

Measurement of the Relationship Between the OCT Signals and Blood Glucose Concentration on Hairless Rats

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ARTICLE INFO

Received: 📅 April 16, 2025

Published: 📅 May 09, 2025

Citation: Fengcheng Wei, Sara Okamoto and Masato Ohmi. Measurement of the Relationship Between the OCT Signals and Blood Glucose Concentration on Hairless Rats. Biomed J Sci & Tech Res 61(5)-2025. BJSTR.MS.ID.009655.

ABSTRACT

The number of diabetic patients is increasing worldwide, but blood glucose levels are still measured daily by diabetic patients through an invasive method. The pain of blood sampling and the process of hemostasis place a burden on diabetic patients. Based on previous research, we investigated the relationship between blood glucose levels and attenuation of interference signals of optical coherence tomography (OCT). In the experiment, we performed a blood glucose tolerance experiment using hairless rats and compared the results of blood glucose levels with the attenuation coefficient of the OCT signal. We found that the attenuation coefficient of the OCT signal tends to decrease with increasing blood glucose concentration. Furthermore, in order to improve the accuracy of the results of the experiment, we should take more OCT signals, then calculate the average of data.

Keywords: OCT; Blood Glucose Tolerance Experiment; Diabetes; Biomedical Engineering

Introduction

Diabetes is a chronic disease in which blood glucose levels rise above the appropriate range. Although it is rarely life-threatening, it can cause arteriosclerosis in blood vessels throughout the body, resulting in complications in various organs if we ignore it. The number of diabetes patients worldwide is increasing unstopably, and is estimated to exceed 380 million this year, with a particularly rapid increase predicted in Asia, including Japan [1]. Diabetic patients need to measure their own blood glucose levels several times a day. Currently, small blood glucose monitors based on enzyme colorimetry and enzyme electrode methods are mainly used to measure blood glucose levels. Both methods are invasive and require blood sampling [2]. Therefore, various non-invasive blood glucose measurement methods have been proposed and are being researched. For example, there have been attempts to measure blood glucose levels using oral mucosa by an ATR prism and attempts to measure anterior aqueous humor using Raman spectroscopy and polarimetry [3]. Among them, KV Lar-

rin, et al. [4] used optical coherence tomography (OCT) technology to evaluate blood glucose levels based on changes in the attenuation coefficient of OCT signals in the dermis layer of pig and rabbit skin [4]. Furthermore, Miura, et al. [5] used OCT to measure diffuse reflected light that penetrated the dermis layer of human skin and clarified that there is a correlation between the signal attenuation coefficient of the reflected light and blood glucose levels [6]. OCT technology is widely used in the field of bio measurement. Jing, et al. [7] used OCT to successfully observe, photograph, and measure the fine structure of the rat brain. Ohmi, et al. [8] also succeeded in measuring skin allergy reactions using guinea pigs. Wei, et al. [8] further applied OCT technology to in-vivo observation and evaluation of surgical sutures. OCT is expected to be useful in measuring skin tissue. Mice, rats, and guinea pigs are widely used as experimental animal especially on the research of skin. Hairless rat skin has a skin structure like human skin and is easy to manipulate in experiments. Based on our successful experience with animal experiments using hairless rats [9], we conducted this study using hairless rats as experimental subjects to verify the

specific relationship between the OCT signal attenuation coefficient and blood glucose concentration.

Materials and Methods

Experimental Animals and Anesthesia Methods

2 hairless rats (HWY/Slc, male, 1 year old) were anesthetized with medetomidine chloride (0.15 mg/0.15 ml/kg), midazolam (2 mg/0.4 ml/kg), butorphanol tartrate (2.5 mg/0.5 ml/kg), and saline (1.45 ml/kg).

Blood Glucose Tolerance Test and OCT Measurement

After sedation, blood was collected from the tail vein using a blood glucose measuring device (Oshieguruko, Foracare Japan Co., Ltd.) and blood glucose levels were measured. Then, glucose solution was administered intraperitoneally to each animal at 3 g/kg body weight. Thereafter, blood glucose levels were measured at 15, 30, 45, 60, and 90 minutes in the same way as before glucose administration. The OCT used is a THORLABS TELESTO320 Spectral Domain OCT. The specifications of this imaging device are a central wavelength of 1300

nm, a maximum imaging depth of 3.5 / 2.6 mm (water / air), an axial resolution of 5.5 / 4.2 μm (water / air), a horizontal resolution of 13.0 μm , and a sensitivity of 93-109 dB. This time, using this device, 2D images were taken at three locations immediately after each blood glucose measurement, and as shown in Figure 1, the signal intensities of the five left and right lines at the 150th, 300th, and 450th A-line (counting from the left) were averaged along the X-axis and calculated. An example of A-line data is shown in Figure 2. After averaging 10 A-lines as in Figure 1, data like that in Figure 2 is obtained. The area with the highest signal intensity (orange arrow) is the stratum corneum of the epidermis. A second peak (black arrow) can be confirmed below the stratum corneum. The area between the two peaks is the epidermis layer. In order to calculate the OCT attenuation coefficient this time, we aimed to obtain data from the dermis layer below the epidermis layer, so we confirmed the area between the second peak and the subcutaneous tissue where signal attenuation is almost non-existent (red arrow) and extracted the data. curve slope should be taken from dermis layer which is on the right side of epidermal layer on this picture. After dermis layer is subcutaneous tissues where the OCT signal slope approaches 0.

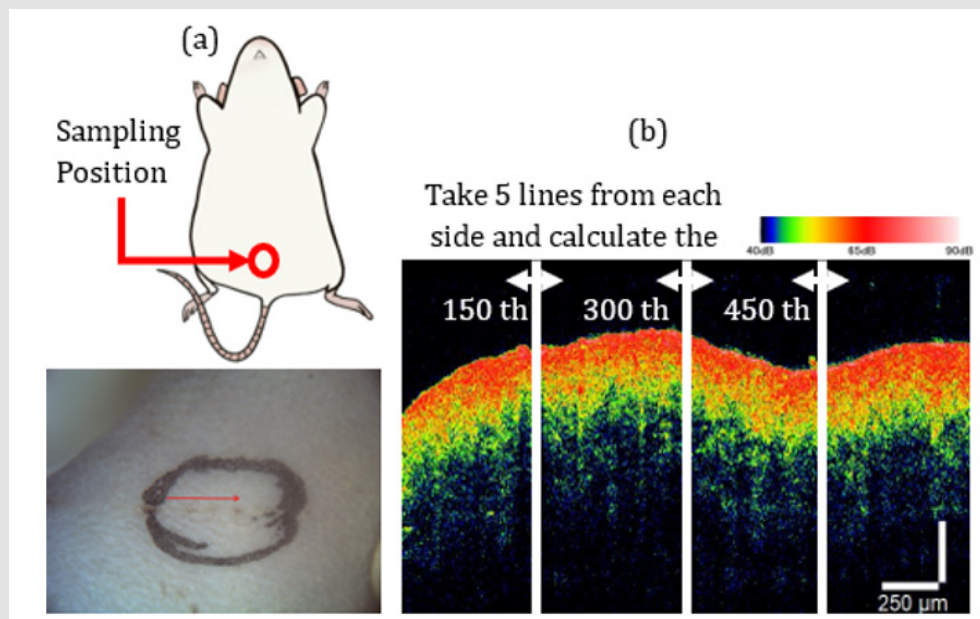


Figure 1: shows the sampling method.

- We achieved the OCT tomographic from the lower abdomen of rats
- Shows an example of OCT tomographic, the 150th, 300th, 450th A-lines (counting from the left) were selected, and we took 5 A-lines from each side of each line to calculate average A-line each contains 10 A-lines data. Then we calculated the curve slope of averaged A-line.

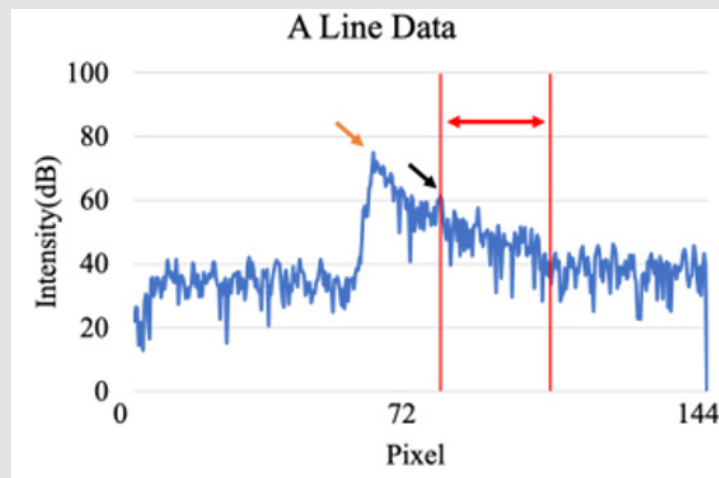


Figure 2: A sample of averaged A-line data. The epidermal layer can be confirmed between 2 OCT signal peaks shown by orange arrow and black arrow. In this research, the data used to calculate curve slope should be taken from dermis layer which is on the right side of epidermal layer on this picture. After dermis layer is subcutaneous tissues where the OCT signal slope approaches 0.

Result

Blood Glucose Tolerance Test

The blood glucose measurement results are shown in Figure 3. The horizontal axis is the time elapsed since glucose injection, and the vertical axis is the blood glucose concentration. The first hairless

rat is shown in yellow, and the second in gray. In both hairless rats, the blood glucose level rose sharply after glucose was injected. The tendency to rise subsided after 30 minutes, but it was found to remain stable at around 500-600 mg/dl up to 90 minutes. Due to individual differences, it was also found that there was a difference of 50-100 mg/dl between the two rats at the same period.

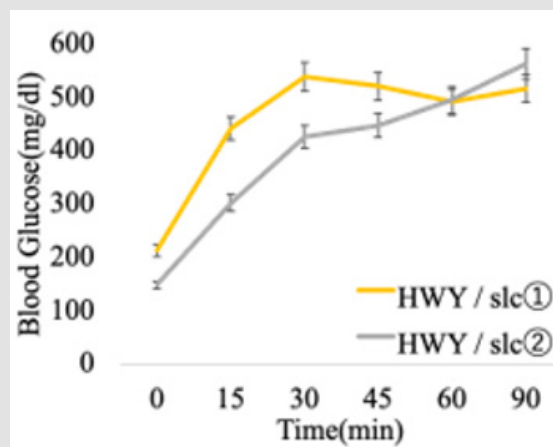


Figure 3: Shows the result of blood glucose. Both rats shown intensive increase from 0 min to 30 min and stabilized at 500-600 mg/dl till 90 min.

Attenuation Coefficient of the OCT Signal

The slope of the OCT attenuation signal was calculated. The results are shown in Figure 4, adjusted to absolute values because the values are in minus and difficult to see. The horizontal axis is the data

of each A-line, and the vertical axis is the absolute value of the slope of the OCT attenuation signal. In the case of 0 min, the 150th line of the first animal and the 150th and 450th lines of the second animal are close to a value of 1. In contrast, in the case of 90 min, they were all lowered to between 0.5 and 1. It is thought that the higher the abso-

lute value of the attenuation coefficient, the lower the blood glucose level tends to be. Between 0 and 30 min, the values of the first animal were generally lower than those of the second animal. This also suggests that the higher the absolute value of the attenuation coefficient, the lower the blood glucose level. In addition, differences appeared

due to differences in the measured individuals. Differences appeared even when the measurement points were slightly apart even in the same tomographic image. It was suggested that the number of measurements should be increased, and experiments should be conducted.

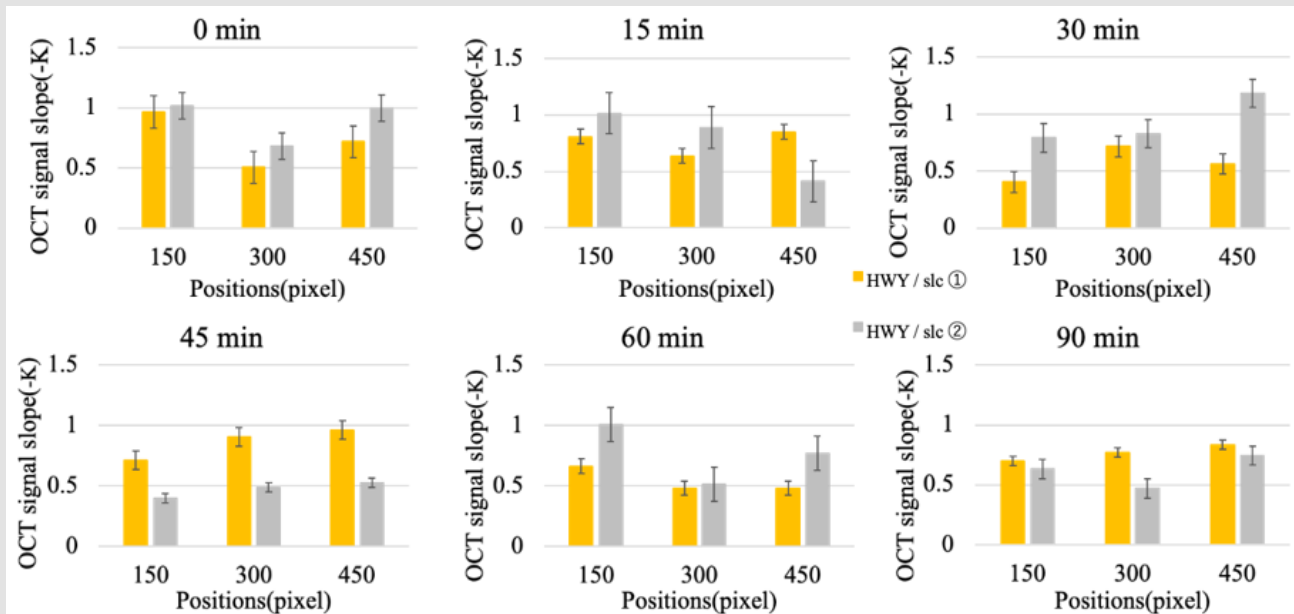


Figure 4: Shows the result of the slope of OCT signal from 0 min to 90 min. The slope value of 0 min is higher than 90 min, and the 1st rat (yellow bar) has a lower value than 2nd rat. We inference that the higher the absolute value of the attenuation coefficient, the lower the blood glucose level could be.

Comparison of OCT Signal Attenuation Coefficient and Blood Glucose Level

The average value of the OCT signal attenuation coefficient was calculated and compared with the blood glucose level. The results are shown in Figure 5

- For the first rat and
- For the second rat.

The left vertical axis shows the absolute value of the OCT attenuation signal, the right vertical axis shows the blood glucose level, and the horizontal axis shows the elapsed time. In the first rat, the results at 30 and 60 min show a lower OCT signal than at 0 min. It is assumed that the OCT signal attenuation coefficient tends to decrease with an increase in blood glucose level. Furthermore, in the case of the second

rat, it was clear that the curve of the OCT signal attenuation coefficient decreased with an increase in blood glucose level. This result is also consistent with the results of Miura, et al. [5] Linear regression calculations were also performed, and the results are shown in

- For the first rat and
- For the second rat.

Although there may be some error in both results due to individual differences between rats and the small number of measurement data, a decrease in the attenuation coefficient due to an increase in blood glucose level was confirmed. In addition, the coefficient of determination for the first rat's data was lower than that for the second rat, which is thought to have more accurately represented the linear regression curve.

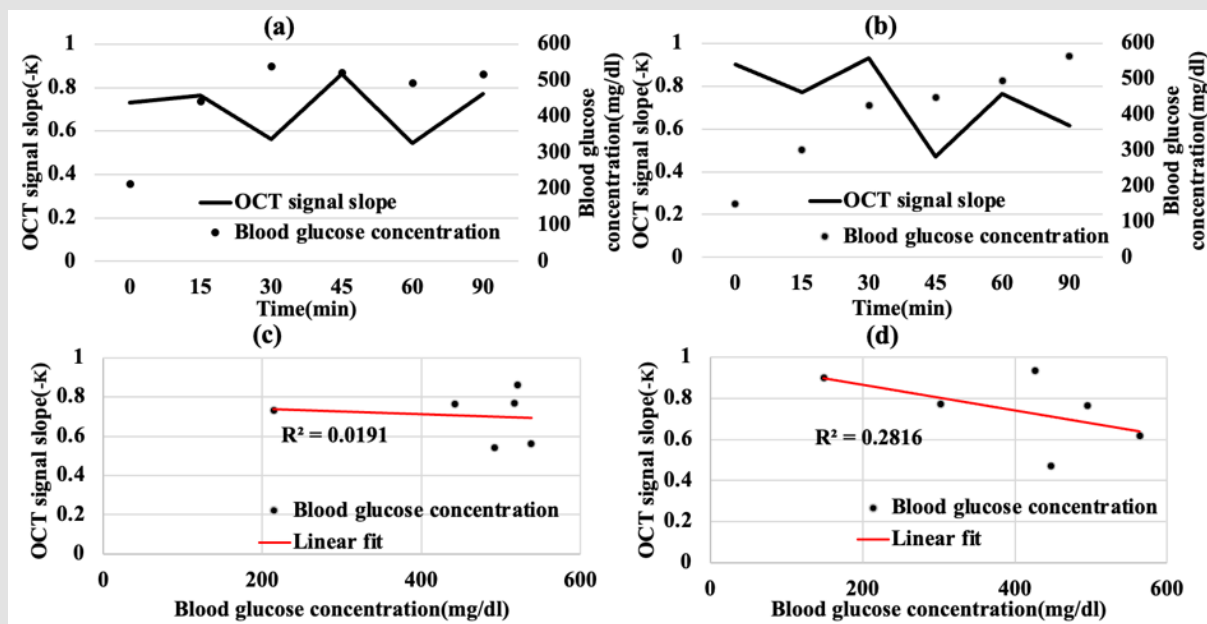


Figure 5: Comparison of OCT signal slope and blood glucose concentration with time lapse. 1st hairless rat shown in (a) and 2nd hairless rat shown in (b). 30 min and 60 min data from 1st hairless rat indicate the OCT signal slope could decrease due to the increasing of blood glucose concentration. And 2nd hairless rat's result from (b) proved this inference. Linear fit between OCT signal slope and blood glucose concentration result are shown in (c) for 1st hairless rat and (d) for 2nd hairless rat. 1st hairless rat has a more accurate coefficient of determination and both 2 rats' result imply the inference of inverse proportion between OCT signal slope and blood glucose concentration.

Conclusion

In this study, we successfully conducted a blood glucose tolerance test using hairless rats. Furthermore, we investigated the relationship between the OCT signal attenuation coefficient and blood glucose concentration in hairless rats and found that there was a strong inverse proportional relationship between the two. However, due to individual differences between rats, blood glucose levels sometimes fluctuated even after the same amount of time had passed. Increasing the number of subjects measured in future experiments will reduce individual differences. It is also highly likely that the number of OCT signal samples is still low. Since the signal coefficient fluctuated significantly back and forth even when the same tomographic image was taken slightly away from the subject, in future experiments we will sample the A-line at the same location 100-200 times to reduce the error.

Acknowledgements

We would like to express our gratitude to Professor Akitoshi Seiyama of the Akita International University for his guidance and encouragement in conducting this research.

Disclosures

The authors have no financial conflicts of interest to declare related in this study.

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ISSN: 2574-1241

DOI: [10.26717/BJSTR.2025.61.009655](https://doi.org/10.26717/BJSTR.2025.61.009655)

Fengcheng Wei. Biomed J Sci & Tech Res



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