Skin Mini-Erosion Sampling Technique: Feasibility Study with Regard to Serial Glucose Measurement

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Purpose. To describe a dermally non-invasive serial sampling technique and to test its clinical feasibility with regard to glucose measurement.

Methods. A standardized skin mini-erosion devoid of the epidermal barrier, and covered by an artificial one, was formed by a suctioning technique. Interstitial fluid (IF) was extracted serially by brief application of negative pressure, and its glucose content compared with that in capillary or venous blood samples.

Results. The procedure caused no discomfort. The epidermis regenerated rapidly after experimentation. There were no complications. In non-diabetic subjects (n = 13) the mean of all IF values measured daily for 6 days was 6.2 ± 0.1 mmol/l (±SE). The corresponding capillary blood glucose value was 5.6 ± 0.1 mmol/l, and the venous glucose value was 5.4 ± 0.1 mmol/l. The differences between IF glucose values and invasive control values remained within narrow limits throughout. The 2SD limits of agreement for the differences were 1.44 mmol/l (IF vs. capillary blood samples) and 1.76 mmol/l (IF vs venous samples) respectively. The OGTT curves suggested glucose kinetics to be similar in IF and in capillary blood. In diabetic subjects, the mean of IF values determined serially during one day was 15.3 ± 1.0 mmol/l (range, 6.7–21.8 mmol/l), and the corresponding mean capillary value was 12.0 ± 0.9 mmol/l (range, 3.3–17.2 mmol/l). The ICC for all paired photometric observations was 0.948.

Conclusions. The results suggest the new sampling technique to be a feasible approach for clinical and experimental purposes. A functionally integrated sampling patch is entering the clinical testing stage.

KEY WORDS: transdermal access; skin erosion; transdermal; dermal interstitial fluid; sampling; glucose; monitoring; diabetes mellitus; pharmacokinetics.

INTRODUCTION

Most of the disadvantages of invasive sampling procedures (pain, bleeding, messy procedure, soreness, infection, recannulation required, need of specialist personnel, occupational risk) emanate from the required penetration of the composite dermal tissue by a needle, cannula or mini-lancet. The demand for self-monitoring of glucose by serial sampling has provided the impetus for the development of alternative methods where dermal penetration is avoided or at least minimized (1–3). The problems of reliably eliminating the variation in transdermal passage induced by the epidermis and its barrier, or serially extracting minute sample volumes from dermal or subcutaneous tissue have proved difficult to overcome. The techniques are at different stages of development and none has as yet been implemented clinically on a large scale.

One of the aims of our research is to develop a more generally applicable clinical and experimental alternative to the invasive route. In order to avoid dermal invasivity we form a skin mini-erosion with a mild suctioning technique, thus providing dermal access for the purpose of systemic drug delivery or sampling (4–7). As only the epidermal barrier with adjoining epidermal cells is split off, the dermis remains intact without penetration, and the epidermis regenerates readily after the intervention. This painless and bloodless technique has been tested as a means of systemic drug delivery (7), using a simpler mechanical device pre-filled with drug (Morphine Cellpatch$_R$, Epiport Pain Relief AB, Sweden), and the clinical trials required for drug registration in Sweden have been completed. This device not only provides an integrated means of erosion formation and drug delivery but, being sealed to the skin throughout, it also acts as an artificial (epidermal) barrier. For sampling, the brief application of negative pressure via the device has been shown to extract a volume of IF fully adequate for analytical purposes. The procedure becomes repeatable since local dehydration is avoided, the device itself constituting an artificial seal replacing the normal epidermal barrier. Access to IF in this way may allow measurement of the entire range of molecules entering the dermal interstitial space. In developing this technique, glucose measurement is the first clinical priority.

The present study is one of three parallel studies carried out at the universities of Lund and Oxford, where the basic feasibility of the suction sampling technique was tested in serial measurements both in diabetic and non-diabetic adults (8) and in in newborn infants (9). In this study, we assessed how many days representative glucose measurements might be obtained from IF sampled from a given occlusive mini-erosion, and whether serial IF samples during a single day would yield representative glucose values. Diabetic subjects were entered into this latter experiment only. We measured IF glucose content photometrically and compared the results with those of photometric and enzymatic measurements in blood sampled with conventional invasive techniques.

METHODS

Subjects

The study was undertaken in volunteers after approval by the Ethics Committee of Lund University and in accordance with the principles of the Declaration of Helsinki. The participants were 13 non-smoking non-diabetic subjects (four women), aged 23–43 years (mean, 26 years) and eight randomly selected patients with diabetes mellitus (two women), aged 30–55 years (mean, 42 years), seven of whom had had diabetes for 7–22 years while the eighth had only recently been diagnosed. All but one were on insulin treatment. Two of them were smokers. The patients were admitted to the Diabetes Day Care Center, University Hospital, Malmö, for treatment of poor diabetes

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ABBREVIATIONS: IF, dermal interstitial fluid; OGTT, oral glucose tolerance test; ICC, interclass correlation coefficient.

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control. All experimental procedures took place at room temperature (68–72°F).

Mini-erosion Formation, IF Sampling and Glucose Measurement

A small suction cup (6 mm diameter) with an acrylic adhesive flange (3M, USA) was airtightly connected to a volume expander. The expander (Epiport, Sweden) consisted of a 5 ml cylinder with a piston that could be retracted and locked at an expansion corresponding to a pressure of 180 mmHg below atmospheric. This allowed negative pressure to be maintained for as long as desired. The device was adhesively applied to skin on the abdomen below and lateral to the umbilicus, and suction was activated, see Fig. 1A. The device was warmed to about 38°C by means of a chemical warming pad (Ingkro, Sweden). An epidermal vesicle (6 mm diameter) was formed and removed in 15–70 minutes, exposing a mini-erosion. During each sampling, a similar suction cup was applied over the erosion, and IF was extracted by suctioning at 180 mmHg below atmospheric pressure for 20 minutes (per sampling), see Fig. 1B. The cup was perforated and a 5 μl fraction of the fluid sample was transferred to a microcuvette (B- Glucose microcuvette, HemoCue, Sweden) by capillary action (Fig. 1C) and then measured with a portable photometer (HemoCue). Except for the measurements made in IF, the recommendations of the manufacturer were strictly followed. Excess IF was absorbed with a piece of gauze before applying and after disengaging the sampling suction cup. Between each sampling procedure and for 4 days after the end of experimentation, the mini-erosion was covered by a small piece of adhesive film (Tegaderm, 3M, USA).

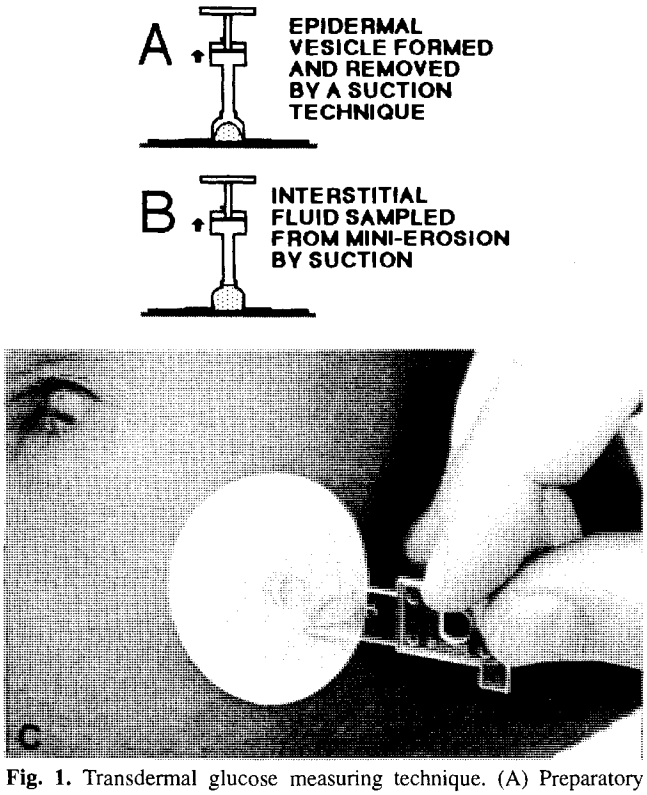

Fig. 1. Transdermal glucose measuring technique. (A) Preparatory step: vesication by suctioning and removal of vesicle using tweezers. (B) Sampling interstitial fluid. In between the sampling procedures the mini-erosion is covered by an adhesive patch. (C) The fluid sample is absorbed into a microcuvette which is measured with a portable photometer.

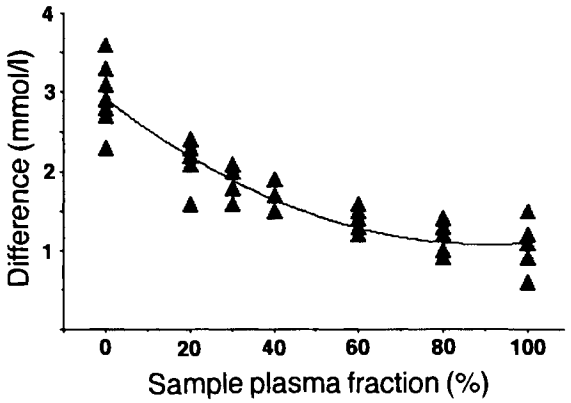

Fig. 2. Glucose determined photometrically and enzymatically in a plasma dilution series where each step of the series contained the same glucose concentration. Differences between photometrically and enzymatically measured glucose values are given.

In the clinically applied microcuvette technique, glucose is measured in hemolyzed blood by means of a modified glucose dehydrogenase method where the reagents, including the haemolysing agent, saponin, and a chromogenic substance, are deposited on the inner walls of the capillary slit in the microcuvette. The chemical reaction yields a coloured compound, the end-point value of which is proportional to the glucose concentration in the haemolysed blood and can be read off on the photometer after 90–240 sec. The technique is calibrated for use on whole blood. The precision of the technique (within-run and between-day) is quite acceptable (10). The coloured compound is distributed in the sample water fraction and not in its protein fraction. A large content of protein will decrease the total concentration of coloured compound in the sample, and vice versa. Since the protein content in IF is lower than that in haemolysed whole blood, a simple experimental assessment was made of the relationship between measurements made in capillary blood and IF. Fresh plasma was sampled from 7 healthy subjects, each plasma sample being diluted stepwise with a glucose solution (5,5 mmol/l). The diluted samples were thoroughly mixed and measured using the HemoCue microcuvette and a protein insensitive enzymatic method adapted to an Instrumentation Laboratory Monarch centrifugal analyser (Warrington, Cheshire, UK). The curve for the relationship is shown in Fig. 2. Obviously the lack of correspondence between haemolysed blood (processed blood sample) and whole plasma, and between interstitial fluid (IF sample) and diluted plasma does not allow direct extrapolation. The findings may mean, however, that the measurements in IF with the HemoCue technique will yield systematically higher values.

Invasive Glucose Measurements

Capillary blood was obtained by pricking finger pulp skin with a lancet (Minilancet, Clean Chemicals Sweden AB). The blood was absorbed into a HemoCue microcuvette and measured with a photometer as described above. Venous blood from a cannulated antecubital vein was collected in heparinized vacutainer tubes and centrifuged for 10 min at 3000 rpm at 4°C. After separation, the plasma glucose content was measured

(enzymatically) with a Beckmann Glucose Analyzer (Beckmann Instruments, Fullerton, CA). The capillary and venous blood was sampled approximately 10 min after starting the transdermal extraction.

Experiments

In all subjects, general observations (discomfort, local findings, fluid sample qualities) were made in connection with each step of the procedure.

(A) All non-diabetic subjects underwent daily (paired) measurements of transdermal and capillary glucose using one mini-erosion for 6 days. In 8 of the 13 subjects, plasma glucose content was also measured. (B) Five of the 13 subjects underwent a standard 75 g oral glucose tolerance test (OGTT) after fasting overnight (on day 3). Glucose was measured in IF and in capillary blood at -30, 0, 30, 60, 90 and 120 minutes. In one subject a series of glucose measurements were made during 3 hours without glucose ingestion. (C) In the 8 diabetic patients, glucose measurements in IF and capillary blood were made 3 times during one day (after overnight fasting, after exercise and after lunch).

Calculations and Statistical Analysis

The limits of agreement were defined as twice the standard deviations of the differences between two sets of values over the 6 days of the experiment. For estimating correspondence between all IF and capillary measurements, a two-way ANOVA for repeated measurements independent of day was used to obtain the interclass correlation coefficient (ICC).

RESULTS

General Observations

Neither formation of the mini-erosions nor the sampling caused discomfort. A light tingle was usually felt at some point during formation of the epidermal vesicle. There were no complications. The lowest rates of effective fluid formation were noted on day 1. Only on day 6 were there occasional signs of decreased extraction, three of the 13 samples being found to produce less than about 5 μl fluid. Confluent petechiae were observed in the erosion in all cases. The fluid sample was always clear with no visible trace of blood. There were no signs of inflammation in adjacent skin. After the experimentation epidermal regeneration was rapid in all cases. A trace of pigmentation, resembling that seen transiently after transdermal drug delivery, was noted in all cases. It was considered trivial by all subjects.

Glucose Measurements in IF vs Capillary and Venous Blood

In the 13 non-diabetic subjects the mean of all glucose values in IF (day 1 to day 6), was 6.2 ± 0.1 mmol/l (±SE), and the corresponding mean capillary glucose value was 5.6 ± 0.1 mmol/1. The mean venous blood glucose value was 5.4 ± 0.1 mmol/1. The differences between all the paired IF and capillary blood glucose values measured during the 6 days,

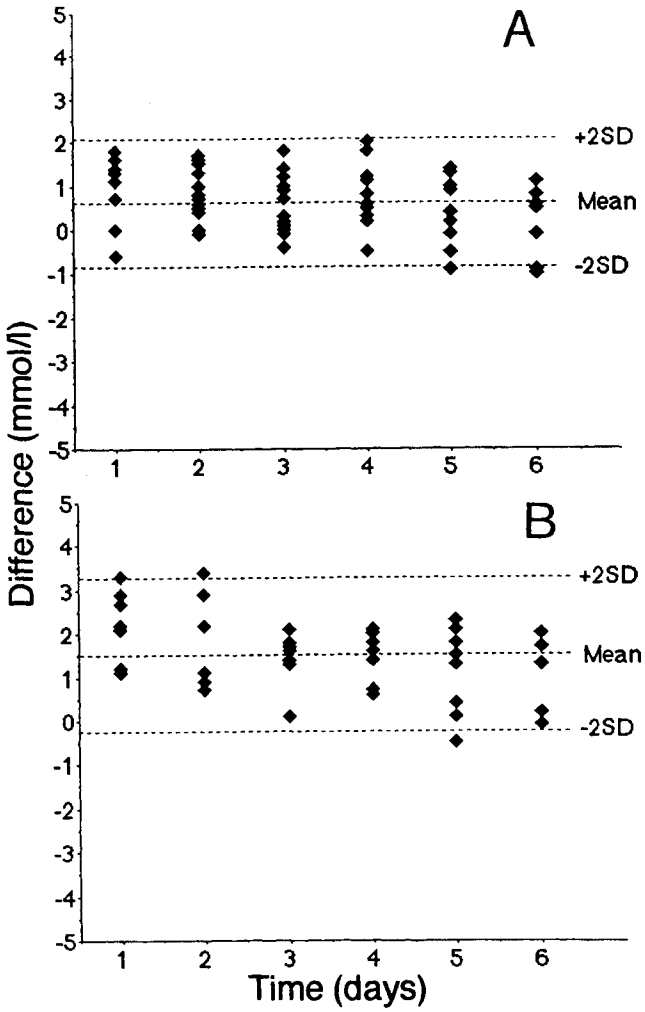

Fig. 3. (A) Difference between interstitial fluid (IF) glucose values and capillary blood glucose values on day to day basis (photometric measurements). (B) Difference between interstitial fluid (IF) glucose values and venous blood glucose values on day to day basis (photometry vs enzymatic).

with limits of agreement, are given in Fig. 3A. The 2SD limit was 1.44 mmol/l. The corresponding differences for the venous glucose values are given in Fig 3B; the 2SD limit was 1.76 mmol/l. The glucose values measured in the (effluent) venous blood also reflects glycolysis occurring during the delay before routine processing at the hospital laboratory could be undertaken. This was the case on day 1 and day 2 in particular. The OGTT curves are shown in Fig. 4. The IF glucose values during the 3-hour steady state period without glucose ingestion were 6.3, 7.1, 6.4, 6.5, 6.4, 6.8 and 6.5 mmol/l. In the diabetic subjects, the mean of all IF values was 15.3 ± 1.0 mmol/l (range, 6.7–21.8 mmol/1), and the corresponding capillary values were 12.0 ± 0.9 mmol/l (range, 3.3–17.2 mmol/1). A scatter plot of all IF and capillary values measured in both non-diabetic and diabetic subjects is shown in Fig. 5. The overall range of measured values was 4.4–21.8 mmol/l in IF and 3.3–17.2 mmol/l in capillary blood. The ICC for the observations was 0.948.

DISCUSSION

The basis for the new sampling method is the suction blister experimental technique. This technique has been extensively used

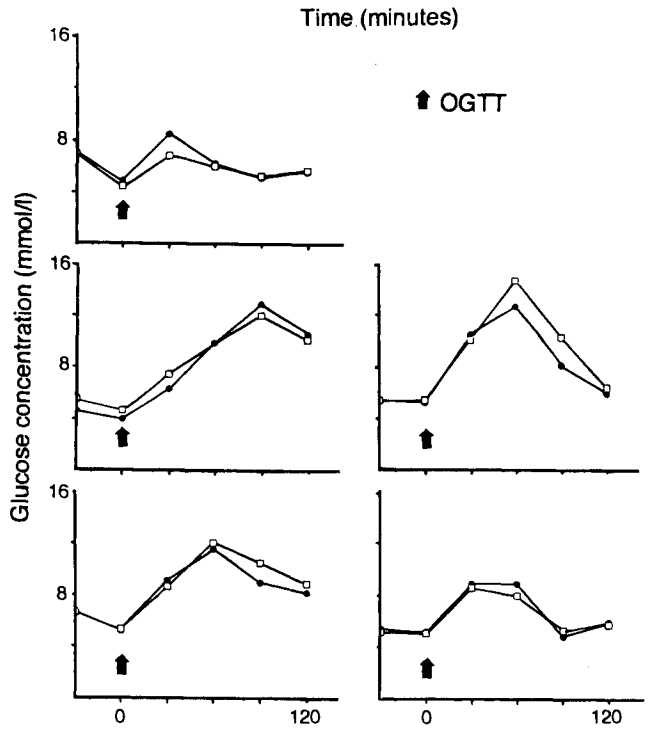

Fig. 4. Oral glucose tolerance test curves in 5 non-diabetic subjects (photometric measurements). □: IF glucose values; ●: Capillary blood glucose values.

in such fields as dermatology (11) and pharmacokinetics (12). In order to allow serial sampling the contents from several vesicles on each subject under investigation are commonly used. Patients afflicted with a number of dermatological diseases, diabetes (13), and an edematous limb after vascular reconstruction (14) have been studied. In spite of the fact that the epidermal barrier is removed, no complications of note have ever been reported. This indicates the efficacy of the dermal protective/regenerative response induced by the suctioning stimulus, findings that are corroborated by our own experimental and clinical experience.

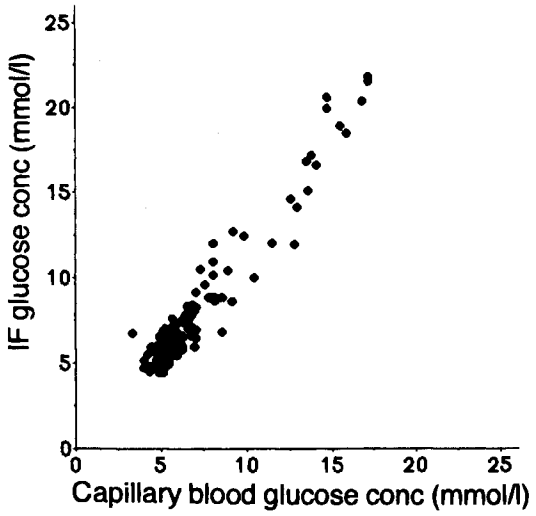

Fig. 5. Plot of all paired photometric measurements of glucose in IF and capillary blood.

The suction induced split always occurs through the lamina lucida of the epidermal basement membrane, independent of the thickness of the overlying epidermal barrier (11) and the dermis, including the dermal microcirculatory network, remains almost completely intact (11,15–17). We have validated use of the mini-erosion for dermal access in a series of studies (4–7,15–19). Of interest are our findings concerning the microvascular aspects of the dermal protective/regenerative response (5,16–19). Taken together, these findings indicated the presence of a marked hyperemic reaction with sudden onset during suctioning and formation of the vesicle, tapering off only gradually over days (16–18), and which was little changed in the presence of an added element of pronounced inflammation (u.v.-irradiation stimulus) (19). Re-epithelialization occurred rapidly and was not retarded by the u.v.-irradiation (19). Oxygen measurements showed no sign of tissue hypoxia in the erosion (5,19). Phagocytic and chemotactic leukocytes are the first line of defense against infection in injured tissue.These cells have an increased demand for nutrients (20), and their killing capacity and tissue oxygen tension are directly related (21,22). Thus, the hyperemia and the normal tissue oxygenation that are present in the mini-erosion ensure a swift and efficient response to invasion of bacteria. The occlusive environment, which is a necessary feature of our technique, prevents contamination as well as dehydration, and facilitates re-epithelialization (23). Suction, applied even to the sensitive tissues of an ischemic skin wound, may enhance healing (24,25). Outwards drainage, which occurs spontaneouly from any erosion, is the established treatment of a locus of infection in the skin. The presence of the lamina densa of the epidermal basement membrane facilitates re-epithelialization (26), which occurs rapidly and completely once the stimulus produced by the serial sampling procedure is brought to an end. By contrast, skin blisters produced by compression or burns, may be similar in appearance but are characherized by extensive microcirculatory damage, local ischemia and necrosis and constitute a *locus minoris resistentiae.* Use of suction "blister" in the present context is thus a misnomer since the major clinical properties of the application are just the opposite of those found in traumatic blisters. Since there is no loss of continuity of composite tissue, no scar forms (11). The slight pigmentation disappears with time (7,11). There is consensus in the literature (11) that suction induced skin vesication is painless. Our own findings, and those of our collaborators (8,9), suggest this also to be the case in serial sampling by suctioning.

We found that sampling could proceed serially during one day (in non-diabetic and diabetic subjects) and even for days (in non-diabetic subjects) on one and the same erosion, findings consistent with those of our collaborators (8, 9). In the parallel diabetic subject study (8), it was shown that IF could be sampled serially during OGTT on day 1 and on day 3 after forming an erosion. The epidermis regenerated readily and there were no complications. These findings suggest the presence of an adequate dermal protective/regenerative response even in diabetic subjects (13,14,27). Significant diabetic skin microangiopathy may increase the risk of infection in connection with invasive sampling. The question as to whether the absence of dermal invasivity in our technique will affect the infection rate differently in such patients may be studied using an optimized suction sampling technique. In the study in neonates (28–42 gestational weeks) (9), it was shown that erosions formed reproducibly, and that the infants could be sampled 4 times a day at least for 3 days from one and the same erosion. They were not disturbed

by the intervention, the epidermis regenerated readily and there were no complications. Our collaborators found the IF extraction rates to range from 1.7 μl/min to 3.7 μl/min (8,9). Samples extracted during 10 min allowed monitoring of complex metabolic events during OGTT (8). The simple, reproducible serial access to relatively voluminous IF samples is an important advantage of our approach (8).

The fluid obtained from the epidermal vesicle appears to be comparable to interstitial fluid (11,28,29). The composition of IF repeatedly extracted from one and the same erosion is likely to reflect a state of mild inflammation. Epidermal vesicle glucose concentrations have been found to closely parallel venous glucose concentrations over a wide range of values (27). Glucose equilibrates freely into the interstitial space. The paired glucose values in IF and blood were similar during each of the OGTT courses shown in Fig. 4, and the difference between the two sets of glucose values shown in Figs. 3A and 3B remained within narrow ranges in the non-diabetic subjects during the six days of sampling at a single site. Thus, there were no signs of increasing divergence between glucose concentrations in IF and blood over time. The consistent, small positive shift in the IF values underscores the need to calibrate the colorimetry on IF rather than blood. Even without correction for this systematic difference, the observed 2SD limits of agreement—with glucose values in a normal range—are consistent with clinical utility, as are the findings in diabetic subjects, covering measurements over a wide range of glucose values. The OGTT curves suggest glucose kinetics to be similar in IF and in capillary blood. These results are corroborated in our other feasibility study where glucose concentrations in IF, measured by an enzymatic technique, were found to closely parallel those in arterialized venous blood during OGTT (8). Moreover, the scatter plot findings provide an indication that photometry may be suitable as a measuring technique.

The basic feasibility of using a new dermally non-invasive sampling technique has been documented in a step by step approach. A functionally integrated Sampling Cellpatch (Epiport, Sweden) is now entering the clinical trials phase. The indications and contraindications for this technology will be evaluated, primarily concerning its clinical use for serial glucose measurement in newborn infants and diabetic subjects. On-site automatic monitoring over several days may become feasible using this technology. The technique also has potential as a sampling tool for other substances of analytical interest in clinical medicine (8), and it may find applications in pharmacokinetic studies (12) for measuring drug concentrations in the peripheral compartment and drug tissue penetration.

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REFERENCES

1. B. H. Ginsberg. An overview of minimally invasive technologies. *Clin. Chem.* **38**:1596–1600 (1992).
2. P. Glikfeld, R. S. Hinz, and R. H. Guy. Noninvasive sampling of biological fluids by iontophoresis. *Pharm. Res.* **6**:988–90 (1989).
3. F. J. Service, P. C. O'Brien, S. D. Wise, S. Ness, and S. M. LeBlanc. Dermal interstitial glucose as an indicator of ambient glycemia. *Diabetes Care* **20**:1426–9 (1997).
4. P. Svedman, S. Lundin, and Ch. Svedman. Administration of antidiuretic peptide (DDAVP) by way of suction deepithelialised skin. *Lancet* **337**:1506–9 (1991).
5. P. Svedman, M. Lundquist, and Ch. Svedman. Oxygen tension on the surface of suction blister wounds measured with a polarographic technique in healthy volunteers. *Vasc. Surg.* **26**:440–50 (1991).
6. D. Westerling, P. Höglund, S. Lundin, and P. Svedman. Transdermal administration of morphine to healthy subjects. *Br. J. clin. Pharmac.* **37**:571–6 (1994).
7. P. Svedman, S. Lundin, P. Höglund, C. Hammarlund, C. Malmros, and N. Pantzar. Passive drug diffusion via standardized skin mini-erosion; methodological aspects and clinical findings with new device. *Pharm. Res.* **13**:1353–8 (1996).
8. Ch. Svedman, J. S. Samra, M. L. Clarke, J. C. Levy, and K. N. Frayn. Skin mini-erosion technique for determining metabolic response in terms of interstitial fluid variables, tested in diabetic and non-diabetic subjects undergoing OGTT. *Scand. J. Clin. Lab. Invest.* Submitted.
9. I. Hansen, K. Håkansson, S. Polberger, N. Svenningsen, and P. Svedman. Dermally non-invasive suctioning technique for painless serial sampling of interstitial fluid (ISF) in newborn infants. *Abstract, The American Pediatric Society Meeting, New Orleans, May 1998.* Submitted.
10. P. A. M. Weiss, F. Kainer, M. C. H. Hauesler, P. Pürstner, and R. Urash. Schnellmetode zum Diabetesscreening in der Schwangershaft. *Geburtsh. u. Frauenheilk.* **54**:697–701 (1994).
11. U. Kiistala. *The suction blister method for the in vivo separation of epidermis from dermis in human skin. Dissertation thesis,* Helsinki, 1976.
12. K. B. Hellum, A. Schreiner, A. Digranes, and I. Bergman. Skin blisters produced by suction: a new model for studies of penetration of antibiotics in humans. In W. Siegenthaler and R. Lüthy (eds.), *Current chemotherapy: proceedings of the 10th International Congress of Chemotherapy,* Zürich, 1977. Washington, American Society of Microbiology, 1978, pp 620–6.
13. J. E. Bernstein, L. E. Levine, M. M. Medecina, C. M. Yung, and K. Soltani. Reduced threshold to suction-induced blister formation in insulin-dependent diabetics. *J. Am. Acad. Dermatol.* **8**:790–1 (1983).
14. R. Haaverstad, I. Romslo, S. Larsen, and H.O. Myhre. Protein concentration of subcutaneous interstitial fluid in the human leg. A comparison between the wick technique and the blister suction technique. *Microcirc.* **16**:111–7 (1996).
15. N. Kutlu and P. Svedman. Suction blister wounding, morphological and functional aspects. *Vasc. Surg.* **26**:388–99 (1992).
16. N. Kutlu and P. Svedman. The superficial dermal microcirculation in suction blister wounds in healthy volunteers. *Vasc. Surg.* **26**:200–12 (1992).
17. N. Kutlu and P. Svedman. The microcirculation i suction blister wounds on the upper and lower limb in healthy volunteers. *Vasc. Surg.* **26**:706–11 (1992).
18. P. Svedman, C. Svedman, and T. Njalsson. Epithelialization and blood flow in suction blister wounds on healthy volunteers. *J. Invest. Surg.* **4**:175–189 (1991).
19. C. Svedman, C. Hammarlund, N. Kutlu, and P. Svedman. A suction blister wound model exposed to u.v. irradiation: a burn wound model for use in humans. *Burns* **17**:41–6 (1991).
20. D. J. Weisdorf, P. R. Craddock, and H. S. Jacob. Glycogenolysis versus glucose transport in human granulocytes: differential activation in phagocytosis and chemotaxis. *Blood* **60**:888–93 (1982).
21. L. Beaman and B. L. Beaman. The role of oxygen and its derivates in microbial pathogenesis and host defense. *Annu. Microbiol.* **38**:27–48 (1984).
22. D. C. Hohn. Oxygen and leucocyte microbial killing. In J. C. Davis and T. K. Hunt (eds.), *Hyperbaric oxygen therapy.* Bethesda: Undersea Medical Society, 1977, pp 101–10.
23. C. D. Hinman and H. Maibach. Effect of air exposure and occlu-

sion on experimental human skin wounds. *Nature* **200**:377–8 (1963).
24. P. Svedman. Irrigation treatment of leg ulcers. *Lancet* **II**:532–4 (1983).
25. B. Arnljots and P. Svedman. Irrigation treatment in the split thickness skin grafting of difficult ulcers. *Scand. J. Plast. Reconstr. Surg.* **15**:35–40 (1985).
26. B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J. D. Watson. *Molecular biology of the cell.* Garland Publishing, Inc. New York & London, 1989.
27. B. M. Jensen, P. Bjerring, J. S. Christiansen, and H. Ørskov. Glucose content in human skin: relationship with blood glucose levels. *Scand. J. Clin. Lab. Invest.* **55**:427–32 (1995).
28. B. J. Vermeer, F. C. Reman, and C. M. van Gent. The determination of lipids and proteins in suction blister fluid. *J. Invest. Dermatol.* **73**:303–5 (1979).
29. S. Groth and B. Staberg. Suction blisters of the skin: a compartment with physiological, interstitium-like properties. *Scand. J. Clin. Lab. Invest.* **44**:311–6 (1984).