1. ABSTRACT

We investigated the influence of the adipose tissue thickness (ATT) on near-infrared spectroscopy (NIRS) measurements of the absorption coefficient ($\mu_a$), the reduced scattering coefficient ($\mu_s'$) and changes in concentrations of oxyhemoglobin ([O$_2$Hb]) and deoxyhemoglobin ([Hb]). We used a frequency domain spectrometer and a special probe to generate maps of these parameters on the human calf during venous occlusion. For ATT below 6 mm $\mu_a$ remained constant, whereas for ATT between 6 and 14 mm $\mu_a$ decreased quickly and became almost constant again for ATT larger than 14 mm. $\mu_s'$ was not significantly altered by the ATT but the values showed a high variability between subjects. We found significantly different changes in both the [O$_2$Hb] and the [Hb] between the proximal and distal locations of measurement. Although ATT influences the recovery of the optical properties of the underlying tissue, these differences depending on the location cannot be sufficiently explained by the ATT for the following reasons. The ATT varied little within one subject (mean difference 0.88 ± 1.80 mm). The inter-subject variability was 5 times higher. For a given ATT within one subject we observed different values for changes in [O$_2$Hb] and [Hb] depending on the measurement location. Moreover for a smaller ATT the difference between the values of $\Delta$[O$_2$Hb] and $\Delta$[Hb] proximal versus distal were more distinct. The thinner the overlying tissue (ATT) the higher is the proportion of muscle tissue in the probed tissue volume. Therefore these differences are most likely coming from the muscle tissue rather than the ATT. This indicates that although the ATT has an evident influence on the measurement of optical

* Ursula Wolf, Laboratory for Fluorescence Dynamics, Department of Physics, University of Illinois at Urbana-Champaign, 1110 W. Green St., Urbana, IL 61801-3080, USA Tel: ++1 217 333 3525 Fax: ++1 217 244 7187

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parameters and hemodynamics and should therefore be recognized when performing NIRS measurements, other factors will have to be considered as well.

1. INTRODUCTION

In this study we have investigated the influence of the adipose tissue thickness (ATT) on non-invasive near infrared spectroscopy (NIRS) measurements. We generated maps of optical parameters, the absorption coefficient (\(\mu_a\)) and reduced scattering coefficient (\(\mu_s'\)) and changes in concentrations of oxyhemoglobin (\(\Delta[O_2Hb]\)) and deoxyhemoglobin (\(\Delta[HHb]\)) on the human calf during venous occlusion measuring at different locations simultaneously. Preliminary data were previously reported\(^1\). We found that \(\Delta[O_2Hb]\) and \(\Delta[HHb]\) varied depending on the measurement location. A high variability of changes in \([O_2Hb], [HHb]\), oxygen consumption, blood flow and of optical density (OD) was described by several authors\(^2\)\(^4\). Their studies were performed as single location measurements and they thought the ATT to be the reason for the high intersubject variability. However, our results revealed the differences in the changes in \([O_2Hb]\) and \([HHb]\) not only between but also within subjects even though the ATT varied little within individuals. Our goal was to study the relationship between the optical and hemodynamic parameters and the ATT.

2. MATERIAL AND METHODS

We used a frequency domain spectrometer (Oxy-Imager, ISS, Champaign, IL). The principle of the frequency-domain spectrometer is described in detail elsewhere. Our instrument operates at 758nm and 830nm. The light generated by 32 laser diodes (16 per wavelength) is intensity modulated at a frequency of 110 MHz. The light from the instrument to the tissue and back to the instrument is guided through optical fibers. The incoming light is collected in 4 photomultiplier tubes (PMT) detectors, demodulated and its mean intensity (DC), modulation amplitude (AC) and phase (\(\Phi\)) are determined. The output signals from the PMTs are sent to a computer for data processing.

The probe covers an area of 18.5x6 cm and its geometry is shown in figure 1.

![Figure 1. Sketch of the sensor: Large circles are detector locations, black dots are source locations. The detector fibers are located on the middle line of the sensor. The source fibers are arranged around the detector fibers. The interoptode distance to the outer 10 source fibers is 3.0 cm. In the center of the probe are different interoptode distances, 2.4 cm and 3.5 cm in a symmetrical parallel arrangement.](image-url)
Maps over the entire region were generated simultaneously at a sample rate of 6 Hz. The pneumatic cuff pressure curve was recorded (Cole Parmer digital manometer). ATT was measured at 9 representative locations with a skinfold caliper (Lange).

Subjects were studied in a supine position with the legs suspended just above the level of the heart to provide a quick venous drainage. Subjects rested for 15 minutes prior to the measurements. The probe was placed over the lateral part (lateral gastrocnemius muscle) of the subject's calf. A pneumatic cuff was wrapped around the subject's thigh. Venous occlusion was achieved by inflating the cuff within 2 seconds to a pressure of 60 mmHg. Venous occlusion was held for one minute, after which the pressure was quickly released. The rest period between the venous occlusions was 2 minutes. 5 cycles of venous occlusion-rest period were performed. Preceding and following these cycles a two-minute baseline measurement was recorded. The protocol was approved by the Institutional Review Board of our university.

The symmetrical arrangement of the two different source detector distances in the middle part of the sensor allows the calculation of \( \mu_a \) and \( \mu_s' \) of the tissue at three locations in the inner part of the probe. From \( \mu_a \) and \( \mu_s' \) we calculated the differential pathlength factor (DPF) for three areas. Quantitative values of \( \Delta[O_2Hb] \) and \( \Delta[HHb] \) in the different locations were calculated from the attenuation changes by the DPF method. To test for the statistical significance we used the Mann-Whitney U test for independent and the Wilcoxon test for paired data.

Fifteen subjects, 9 female, 6 male with an age range of 26-37 years were included in the study. Both calves of each individual were measured (30 legs). Written informed consent was obtained from all subjects prior to the experiments.

3. RESULTS

![Figure 2](image-url)

Figure 2. The diagrams show \( \mu_a \) (a) and \( \mu_s' \) (b) of the tissue depending on the adipose tissue thickness. For an ATT lower than 6 mm no change in \( \mu_a \) was observed. For an ATT higher than 6 mm \( \mu_a \) decreased constantly, whereby the most distinct changes were observed in the ATT range between 6 and 14 mm. The decrease stopped above 14 mm ATT. \( \mu_s' \) is almost constant over the range of the ATT. The variability of the \( \mu_s' \) values is relatively large for a given ATT.
Figure 2a shows the dependence of the $\mu a$ at 830 nm on the ATT. In figure 2b the relationship between $\mu s'$ at 830 nm and the ATT can be seen. A similar pattern was also found for 758 nm.

The distribution of the ATT over the subjects' calves showed slightly higher values in the distal compared to proximal area. The difference was significant. The inter-individual differences in the ATT were about 5 times larger than the intra-individual differences. The mean ATT for the 2 areas over 28 legs is given in Table 1.

Changes in $[O_2Hb]$ during venous occlusion were significantly larger in the proximal area compared to the distal area ($p<0.0001$). Also the changes in $[HHb]$ proximal versus distal showed significantly different values ($p<0.0001$). The mean of the changes in $[O_2Hb]$ and $[HHb]$ over all legs is given in Table 1.

Table 1. Shows the mean over all legs of $[O_2Hb]$, $[HHb]$ and ATT over 30 legs at the proximal and distal area of the calf ($^*p=0.02$, $^{**}p<0.0005$).

<table>
<thead>
<tr>
<th></th>
<th>Proximal</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta [O_2Hb] \pm SD$</td>
<td>$7.30 \pm 3.79^{**}$</td>
<td>$5.05 \pm 2.86^{**}$</td>
</tr>
<tr>
<td>$\Delta [HHb] \pm SD$</td>
<td>$2.31 \pm 1.07^{**}$</td>
<td>$1.26 \pm 1.11^{**}$</td>
</tr>
<tr>
<td>ATT $\pm SD$</td>
<td>$9.7 \pm 4.38^*$</td>
<td>$10.6 \pm 4.56^*$</td>
</tr>
</tbody>
</table>

In figure 3 the correlation between the mean $\Delta [O_2Hb]$ of each subject at the proximal and distal locations and the ATT for all subjects is presented.

![Figure 3](image)

Figure 3. Relationship between the mean $\Delta [O_2Hb]$ of each subject at the proximal (squares) and distal (triangles) locations and the ATT for all subjects. The values for the $\Delta [O_2Hb]$ are higher in the proximal than in the distal part. It can clearly be seen that for a given ATT the values for the $\Delta [O_2Hb]$ vary.

4. DISCUSSION

We performed maps of hemodynamics during venous occlusion where we observed different values for $\Delta [O_2Hb]$ and $\Delta [HHb]$ depending on the point of measurement. To address the question whether these differences are truly generated by local variations in
hemodynamics or by the overlying tissue we investigated the influence of the ATT on $\mu_a$ and $\mu_s'$. From our measurement we found a clear influence of the ATT on the $\mu_a$ at both wavelengths, 758 and 830 nm. With increasing ATT $\mu_a$ decreased revealing a sharp decline for ATT between 6 and 14 mm. For ATT larger than 14 mm $\mu_a$ was almost the same.

For superficial layers below 6 mm we obtained steady values for $\mu_a$. Our findings are in accordance with results from Franceschini$^9$ whose study on a two-layer phantom model is relevant to our data because they used the same optical properties, approximately the same source detector distances and the multi-distance approach as in our study. They showed that superficial layers up to 6 mm do not influence the recovery of the optical properties of the underlying tissue. For superficial layers of more than 16 mm they found that only the optical properties of these layers could be detected. The $\mu_s'$ values are virtually independent of the ATT. The variability of the $\mu_s'$ values is relatively large, which may hide a possible difference between muscle tissue (for ATT smaller than 6 mm) and adipose tissue (for ATT larger than 14 mm). Our data are in agreement with the literature concerning the size of the values as well as their variability$^{10}$.

Homma$^4$ did studies on the human lower leg using a continuous wave instrument and could therefore not separate $\mu_a$ and $\mu_s'$. They determined the optical density (OD) and found a negative correlation between ATT and OD. Since with increasing ATT we observed a constant $\mu_s'$ and decreasing $\mu_a$, which corresponds to a decreasing OD, our findings are comparable and coincide with their results. Niwayama$^11$, measuring the human forearm, described with increasing ATT a decrease in mean optical pathlength in the muscle, while it increased in the adipose tissue layer, thus being more sensitive to the latter. This again confirms our results, since the adipose tissue has a lower absorption than the muscle tissue. Homma$^4$ and Niwayama$^11$ attribute the difference in absorption to the lower concentration of the main chromophores $O_2$Hb and HHb in the adipose tissue.

Binzoni$^{12}$ investigated the hemodynamics in the human calf and found that with increasing ATT absolute values for $[O_2$Hb] decreased while $[HHb]$ did not change much. They commented that with increasing ATT the volume, which is probed contains less muscle and more adipose tissue. A decreasing $\Delta[O_2$Hb] with increasing ATT would therefore indicate that the adipose tissue contains less $[O_2$Hb] than the muscle tissue.

All mentioned studies were performed by single location measurements over the tissue of interest. Our measurements were probing several locations simultaneously and thus we obtained maps of hemodynamic changes in the proximal and distal part of the lateral human calf. Although we observed on the one hand an influence of the ATT on $\mu_a$, we measured on the other hand for a given ATT within one subject different values for $\Delta O_2$Hb and $\Delta HHb$ depending on the measurement location. Moreover for a smaller ATT the difference between the values of $\Delta[O_2$Hb] and $\Delta[HHb]$ proximal versus $\Delta[O_2$Hb] and $\Delta[HHb]$ distal were more distinct. The smaller the overlying tissue the greater the proportion of muscle tissue in the probed tissue volume. Therefore these differences are most likely to come from the muscle tissue. This indicates that although the ATT has an evident influence on the measurement of hemodynamics and should therefore be taken into consideration when performing measurements not all the effects described in this study can be explained by it. Other factors in the human tissue such as structural and functional parameters may play an important role.
CONCLUSION

The ATT has an influence on μa and therefore on Δ[O₂Hb] and Δ[Hb]. The ATT does not influence μs. The ATT on the human calf varied little within a subject while the variations in Δ[O₂Hb] and Δ[Hb] with respect to the location were significant. To explain these effects other parameters (anatomical and physiological) than ATT should be considered.

ACKNOWLEDGEMENT

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REFERENCES