Reverse Iontophoresis: A New Approach to Measure Blood Glucose Level

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ABSTRACT

This study focused on the investigation of the possibility of monitoring blood glucose levels in humans by non-invasively extracting glucose and lactate from blood through the skin using reverse iontophoresis. *In vitro* reverse iontophoresis studies have indicated that the optimum switching mode for reverse iontophoresis of lactate and glucose are continuous direct current and direct current with electrode polarity reversal every 15 minutes, respectively. The application of a current combined with electrode polarity reversal every 15 minutes has been suggested for use in humans. The reverse iontophoresis technique was applied to 10 healthy volunteers. Glucose and lactate were successfully extracted through the subjects' skin into the methylcellulose gel of the electrodes. A fair-good correlation ($r^2 = 0.62$) between the subject's blood glucose level and the ratio of glucose to lactate levels in the collection gels was observed after two outliers were removed from the regression equation. The result suggests that it may be possible to non-invasively monitor the blood glucose levels using this new approach free of the need for calibration with a blood sample.

***Key words*:** reverse iontophoresis, iontophoresis, glucose, monitoring, non-invasive.

1. INTRODUCTION

Epidemiological studies have shown that the prevalence of diabetes is steadily increasing and is a widespread problem in modern society (Wild, Roglic, Green, Sicree & King, 2004; Amos, McCarty & Zimmet, 1997; King & Rewers, 1993). The number of cases of diabetes worldwide in 2000 among adults ≥ 20 years of age was estimated to be 171 million (Wild et al., 2004) and in 2003 this was estimated to be 194 million (International Diabetes Federation, 2003). By 2025, the number of people with diabetes is expected to exceed 333 million (International Diabetes Federation, 2003).

Diabetes increases the risk of ill health and shortens life. It is the leading cause of blindness and visual impairment (International Diabetes Federation, 2003; Evans, 1995), amputation (Davis, Kuznicki, Praveen & Sferra, 2004; International Diabetes Federation, 2003), cardiovascular disease (International Diabetes Federation, 2003; Laing et al., 1999a, 1999b; Stephenson, Kenny, Stevens, Fuller & Lee, 1995), stroke (International Diabetes Federation, 2003; Stephenson et al., 1995; Bell, 1994) and kidney failure (International Diabetes Federation, 2003; Brancati et al., 1997; Wang, Head, Stevens & Fuller, 1996; Stephenson et al., 1995).

Evidence has shown that tight monitoring and control of blood glucose levels can reduce the prevalence of complications in both Type 1 and Type 2 diabetes

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(The UK prospective diabetes study group, 1998a, 1998b, 1998c, 1998d). To date, most Type 1 and Type 2 diabetic patients measure their own blood glucose several times a day by obtaining finger-prick capillary samples and applying the blood to a reagent strip for analysis in a portable meter (Pickup, 2003). However, this method is painful, cumbersome, aesthetically unpleasant and inconvenient. More importantly, this method is not a continuous monitoring method and therefore blood glucose monitoring cannot be performed during sleeping and whilst the subject is occupied, such as during driving a motor vehicle. This provides considerable impetus for the development of non-invasive methods for continuous blood glucose monitoring.

Skin provides a unique gateway for non-invasive transdermal monitoring. Recently, the reverse iontophoresis technique has been used for patient monitoring (Degim, Ilbasmis, Dundaroz & Oguz, 2003; Potts, Tamada & Tierney, 2002; Pitzer et al., 2001; Tierney et al., 2001; Tamada et al., 1999; Rao et al., 1995) and non-invasive diagnosis (Merino, Lopez, Hochstrasser & Guy, 1999; Mize, Buttery, Daddona, Morales & Cormier, 1997; Numajiri et al., 1993). Reverse iontophoresis refers to the passage of a low level of current through the skin to promote the transport of both charged and neutral molecules (Merino, Kalia & Guy, 1997). The major mechanisms are either the electromigration of charged species to the electrode of opposite polarity, electro-osmosis of neutral molecules to the cathode or anode, or a combination of these two processes. Topically, the most successful reverse iontophoresis application for patient monitoring has been non-invasive and continuous blood glucose detection (Potts et al., 2002; Tierney et al., 2001; Rao et al., 1995). GlucoWatch® biographer is the best example of a commercial product utilising the techniques of reverse iontophoresis and biosensor to non-invasively and continuously monitor blood glucose. However, GlucoWatch® still has some problems, such as it does not reliably detect hypoglycaemia (The Diabetes Research in Children Network (DirecNet) Study Group, 2004). Also, before each use, GlucoWatch® must be calibrated with a blood sample assayed in the conventional way. This provides considerable impetus for the improvement of the existing system or the development of a new system, which is free of the need for calibration with a blood sample, to non-invasively and continuously monitor blood glucose level.

Lactate is known to be a major regulatory substrate in carbohydrate (e.g. glucose) metabolism. Therefore, lactate may be also a good parameter to monitor blood glucose level. Until now, no research has been done on investigating reverse iontophoresis of lactate in humans. Therefore, in this study, glucose and lactate were used as parameters to establish a new approach free of the need for calibration with a blood sample to measure blood glucose level in humans by reverse iontophoresis.

Hydrogen and hydroxyl ions are the two ionic species inherently present in the skin. Use of constant direct current (DC) may localise hydroxyl ions at the anodal skin region and hydrogen ions at the cathodal skin region. This process can cause a polarization of the skin which leads to stinging and erythrema (Howard, Drake & Kellogg, 1995). The use of pulsed DC, a constant DC delivered

periodically, has been suggested to prevent skin polarization (Chien, Lelawongs, Siddiqui, Sun & Shi, 1990). During the "off time," the skin naturally depolarizes itself and returns to its near initial electric condition. The use of a bipolar current, a constant DC which changes its current flow direction periodically, has also been suggested to overcome skin polarization (Tomohira, Machida, Onishi & Nagai, 1997; Howard et al., 1995). Tomohira et al. (1997) used an *in vivo* rat abdominal skin model to study the effect of electrode polarity switching (i.e. bipolar DC current profile) in iontophoresis of insulin and calcitonin. They found that bipolar DC current profile could enhance the absorption of the insulin and calcitonin. Hirvonen, Hueber and Guy (1995) used a diffusion cell with mouse skin to study the impact of different applied current profiles in regulating the permeation of two charged amino acids, lysine and glutamic acid. They found that bipolar current profiles and constant DC current profile resulted in comparable transport rates, which are higher than that of pulsed DC current profile. Thus, in order to achieve a high transport rate, it is better to use bipolar current profile or constant DC current profile rather than pulsed DC current profile. However, constant DC is thought to cause a polarization of the skin which leads to stinging and erythrema. Therefore, a bipolar current profile is used in this study as it can provide a high transport rate and reduce skin irritation.

The aims of this current work were to establish the optimum switching mode for reverse iontophoresis of both glucose and lactate and to study the reverse iontophoretic extraction of glucose and lactate across human skin barriers. The effects of switching mode for reverse iontophoresis of glucose and lactate were investigated using *in vitro* diffusion cells. The best switching mode was applied to healthy human volunteers, and glucose and lactate was extracted through their skin using a specially-designed transdermal reverse iontophoresis collection device. The ratio of glucose to lactate levels in collection gels and the glucose level in healthy volunteers' blood were compared.

2. MATERIALS AND METHODS

2.1 Constant current source

The constant current source, designed and developed by the authors (Ching, Camilleri & Connolly, 2005), was used to deliver iontophoretic current for both *in vitro* and humans reverse iontophoresis experiments.

2.2 *In vitro* reverse iontophoresis experiments

N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES), sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl) and lactate were purchased from Sigma Chemical Company (St. Louis, MO). Glucose was purchased from BDH Limited (Poole, England). Lactate reagents (LC 2389) and glucose reagents (GL 26233) were purchased from Randox Laboratories Limited

(Antrim, UK). De-ionized water (resistivity ≥ $18x10^6$ Ωcm) that had been purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to prepare all solutions. Nanoporous membrane with a net negative charge at pH 7 was used as the artificial skin model (Spectra/Por® CE (cellulose ester) Dialysis Membranes MWCO: 500, Spectrum Laboratories, Inc., Canada).

Silver-silver chloride (Ag/AgCl) electrodes were prepared by chloridizing silver wire (1mm diameter, 25mm length, 99.99% pure; Aldrich Chemical Company Inc., Milwaukee, WI) immersed in 0.1M HCl solution (Pt-cathode) for 90 minutes at an applied current of 314μA.

All experiments were performed using diffusion cells (Connolly, Cotton & Morin, 2002), in which both electrode chambers were located on the same surface side of a nanoporous membrane. These chambers were filled with 350μl of 25mM, pH 7.4, HEPES buffer containing 133mM NaCl. The lower chamber of the diffusion cell contained an electrolyte solution comprising 133mM NaCl, buffered to pH 7.4 with 25mM HEPES, and either 5mM glucose or 10mM lactate. Each electrode chamber contained a Ag/AgCl electrode. The surface area of the nanoporous membrane exposed to the electrode in each chamber was $0.2cm^2$ and the electrode chambers were 11mm apart. An iontophoretic current of $0.3mAcm^{-2}$ at 4 different switching manners (the polarity of electrodes reversing at intervals of 5, 10 and 15 minutes, or without reversing) was passed for 60 minutes via the Ag/AgCl electrodes. The entire content of the electrode chambers were removed at the end of the experiment to quantify the amount of glucose or lactate extracted. For the control experiments, all the experimental arrangements and procedures were the same as that described in reverse iontophoresis experiments except no current was applied in the control experiments.

Spectrometric assay, using a spectrometer (Multiskan Ascent®, Labsystems Oy, Finland), was used to quantify the amount of glucose (determined by Glucose reagents) or lactate (determined by Lactate reagents) extracted through the nanoporous membrane. Excellent linear relationships ($r^2 > 0.99$ for both cases) between lactate/glucose concentrations and their relative absorbance were found, allowing the lactate/glucose concentrations to be calculated simply by linear regressions.

2.3 Humans reverse iontophoresis experiments

Sodium phosphate monobasic (USP grade) and NaOH were purchased from Sigma Chemical Company (St. Louis, MO). Methylcellulose (MC) was purchased from The DOW Chemical Company (USA). 0.1M phosphate buffer solution (PBS) was prepared by dissolving 1.1998g of sodium phosphate monobasic in 100ml de-ionized water and adjusting the pH to 7.4 using a 40% w/v solution of NaOH. 4% MC gel was prepared by mixing 4g of MC with 100ml of 0.1M PBS. De-ionized water (resistivity ≥ $18x10^6$ Ωcm) that had been purified by a Millipore System (Milli-Q UFplus; Bedford, MA) was used to prepare all solutions.

Circular-shaped (11.3mm diameter) screen-printed Ag/AgCl electrodes (SPE) were fabricated and used in this study. Each SPE contains 90μl MC (4%) gel which acts as a conductive media for the transmission of iontophoretic current from the

SPE to the human skin and also acts as a collector for the storage of the extracted lactate and glucose.

Ten healthy human volunteers (8 men and 2 women), aged between 22 and 35 years (mean = 26.8, SD = 3.7), with no history of dermatological disease participated in this study. The subjects were required to maintain the sites under investigation free from application of any cosmetic topical formulations for at least 5 days before the study. The study was approved by the Strathclyde University Ethics Committee and informed consent was obtained from each volunteer.

The areas of skin of the subject's inner forearm where SPEs to be located were prepared by briskly rubbing the areas for 6-8 seconds with alcohol prep pads to remove dry skin, oils and other contaminants. The areas were then allowed to dry thoroughly. Then, the four SPEs were positioned on the subject's inner forearms and fixed in position with surgical tapes. Each pair of SPEs was about 23mm apart between the electrode centres and the two pairs of SPEs were about 50mm apart. An iontophoretic current of 0.3mA, with polarity of SPE reversing at intervals of 15 minutes, was passed between one pair of SPEs in conditions of room temperature for a period of 60 minutes. The second pair of SPEs was used as control with no current passing through it. At the end of the experiment, 20μl MC gel was carefully pipetted from each SPE and stored separately in microcentrifuge tubes at 4°C for later quantification of lactate and glucose. The subject's blood glucose level was also measured before experiments by a portable glucose meter (FreeStyle Blood Glucose Monitoring System, TheraSense Ltd., UK).

180μl of 0.1M PBS was pipetted to each microcentrifuge tube containing the extracted 20μl MC gel. The mixtures were then mixed well. From those mixtures, the amount of lactate (determined by Lactate reagents) and glucose (determined by Glucose reagents) extracted through the human skin was determined by spectrometric assay using spectrometer (Multiskan Ascent®, Labsystems Oy, Finland). Excellent linear relationships ($r^2 > 0.98$ for both cases) between lactate/glucose concentrations and their relative absorbance were found, allowing the lactate/glucose concentrations to be calculated simply by linear regressions.

3. RESULTS

In vitro diffusion studies were performed using a nanoporous membrane with and without application of iontophoretic current. It was demonstrated that the passage of a current facilitates movement of lactate and glucose across the nanoporous membrane. The optimum switching mode for reverse iontophoresis of lactate and glucose are DC and DC with electrode polarity reversal every 15 minutes, respectively (see Figure 1).

In the *in vitro* lactate diffusion studies, it was found that there is a significant difference between the four switching modes (one-way ANOVA: $p < 0.001$). Lactate extraction at the anode during continuous current passage (no polarity reversal) was found to be significantly higher than at all other switching modes

(LSD post-hoc multiple comparisons: $p < 0.001$ in all cases), except at 15 minute electrode polarity reversing. Conversely, lactate extraction at 15 minute electrode polarity reversing was found to be significantly higher (LSD post-hoc multiple comparisons: $p < 0.001$ in both cases) than that at 5 and 10 minutes electrode polarity reversing. It was also found that there was a significant higher lactate extraction at 5, 10 and 15 minute electrode polarity reversing than in the control (LSD post-hoc multiple comparisons: $p < 0.05$ in all cases).

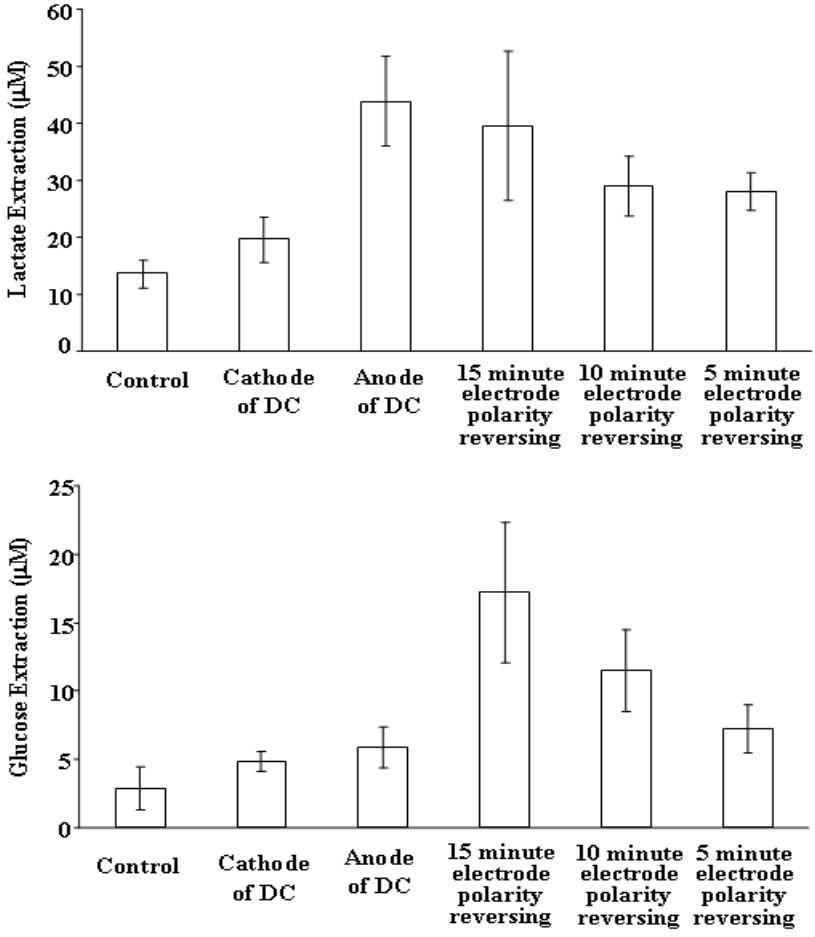

Figure 1. Reverse iontophoresis extraction of (a) lactate and (b) glucose (mean ± SD; n≥12 for each bar), as a function of switching modes. The iontophoretic current was 0.3 mA/cm^2. The electrolyte in the electrode chambers of the diffusion cell was 25mM, pH 7.4, HEPES buffer containing 133mM NaCl. The lower chamber of the diffusion cell was filled with an electrolyte solution comprising 133mM NaCl, buffered to pH 7.4 with 25mM HEPES, and either10mM lactate or 5mM glucose.

In the *in vitro* glucose diffusion studies, a significant difference was found between the four switching modes (one-way ANOVA: $p < 0.001$). It was found that glucose extraction at 15 minute electrode polarity reversal was significantly higher than that at all other switching modes (LSD post-hoc multiple comparisons: $p < 0.001$ in all cases). A significantly higher glucose extraction was also found at 5, 10 and 15 minutes electrode polarity reversals than the control (LSD post-hoc multiple comparisons: $p < 0.001$ in all cases). Obviously, anodal extraction was greater than cathodal extraction, but it was not significant.

Table 1. *The results of the real blood glucose level and the ratio of extracted glucose level to extracted lactate level of the 10 healthy volunteers*

	Real Blood Glucose Level (mmol)	Ratio of Extracted Glucose Level to Extracted Lactate Level
Subject A	4.6	0.33
Subject B	4.8	0.46
Subject C	4.6	0.27
Subject D	4.3	0.50
Subject E	5.3	0.38
Subject F	5.1	0.43
Subject G	4.6	0.43
Subject H	4.6	0.53
Subject I	4.2	0.63
Subject J	4.7	0.44
Mean	4.7	0.44
SD	0.3	0.10

During the human studies, lactate and glucose were successfully extracted from the subject's skin using reverse iontophoresis. Only one subject reported the experience of a very weak tingling sensation as the current was brought to 0.3mA at the start and when the electrode polarity reversed. At time 0 minute of current passage, she experienced a tingling sensation which lasted no longer than two seconds. At time 15 minutes of current passage (i.e. the occasion of electrode polarity reversed), she again experienced a tingling sensation which also lasted no longer than two seconds. Subsequently, she did not experience any tingling sensations until the end of the experiment. Conversely, four subjects had very mild erythema at the reverse iontophoresis site and this lasted for 15-30 minutes after termination of current flow.

The results of the human studies are summarized in Table 1 and Figure 2. It was found that diffusion of lactate and glucose across the subject's skin into the MC gel of the SPE took place. This was proved by the presence of small amounts of lactate and glucose at the control groups. On the other hand, for a pair of electrodes of the control/reverse iontophoresis groups, the lactate or glucose extraction at one of the electrodes was found to be statistically the same (independent t-test) as at the other electrode (see Figure 2). Also, reverse

iontophoresis was found to significantly promote more glucose extraction (around 4 times) and lactate extraction (around 2.5 times) than diffusion alone (independent t-test: $p < 0.001$ in both cases).

The blood glucose levels of 10 healthy volunteers and the ratio of extracted glucose levels to extracted lactate levels were also compared (see Figure 3) and no correlation was found ($r^2 = 0.11$). The r^2 values were found to be higher when two outliers were removed from the regression equation ($r^2 = 0.62$).

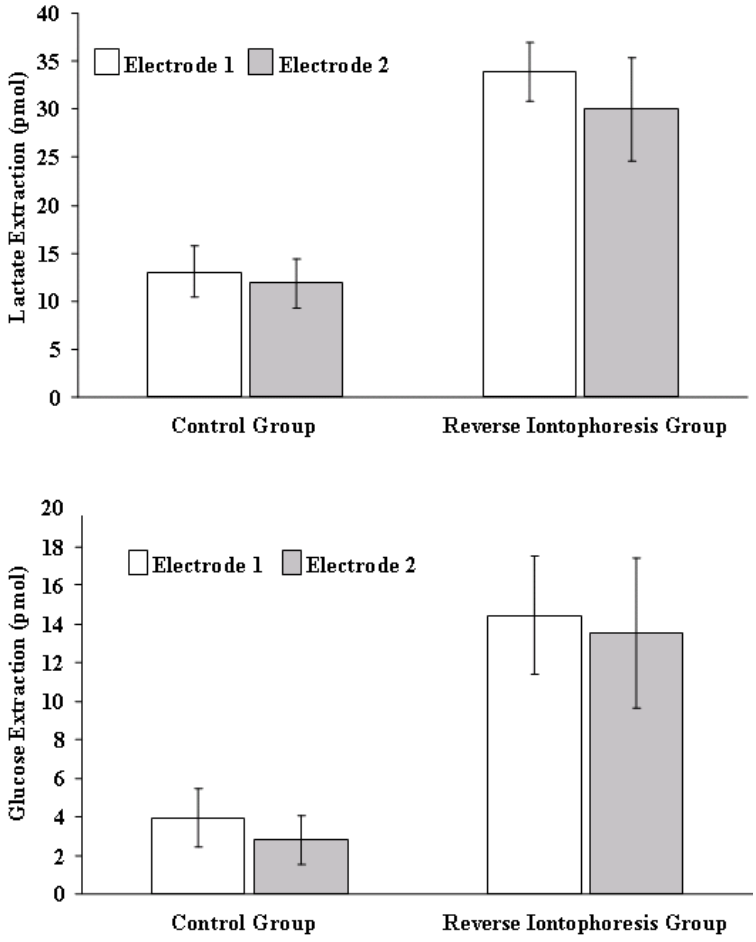

Figure 2. Long duration bipolar direct current (current density of 0.3 mA/cm^2, polarity of electrode reversing at intervals of 15 minutes, experimental time of 60 minutes) on human transdermal extraction of (a) lactate and (b) glucose (mean ± SD; n=10 for each bar). Extraction of lactate or glucose by reverse iontophoresis was significantly higher ($p<0.001$ for both cases) than that in the control.

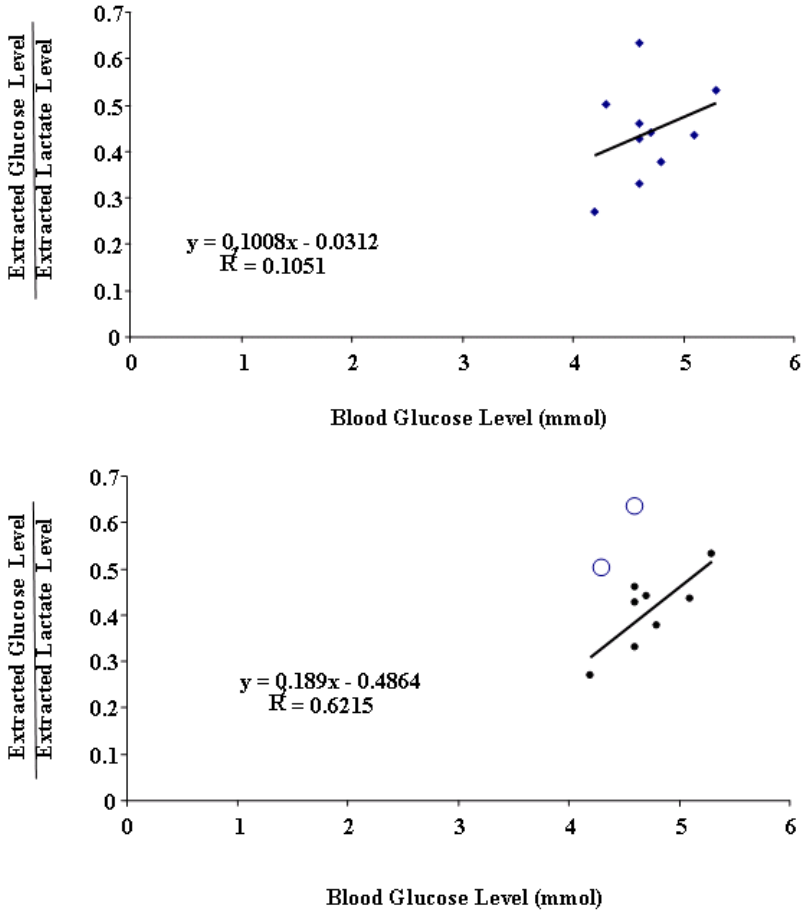

Figure 3. Comparison of real blood glucose levels of healthy subjects and the ratio of glucose to lactate levels in collection gels after reverse iontophoresis. (a) Correlation analysis includes outliers (n = 10). (b) Correlation analysis excludes outliers (n = 8). Outliers (dashed circle in Figure b) markedly affect the correlation and this can be seen in the equations of the correlation lines.

4. DISCUSSION

In the *in vitro* diffusion studies, in order to avoid the large sample-to-sample differences, cellulose ester nanoporous membrane was used to substitute for human or animal skin samples. This allowed the electrical properties of the electrodes and performance of the constant current source for reverse iontophoresis of lactate and glucose to be studied without the non-uniformities introduced by large sample-to-sample variation in animal tissue. Moreover, the availability of excised

human or animal skin samples is becoming limited for legal, ethical and safety reasons. Though excised healthy human skin samples can be obtained, they may be of differing ages, body site or race. Also, many substances in excised human or animal skin samples may cause interference for the investigation of the characteristics of an extraction system during reverse iontophoresis and these effects are relatively unpredictable. Thus basic electrical characterisation studies are preferably conducted in as controlled an environment as possible before skin studies commence.

Glucose is an uncharged molecule and therefore, in the *in vitro* glucose diffusion studies, electro-osmosis is the main mechanism for glucose extraction during reverse iontophoresis. Because the efficiency of electro-osmotic flow is weakly dependent upon current density in the range of 0.14 – 0.55 mA/cm^2 (Delgado-Charro & Guy, 1994), a current density for iontophoresis of 0.3 mA/cm^2, close to the value recommended as an upper limit by other workers (Ledger, 1992), was used.

Electro-osmotic flow is always in the same direction as the flow of counter ions. Since human skin is negatively charged under physiological conditions (Burnette and Ongpipattanakul, 1987), the counter ions are cations and the electro-osmotic flow is thus from anode to cathode (Pikal, 2001; Guy et al., 2000). The nanoporous membrane used in the diffusion cell is negatively charged. Therefore, electro-osmotic flow in this model system should be from anode to cathode during direct current reverse iontophoresis and thus more glucose should be found at the cathode rather than at the anode. However, in this model system, extraction of glucose at the anode was greater than that at the cathode during direct current reverse iontophoresis but this was not significant. This result was not in agreement with other researchers (Rao, Glikfeld & Guy, 1993; Rao et al., 1995). Anodal extraction being greater than cathodal extraction may be due to neutral or positively charged pores existing in the membrane (Pikal & Shah, 1990). It is also possible that some masking of negative charge in the nanoporous membrane or some membrane polarisation occurred which reduced the effect of cathodal electro-osmosis.

As can be seen in Figure 1b, the amount of glucose extracted in the model system at either 10 or 15 minutes switching times was significantly more than that at continuous current passage without switching (i.e. cathode and anode held constant) but this was inconsistent with the results reported by Santi and Guy in 1996. They found that total mannitol extractions were statistically equivalent for the continuous current passage with or without switching, albeit that a different sugar molecule was studied. The glucose result in this study for switching of polarities may be due to the electrode polarity reversal allowing depolarisation of the membrane (Banga & Chien, 1988). During continuous current passage without switching, sodium and chloride ions pass across the membrane to the cathode and anode, respectively. Continuous transport of these ions possibly leads to the accumulation of the ions at the membrane and the inherent fixed negative charge of the membrane is therefore shielded. This reduction of the electric field in the current conducting pathways diminishes counter-ion migration and therefore

reduces the level of electro-osmotic flow – the key mechanism of transport for uncharged molecules. Accumulation of ions could also cause membrane polarisation, reducing the level of electro-osmosis as well (Lawler, Davis & Griffith, 1960). For current passage with periodic electrode polarity switching, the level of masking of negative charge in the nanoporous membrane or membrane polarisation can be reduced once the polarity of the electrodes is reversed (Banga & Chien, 1988).

Based on the findings, it is postulated that 15 minute electrode polarity reversing is the optimum switching mode as it extracts significantly more glucose than the control (at least > 3.9 times) and continuous current passage without switching (at least > 3.1 times) within the studied iontophoresis application time.

In the *in vitro* lactate diffusion studies, results showed that reverse iontophoresis could facilitate lactate extraction and the switching mode governed the amount of lactate to be extracted. Lactate, a negatively charged molecule, is mainly extracted by electromigration during reverse iontophoresis. Based on the general principle of electricity, like charges repelling each other and opposite charges attracting each other, therefore lactate passed across the nanoporous membrane to the anode during continuous current passage without switching. Therefore, more lactate could be detected at the anode than at the cathode.

Clearly, the time of the application of positive polarity to any chamber during periodic electrode polarity reversal is half of that of the continuous current passage conditions. Therefore, continuous direct current promotes more lactate extraction than direct current with electrode polarity reversal. However, the differences between continuous current application and polarity reversal did not lead to halving of the lactate extraction when polarity reversal was compared to continuous current conditions (see Figure 1a). The possible reason for that might be due to membrane depolarisation effects once the polarity of the electrodes was reversed (Banga & Chien, 1988). Such depolarisation process might have helped to maintain the transport properties of the membrane. On the other hand, for continuous current passage, the membrane will become more negative in the anode chamber with the accumulation of chloride and lactate ions.

Even though continuous current passage without switching promoted more lactate extraction compared with other switching modes, in practical devices for patient use it could cause erythrema and stinging (Howard et al., 1995). Periodic electrode polarity reversal could help to depolarise skin at regular intervals (Banga and Chien, 1988). In addition skin damage and irritation is minimised with polarity reversal (Tomohira et al., 1997). Because there was no significant difference between the amounts of lactate extracted by continuous current application without switching and current application combined with electrode polarity reversal every 15 minutes, 15 minutes electrode polarity reversal during current application was selected for healthy volunteer trials as this still gave good lactate extraction in the model system and some gains could still be made such as minimising skin damage and irritation.

In the human studies, 40 % of the subjects still had very mild erythema even though bipolar direct current had been employed in reverse iontophoresis. This was

probably due to the duration of the electrode polarity reversal times being too long. However, as shown in Figure 1, if the duration of the electrode polarity reversal time is set too short, only a relatively small amount of lactate or glucose can be extracted. Therefore, there may still be opportunities in future studies to further optimise switching times of electrode polarities which could directly reduce some aspects of skin irritation.

As shown in Figure 2, lactate or glucose extraction at one of the electrodes being statistically the same as at the other electrode. This finding suggested that the initial polarity of the electrode was not important for reverse iontophoresis with polarity reversal which would be the logical expectation if both chambers of a device get to be both anode and cathode for equal duration during extraction.

Not many researchers have investigated glucose extraction by reverse iontophoresis in human subjects (Sieg, Guy & Delgado-Charro, 2004a, 2004b; Sieg, Guy & Delgado-Charro, 2003; Rao et al., 1995) and no researchers have studied the effects of reverse iontophoresis on lactate extraction both in human subjects and *in vitro*. Several papers from the past twenty years (Sieg et al., 2004a, 2004b; Sieg et al., 2003; Rao et al., 1995) have been found for the reverse iontophoresis of glucose in humans, but only one relevant paper (Rao et al., 1995) was found which could be used for comparison with our findings. On the other hand, no relevant paper was found for the reverse iontophoresis of lactate in human subjects. Rao et al., (1995) passed a direct current (0.25 mA/cm^2) across the human skin (ventral forearm) of subjects for a period of 60 minutes to extract glucose through the skin and they found that around 5.83 nmol of glucose could be extracted at the cathode. However, our findings are not in agreement with their findings. In this study (i.e. current density = 0.3 mA/cm^2, time of current application = 60 minutes, electrode polarity reversal = every 15 minutes), it was found that around 13.95 nmol of glucose could be extracted by long duration bipolar direct current. The possible explanation might be that we used a higher current density (0.3 mA/cm^2) compared to Rao et al., (1995). More importantly, we used a bipolar direct current rather than a direct current. Bipolar direct current can prevent skin polarisation (Banga & Chien, 1988) and skin polarisation can reduce the level of electro-osmosis (Lawler et al., 1960). Because glucose is mainly extracted out of the skin by electro-osmosis and bipolar direct current can prevent the reduction in the level of electro-osmosis, bipolar direct current can promote more glucose extraction than direct current.

Although no data could be used for comparison with our findings of human transdermal extraction of lactate, our findings suggested that reverse iontophoresis significantly promoted more lactate extraction (around 2.5 times) than diffusion alone.

In a nutshell, both glucose and lactate can be effectively and non-invasively extracted through human skin by the technique of reverse iontophoresis and this provides a potential for the development of a continuous and non-invasive system to monitor the blood glucose level. To arrive at this a calibration of internal versus external glucose and lactate will be required for each individual subject. Because of the known skin to skin permeability variations between humans, it is expected that there will be a poor correlation across humans between blood glucose and extracted

glucose levels and blood lactate and extracted lactate levels. This was overcome in the GlucoWatch® and a calibration was possible for each individual user. Each user was able to calibrate the device by utilising a single finger stick internal blood glucose sample which the device correlated with the reverse iontophoresis glucose level of the wearer at a single point in time (Tamada et al., 1999). To avoid this invasive step, a new approach is investigated. We haver endeavoured to correlate the extraction ratio (glucose/lactate) with the real blood glucose level.

As shown in Figure 3, no relationship was found between the subject's blood glucose level and the ratio of glucose to lactate levels in collection gels. However, improved correlations were obtained (see Figure 3b) if the two outliers were removed from the regression equations during correlation analysis. This was probably due to the small sample size (only 10 healthy volunteers in this study) leading to high variations. It can be seen that the two outliers removed from the correlation on the real blood lactate level with the extracted lactate level, were far away from the linear regression line. In correlation analysis, many factors can affect the correlation coefficients (r^2), such as sample size, the types of variable being studied, and so on. Better correlations without the elimination of outliers from the linear regression lines can be obtained if the sample size is big enough so that the variations of data are low as well as the data being more random. In addition, different kinds of data (healthy subjects data at resting condition in this study, healthy subjects data at exercising condition in a future study, and so on) could be included in the correlations analysis. This will provide a wider range of data making the correlations analysis more reliable. However, it may be that no such correlation will exist in a wider sample.

Despite promising early results, the correlation between the subject's blood glucose level and the ratio of glucose to lactate levels in collection gels was still not too high. Hence, it is necessary to test this method using larger subject groups in order to achieve a better correlation between the subject's blood glucose level and the ratio of glucose to lactate levels in collection gels.

5. CONCLUSION

The application of a current can facilitate movement of both glucose and lactate across the nanoporous membrane and the optimum switching mode for reverse iontophoresis of lactate and glucose are DC and DC with electrode polarity reversal every 15 minutes, respectively. However, the application of current combined with electrode polarity reversal every 15 minutes is recommended to be used in clinical situations as lactate extraction under these conditions was still good and skin irritation may be minimised. On the other hand, long duration bipolar direct current with 15 minutes electrode polarity reversal can also facilitate both glucose and lactate extraction across the human skin *in vivo*. A fair-good degree of relationship was found between the subject's blood glucose level and the ratio of glucose to lactate levels in collection gels after two outliers were removed from the

regression equation. This possibly permits a non-invasive glucose sampling methodology free of the need for calibration with a blood sample.

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REFERENCES

Amos, A. F., McCarty, D. J., & Zimmet, P. (1997). The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabetic Medicine*, *14*(Suppl. 5), S1-S85.

Banga, A. K., & Chien, Y. W. (1988). Iontophoretic delivery of drugs: Fundamentals, developments and biomedical applications. *Journal of Controlled Release*, *7*(1), 1-24.

Bell, D. S. H. (1994). Stroke in the diabetic patient. *Diabetes Care*, *17*(3), 213-219.

Brancati, F. L., Whelton, P. K., Randall, B. L., Neaton, J. D., Stamler, J., & Klag, M. J. (1997). Risk of end-stage renal disease in diabetes mellitus: a prospective cohort study of men screened for MRFIT. Multiple Risk Factor Intervention Trial. *Journal of the American Medical Association*, *278*(23), 2069-2074.

Burnette, R. R., & Ongpipattanakul, B. (1987). Characterization of the perm selective properties of excised human skin during iontophoresis. *Journal of Pharmaceutical Sciences*, *76*(10), 765-773.

Chien, Y. W., Lelawongs, P., Siddiqui, O., Sun, Y., & Shi, W. M. (1990). Facilitated transdermal delivery of therapeutic peptides and proteins by iontophoretic delivery devices. *Journal of Controlled Release*, *13*(2-3), 263-278.

Ching, C. T. S., Camilleri, I., & Connolly, P. (2005). A low-cost, programmable device for versatile current delivery in iontophoresis applications. *Sensors and Actuators B*, *106*(2), 534-540.

Connolly, P., Cotton, C., & Morin, F. (2002). Opportunities at the Skin Interface for Continuous Patient Monitoring: A Reverse Iontophoresis Model tested on Lactate and Glucose. *IEEE Transactions in Nanobioscience*, *1*(1), 37-41.

Davis, B. L., Kuznicki, J., Praveen, S. S., & Sferra, J. J. (2004). Lower-extremity amputations in patients with diabetes: pre- and post-surgical decisions related to successful rehabilitation. *Diabetes-Metabolism Research and Reviews*, *20*(Suppl 1), S45-S50.

Degim, I. T., Ilbasmis, S., Dundaroz, R., & Oguz, Y. (2003). Reverse iontophoresis: a non-invasive technique for measuring blood urea level. *Pediatric Nephrology*, *18*(10), 1032-1037.

Delgado-Charro, M. B., & Guy, R. H. (1994). Characterization of convective solvent flow during iontophoresis. *Pharmaceutical Research*, *11*(7), 929-935.

Evans, J. (1995). *Causes of blindness and partial sight in England and Wales 1990-1991*. London, UK: HMSO.

Guy, R. H., Kalia, Y. N., Delgado-Charro, M. B., Merino, V., Lopez, A., & Marro, D. (2000). Iontophoresis: electrorepulsion and electroosmosis. *Journal of Controlled Release*, *64*(1-3), 129-132.

Hirvonen, J., Hueber, F., & Guy, R. H. (1995). Current profile regulates iontophoretic delivery of amino acids across the skin. *Journal of Controlled Release*, *37*(3), 239-249.

Howard, J. P., Drake, T. R., & Kellogg, D. L. (1995). Effects of alternating current iontophoresis on drug delivery. *Archives of Physical Medicine and Rehabilitation*, *76*(5), 463-466.

International Diabetes Federation (2003). *Diabetes Atlas 2003*. (2nd ed.), Brussels, Belgium: Belgium International Diabetes Federation.

King, H., & Rewers, M. (1993). Global estimates for prevalence of diabetes mellitus and impaired glucose tolerance in adults. *WHO Ad Hoc Diabetes Reporting Group. Diabetes Care*, *16*(1), 157-177.

Laing, S. P., Swerdlow, A. J., Slater, S. D., Botha, J. L., Burden, A. C., Waugh, N. R., Smith, A. W., Hill, R. D., Bingley, P. J., Patterson, C. C., Qiao, Z., & Keen, H. (1999a). The British Diabetic Association Cohort Study, I: all-cause mortality in patients with insulin-treated diabetes mellitus. *Diabetic Medicine*, *16*(6), 459-465.

Laing, S. P., Swerdlow, A. J., Slater, S. D., Botha, J. L., Burden, A. C., Waugh, N. R., Smith, A. W., Hill, R. D., Bingley, P. J., Patterson, C. C., Qiao, Z., & Keen, H. (1999b). The British Diabetic Association Cohort Study, II: cause-specific mortality in patients with insulin-treated diabetes mellitus. *Diabetic Medicine*, *16*(6), 466-471.

Lawler, J. C., Davis, M. J., & Griffith, E. (1960). Electrical characteristics of the skin: The impedance of the surface sheath and deep tissues. *Journal of Investigative Dermatology*, *34*, 301-308.

Ledger, P. W. (1992). Skin biological issues in electrically enhanced transdermal delivery. *Advanced Drug Delivery Reviews*, *9*(2-3), 289-307.

Merino, V., Kalia, Y. N., & Guy, R. H. (1997). Transdermal therapy and diagnosis by iontophoresis. *Trends in Biotechnology*, *15*(8), 288-290.

Merino, V., Lopez, A., Hochstrasser, D., & Guy, R.H. (1999). Noninvasive sampling of phenylalanine by reverse iontophoresis. *Journal of Controlled Release*, *61*(1-2), 65-69.

Mize, N. K., Buttery, M., Daddona, P., Morales, C., & Cormier, M. (1997). Reverse iontophoresis: monitoring prostaglandin E2 associated with cutaneous inflammation *in vivo*. *Experimental Dermatology*, *6*(6), 298-302.

Numajiri, S., Sakurai, H., Sugibayashi, K., Morimoto, Y., Omiya, H., Takenaka, H., & Akiyama, N. (1993). Comparison of depolarizing and direct current systems on iontophoretic enhancement of transport of sodium benzoate through human and hairless rat skin. *Journal of Pharmacy and Pharmacology*, *45*(7), 610-613.

Pickup, J. (2003). Diabetic control and its measurements. In: J. C. Pickup & G. Williams (Eds.), *Textbook of Diabetes* (3rd ed., pp. 34.1–34.17). Oxford, UK: Blackwell Science.

Pikal, M. J. (2001). The role of electroosmotic flow in transdermal iontophoresis. *Advanced Drug Delivery Reviews*, *46*(1-3), 281-305.

Pikal, M. J., & Shah, S. (1990). Transport mechanisms in iontophoresis. III. An experimental study of the contributions of electroosmotic flow and permeability change in transport of low and high molecular weight solutes. *Pharmaceutical Research*, *7*(3), 222-229.

Pitzer, K. R., Desai, S., Dunn, T., Edelman, S., Jayalakshmi, Y., Kennedy, J., Tamada, J. A., & Potts, R. O. (2001). Detection of hypoglycaemia with the GlucoWatch biographer. *Diabetes Care*, *24*(5), 881-885.

Potts, R. O., Tamada, J. A., & Tierney, M. J. (2002). Glucose monitoring by reverse iontophoresis. *Diabetes Metabolism Research and Reviews*, *18*(Suppl 1), S49-53.

Rao, G., Glikfeld, P., & Guy, R. H. (1993). Reverse iontophoresis: Development of a noninvasive approach for glucose monitoring. *Pharmaceutical Research*, *10*(12), 1751-1755.

Rao, G., Guy, R. H., Glikfeld, P., LaCourse, W. R., Leung, L., Tamada, J., Potts, R. O., & Azimi, N. (1995). Reverse iontophoresis: Noninvasive glucose monitoring *in vivo* in human. *Pharmaceutical Research*, *12*(12), 1869-1873.

Santi, P., & Guy, R. H. (1996). Reverse iontophoresis — parameters determining electroosmotic flow. I. PH and ionic strength. *Journal of Controlled Release*, *38*(2-3), 159-165.

Sieg, A., Guy, R. H., & Delgado-Charro, M. B. (2003). Reverse iontophoresis for noninvasive glucose monitoring: The internal standard concept. *Journal of Pharmaceutical Sciences*, *92*(11), 2295-2302.

Sieg, A., Guy, R. H., & Delgado-Charro, M. B. (2004a). Simultaneous extraction of urea and glucose by reverse iontophoresis *in vivo*. *Pharmaceutical Research*, *21*(10), 1805-1810.

Sieg, A., Guy, R. H., & Delgado-Charro, M. B. (2004b). Noninvasive glucose monitoring by reverse iontophoresis *in vivo*: Application of the internal standard concept. *Clinical Chemistry*, *50*(8), 1383-1390.

Stephenson, J. M., Kenny, S., Stevens, L. K., Fuller, J. H., & Lee, E. (1995). Proteinuria and mortality in diabetes: the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetic Medicine*, *12*(2), 149-155.

Tamada, J. A., Garg, S., Jovanovic, L., Pitzer, K. R., Fermi, S., & Potts, R. O. (1999). Noninvasive glucose monitoring: Comprehensive clinical results. *JAMA*, *282*(19), 1839-1844.

The Diabetes Research in Children Network (DirecNet) Study Group (2004). Accuracy of the GlucoWatch G2 Biographer and the Continuous Glucose Monitoring System During Hypoglycemia: Experience of the Diabetes Research in Children Network. *Diabetes Care*, *27*(3), 722-726.

The UK Prospective Diabetes Study Group (UKPDS) Group (1998a). Intensive blood-glucose control with sulphonylureas or insulin compared with

conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet*, *352*(9131), 837-853.

The UK Prospective Diabetes Study Group (UKPDS) Group (1998b). Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet*, *352*(9131), 854-865.

The UK Prospective Diabetes Study Group (1998c). Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. *British Medical Journal*, *317*(7160), 713-720.

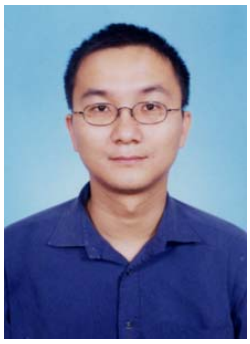
The UK Prospective Diabetes Study Group (1998d). Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *British Medical Journal*, *317*(7160), 703-713.

Tierney, M. J., Tamada, J. A., Potts, R. O., Jovanovic, L., Garg, S., & Cygnus Research Team (2001). Clinical evaluation of the GlucoWatch® biographer: a continual, non-invasive glucose monitor for patients with diabetes. *Biosensors & Bioelectronics*, *16*(9-12), 621-629.

Tomohira, Y., Machida, Y., Onishi, H., & Nagai, T. (1997). Iontophoretic transdermal absorption of insulin and calcitonin in rats with newly-devised switching technique and addition of urea. *International Journal of Pharmaceutics*, *155*(2), 231-239.

Wang, S. L., Head, J., Stevens, L., & Fuller, J. H. (1996). Excess mortality and its relation to hypertension and proteinuria in diabetic patients. The World Health Organization Multinational Study of Vascular Disease in Diabetes. *Diabetes Care*, *19*(4), 305-312.

Wild, S., Roglic, G., Green, A., Sicree, R., & King, H. (2004). Global Prevalence of Diabetes. Estimates for the year 2000 and projections for 2030. *Diabetes Care*, *27*(5), 1047-1053.



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