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Could Measuring Blood Glucose Level help in Early Detection of Venous Flap Congestion in Albino rats ?

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Abstract: Nowadays, a reliable method for reconstructive surgery is flap surgery. With its help, the body is able to mend almost any damage to its vascularized tissues. The current study set out to determine whether measuring flap blood glucose was a sensitive and dependable predictor for venous congestion early detection.
Methods: Thirteen male Sprague-Dawley rats (n=26) were divided into two groups for the purpose of this experiment: one group received vertical rectus abdominis muscle flaps with upper pedicles while the other group did not. One group received venous occlusion of the pedicle, and the other group received no such treatment (Control group). We measured the interstitial glucose in the flaps every ten minutes and checked the rats' glycemia in the caudal vein of their foot at regular ten-minute intervals from the start to the finish of the experiment.
Results: At 0 minutes, 20 minutes, and 30 minutes, the flap glucose level showed a statistically significant decrease compared to the foot glucose level (p=0.01, p=0.005, p=0.002 respectively). At 10 minutes, 40 minutes, 50 minutes, and 1 hour, the flap glucose level showed a high statistically significant decrease compared to the 10 minute foot glucose level (p-value < 0.001). The mean flap glucose level in the cases group decreased significantly (p-value < 0.001) compared to the mean foot glucose level (mean = 228.1 ± 65.1, range = 114.7 - 342.1), as compared to the mean foot glucose level (mean = 179.7 ± 54.3, range = 89.7 - 297.2). With an AUC of 0.7 and a p-value of 0.054, the ROC curve demonstrated that the mean glucose level could discriminate between the flap glucose level and the foot glucose level in the cases group at a cutoff level of 187.2, with a sensitivity of 69.2%, specificity of 69.2%, PPV of 69.2%, and NPV of 69.2%.
Conclusion: The potential for non-invasive and cost-effective early management in cases of venous congestion in tissue flaps has been suggested by monitoring blood glucose levels.

Keywords: *Early Detection, Venous Flap, Congestion, Blood Glucose Level, Albino rat..*

Introduction

There is now a reliable method for reconstructive flap surgery. Abnormalities induced by trauma or surgery can be fixed almost anywhere on the body with vascularized tissue. [1]. When doing regional or free tissue transplants, venous congestion is a common cause of flap failure [2]. It is well-documented that venous

thrombosis causes congestion, which is more harmful to the flap and occurs less frequently than ischemia caused by arterial thrombosis [3].
One of the many reasons this problem arises is that veins are sensitive and have thin walls, making them vulnerable to harm from things like pressure or shock. Vein spasm, venous thrombosis, pedicle torsion, local edema, venous lack of repair, venous spasm, and countless other medical disorders are examples of this [4].
The microcirculatory alterations caused by venous insufficiency are long-lasting, therefore prompt diagnosis and treatment are crucial [5]. Reason being, as the interval between the complication's incidence and discovery grows longer, the likelihood of flap salvage lowers. [6].
Color, temperature, turgor, capillary refill, skin scratch test, pinprick/ultrasonic Doppler, and general postoperative health are some of the traditional methods for monitoring flaps to ascertain vascular perfusion. In order to understand the findings, clinical expertise is required. [7,8].
Clinical cases of congestive flaps were associated with a decreased blood glucose level, according to Sakakibara et al. [9]. According to Hara et al. [10], who described blood glucose testing for flap monitoring, the ideal threshold value for detecting venous thrombosis was determined to be 62 mg/dl. This value had a sensitivity of 88% and a specificity of 82%. Glucose monitoring in flap capillaries is a simple, fast, and cheap method to detect microvascular complications and reduce flap failure. [11].
Recent studies attempted to determine whether adipose tissue glucose levels may be used as a sensitive and specific marker of adipose venous congestion. This study was conducted at the Plastic and Reconstructive Surgery Department of the Zagazig University Hand and Microsurgery Center (ZUHMC) to evaluate the sensitivity and reliability of measuring flap blood glucose for early diagnosis of venous congestion.

2-Methods:

Materials and Methods
The experimental investigation involved 26 male Sprague-Dawley rats ranging in weight from 250 to 350 grams. Zagazig University's Faculty of Medicine's Institutional Animal Care and Use Committee gave its approval for the study (ZU-IACUC/3/F/15/2023) to run from January 2023 through March 2024. The experiment was conducted in accordance with the Declarations of Helsinki and the regulations of the European Community regarding the use of animals in experiments.
Methods:
The experiment's handling of the rats was completely humane and ethical. Before the rats were prepared for the procedure, they were given an intra-peritoneal injection of a mixture of ketamine and xylazine at a dosage of 0.1 mL/100 g of rat weight.
In Group (1) Intervention (n=13): After shaving their abdomens and marking the vertical Rectus Abdominis Muscle Flap (VRAM), the rats were placed in a supine position. Using thermal plates, we positioned the rats in dorsal decubitus and executed abdominal trichotomy. The VRAM flap was dissected **(fig 1 A, B)**. In rats, the rectus abdominis muscle's dominant pedicle, the superior epigastric vessels, were the only ones to pediculate the flap. To prevent heat loss and visceral dryness, a plastic field was placed over the abdominal cavity. Utilizing microsurgical tools and operating under four-time magnification, we carefully separated the pedicle that houses the upper epigastric veins. We punctured a vein in the back of the flap to measure the interstitial glucose every 10 minutes from the beginning (before clamping and dividing the superior epigastric vein) to the end (60 minutes). We additionally monitored the rats' caudal vein in the foot for glycemia at the same intervals. We used the MediSense Optimum glucose monitor to measure blood sugar levels **(fig 1C, D)**.
We ended the experiment with euthanasia by administering a lethal dose of isoflurane, which can cause cardiac arrest, followed by exsanguination. Regarding the ethical considerations that pertain to animal experimentation, we adhered to all biosafety standards.

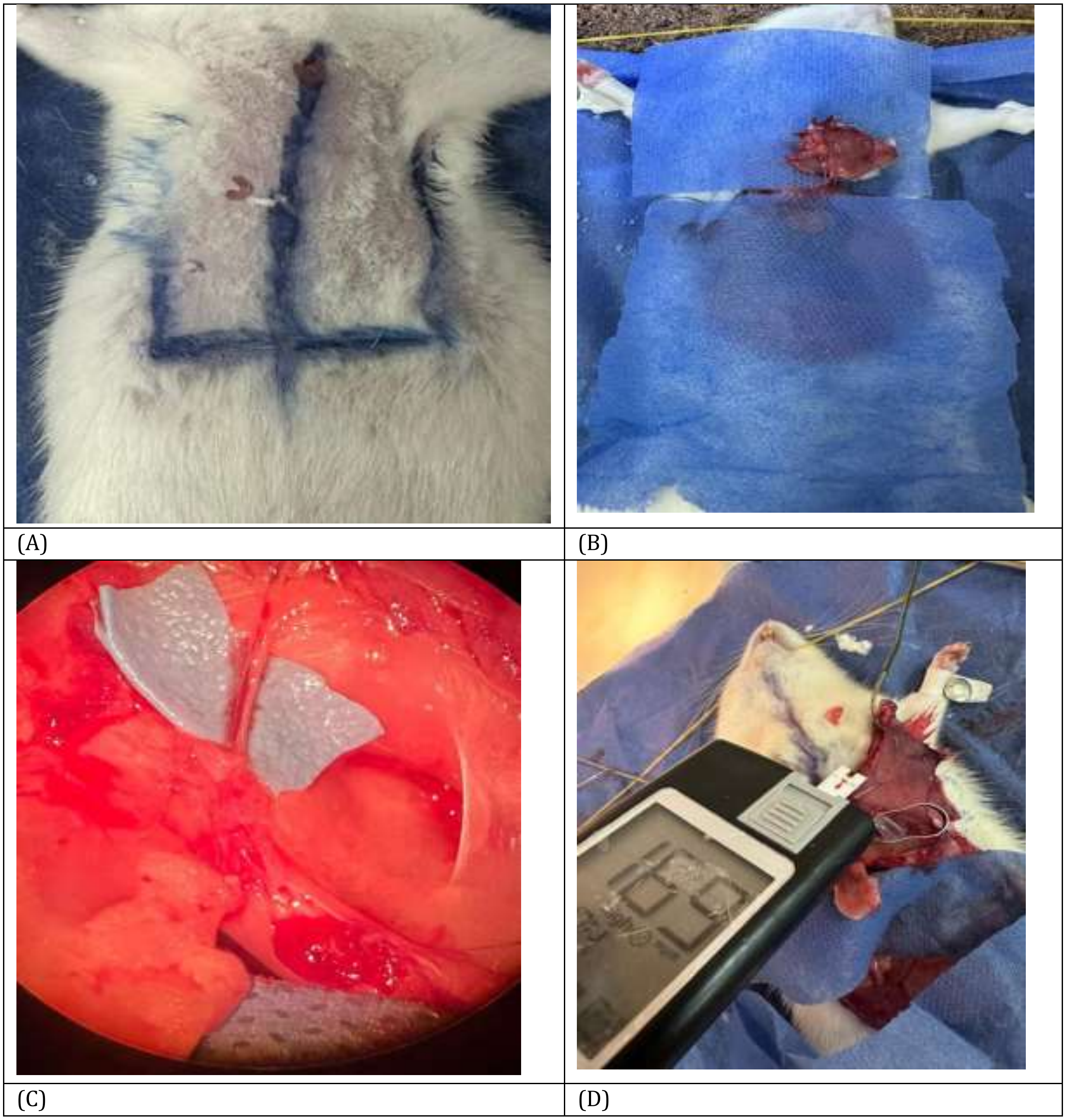

Figure 1: A) placing the rats in dorsal decubitus on thermal plates and performing abdominal trichotomy, B) dissection of the VRAM flap and pediculation of the flab, C) dissection of the pedicle containing the upper epigastric vessels, D) measurement of interstitial glucose in the flaps

Control group 2 control (n=13): We did the same steps but we didn't ligate the vein of the flap pedicle Then we measured the flap blood glucose level every 10 minutes comparing to the Rat's caudal vein Statistical analysis was carried out using a model of generalized estimating equation25. The study involved comparing measurement times and groups (congestive flap, control flap, and systemic control), as well as the interaction between these terms.

Glycemic readings were presented as the mean plus or minus the standard deviation. We performed diagnostic analysis using cutoff thresholds determined by the Receiver Operating Characteristic (ROC) curve. In this case, we determined the sensitivity and specificity values together with the 95% CIs for both.

Statistical Analysis:
Data was analyzed statistically using SPSS version 28 (IBM Co., Armonk, NY, USA). The quantitative data was shown as mean, standard deviation, and range. A statistically significant result was defined as a P-value less than 0.05, and categorical variables were examined using the Chi-square test or independent t-test, which were expressed as frequency and percentage (%).

Results

There was a significant drop in the flap glucose level at 0, 20, and 30 minutes compared to the foot glucose level at the same times (p=0.01, p=0.005, and p=0.002 respectively). In the cases group, there was a high drop in the flap glucose level at 10, 40, 50, and 1 hour compared to the 10 minute foot glucose level at the same times (p-value < 0.001). (Table 1).
In the cases group, the mean flap glucose level decreased significantly (p-value < 0.001) compared to the mean foot glucose level (mean = 228.1 ± 65.1, range = 114.7 - 342.1). (Table 2).
We found that at a cutoff level of 187.2, the mean glucose level could discriminate between the flap glucose level and the foot glucose level in the cases group with a sensitivity of 69.2%, specificity of 69.2%, positive predictive value (PPV) of 69.2%, and negative predictive value (NPV) of 69.2% (AUC = 0.7 & p-value = 0.054). (Table 3, Figure 2).
At 30 minutes, 40 minutes, 50 minutes, and 1 hour, there was a statistically significant drop in the flap glucose level compared to the foot glucose level (p=0.001, p=0.001, p=0.007, p=0.001 respectively). Similarly, at 0 minutes, 10 minutes, and 20 minutes, there was a highly significant drop in the flap glucose level compared to the corresponding foot glucose levels (0 minutes, 10 minutes, and 20 minutes) (p-value < 0.001) in the control group. (Table 4).

Table (1): comparison of glucose level between foot and flap in Cases group.

Cases group		Foot (N = 13)	Flap (N = 13)	t	P-value
Zero	**Mean ±SD**	224.8 ± 37.4	184.7 ± 47.9	**3.06**	**0.01 S**
	Range	178 -289	109 -270		
10 min	**Mean ±SD**	230.9 ± 56.4	189.8 ± 60.2	**4.8**	**< 0.001 HS**
	Range	120 -327	97 -315		
20 min	**Mean ±SD**	231 ± 70.9	186.3 ± 54.3	**3.4**	**0.005 S**
	Range	125 -366	109 -310		
30 min	**Mean ±SD**	228 ± 78.1	178.5 ± 52.7	**3.9**	**0.002 S**
	Range	109 -384	103 -291		
40 min	**Mean ±SD**	232.8 ± 80.1	180.9 ± 64.1	**5.3**	**< 0.001 HS**
	Range	102 -399	75 -306		
50 min	**Mean ±SD**	229.2 ± 82.4	175.5 ± 68.2	**5.7**	**< 0.001 HS**
	Range	75 -389	55 -299		
1 hour	**Mean ±SD**	219.6 ± 75.4	162.5 ± 64.5	**6.7**	**< 0.001 HS**
	Range	94 -337	50 -302		

S: p-value < 0.05 is considered non-significant. t: paired sample T test.
HS: p-value < 0.001 is considered highly significant.

Table (2): comparison of mean glucose level between foot and flap in Cases group.

Cases group		Foot (N = 13)	Flap (N = 13)	t	P-value
Mean glucose level	**Mean ±SD**	228.1 ± 65.1	179.7 ± 54.3	**6.3**	**< 0.001 HS**
	Range	114.7 - 342.1	89.7 – 297.2		

t: paired sample T test. **HS: p-value < 0.001 is considered highly significant.**

Table (3): Diagnostic performance of mean glucose level in cases group.

	Cut off	AUC	Sensitivity	Specificity	PPV	NPV	p-value
Mean glucose level	**< 187.2**	**0.7**	**69.2%**	**69.2%**	**69.2%**	**69.2%**	**0.054**

PPV: positive predictive value. **AUC: Area under curve**
NPV: negative predictive value.

Table (4): comparison of glucose level between foot and flap in Control group.

Control group		Foot (N = 13)	Flap (N = 13)	T	P-value
Zero	**Mean ±SD**	220.5 ± 34.6	213.6 ± 32.7	**6.9**	**< 0.001 HS**
	Range	172 -287	168 -276		
10 min	**Mean ±SD**	212.6 ± 32.4	205.5 ± 32.2	**8.4**	**< 0.001 HS**
	Range	166 -269	159 -260		
20 min	**Mean ±SD**	205.6 ± 31	198.8 ± 29.7	**801**	**< 0.001 HS**
	Range	159 -250	153 -238		
30 min	**Mean ±SD**	200.4 ± 31.9	191.7 ± 28.8	**4.4**	**0.001 S**
	Range	151 -255	148 -230		
40 min	**Mean ±SD**	195.6 ± 31.8	186.5 ± 29.8	**4.2**	**0.001 S**
	Range	145 -245	142 -232		
50 min	**Mean ±SD**	191.4 ± 32.7	182.8 ± 28.3	**3.2**	**0.007 S**
	Range	140 -240	137 -220		
1 hour	**Mean ±SD**	188.2 ± 32.9	182.8 ± 27.1	**4.5**	**0.001 S**
	Range	135 -236	142 -218		

S: p-value < 0.05 is considered non-significant.
T: Independent sample T test.
HS: p-value < 0.001 is considered highly significant.

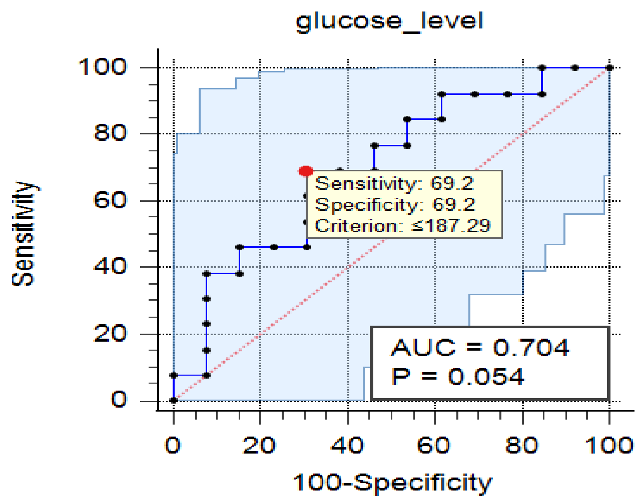

Figure 2: ROC curve between Foot and Flap in Cases group as regard mean glucose level.

Discussion

The purpose of that research was to find out how well flap blood glucose measurement could detect venous congestion in its early stages.

At0,10,20,30,40,50, and 1 hour, there were no statistically significant variations in foot blood glucose levels between the Cases and Control groups, according to the present study. Both sets of participants had similar average blood glucose readings. Additionally, at40,50, and 1 hour, there were no statistically significant changes between the Cases and Control groups in terms of foot blood glucose levels. There did not appear to be a significant difference in glucose levels across the groups, according to the results.

Extremely low blood glucose levels were rapidly attained, as demonstrated in the present investigation and in a previous work by Mochizuki et al. [12]. There was no statistically significant difference ($p = 0.379$) in the amount of time it took for the flap blood glucose to reach an off-scale low after venous obstruction. In both groups, loop blood glucose levels returned to systemic values within fifteen minutes or less following venous declamping.

Additionally, it was noted by Hara et al. [10] that low blood glucose levels were associated with early venous thrombosis. Determination of low capillary blood glucose levels before the occurrence of flap discolouration.

The average range of zero-minute blood glucose levels in the cases group was 194.7 ± 47.9 in this study, whereas in the control group it was 189.8 ± 60.2. The Cases group had an average blood glucose range of 186.3 ± 54.3 minutes after a meal, and an average range of 178.5 ± 52.7 minutes after a snack. In the Cases group, the average range of blood glucose levels after 40 minutes was 180.9 ± 64.1, while in the Control group, it was 186.5 ± 29.8. In the Cases group, the average 50-minute blood glucose range was 175.5 ± 68.2, whereas in the Control group, it was 182.8 ± 28.3. In the Cases group, the average 1-hour blood glucose range was 219.6 ± 75.4, while in the Control group, it was 188.2 ± 32.9. Results showed no statistically significant difference in flap blood glucose levels between the two groups regardless of time or place.

Consistent with the results of the present study, Mochizuki et al. [12] demonstrated that hyperglycemic rats with venous obstruction had blood glucose levels that were higher than the systemic blood at each time point. The researchers found that the two values separated at 10 minutes after vein clamping, and that the flap blood glucose level had returned to the systemic value at 5 minutes after declamping ($p > 0.05$ at 55, 60, 65, and 70 minutes).

In addition, Mochizuki et al. [12] found no statistically significant difference between the two groups (flap congestion groups with and without glucose preloading; $p = 0.379$) regarding the time it took for the flap blood glucose level to decline below 20 mg/dl following vein clamping. However, after declamping, the hyperglycemic rat group returned to systemic blood glucose levels much faster than the normoglycemic rat group (p = 0.028). Glucose levels in the flap, which stand in for the flap venous system, were unaffected by systemic blood glucose levels.

Additionally, in order to monitor the advancement of the flaps, Choudhary et al. [11] utilized a Medtronic glucometer, which is a commonly used device for measuring capillary blood glucose levels. For the simple and fast procedure, just little amounts of blood (10-20 μL) are required. More traditional ways of monitoring flaps, including observing their color, turgor, or employing the pinprick test, are replaced by this more quantitative method. In addition to reflecting the adequacy of flap perfusion, it allows for objective comparisons among experiments.

As our research demonstrated In the cases group, there was a statistically significant drop in flap glucose levels at 0, 20, and 30 minutes compared to the corresponding foot glucose levels at 0, 20, and 30 minutes (p=0.01, p=0.005, and p=0.002 respectively). Additionally, there was a highly significant drop at 10, 40, 50, and 1 hour compared to the 10 minute foot glucose levels at 10 minutes (p-value < 0.001). Glucose monitoring devices may be useful for keeping tabs on patients' glucose levels, according to these results.

Consistent with this, Berlim et al. [13] found that glucose levels in the clogged flap started to drop sharply 15 minutes after venous obstruction. A sensitivity of 100% (95% CI 83.99-100%) and specificity of 90% (95% CI 69.90-97.21%), when using a glycemic difference of at least 20mg/dl between the flap and systemic blood 30 minutes after occlusion as a diagnostic criterion, were achieved for the diagnosis of flap congestion.

Furthermore, it was noted by Berlim et al. [13] that all animals maintained similar systemic glucose levels throughout the experiment. After venous occlusion, glucose levels decreased significantly in one group while remaining unchanged in the other. Compared to the control and systemic blood groups, the intervention group's flaps showed significantly lower glucose levels fifteen minutes after venous closure.

The flap blood glucose method was demonstrated by Anwar et al. [14] to be user-friendly for patients, nurses, and residents, and it is effective even when dealing with intraoral flaps, which are infamously difficult to track using merely color or temperature as markers. One of its limitations is that it can't be used for ischemic flaps or buried flaps since it can't take enough blood to measure the glucose level. Because of metabolic problems, poor cellular glucose uptake, and unstable blood glucose levels, diabetes patients may also experience an unpredictable reaction to venous congestion in their flap blood glucose.

With an AUC of 0.7 and a p-value of 0.054, the present study demonstrated that the mean glucose level could be utilized to differentiate between the flap glucose level and the foot glucose level in the cases group at a cutoff level of 187.2, with a sensitivity of 69.2%, specificity of 69.2%, PPV of 69.2%, and NPV of 69.2%. The study performed ROC curve analysis to draw these conclusions. When comparing the mean flap glucose level (mean = 179.7 ± 54.3, range = 89.7 - 297.2) with the mean foot glucose level (mean = 228.1 ± 65.1, range = 114.7 - 342.1) in the Cases group, there was a highly significant (p-value < 0.001) drop.

Consistent with the results of Berlim et al. [13], who found that glycemic levels alone were not a reliable diagnostic tool for flap congestion, the current investigation found that diagnostic criteria that included comparing glucose levels were more accurate. The comparative criteria' area under the curve (ROC), sensitivity and specificity levels, and overall accuracy all point to their superiority.

Henault et al. [15] examined 37 people's free flaps. We used cutoff values of 69.37 mg/dL for tissue glucose and 57.66 mg/dL for lactate to evaluate hypoperfusion. They were able to diagnose hypoperfusion approximately 5.4 hours before to the actual diagnosis using the clinical examination, and they attained a sensitivity of 98.5% and a specificity of 99.5% utilizing these criteria.

Flap glucose and tissue oximetry levels were compared in the study by Akita et al. [16]. In order to ensure that the flaps were functioning properly, they used indices to compare the numbers recorded there with those from an unoperated part of the body. Tissue oximetry enabled a faster diagnosis, while all assessments were helpful in that study.

In their study on flap monitoring using blood glucose monitoring, Hara et al. [10] found that a threshold value of 62 mg/dl had an 88% sensitivity and 82% specificity for detecting venous thrombosis.

The ongoing consumption of glucose from stagnant blood for tissue metabolism in venous thrombosis led Sakakibara et al. [9] to postulate a decreased flap blood glucose content.
Multiple investigations have shown that ischemia is associated with lower blood glucose levels, and that this drop is more extensive and faster than the drop observed in patients with congestion. Ischemia also leads to a more rapid and substantial rise in lactate concentration than congestion. Based on these findings, it seems that elevated anaerobic respiration in tissues and a shortage of glucose supply are the two main causes of decreased blood glucose in ischemia and congestion.
Sakakibara et al. [9] found that flaps with vascular difficulties exhibited a decrease in blood glucose, demonstrating that the glucose checker's sensitivity is sufficient to detect venous abnormalities.
However, the specificity of the approach is unknown since it has not been tested on undamaged free flaps yet.

Conclusion

This experimental work offers important insights into possible diagnostic procedures for evaluating the viability of venous flaps by investigating the reliability of early identification of congestion by measuring blood glucose levels in albino rats. One non-invasive and cost-effective way to detect venous congestion in tissue flaps is to monitor blood glucose levels, according to the research. This could be a potential indicator for early intervention.

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