

# INFLUENCE OF ADIPOSE TISSUE THICKNESS ON NEAR INFRARED SPECTROSCOPIC SIGNALS IN THE MEASUREMENT OF HUMAN MUSCLE

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## ABSTRACT

The aim of this study was to clarify the influence of subcutaneous adipose tissue (AT) thickness on near-infrared (NIR) optical density and the penetration depth of light in muscle tissue *in vivo*. The thickness of adipose tissue in the leg was measured using ultrasonography in 12 young subjects. Optical densities (OD) at 775, 807, and 827 nm were measured when the distance between the light source and the detector was increased from 20 to 100 mm in 10 mm steps. Ultrasonography showed that AT thickness ranged from 4 to 10 mm. The OD increased with increasing distance between the light source and the detector in all subjects. At the same distance between the light source and the detector (30 mm), the OD values correlated negatively with AT thickness ( $r = -0.79, -0.82, \text{ and } -0.79$  at 775, 807, and 827 nm, respectively). Ultrasonography also showed that only the extensor hallucis longus muscle (EHL), which is under the extensor digitorum longus muscle (EDL), was activated during the flexion of the big toe. In order to evaluate the penetration depth of NIR light, the depth of EHL was measured and the OD observed before and during flexion. When the distance between the light source and detector was set at 30 mm, the OD values before exercise ranged from 0.36 to 3.18 at 775 nm, from 0.19 to 2.43 at 807 nm, and from 0.15 to 1.60 at 827 nm. Changes in OD during exercise were detectable for all subjects, and the EHLs of the subjects were located 10.0 to 20.2 mm under the detector. However, when the light source-detector distance was set at 20 mm, changes in OD during exercise were detectable for only 2 subjects, whose AT thicknesses were 4.0 or 5.0 mm, and the EHLs of the subjects were 10.0 or 11.7 mm deep. At a distance of 40 mm, 9 out of 12 subjects showed changes in OD, and their AT thicknesses and EHL locations ranged from 6.4 to 10.0 mm and from 11.4 to 20.4 mm deep, respectively. However, at this distance, OD reached our instrumental limit (4.82) and no OD change could be detected in three subjects with small AT values (4.0, 4.0, and 5.3 mm). These findings suggested that the thinner the AT, the higher the OD when the light source and detector are set a certain distance apart, and that NIR light penetrates the muscle tissue at least deep enough to reach half the distance between the light source and the detector when the AT thickness ranges from 4 to 10 mm. © 1996 Society of Photo-Optical Instrumentation Engineers.

**Keywords** near infrared spectroscopy; adipose tissue; skeletal muscle; penetration depth.

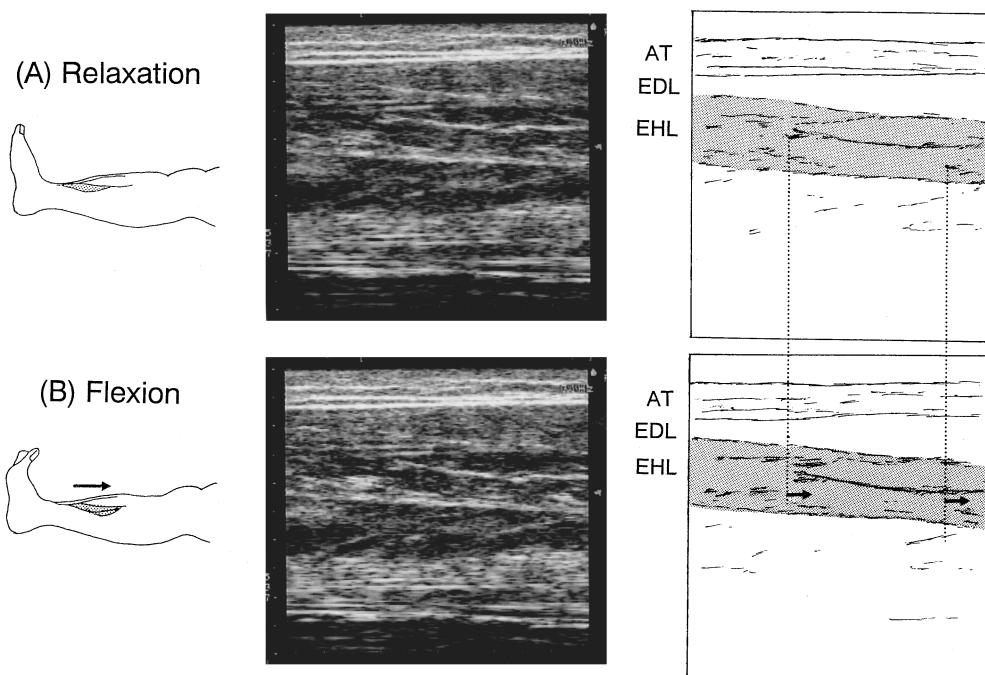
## 1 INTRODUCTION

Near infrared spectroscopy (NIRS) has been recognized as a useful instrument for evaluating changes in the concentrations of oxygenated hemoglobin [HbO<sub>2</sub>], deoxygenated hemoglobin [Hb] and total hemoglobin [HbT] in brain<sup>1-3</sup> and muscle tissue<sup>4-6</sup> *in vivo*. These physiologically and clinically valuable parameters can be detected noninvasively by measuring the change in NIR optical density within the tissue by simply attaching small optical fibers to the skin. However, the propagation of light within tissue is known to be complex, and this has made it difficult to quantify these parameters. Much effort

has been expended in clarifying the optical properties of and path length of light in tissue. Studies using time- or phase-resolved spectroscopy have shown that the differential path length factor (DPF), which is calculated by dividing the mean path length by the distance between the light source and the detector, differs between the forearm, calf, and head.<sup>7,8</sup> Gender<sup>7,8</sup> and considerable intersubject<sup>9</sup> differences have also been found in the DPF values for specific tissues. These findings suggest that anatomical variation affects the path length of the light.

The thickness of subcutaneous adipose tissue (AT) is known to vary greatly in humans. Therefore, it is necessary to consider the influence of AT

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**Fig. 1** Ultrasonographic image of adipose tissue (AT), the extensor digitorum longus (EDL), and extensor hallucis longus (EHL).

on NIR optical density, particularly when estimating the oxygenation state in skeletal muscle tissue.<sup>10</sup>

The aim of this study was to clarify the influence of AT thickness on NIR optical density. We measured AT thickness using ultrasonography, and investigated the optical densities at varying distances between the light source and the detector. The influence of AT thickness on the penetration depth was also investigated by monitoring the changes in NIR optical densities for the extensor hallucis longus muscle (EHL). The EHL is under the extensor digitorum longus muscle (EDL) and is solely activated during flexion of the big toe. The penetration depth was assessed by investigating the relationship between changes in NIR signal during the exercise and the depth of the EHL.

## 2 METHODS

### 2.1 SUBJECTS

Five young men (mean  $\pm$  S.D. of  $24.8 \pm 3.0$  yr) and 7 women ( $21.7 \pm 1.5$  yr) participated in this study after giving their informed consent. None of them had any medical problems.

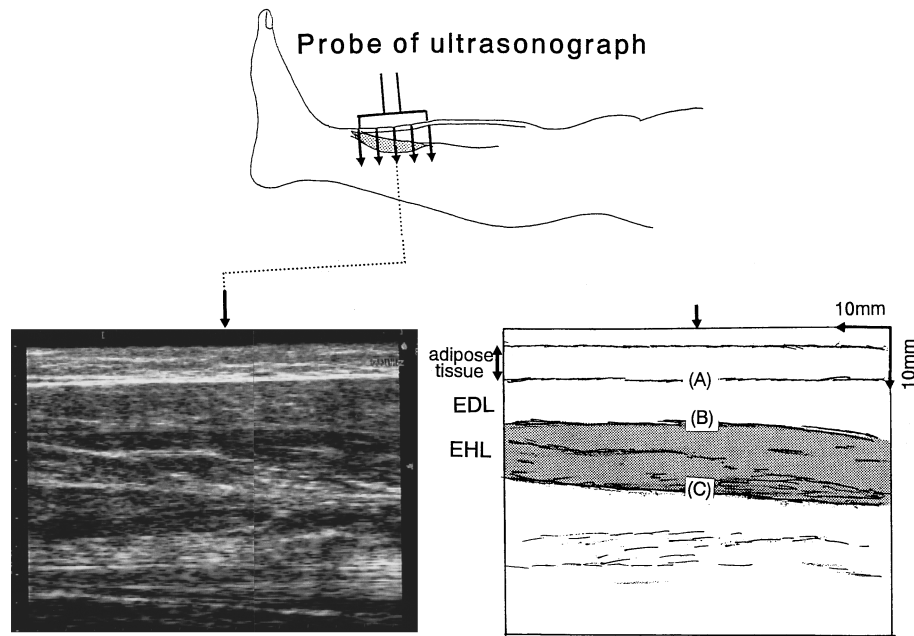
### 2.2 EXPERIMENTAL PROCEDURE

Each subject sat on a chair without flexing the knee joint. First, an ultrasound scan was conducted to determine the best site for measurement. A sagittal ultrasonographic image of the leg was obtained using a B-mode ultrasound apparatus (Aloka Echo Camera SSD-500, Tokyo, Japan) with a 7.5-MHz

transducer. The transducer was held over the site at which the clearest ultrasonographic image of the EHL was obtained, and a mark was made at this position. The subjects then repeatedly flexed the big toe. As shown in Figure 1, only the EHL muscle was activated when the subjects flexed only the big toe, whereas both the EHL and EDL were activated when the subjects flexed all their toes. So that they would activate only the EHL, the subjects practiced the exercise while watching a monitor image of the ultrasonograph until they could flex only the big toe. Once this had been accomplished, the NIR light source and detector were placed over the site at which the clearest ultrasonograph was obtained. Changes in NIR optical density were then measured during a 120 s preexercise control period, a 60 s flexion period, and a 60 s postexercise period. The NIR optical density measurements were repeated while varying the distance between the light source and the detector from 20 to 100 mm in 10-mm steps. The order of the measurements with respect to the distance between the fibers was randomized. An interval of a few minutes was left between the measurements to allow the NIR light level to recover from the previous exercise period.

### 2.3 DATA ANALYSIS

The ultrasonographic image was frozen on the display and was then printed using a graphic printer (Aloka Echo Copier SSZ-305, Tokyo, Japan). On the print, the AT thickness was measured from the skin surface to the fat/muscle tissue boundary and the



**Fig. 2** Measurement of adipose tissue thickness and the depth of EHL. (A) Adipose tissue thickness, distance from the skin surface to the fat/muscle tissue boundary, (B) the depth of EHL, distance from the skin surface to EDL/EHL interface, and (C) end of EHL.

depth of the EHL was measured from the skin surface to the EDL/EHL interface and to the end of the EHL as shown in Figure 2.

**2.4 MEASUREMENT OF NIR OPTICAL DENSITY**

Optical density was defined as equal to  $\log_{10} I_0 / I_t$  where  $I_0$  is the measured intensity and  $I_t$  is the reference.

NIR optical densities at 775, 807, and 827 nm were measured using an O<sub>2</sub> monitor (OM-100A, Shimadzu, Kyoto, Japan). The changes in HbO<sub>2</sub>, Hb, and HbT were calculated by the least-squares method as follows,

$$\Delta HbO_2 = -2.89 \Delta_{OD775} + 1.23 \Delta_{OD807} + 2.30 \Delta_{OD827}$$

$$\Delta Hb = 3.01 \Delta_{OD775} - 0.85 \Delta_{OD807} - 1.82 \Delta_{OD827}$$

$$\Delta HbT = 0.12 \Delta_{OD775} + 0.38 \Delta_{OD807} + 0.48 \Delta_{OD827}$$

The light source and the detector were placed on the leg and the distance between the fibers was varied from 20 to 100 mm, with the mark on the leg kept as the central point.

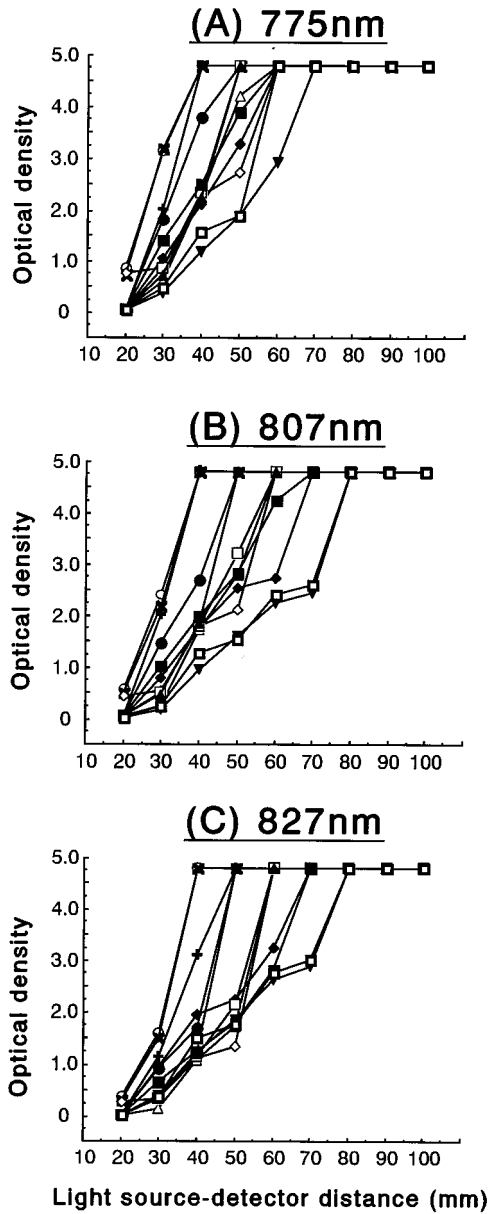
**3 RESULTS**

Table 1 shows the AT thickness and the depth of the upper and lower parts of the EHL. The AT

**Table 1** Adipose tissue (AT) thickness and depth of extensor hallucis longus (EHL).

Distance from the center of EHL (mm)	-40	-30	-20	-10	0 (center)	10	20	30	40
AT thickness	6.9	6.9	7.0	7.0	7.0	7.1	7.1	7.1	7.1
	(4-10)	(4-10)	(4-10)	(4-10)	(4-10)	(4-10)	(4-10)	(4-10)	(4-10)
Depth of EDL/EHL interface	12.1	12.3	13.3	14.0	14.3	14.7	15.8	17.2	18.3
	(7-17)	(7-17)	(10-18)	(10-20)	(10-21)	(10-21)	(10-22)	(10-24)	(10-25)
Depth of end of EHL	23.3	24.0	25.3	26.8	27.6	28.5	29.0	29.7	30.4
	(13-34)	(14-35)	(17-36)	(19-36)	(18-36)	(19-38)	(21-38)	(23-37)	(24-37)

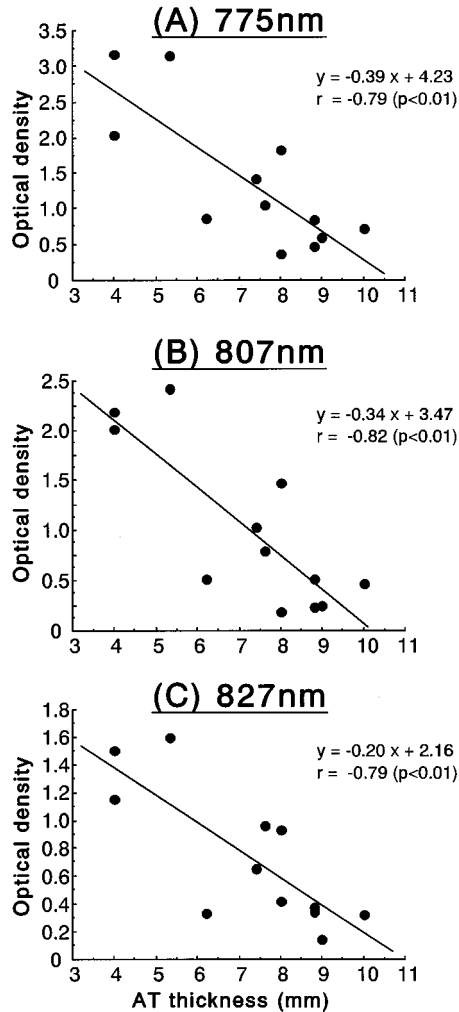
Note: Values are mean (range) for all subjects. A negative value for the distance indicates a measurement taken nearer the foot; a positive value indicates one taken nearer the ankle.



**Fig. 3** Relation between the distance of the light source-detector and optical density at (A) 775, (B) 807, and (C) 827 nm in all subjects.

thickness ranged from 4 to 10 mm. The depth of the EDL/EHL interface ranged from 10 to 21 mm deep at the center of the measurement site among 12 subjects.

Figure 3 shows the relationship between the distance of the light source from the detector and OD at 775, 807, and 827 nm in all subjects. The OD at all wavelengths increased with increasing distance of the light source from the detector and reached full scale (= 4.82) when this distance was 40 to 80 mm in each subject. At a distance of 40 mm, 3 male subjects reached full OD scale at 775 and 807 nm, and two subjects reached it at 827 nm. The number of

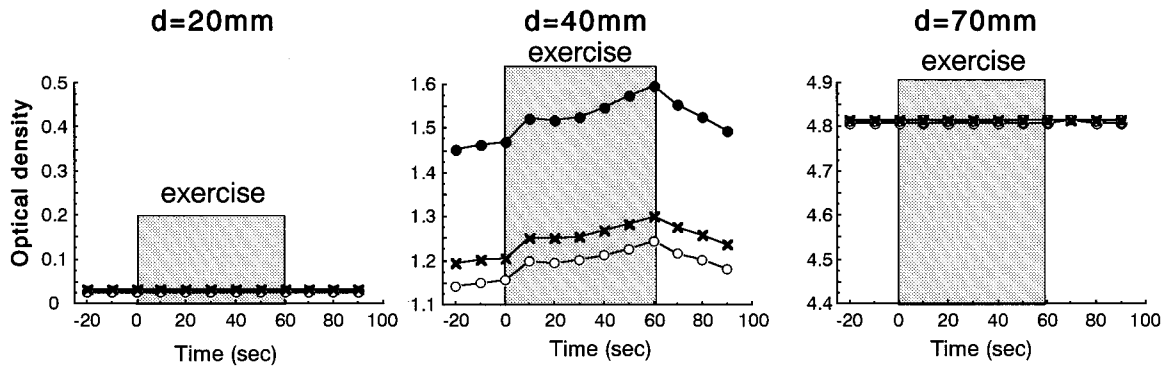


**Fig. 4** Relationship between the thickness of adipose tissue and the optical density (OD) at a distance of 30 mm. (A) 775 nm, (B) 807, and (C) 827 nm.

subjects who reached the full OD scale increased with increasing distance of the light source from the detector, and all subjects reached full scale at 70 mm when the wavelength was 775 nm and at 80 mm for wavelengths of 807 and 827 nm.

Figure 4 demonstrates the relationship between AT thickness and OD at a distance of 30 mm between the light source and the detector. At this distance, the OD ranged from 0.36 to 3.18 at 775 nm, from 0.19 to 2.43 at 807 nm, and from 0.15 to 1.60 at 827 nm, and no subject reached full-scale OD at all three wavelengths. The OD correlated negatively with AT thickness at all wavelengths (775 nm:  $r = -0.79$ , 807 nm:  $r = -0.82$ , 827 nm:  $r = -0.79$ , all  $p$  values  $< 0.01$ ).

Figure 5 shows representative OD values under control conditions and changes during exercise measured at distances of 20, 40, and 70 mm between the light source and the detector. In this subject, OD values at all wavelengths reached full scale



**Fig. 5** Representative changes in optical density under control conditions and exercise at 20, 40, and 70-mm distance of a light source-detector. ●, 775 nm; ○, 807 nm; and ×, 827 nm.

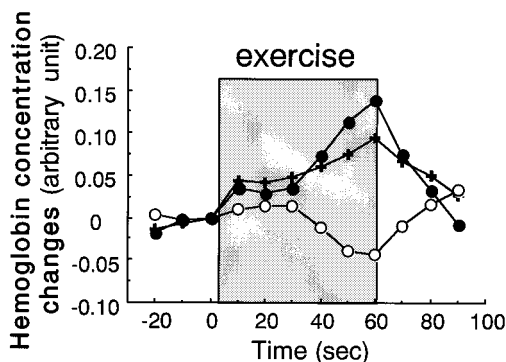
at a distance of 70 mm. No changes were observed during exercise at distances of 20 and 70 mm. On the other hand, a marked change was observed with exercise when the distance was 40 mm. The changes in HbO<sub>2</sub>, Hb, and HbT from the pre-exercise levels are shown in Figure 6. HbO<sub>2</sub> decreased and Hb increased with the exercise time. These changes were thought to reflect the activation of the EHL. When the distance between the light source and the detector was 20 mm, these changes in HbO<sub>2</sub> and Hb were found only in two subjects (16.7%). At a distance of 30 mm, all the subjects (100%) showed these changes. At a distance of 40 mm, 9 subjects (75.0%) showed changes in HbO<sub>2</sub> and Hb.

Figure 7 shows the relationship between the AT thickness and the depth of the EHL for subjects who exhibited changes in HbO<sub>2</sub> and Hb induced by exercise at each distance of the source from the detector (20, 30, and 40 mm). The AT thickness (abscissa) and depth of the EHL (ordinate) were obtained by calculating mean values of several measurements at each distance point between the light source and the detector for each subject. At a

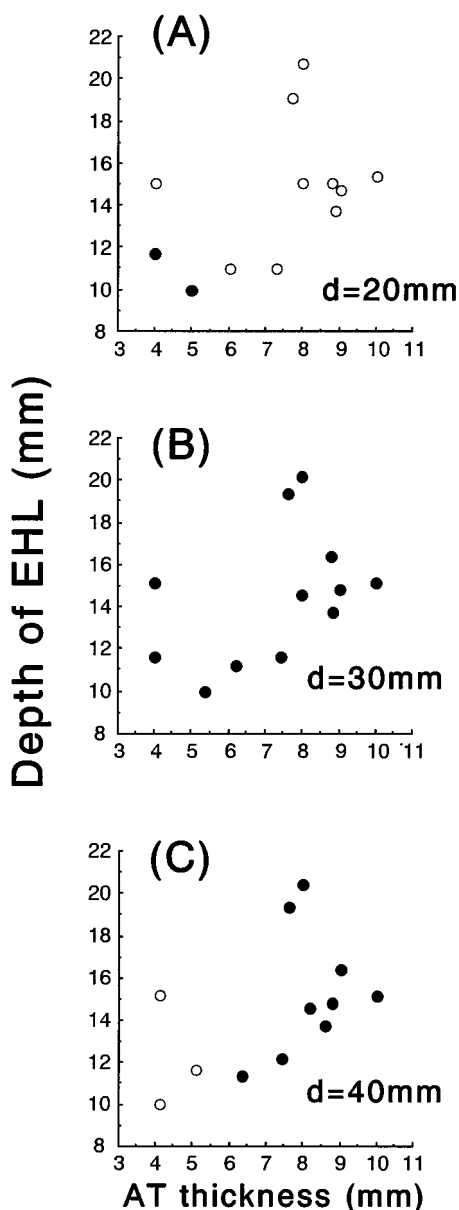
distance of 20 mm between the light source and the detector, changes in HbO<sub>2</sub> and Hb were found in two subjects whose EHLs were located at depths of 10.0 and 11.7 mm and whose AT thicknesses were 4.0 and 5.0 mm, respectively. Changes in HbO<sub>2</sub> and Hb during exercise were found in all subjects when the light source-detector distance was 30 mm. In this case, the EHLs were located at depths of 10.0 to 20.2 mm and the AT thicknesses ranged from 4.0 to 10.0 mm. Changes in HbO<sub>2</sub> and Hb were also found in 9 subjects whose EHLs were 11.4 to 20.4 mm deep and whose AT thicknesses ranged from 6.4 to 10.0 mm when the light source-detector distance was 40 mm. However, in subjects with small AT thicknesses (4.0, 4.0, and 5.3 mm), the OD reached full scale and changes in OD could not be detected. These results are summarized in Table 2. Averaged AT thickness and depth of the EHL were lower at a distance of 20 mm between the light source and detector compared to distances of 30 and 40 mm. Averaged OD values increased with the distance between light source and detector at all wavelengths.

**4 DISCUSSION**

Using continuous spectroscopy, we found intersubject differences in changes in OD with increasing distance between the light source and the detector. The AT thickness varied from 4 to 10 mm in these subjects. If anatomical variations do not affect OD, changes in OD with increasing distance should be the same in all subjects. Therefore, this intersubject variation suggests that variations in anatomy and/or tissue composition affect NIR light propagation within the tissue. In the present study, the AT thickness correlated negatively with OD at all wavelengths. This suggests that the thinner the AT, the higher the OD when the light source-detector distance is set at 30 mm. Since we used continuous NIR spectroscopy, we cannot estimate the absorption ( $\mu_a$ ) and scattering coefficients ( $\mu'_s$ ) separately,



**Fig. 6** HbO<sub>2</sub> (○), Hb (●), and HbT (+) changes calculated from NIR light attenuation.



**Fig. 7** Relationship between the thickness of AT and the EHL depth of the subjects who showed changes in  $\text{HbO}_2$  and Hb followed by exercise at 20, 30, and 40 mm from a light source-detector. ●, the subjects who exhibited changes in  $\text{HbO}_2$  and Hb during exercise; ○, the subjects who exhibited no changes in  $\text{HbO}_2$  and Hb during exercise.

and therefore cannot conclude how  $\mu_a$  and  $\mu'_s$  would change with increasing AT thickness. According to Beauvoit et al.,<sup>11</sup> the  $\mu_a$  at 780 nm in white adipose tissue of rats is very low ( $0.04\text{ cm}^{-1}$ ) and its  $\mu'_s$  is very high ( $18.6\text{ cm}^{-1}$ ). Conversely,  $\mu_a$  in rat skeletal muscle is relatively high ( $0.15\text{ cm}^{-1}$ ) and  $\mu'_s$  is low ( $6.5\text{ cm}^{-1}$ ). On the basis of these results, it appears that when AT thickness is small and the muscle is located relatively near the skin surface, light absorption is high and light scat-

tering is low. Therefore, a high OD, which was found in subjects with a small AT thickness in the present study, may arise from high absorption by the muscle tissue. Conversely, a low OD, which was found in subjects with a thick AT layer, may arise from low absorption of light by the muscle tissue. It may therefore be that the absorption of light by muscle tissue has a greater effect on the OD at a certain distance than the scattering caused by the thickness of the AT.

Cui et al.<sup>12</sup> suggested that the mean penetration depth was approximately equal to half the distance between the light source and the detector, using intralipid emulsions as a phantom medium. However, in human tissue, the mean and effective penetration depths are also thought to vary according to AT thickness. We therefore tried to estimate the NIR penetration depth by measuring changes in OD during contraction of a muscle located at a depth of approximately 10 to 20 mm. We concluded that NIR light reached the contracting muscle when we found a decrease in  $\text{HbO}_2$  and an increase in Hb, which was calculated from light changes during contraction. Our results showed that when the distance between the light source and the detector was 20, 30, and 40 mm, NIR light changes were detectable where the muscle lay 10.0 to 11.7 mm, 10.0 to 20.2 mm, and 11.4 to 20.4 mm deep, respectively. These results suggest that NIR light penetrates the muscle tissue at least deep enough to reach half the distance between the light source and the detector. This is in agreement with previous studies<sup>12,13</sup> which showed that the penetration depth increased with increasing source-detector distance.

However, at 40 mm distance between the light source and the detector in the present study, the OD reached our instrumental limit and we could not detect OD changes during exercise in 3 subjects with small AT thicknesses (4.0, 4.0, and 5.3 mm). Since light absorption by muscle tissue becomes higher when AT thickness is small and muscle tissue is located relatively near the surface, it becomes difficult to collect the NIR light passing through the muscle in subjects whose AT thickness is small. These data suggest that AT thickness and depth of the muscle tissue should be measured in advance; then the light source and detector should be set at an optimal distance. To estimate absolute NIR signal changes in muscle tissue by using time or phase-resolved spectroscopy, the influence of AT on NIR signal should be considered. A technique to compensate for this influence seems to be necessary for future studies.

In summary, the present study demonstrates the influence of AT thickness on the OD of NIR *in vivo*. Our results suggest that the OD is higher in subjects with thin AT layers. It appears that NIR light penetrates the muscle tissue at least deep enough to reach half the distance between the light source and

**Table 2** Optical density, AT thickness, and depth of the EHL in subjects who showed changes in NIR signal due to exercise at distances between the light source and detector of 20, 30, and 40 mm.

Light source-detector distance (mm)	n	Optical density (nm)			AT thickness (mm)	Depth of EHL (mm)
		775	807	827		
20	2	0.792	0.529	0.345	4.5	10.9
	(16.7%)	(0.72, 0.86)	(0.49, 0.56)	(0.31, 0.38)	(4.0, 5.0)	(10.0, 11.7)
30	12	1.377	1.011	0.732	7.3	14.5
	(100%)	(0.36–3.18)	(0.19–2.43)	(0.15–1.60)	(4.0–10.0)	(10.0–20.2)
40	9	2.258	1.767	1.355	8.2	15.4
	(75.0%)	(0.36–3.18)	(0.19–2.43)	(0.15–1.60)	(6.4–10.0)	(11.4–20.4)

Notes: n, number of subjects who showed changes in NIR signal due to exercise (the ratio to all the subjects, n=12); AT, adipose tissue; EHL, extensor hallucis longus. Values are mean (range) for the subjects who showed NIR changes at each light source-detector distance.

the detector when the AT thickness ranges from 4 to 10 mm.

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