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Correlation between Red Blood Cell Aggregation and Blood Glucose Level

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***Abstract*—High blood glucose level (BGL) is related to the high viscosity of blood. Red blood cells (RBCs) tend to gather each other in the condition of high viscosity. Backscattered echoes become high as RBCs aggregate. Therefore, the intensity of backscattered echo is anticipated to be high as the BGL increases. In the present study, we investigated the relationship between RBC aggregations by ultrasound and the BGL for the development of non-invasive BGL measurement methods. Ultrasonic backscattering echoes for dorsal hand vein and BGLs were repeatedly measured from fasting to 190 min after injecting 40-g glucose for a healthy subject. The brightness of the B-mode image increased as the BGL increased. The brightnesses of B-mode image and the BGLs after 160-190 min were less than those at fasting. The relationship between the brightness of the B-mode image and BGL was almost linear, although the hysteresis characteristic was observed. This is because multiple factors other than BGL are closely related to the RBC aggregations.**

Keywords—red blood cell, aggregation, blood glucose level, ultrasound scattering

I. Introduction

The red blood cell (RBC) aggregation is a reversible phenomenon in which RBCs, which are the main components of blood, adhere to each other [1]. Blood viscosity, hematocrit, blood coagulation factors, immunoglobulins, acute phase reactive proteins, and so on are factors directly related to the incidence. In particular, blood viscosity is highly related to the blood glucose level (BGL) and it increases particularly at low shear rates of the blood flow [2]. Therefore, there may be a correlation between the degree of RBC aggregation and the BGL. However, there are very little experimental works on this relationship. In addition, in B-mode images acquired by ultrasonic diagnostic apparatuses, high-intensity haze echoes called "smoke-like echoes" are observed in the low-intensity blood vessel lumen depending on the degree of RBC aggregation [3].

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We have studied a non-invasive and quantitative method to evaluate the degree of RBC aggregation by estimating the scatterer size of RBCs. Fukushima *et al.* measured the power spectrum of ultrasound waves scattered by a single RBC and RBC aggregates *in vivo*, and developed an evaluation method of the degree of RBC aggregation by estimating the size of the scatterers [4]. Kurokawa *et al.* improved the calculation procedure of the power spectrum for the scatterer size estimation [5]. Sakaki *et al.* examined the relationship between the BGL and RBC parameters ultrasonically measured in *in vivo* in healthy subjects and diabetics [6]. However, there were problems that avascularization, which realizes low shear rates of the blood flow, could not be surely and instantly started and that the measurement interval of BGLs by the blood glucose measuring device accompanying puncture was too long to follow the temporal change of BGLs.

The objective is to clarify the relationship between information obtained from observations of RBC aggregation by ultrasound and events in the body. In the present study, we experimentally examined the correlation between BGL and RBC aggregation before and after glucose ingestion.

II. Method

A. Measurement of blood glucose level

BGL was measured using the blood glucose measuring device (FreeStyle Libre Continuous Glucose Monitoring (FSL-CGM) (Abbot Diabetes Care Inc., Alameda, CA, USA). The FSL-CGM sensor was attached to the back of the upper arm. However, the measured value is delayed to the BGL since it measures not the BGL itself but the glucose level of the interstitial fluid. In addition, it is considered that the FSL-CGM sensor has a systematic error depending on the production lot. We compared the glucose levels measured by the FSL-CGM system with those by the self-measurement of blood glucose (SMBG) level using blood glucose measuring device (FreeStyle Freedom Lite; NIPRO Corp., Osaka, Japan) on healthy subjects

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in state of fasting and after ingesting glucose, and examined the time lag between those devices.

B. Measurement using ultrasound

The ultrasonic backscatter power from blood is proportional to the average aggregate volume under the constant hematocrit [7]. The brightness in the B-mode image becomes higher as the scatterer size increases. Thus, the change in RBCs from a single cell with a diameter of about 8 μm to large aggregates with diameter of about 50 μm can be confirmed as an increase in the brightness changes.

For the measurement of B-mode images using ultrasound, an ultrasound diagnostic apparatus (UD-8000; Tomey Corp., Nagoya, Japan) was used with a mechanical sector ultrasound probe (IP210; Tomey Corp., Nagoya, Japan) operating at the center frequency of 40 MHz and whose focal length is 9 mm. The wavelength corresponds to approximately 38 μm. The sampling frequency was 240 MHz. A dorsal hand vein was chosen as the measurement target because of the large propagation attenuation in the living body. The focal point of the probe was adjusted to the center of the blood vessel lumen.

Since the measurement target was the dorsal vein of the hand, one point closer to the heart than the measurement region was pressed from above using the tip of a bar-shaped instrument with a 5-mm diameter for avascularization. The region with the same width in the neighborhood of the focal point of the probe was used for luminance analysis of the B-mode images, and the same region was selected within a series of experiments.

C. The relationship between blood glucose level and RBC aggregation

We investigated the relationship between BGL and RBC aggregation. BGLs were acquired using the wearable sensor, and B-mode images were acquired using ultrasound. B-mode images were measured twice on an empty stomach after fasting more than 10 hours, and 12 times over 190 minutes after ingesting 40-g glucose. The first measurement was 10 minutes after glucose ingestion. The measurements up to the 7th (after 70 minutes) were 10-minute intervals, then those up to the 10th (after 130 minutes) were 20-minute intervals, and then those up to the 12th (after 190 minutes) were 30-minute intervals.

19 frames of B-mode images were measured in each measurement. 7 frames were acquired before avascularization and the rest were acquired after that. B-mode images were acquired at 10-second intervals. In order to advance RBC aggregation, the low shear rate of blood flow was realized by avascularization.

The subject was a healthy man in his 40s.

III. Results

A. Comparison of blood glucose level measured by CGM systems and SMBG

By comparing the glucose levels measured by the CGM system and the SMBG, the glucose levels by the CGM system had a time delay of 10 minutes compared to BGLs by the SMBG. Therefore, the measured glucose levels obtained by the CGM system were treated as BGLs 10 minutes ago.

In order to correct the effects of systematic errors among production lots, the glucose level obtained by the CGM system was compared with those by the SMBG twice, just before the start of the ultrasound measurement and just after the end of all ultrasound measurements. As the result, the glucose level measured by the CGM system was approximately 20 mg/dl larger than those by the SMBG. Therefore, the BGL in each measurement was reduced by 20 mg/dl.

B. Changes in blood glucose level and image brightness over time

Figure 1(a) shows temporal changes in B-mode images in the vein of the subject. The horizontal axis in the figure is the elapsed time since glucose ingestion. From top to bottom, four images before avascularization, at 5, 25, and 85 seconds from the start of the avascularization are shown. Regardless of the elapsed time from glucose ingestion, the image brightness increased at avascularization, and the brightness further increased as the times at avascularization became longer. This is caused by the increases in the size of the scatterer because of the RBC aggregation. The BGLs are shown in Fig. 1(b). The BGL rose greatly after glucose ingestion, became maximum after 40 minutes from the ingestion, and then decreased. The BGL change became gentle as time passed after ingestion. The image brightness was highest after 40 minutes from the glucose ingestion regardless of the elapsed time of avascularization including pre-avascularization. In addition, after 160 minutes from the glucose ingestion, both image brightness and BGL were lower than those before glucose ingestion.

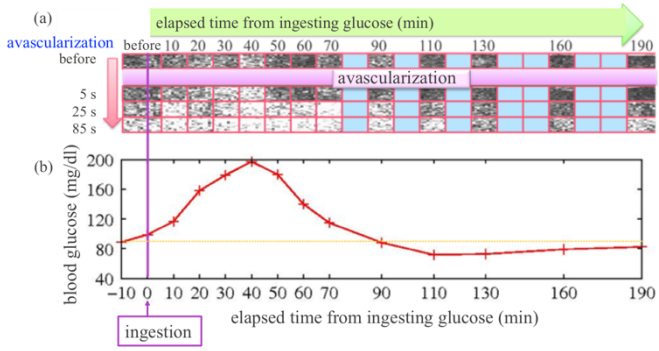

Fig. 1. Temporal changes of (a) B-mode images and (b) blood glucose levels.

C. Relationship between blood glucose level and the average luminance value

Using the image processing software ImageJ (National Institute of Health, Bethesda, MD, USA), the average luminance value of each image was calculated at each avascularization time. Figure 2 shows their relationships.

Positive correlations were observed at 5 and 25 seconds after avascularization. On the other hand, the average luminance values were almost constant regardless of the BGLs before avascularization and 85 seconds after avascularization. However, even for the data having a positive correlation, the image brightness corresponding to the same BGL did not necessarily take close values when the BGL increased or decreased. In most results, the BGL and brightness after 110 minutes from glucose ingestion were lower than those on fasting.

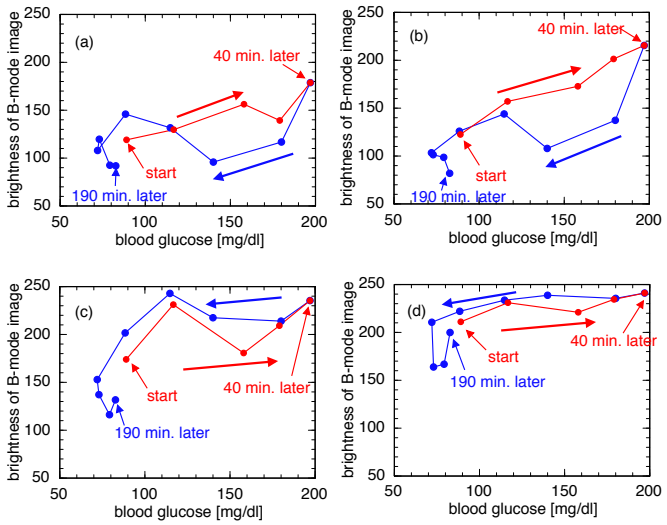

Fig. 2. Relationships between brightness of B-mode images and blood glucose levels for the subject. (a) before avascularization, (b) 5 s, (c) 25 s, and (d) 85 s after avascularization.

IV. Discussion

In Fig. 1, the brightness of the intravascular B-mode image increased with the passage of the avascularization time regardless of the elapsed time of the glucose ingestion. This could be caused by increases in the degree of RBC aggregation due to continuous low shear rate conditions. In addition, the brightness became peak around 40 minutes after glucose ingestion despite with or without avascularization. It means that the degree of RBC aggregation tends to increase after 40 minutes from glucose ingestion. Immediately after glucose ingestion, absorption of glucose into the body begins and BGL rapidly rises. The change from rising to fall after 40 minutes from glucose ingestion could be caused by that the factors such as insulin, which antagonize the increases of BGL, became more active.

The decrease in BGL gradually slowed down. BGLs were lower than that before glucose ingestion after 160 minutes, and there was no significant change until 190 minutes. This is also due to the undershoot of BGL caused by factors such as insulin. The brightness of the ultrasonic image was similarly undershot and was lower than that on fasting.

In Figs. 2(b) and (c), there was a positive correlation between the BGLs and the brightness values of the B-mode image. However, they did not linearly correspond. This might be caused by that the period in which the image brightness change becomes large after glucose ingestion is different from that of BGL due to any conditions other than BGL. The BGL and the image brightness decreased after 40 minutes from the glucose ingestion, but the brightness changed earlier (Fig. 2 (b)). Therefore, a time lag occurred between the change in BGL and that in the degree of RBC aggregation due to some causes.

V. Conclusion

In the present paper, in order to clarify the correlation between BGL and RBC aggregation, we investigated the changes in BGL and RBC aggregate caused by glucose ingestion in a healthy subject, and discussed their relationships.

The experimental results showed that there was a positive correlation between BGL and RBC aggregation at a certain time after avascularization, indicating the possibility of BGL measurement by ultrasonic measurement. However, the brightness values for the same BGL were different between ascending and descending of them. The factors other than BGL could be related to the RBC aggregates.

In the present study, the relative relationships between BGL and RBC aggregation were shown, but they did not correspond one-to-one even in consecutive measurements on the same subject. In the future, it will be necessary to examine differences in the rate of rising and fall between them and factors other than BGL (blood pressure, hematocrit, HbA1c, etc.). In addition, it is necessary to investigate the reproducibility of the same subject and the differences among subjects in detail.

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