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Attempt for Noninvasive Evaluation of *in vivo* Triglyceride in Blood*

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***Abstract*— To find an early symptom of postprandial hyperlipidemia, we developed a technique to measure the change in triglyceride noninvasively. We examined the feasibility to measure the change in the triglyceride concentration as the change in the scattering coefficient of the blood. In an experiment, good correlation was obtained between the change in the triglyceride after a meal and the optical change. This suggested the feasibility of the noninvasive measurement of the triglyceride in blood.**

I. Introduction

In recent years, the problem of the excessive fat in blood after meals (postprandial hyperlipidemia) is attracting public attention as a risk factor for atherosclerosis [1]. For the diagnosis of postprandial hyperlipidemia, it is necessary to observe the change in blood lipid levels for 6–8 hours after a meal. In other words, the subject must be restrained in 6–8 hours for multiple blood sampling. It is not practical to carry out such a test in non-clinical settings. To answer this demand, we have developed an optical technique to measure the triglyceride *in vivo* without drawing blood.

Lipid components in the blood are hydrophobic and are found in the form of suspended particles in the blood. They are the lipoproteins covered with amphipathic phosphatide. The lipoproteins can be classified by their diameters into chylomicrons, VLDL, LDL and HDL. These lipoproteins are present in the blood as scattering particles for the light. Therefore, we can evaluate the quantity of the lipoprotein by measuring the scattering characteristics.

Some optical techniques have been developed to evaluate the lipoprotein level in the blood. The dynamic light scattering (DLS) technique is one of them [2]. This technique is useful to obtain the particle size distribution. However, the sample preparation and the instrument of this technique are often complicated, and it is not suitable for a simple measurement. On the other hand, if we focus on the scattering measurement of the large particles such as the chylomicron and VLDL, we can expect real-time assessment of triglyceride with a much simpler device than DLS. In this study, we examined the possibility to measure the concentration of the lipoprotein from 0.1 to 1 micrometer diameter in the blood *in vivo* by the optical scattering measurement from outside the body.

II. Method

An overview of the measurements is shown in Fig. 1. We illuminate continuous light at a point on the skin, and measure the intensity of the backscattered light (reflectance) at another point with the distance ρ. The distribution of the reflectance $R(\rho)$ is given as [3,4],

$$R(\rho) = S_0 \frac{z_0}{2\pi}\left(\mu_{eff} + \frac{1}{\sqrt{\rho^2 + z_0^2}}\right)\frac{\exp\left(-\mu_{eff}\sqrt{\rho^2 + z_0^2}\right)}{\rho^2 + z_0^2}, \quad (1)$$

where $z_0 = 1 / \mu'_s$ and $\mu_{eff} = [3\mu_a(\mu'_s + \mu_a)]^{1/2}$. S_0, μ'_s and μ_a are the intensity of the light source, the reduced scattering coefficient and the absorption coefficient, respectively. If we measure the reflectance at the two different points ρ_1 and ρ_2, we can estimate the μ'_s of the scattering medium as,

$$\mu'_s = \frac{1}{3\mu_a}\left[\frac{1}{\rho_2 - \rho_1}\ln\frac{\rho_1^2 R(\rho_1)}{\rho_2^2 R(\rho_2)}\right]^2. \quad (2)$$

Therefore, we can quantitatively evaluate the change in the scattering property inside the body by adding another light receiver to the measurement system shown in Fig. 1.

III. Experiment

For the future application to postprandial hyperlipidemia, we attempted to measure the scattering coefficient in the conventional fat-load test. After the fast for 12 hours, we fed

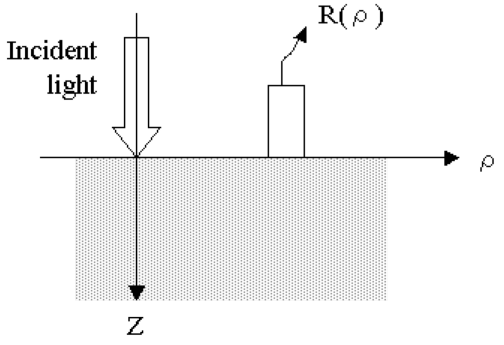

Figure 1. Outline of noninvasive measurement of backscattered light intensity.

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the subject one pack (160 g) of the fat (OFTT cream) designed for a medical fat-load test, and measured the reflectance, or the intensity of the backscattered light. The measurements were made before the fat ingestion and 60, 150, 180, 210, 240, 270, 300, 330 and 360 minutes after the ingestion. As the light source and the detector, we used the Ti:Sapphire laser (Chameleon Ultra II: Coherent Inc.) with 800 nm wavelength and photodiode (FWPR-20-SI, FEMTO Messtechnik GmbH). For comparison, we took blood samples from the vein almost simultaneously with the light measurement, and carried out the chemical hemanalysis. The concentration of triglyceride was measured with automatic analyzing device (H-7170, Hitachi High-Technologies Corp.). In addition, the blood serum was analyzed with a liquid chromatograph (HPLC, LC-20AD, Shimadzu Corp.) using the Superose 6 column.

IV. Examination of Blood Components

First, we attempted to identify the blood components to evaluate the postprandial hyperlipidemia quantitatively. The measurement was carried out with a human subject (male, 39 years old, body-mass index is 30.7) under the authority of the ethics committee for engineering research involving human subject in Hokkaido University with the agreement of the subject. Fig. 2 shows the temporal change of the serum lipids measured by the chemical technique after a meal. With this result we confirmed that the triglyceride showed most apparent change among serum components.

In the gel filtration HPLC, we fractionated the serum lipoprotein according to the particle size. Fig. 3 shows the result obtained with the sample before a meal. The triglyceride was contained mainly in chylomicron and VLDL. Fig. 4 shows the after-meal temporal change of the triglyceride in different lipoproteins. With this result, we confirmed that the triglyceride in chylomicron and VLDL showed most apparent change after the fat-load test.

V. Assessment of Measurement Conditions

In (2), we can expect the higher sensitivity for the μ'_s estimation with the larger $\rho_2-\rho_1$ and the larger $R(\rho_1) / R(\rho_2)$. In other words, the more distance between the two detectors seemed to be the better. However, as can be seen in (1), the measured reflectance $R(\rho)$ decreases exponentially as the distance ρ increases. This leads to the decrease in the SN ratio and makes the measurement less accurate. Furthermore, as the difference between ρ_2 and ρ_1 increases, the target region to estimate μ'_s becomes small because of the following reason. This also makes the measurement less accurate. The light received at the detector in Fig. 1 had propagated in the specific region of the scattering medium, so called the "banana shape" region between the incident and the output points. Using (2) we estimate μ'_s in the overlapping region of the two banana-shape regions of the two detectors. As the distance between the two detectors becomes lager, the overlapping region becomes smaller.

Therefore, to find the appropriate positions of the detectors, we investigated the measurement conditions by varying ρ_1 and ρ_2 within the practical range. In this experiment, we made tissue-equivalent phantoms with known μ'_s using aqueous solution of Intralipid. Fig. 5 shows a typical result, or the change in $R(\rho_1) / R(\rho_2)$ for the given μ'_s. For the calibration

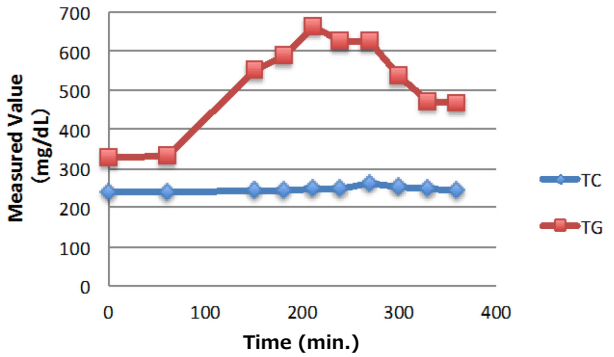

Figure 2. After-meal temporal change of total cholesterol (TC) and trigryceride (TG) in blood serum measured by conventional chemical method.

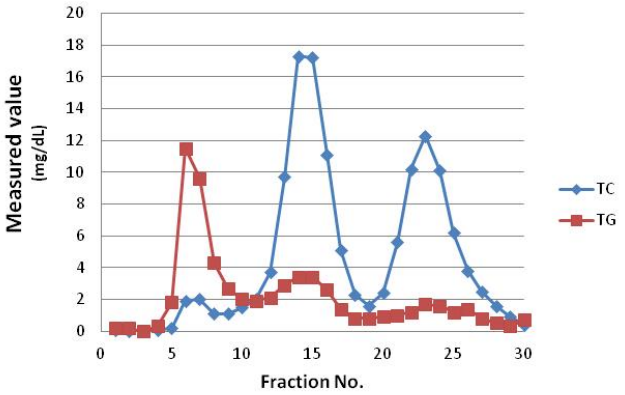

Figure 3. Result of tractionation by HPLC.
Fraction No. 4–9 : CM/VLDL,
Fraction No. 12–18 : LDL,
Fraction No. 20–28 : HDL.

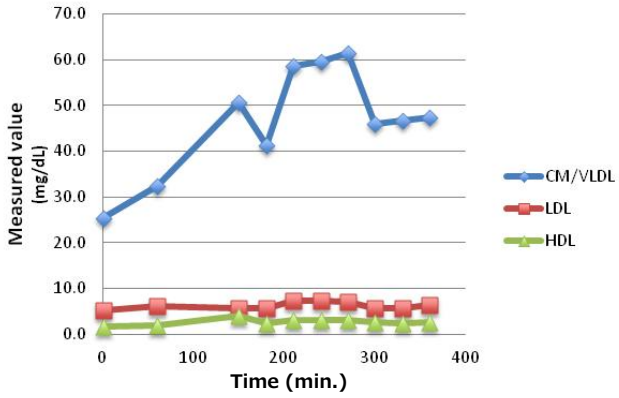

Figure 4. Temporal change of triglyceride in different lipoproteins.

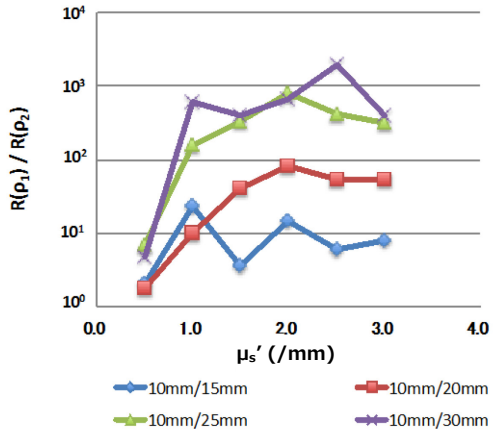

Figure 5. Dependence of of $R(\rho_1) / R(\rho_2)$ on detector position.

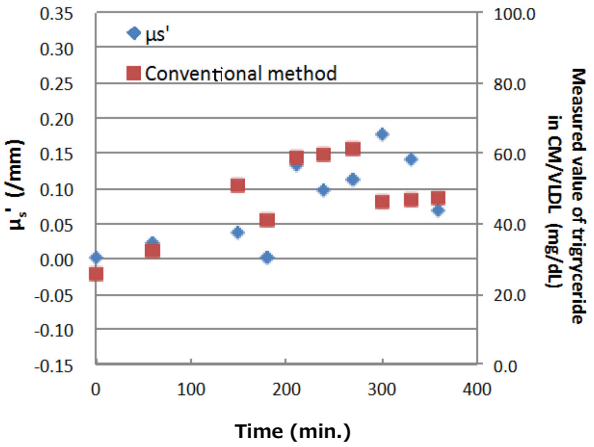

Figure 6. After-meal temporal change of estimated μ'_s and serum triglyceride measured by conventional cheminacl method.

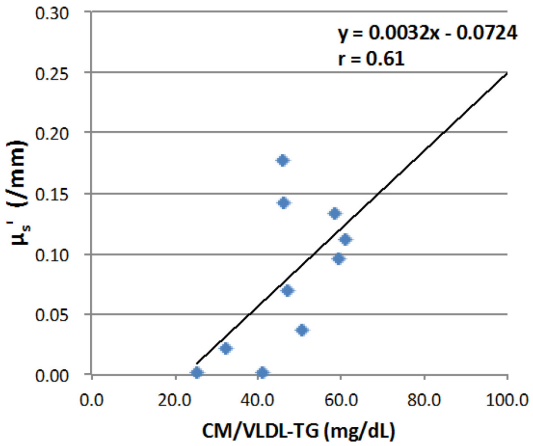

Figure 7. Correlation between estimated μ'_s and serum triglyceride measured by conventional chemical method.

curve to estimate μ'_s from the measured $R(\rho_1) / R(\rho_2)$, stable monotonous change in $R(\rho_1) / R(\rho_2)$ is desirable. From this point of view, the case of $\rho_1 = 10$ mm and $\rho_2 = 20$ mm was the best among the cases examined. In subsequent experiments, we set the detectors at these distances from the light point.

VI. Measurement in Human Subject

The optical measurement was carried out at the same time as the blood component analysis described in Chap. IV. Laser light was guided to the skin surface above the vein at the back of the hand through an optical fiber (100 μm core diameter). Received light was guided through an optical fiber of the same kind to a detector. The value of μ'_s was estimated using the proposed technique. To reduce the effect of the artery/capillary perfusion and other measurement noises, we measured the average of the received intensity for 20 second at each point. For comparison, blood sample was taken at the same time as the optical measurement, and the amount of the triglyceride was measured by the conventional chemical method after the fractionation by HPLC. Fig. 6 shows the after-meal temporal change of the estimated μ'_s and the amount of the triglyceride measured chemically. Fig. 7 shows the correlation of these two. A positive correlation with the coefficient 0.61 was obtained. In the measurement with another subject, the (male, 33 years old, body-mass index is 20.6), the correlation was almost same.

VII. Conclusion

With the view toward the control of excessive fat in blood, a new technique was developed to evaluate the triglyceride in blood noninvasively using light. An equation was derived which gives the reduced scattering coefficient from the measured intensity of backscattered light at two different positions on the body surface. Experimental study was conducted to examine the feasibility of the proposed technique. In this study, we found that the triglyceride in blood was contained mainly in the relatively large lipoproteins, or chylomicron and VLDL. Since the reduced scattering coefficient is dependent more on the larger scatterers, we can expect to evaluate the triglyceride by this coefficient. In the experiment, positive correlations with the amount of triglyceride were confirmed.

Through these analyses, the feasibility of the noninvasive real-time evaluation of triglyceride in blood using the proposed technique was verified. If we can measure the concentration change of triglyceride continuously, we can evaluate the lipid metabolism function quantitatively. It will provide a new possibility in the assessment of not only arteriosclerosis but also liver functions.

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