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Original Article

Monitoring Interstitial Glucose Changes During Ischemia/Reperfusion in Human Cutaneous Free Flaps

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Abstract

Background: **Free tissue transfer has unique post ischemic tissue metabolism that resembles the ischemia/reperfusion model. Glucose regulation during ischemia/reperfusion and effects of the insult to glucose metabolism in various human skin flaps were examined in this study by using the continuous glucose monitoring device.**

Methods: **Seven cutaneous-containing free flaps reconstruction were performed in seven adult patients. Interstitial glucose within the flaps was monitored using a transcutaneous sensor. Interstitial glucose (ISG) was recorded from flap harvesting until one hour following arterial anastomosis. Interstitial glucose concentrations and trends were reported.**

Results: **Six free flaps were successfully monitored according to study protocol. The initial mean ISG in the flap was 111.219± 26.33 (59.5-129) mg/dl. The glucose average rate of fall was 1.04 mg/min after pedicle transection. Average time to reach low plateau phase (47.49± 13.98% of initial ISG) was 87± 42.16 minutes. The mean ischemic time was 165.83± 53.88 (105-237) minutes. After arterial reperfusion, the interstitial glucose rose in the average rate of 0.79 mg/min. The 1-hr post-reperfusion ISG remained stable at 87.5± 6.85% (75.51-94.1) of the initial (p = 0.01). Weak negative correlation between ischemic time and 1-hr post-reperfusion ISG was observed (r = -0.59).**

Conclusions: **Interstitial glucose concentration in human skin flap during ischemia/reperfusion episode is correlated with tissue perfusion. Prolonged ischemia compromises glucose metabolism in the early reperfusion period.**

Keywords: **free flap, monitoring, interstitial glucose, ischemia/reperfusion**

Every free flap has the potential for developing ischemia/reperfusion injury. Free tissue transfer involves an obligatory period of ischemia during the surgical procedures when the pedicle is transected before transferring to the recipient site. In most cases, this normothermic ischemia is tolerated well, and success rate greater than 90% is expected. Although most of the surgeon's attention is directed to the anastomotic site, a technically sound microanastomosis does not guarantee success. Reperfusion must also occur at the level of the microcirculation. Failure at this site is often inflammatory in nature and a result of

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ischemia/reperfusion injury. Scientific interest has now shifted from primarily clinical aspects (e.g., surgical techniques and postoperative monitoring) toward pathophysiological background of microvascular anastomoses[1,2].

Ischemia is defined as a condition of inadequate blood supply to an area of tissue. It is characterized by insufficient oxygen intake and thus a conversion of cellular metabolism to anaerobic pathways[2]. The decrease in tissue oxygen level elevates glycolysis and lactic acid accumulation associated with pH reduction and activation of the complement system subsequently occur[3]. The critical ischemic time, the maximum length of time tissue can tolerate complete ischemia and remain viable once circulation is restored, varies in different tissue types. Skin flap is able to tolerate ischemia longer than myocutaneous flap due to differences between their intrinsic metabolism.

Apart from ischemia itself, another causative factor of tissue loss is the so-called ischemia/reperfusion injury, an inflammatory reaction that takes place after an episode of ischemia when the blood flow is restored. The biochemical and molecular changes that occurred during ischemia predispose to free radical-mediated damage. Abrupt reperfusion causes a burst of reactive oxygen species production in the post-ischemic tissues (particularly in the vascular endothelial cells) resulting in an inflammatory-like response to occur at the onset of reperfusion, such as, endothelial dysfunction (decreased endothelium-dependent vasodilation), decreased endogenous nitric oxide generation, increased superoxide anion generation, and release of proinflammatory cytokines into the interstitium and vascular space[4]. Many researchers have investigated cellular and molecular changes induced by ischemia/reperfusion but only a few did so on the tissue interstitial glucose regulation which is one of the earliest factors that changes during the process of ischemia[2,5,6]. In order to reduce the deleterious effects of ischemia and reperfusion injury, several methods of ischemia tolerance induction were developed including sublethal ischemia[4,7-11], hyperthermia[12], hypothermia, drugs[13,14] and growth factors.

Free tissue transfer has unique postischemic tissue metabolism that resembles the ischemia/reperfusion model[5]. Real time study of glucose regulation in the flap during free tissue transfer is now possible by using a small glucose sensor probe inserted directly into tissues.

It is generally accepted that glucose concentration in interstitial fluid decreases during tissue ischemia. This has been proved by microdialysis technique, which analyzes interstitial fluid taken from the body. Despite its ability of monitoring an intraoral free flap, which is difficult to monitor by clinical[15], this technique is still laborious and expensive so its use is still limited[16].

Interstitial glucose monitoring device has been used widely in diabetic patients to help physicians control the blood glucose level as the data is directly correlate to the plasma glucose concentration. This monitoring device is designed for nearly "continuous" report of the interstitial glucose level and has been experimentally used to monitor flap perfusion after occlusion of the pedicle in a rodent model[17]. Its principle of interstitial glucose detection was described previously elsewhere[18-21]. For the clinical aspect, glucose concentration in the flap during harvesting process and after vascular anastomoses has to be examined to help understanding glucose regulation during ischemia/reperfusion and effects of the insult to glucose metabolism in the flap, thus providing information before using the device as a flap monitor.

This study was conducted to evaluate the changes of interstitial glucose level in the cutaneous portion of various human free flaps throughout ischemia/reperfusion episode by using the continuous glucose monitoring device.

Materials and Methods

Patients and Flaps

From April 2011 to January 2012, adult patients without major co-morbid disease who underwent various soft tissue reconstructions with microvascular free tissue transfer at plastic surgery unit of Lerdsin General Hospital were recruited into study protocol. Seven cutaneous-containing free flaps from seven non-diabetic patients were selected into study with one flap excluded later due to protocol violation (Table 1). All flaps included into this study have thickness of more than 10 mm at the perforator area to accommodate the glucose sensor tip. Flaps used for intraoral reconstruction were not recruited because the sensor may hinder flap inset during operations. The institutional ethic committee approved the protocol.

Table 1 Flaps category

Patient No.	Age (year)	Gender	Flap	Type	Skin Dimension (cm2)
1	30	M	Parascapular	Fasciocutaneous	12x18
2	34	M	ALT (thinned)	Fasciocutaneous	12x20
3	25	M	ALT (thinned)	Fasciocutaneous	9x20
4	32	F	LD	Myocutaneous	12x15
5	67	F	LD	Myocutaneous	12x18
6	77	M	ALT	Fasciocutaneous	16x25
7*	29	F	ALT	Fasciocutaneous	16x26

*patient 7 was excluded from study (sensor prematurely removed)
ALT = anterolateral thigh, LD = latissimus dorsi

Glucose monitoring

The interstitial glucose concentration (ISG) in cutaneous portion of the flap was measured every 5 minutes for 15 minutes before flap raising and then continuously recorded every 5 minutes during pedicle transection, flap setting, and completion of arterial and venous anastomoses using the Medtronic Continuous Glucose Monitoring System (Medtronic Diabetes, Northridge, Calif.). Calibration of ISG with the capillary blood glucose (CBG) was done following the company's recommendation. The sensor was placed transcutaneously with its tip in the subcutaneous tissue. The area of sensor placement was determined preoperatively. The area in which perforator was audible by doppler ultrasound was marked for sensor placement to make sure of its inclusion in the flap territory. This sensor uses a glucose oxidase-based platinum electrode to measure an electrical current that relates to interstitial fluid glucose concentration (Figure1).

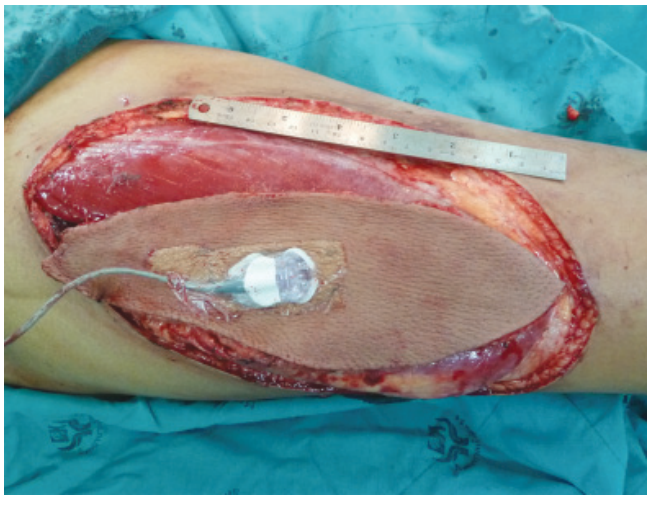

Figure 1 Glucose sensor placement in patient 3. The sensor was placed transcutaneously into the area of audible perforator and left in place during the operation.

After vascular anastomoses, the sensor was left in place for at least 60 minutes and then removed. The data was transferred to computer for analysis.

Statistical Analysis

Initial CBG and ISG was compared using unpaired t-test. Glucose concentrations are expressed as mean ± SD and percentage of the baseline. Glucose concentration was calculated every 5 minutes after pedicle transection until reaching the plateau, and every 5 minutes for at least 60 minutes period after completion of arterial and venous anastomoses (post-reperfusion). The ischemic time was recorded. The glucose rate of change and time to 25%, and 50% glucose reduction were also calculated during ischemia/reperfusion. The post-reperfusion glucose level (PR-ISG) was calculated as percentage of the baseline at the end point of study protocol (one hour after reperfusion). Interstitial glucose in identical flaps at different time points were compared using paired t tests. All *p* values were calculated from two-tailed tests of statistical significance. Correlations (*Pearson r*) between flap dimensions, ischemic time and 1-hr PR-ISG and glucose rate of changes were also determined.

Results

Seven cutaneous-containing free flaps were included in the study and interstitial glucose concentration was monitored. In one flap, the sensor was prematurely removed before vascular anastomosis due to difficulty in flap setting. Data from this flap was

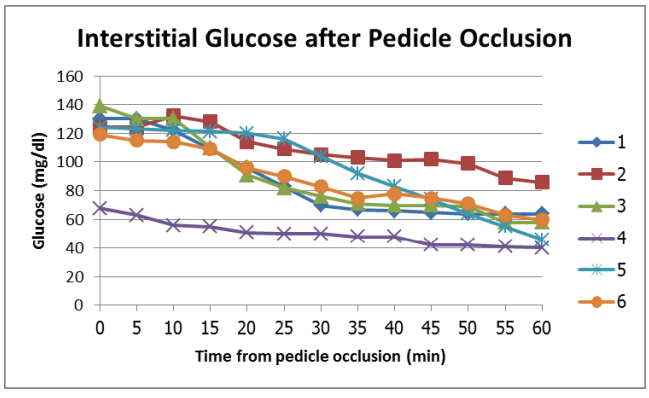

Figure 2 Interstitial glucose after pedicle occlusion. Interstitial glucose fell rapidly following pedicle occlusion and transection.

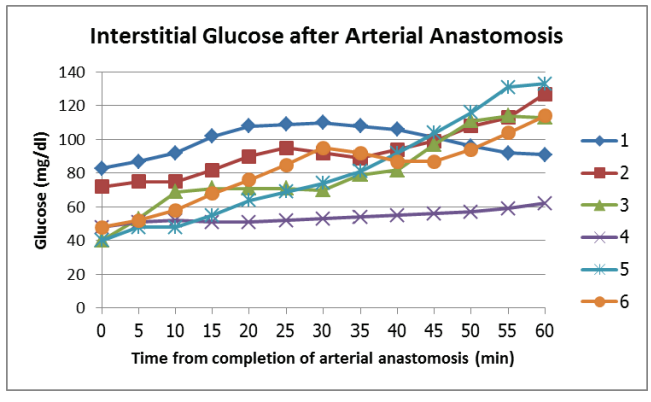

Figure 4 Interstitial glucose after arterial anastomosis. Interstitial glucose rose rapidly following clamp release after completion of arterial anastomosis.

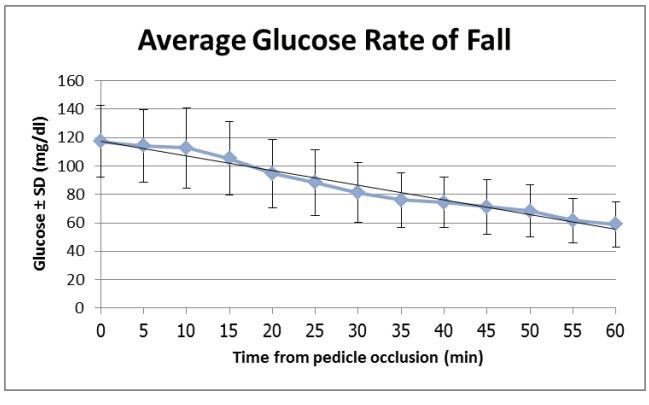

Figure 3 Average glucose rate of fall. Interstitial glucose in all flaps fell at the average rate of 1.04 mg/min after pedicle occlusion and transection.

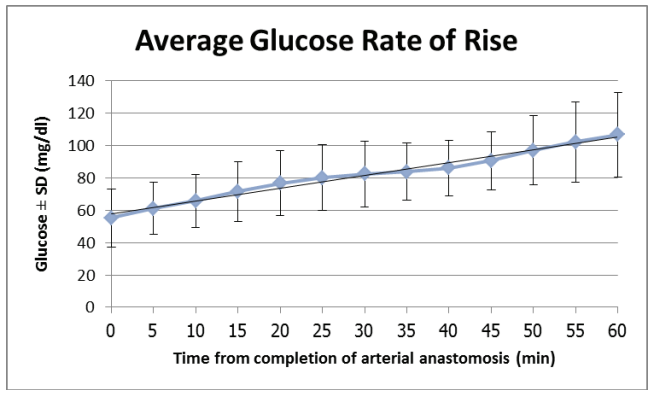

Figure 5 Average glucose rate of rise. Interstitial glucose in all flaps rose at the average rate of 0.79 mg/min after completion of arterial anastomosis.

excluded from the study. In all patients, the initial CBG was euglycemic and intimately correlated with the ISG with no statistical differences ($p = 0.91$). The initial mean ISG in the flap was 111.21 ± 26.33 (59.5-129) mg/dl while the mean CBG was 106.5 ± 26.44 (66-137) mg/dl.

The glucose average rate of fall was 1.04 mg/min after pedicle transection (Figure 2 and 3). Average time to reach plateau phase was 87 ± 42.16 minutes. The ischemic times varied among flaps with a mean of 165.83 ± 53.88 (105-237) minutes . The ISG level in the ischemic flap appeared stable at some low level instead of falling to zero in this study (47.49 ± 13.98% of initial ISG).

Within 5 minutes after arterial reperfusion, the interstitial glucose started to rise in the average rate of 0.79 mg/min (Figure 4 and 5). The 1-hr PR-ISG did not reach the initials ISG level but remained stable at 87.51 ± 6.85% (75.51-94.1) of the initial. This difference between initial ISG and 1-hr PR-ISG reached statistical significance ($p = 0.01$) (Table 2). With variable rates, the interstitial glucose concentrations rose instantly after completion of arterial anastomoses and continuously increased to the second plateau. Venous anastomosis did not influence the rate of rising.

For the time relation aspect, ISG level and flap perfusion had close relationships. That was, when perfusion ceased, ISG dropped immediately within the first five minutes of the device sampling. Conversely, when the flap was re-perfused, ISG also raised in the same time interval.

There was weak negative correlation between ischemic time and 1-hr PR-ISG (correlation coefficient = -0.59). No correlation between ischemic time and glucose rate of rise. Flap dimension and the rate of ISG level change were also not correlated.

Table 2 Flaps parameter

Patient No.	Ischemic time (min)	Base line ISG (mg/dl)	25% time reduction (min)	50% reduction time (min)	Time to plateau (min)	Lowest ISG (mg/dl) (% of baseline)	1-hr PR-ISG (mg/dl) (% of baseline)
1	237	121.5 ± 8.96	7	N/A	35	64 (52.67)	91.75 (75.51)
2	105	129 ± 3.83	34	N/A	72	73 (56.5)	121.42 (94.1)
3	136	127.25±12.25	9	35	65	40 (31.43)	110 (86.44)
4	126	59.5 ± 4.69	49	N/A	104	40 (67.2)	54.33 (91.31)
5	223	121.5 ± 1.29	18	32	87	*<40 (<32.92)	112.5 (92.59)
6	168	108.5 ± 5.07	55	94	159	48 (44.2)	92.33 (85.09)

*ISG level below 40 mg/dl was undetectable by the device and shown as error.
ISG = interstitial glucose, PR-ISG = post-reperfusion interstitial glucose

Discussion

The main finding of the study was that flap ischemia/reperfusion has direct effect on interstitial glucose concentrations in a timely manner as monitored by the continuous glucose monitoring device. A negative correlation between ischemic time and post-reperfusion interstitial glucose concentration at the study end point was also observed.

Flap tissue metabolism was first investigated by biopsies[22,23]. Biopsies from ischemic rat skin flaps revealed low glucose and high lactate concentrations compared to normal skin or viable flap skin[23]. Consequently, the use of microdialysis produced data about local glucose or lactate metabolism during normal perfusion and during ischemia in various tissues and flaps[24-26]. The tested human and animal tissues responded rapidly to vascular occlusion with decreasing glucose and/or increasing lactate levels. As in our series, which specialized in human skin, all ischemic/reperfusion episodes were accompanied by decreasing and increasing glucose concentrations as early as within five minutes. These time correlations have never been shown in a continuous and realtime fashion to date.

Ischemia/reperfusion injury occurs when tissue is reperfused following a prolonged period of ischemia. It is a subject of interest to plastic surgeons involved in replantation, free tissue transfer, and composite tissue allotransplantation, as it can have a significant impact on the overall success of these procedures[3]. Interstitial glucose monitoring provides quantitative evaluation of free flap metabolism. The changes of interstitial glucose concentration during ischemia/reperfusion of various cutaneous flaps help us understand the effect of free-radical damage to the human skin and subcutaneous tissue. We consider our results to be applicable in forming a general view of metabolic status during ischemia and reperfusion.

Another interesting finding was a negative correlation between ischemic time and 1-hr PR-ISG level, which was significantly lower than the pre-ischemic baseline. Prolonged ischemia results in an impairment of glucose metabolism in the flap tissue. This finding might be explained by the "no-reflow" phenomenon, which is a result of ischemia/reperfusion injury. Several theories regarding the final mechanism of "no-reflow" exist, such as intravascular hemoconcentration, swelling of the endothelial cells, leukocyte plugging, and interstitial edema[27]. As of the authors' knowledge, there is still no established critical ischemic time for each individual free flap. By monitoring interstitial glucose in the flap throughout the operation, we may anticipate the degree of ischemia/reperfusion injury insulted to the flap and finally, the outcome after a sound anastomosis.

Two limitations to the present study must be addressed. First, variation of ischemic times among various free flaps caused each subgroup population to be too low. This affected the significance of correlation we observed. We are currently collecting more patients for further study analysis in the near future to address this problem. The second limitation involved the short period of monitor time after completion of vascular anastomosis, that was limited to one to two hours due to lack of sensor data from some particular flaps. This limitation was offset by studies demonstrating that the recovery speed of flap metabolism after

reperfusion seemed to vary without relation to the ischemic time. In rats model, the result of capillary damages from ischemia/reperfusion appeared active for seven to eight hours after reperfusion following the ischemic time of four hours[28]. Another study showed return of baseline perfusion at 3 hours after reperfusion following 12 hours of flap ischemia[29]. However, in porcine tissues analyzed by microdialysis technique, the glucose concentration returned to normal during the 60-minute reperfusion after the ischemic time of 1 hour[25].

Continuous glucose monitoring device is invasive, but the trauma induced to the tissue is comparable to the insertion of a small intravenous cannula. We observed no direct complication from insertion of the glucose sensor. In some instances, the sensor seemed to have lost its ability to collect substances from the interstitium, reflected as signal error. This is compensated by its advantage in high frequency of sampling, i.e. every five minutes. Thus the data was almost continuous.

In conclusion, our study results indicate that interstitial glucose concentration in human skin flap during ischemia/reperfusion episode is correlated with tissue perfusion. Prolonged ischemia compromises glucose metabolism in the early reperfusion period. Monitoring trends of glucose regulation in the flap during operation may specify the critical ischemic time for each flap and thus define a benchmark for the postoperative glucose changes in human free flap cutaneous tissue.

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