h of iontophoresis (17), which were performed before any measurements were made. Rather, it appears that there are possibly a diverse range of biochemical processes ongoing in living tissue (Fig. 4) that are more likely to impact upon the amount of glucose extracted (as, in fact, has been observed).

Iontophoretic extraction is thought to occur via two major pathways, namely the appendageal (sweat glands, hair follicles) and intercellular routes (18). In human skin, sweat glands and hair follicles have been identified as important low-resistance pathways (19); these structures are, at the same time, highly metabolically active (20), and glucose serves as a critical substrate for energy production in these and other skin cells (21). It is possible, therefore, that a fraction of the glucose being extracted through the skin by electroosmosis is "conscripted" for metabolism and this would lead to a significant reduction in the apparent normalized flux.

Additional physiologic factors that may interfere with the iontophoretic sampling of analytes are also highlighted in Fig. 4. As previously mentioned, skin reservoirs of glucose (12) and urea exist but have not been quantified, and the kinetics, with which these reservoirs can be emptied, have only been studied empirically. Thus, despite the 2-h preiontophoresis period, there is no certainty at the moment as to whether the skin depot(s) has (have) been completely depleted. Furthermore, the skin surface is another complicating factor. For example, perspiration may leave an elevated

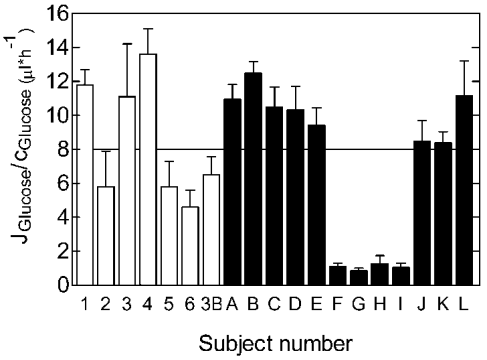

Fig. 2. Normalized reverse iontophoretic extraction fluxes of glucose *in vivo* (mean ± SD) from this study (□) and from a previous investigation (■). The subjects showing more efficient extraction (somewhat arbitrarily set at 8 $\mu l \cdot h^{-1}$) have an average electroosmotic flow of 10.7 (± 1.6) $\mu l \cdot h^{-1}$. For the less efficient group, the mean (± SD) convective flow is 3.3 (± 2.6) $\mu l \cdot h^{-1}$.

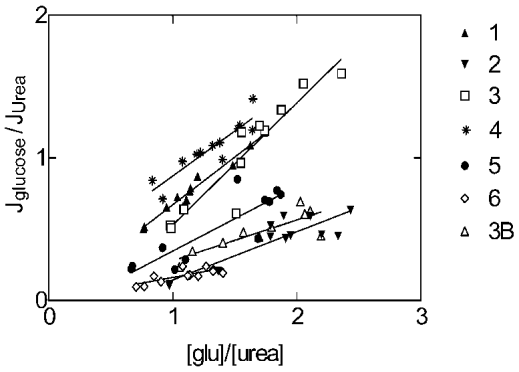

Fig. 3. Linear regressions of the ratio J_{glu}/J_{urea} against the corresponding ratio of their blood concentrations (C_{glu}/C_{urea}). The slope of each line corresponds to the value of K, as defined in Eq. 3.

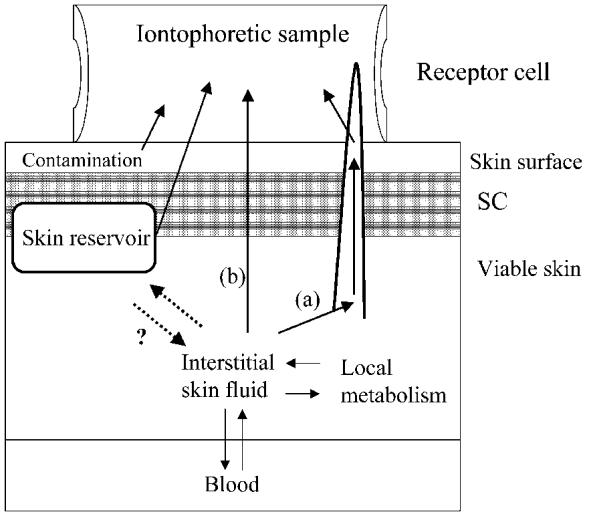

Fig. 4. Schematic diagram of the reverse iontophoresis sampling process. Iontophoresis extracts substances from the interstitial skin fluid (ISF) via the follicular (a) and the stratum corneum intercellular (b) pathways by electroosmosis, electromigration, and enhanced passive transport. Samples from capillary blood are used as references for concentration in the assumption that blood and ISF levels equilibrate relatively fast. ISF levels may also change as a consequence of local metabolism. Furthermore, a skin reservoir may exist due to local metabolism and distribution and/or SC differentiation. This depot, whose magnitude is not necessarily related to the ISF levels, will also be sampled by the application of iontophoresis. The skin surface may also contribute to the extracted sample via, for example, perspiration, microbial activity, or contamination from personal-care products. Finally, local metabolism and/or additional contamination may originate in skin appendages (including hair follicles and sweat glands).

amount of an analyte at the exit of the sweat gland (21,22); contamination of the skin surface by personal care products is also possible (23,24); and the skin microflora can be expected to play a role as well (25) in terms of glucose consumption and the apparent extraction rate of this analyte.

CONCLUSIONS

In summary, urea may prove to be a useful internal standard, in that its systemic concentration is quite stable and its reverse iontophoretic extraction flux proved to be quite constant (of course, this would probably not be the case for patients in severe renal failure who need to undergo regular dialysis). The electrotransport of glucose, however, continued to show inter-individual variability due to other factors that remain to be fully characterized. We would submit, therefore, that the real challenge for calibrating the reverse iontophoretic extraction of glucose via an internal standard is to understand and control the biochemical and metabolic processes occurring on and within the skin that have the potential to significantly perturb the outward movement of the analyte.

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REFERENCES

1. M. J. Tierney, J. A. Tamada, R. O. Potts, L. Jovanovic, and S. Garg, and Cygnus Research Team. Clinical evaluation of the GlucoWatch Biographer: a continual, non-invasive glucose monitor for patients with diabetes. *Biosens. Bioelectron.* **16**:621–629 (2001).
2. M. J. Pikal. The role of electroosmotic flow in transdermal iontophoresis. *Adv. Drug Del. Rev.* **9**:201–237 (1992).
3. G. B. Kasting, R. L. Smith, and E. G. Cooper. Effect of lipid solubility and molecular size on percutaneous absorption. In B. Shroot and H. Schaefer (eds.), *Skin Pharmacokinetics*, Karger AG, Basel, 1987, pp. 138–153.
4. A. Lopez Castellanos, R. H. Guy, and M. B. Delgado-Charro. Effect of area on the iontophoretic transport of phenylalanine and propanolol across the skin. *Int. Symp. Control. Rel. Bioact. Mater* **24**:405–406 (1999).
5. K. R. Pitzer, S. Desai, T. Dunn, S. Edelmann, Y. Yakalakshmi, J. Kennedy, J. A. Tamada, and R. O. Potts. Detection of hypoglycemia with the GlucoWatch Biographer. *Diabetes Care* **24**:881–885 (2001).
6. A. Sieg, R. H. Guy, and M. B. Delgado-Charro. Reverse iontophoresis for noninvasive glucose monitoring: the internal standard concept. *J. Pharm. Sci.* **92**:2295–2302 (2003).
7. A. Sieg, R. H. Guy, and M. B. Delgado-Charro. Non-invasive glucose monitoring by reverse iontophoresis in vivo: application of the internal standard concept, *Clin. Chem.* **50**:1383–1390 (2004).
8. J. A. Tamada and R. O. Potts. Glucose monitoring using electroosmotic transdermal extraction. In B. Berner and S. M. Dinh (eds.), *Electronically Controlled Drug Delivery*, CRC Press, Boca Raton, 1998, pp. 161–173.
9. L. Goeyens, N. Kindermans, M. A. Yusuf, and M. Elskens. A room temperature procedure for the manual determination of urea in seawater, *Estuarine. Coastal and Shelf Science* **47**:415–418 (1998).
10. P. F. Mulvenna and G. Savidge. A modified manual method for the determination of urea in seawater using diacetylmonoxime reagent, *Estuarine. Coastal and Shelf Science* **34**:429–438 (1992).
11. J. H. Zar. Comparing simple linear regression equations. In J. H. Zar (ed.), *Biostatistical Analysis*, Prentice-Hall, Englewood Cliffs, NJ, 1984, pp. 292–327.
12. G. Rao, R. H. Guy, P. Glikfeld, W. R. LaCourse, L. Leung, J. A. Tamada, R. O. Potts, and N. T. Azimi. Reverse iontophoresis: non invasive glucose monitoring in vivo in humans. *Pharm. Res.* **12**:1869–1873 (1995).
13. I. T. Degim, S. Ilbasmis, R. Dundaroz, and Y. Oguz. Reverse iontophoresis: a non-invasive technique for measuring blood urea level. *Pediatr. Nephrol.* **18**:1032–1037 (2003).
14. K. Wellner and W. Wohlrab. Quantitative evaluation of urea in stratum corneum of human skin. *Arch. Dermatol. Res.* **285**:239–240 (1993).
15. A. V. Rawlings, I. R. Scott, C. R. Harding, and P. A. Bowser. Stratum corneum moisturization at the molecular level. *J. Invest. Dermatol.* **103**:731–740 (1994).
16. P. J. Caspers, G. W. Lucassen, E. A. Carter, H. A. Bruining, and G. J. Puppels. In vivo confocal raman microspectroscopy of the skin: noninvasive determination of molecular concentration profiles. *J. Invest. Dermatol.* **116**:434–442 (2001).
17. F. Jeanneret. Etude du liquide interstitial cutané comme matrice alternative lors de suivi thérapeutique: couplage LC-MS pour l'analyse des acides aminées extraits par ionophorèse inverse in vitro et in vivo. Ph.D. Thesis, University of Geneva, 2004.
18. C. Cullander. What are the pathways of iontophoretic current flow through mammalian skin? *Adv. Drug Del. Rev.* **9**:119–135 (1992).
19. O. D. Uitto and H. S. White. Electroosmotic pore transport in human skin. *Pharm. Res.* **20**:646–652 (2003).

20. K. Sato. The physiology, pharmacology, and biochemistry of the eccrine sweat gland. *Rev. Physiol. Biochem. Pharmacol.* **79**:51–131 (1977).
21. R. K. Freinkel. Carbohydrate metabolism of epidermis. In L. A. Goldsmith (ed.), *Physiology, Biochemistry, and Molecular Biology of the Skin*, Oxford University Press, New York, 1993, pp. 452–461.
22. S. W. Brusilow. The permeability of the sweat gland to non-electrolytes. *Bibl. Paed.* **86**:32–40 (1966).
23. E. Proksch. Urea in dermatology. *Deut. med. Wochenschr.* **119**: 1126–1130 (1994).
24. H. Pratzel and K. Geiger. Zur Biochemie der freien Aminosäuren im Stratum Corneum menschlicher Epidermis I. Die Argininase-Reaktion. *Arch. Derm. Res.* **259**:151–156 (1977).
25. R. A. Bojar and K. T. Holland. Review: the human cutaneous microflora and factors controlling colonisation. *World J. Microb. Biot.* **18**:889–903 (2002).