

Identification of human brown/beige adipose tissue using near-infrared time-resolved spectroscopy

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ABSTRACT

Brown/beige adipose tissue (BAT) is expected to contribute to protecting lifestyle-related diseases. The purpose of this study was to examine if near-infrared time-resolved spectroscopy (NIR_{TRS}) is capable of distinguishing BAT from muscle and white adipose tissue (WAT). We analyzed the optical characteristics of tissues in the supraclavicular region, where BAT deposits can be located, and deltoid and abdominal regions in 36 participants (16 men and 20 women) who were apparently healthy individuals, with a median age of 44.5 years, in winter and summer. They also had a median body fat percentage of 28.3% and a deltoid and abdominal adipose tissue thickness of 0.85 cm and 1.71 cm, respectively. The total hemoglobin concentration [total-Hb] and the reduced scattering coefficient (μ_s') were determined using near-infrared time-resolved spectroscopy (NIR_{TRS}) with a 3 cm optode separation for supraclavicular and deltoid regions and a 2 cm optode separation for abdominal region. The results regarding data collected in winter were the following: deltoid ($\mu_s' = 9.6 [9.1, 10.4] \text{ cm}^{-1}$, [total-Hb] = 114.9 [107.0, 127.7] μM); abdominal ($\mu_s' = 9.0 [7.9, 10.1] \text{ cm}^{-1}$, [total-Hb] = 11.2 [8.0, 16.0] μM); and supraclavicular ($\mu_s' = 7.9 [7.2, 8.7] \text{ cm}^{-1}$, [total-Hb] = 60.7 [48.9, 74.7] μM) in winter. Some data are overlapped between groups of muscle and BAT. These results indicated that [total-Hb] and μ_s' show region-specific characteristics. We conclude that using [total-Hb] - μ_s' relationship determined by NIR_{TRS} is a useful strategy to distinguish BAT from other tissues in a simple, rapid, and non-invasive manner.

Keywords: Brown adipose, white adipose, tissue hemoglobin, reduced scattering coefficient, near-infrared spectroscopy.

1. INTRODUCTION

Previously, the brown/beige adipose tissue (BAT) was considered to be deteriorated during the process of maturation; however, recent studies have shown that it exists in adult humans¹⁻⁴. BAT promotes non-shivering thermogenesis under cold environments or dietary intake. This tissue has specific uncoupling proteins (UCP)-1, primarily elicited upon β_3 -adrenergic stimulation, enabling BAT to convert energy to heat by proton leakage through the mitochondrial inner membrane^{5,6}. Human BAT is related to lower body weight^{3,4,7,8} and enhanced glucose tolerance⁹. Daily cold exposure increased the BAT activity and enhanced glucose metabolism not only in healthy subjects^{8,10,11}, but also in obese individuals¹² and patients with type 2 diabetes¹³. Thus, BAT is expected to contribute to preventing or treating obesity and life-style related diseases. In contrast, white adipose tissue (WAT) is characterized by its capacity to store excess energy as triglyceride droplets. It is generally accepted that cold-induced activation of human BAT can be detected by ¹⁸F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) with computed tomography (CT) (¹⁸FDG-PET/CT)^{1,3,6,14}. However, ¹⁸FDG-PET/CT has several limitations, including ionizing radiation and acute cold exposure¹⁵, which makes repeated ¹⁸FDG-PET/CT difficult, specifically longitudinal ones. Near-infrared spectroscopy (NIRS) has been used for measuring O₂-dependent absorption changes in hem red blood cells perfused in biological tissues such as the brain and muscles. Among other noninvasive technologies, NIRS is a relatively new technique for analyzing BAT characteristics¹⁶. The basis for assessing BAT characteristics using time-resolved NIRS (NIR_{TRS}) is that the vascular density, as estimated by total hemoglobin concentration [total-Hb], and mitochondria density, as estimated by reduced scattering coefficient (μ_s'), in the supraclavicular region are higher in BAT than in WAT¹⁷. The purpose of this study was to examine if NIR_{TRS} is capable of distinguishing BAT from muscle and WAT.

2. METHODS

2.1 Participants

We recruited 54 healthy individuals (27 men and 27 women) in the winter by advertising on posters or by direct contact. All individuals also participated in the study during summer. After the participants arrived at the laboratory, the following parameters were measured: body height, body weight, percentage of body fat (%BF), visceral fat area (VFA), and BAT density (BAT-d). Room temperature was regulated from 23 to 27°C using an air conditioner. The study design and protocols were approved by the institutional review board of the Tokyo Medical University (approval no. 2017-199), in accordance with the ethical principles contained in the Declaration of Helsinki. Written informed consent was obtained from all participants.

2.2 Measurements of anthropometric parameters

Body mass index (BMI) was calculated as follows: body weight in kilograms divided by the square of height in meters (kg/m^2). The %BF was estimated by the multifrequency bioelectric impedance method (Inbody 720; InBody Japan, Tokyo, Japan). The VFA, at the abdominal level of L4, was estimated using a bioelectrical impedance analysis (EW-FA90; Panasonic, Osaka, Japan). The subcutaneous adipose thickness of the supraclavicular, deltoid, and abdominal regions were monitored using B-mode ultrasonography (Vscan Dual Probe; GE Vingmed Ultrasound AS, Horten, Norway). The measurement point in the supraclavicular region was the center of the supraclavicular fossa, and in the deltoid region, the belly of the deltoid muscle. The measurement point in the abdomen was fixed as 1.0 cm dorsally and ventrally from the center of the anterior axillary line across the umbilical height, which generally contains the thickest fat layer. The subcutaneous adipose thickness was measured by the investigator using the attached distance measuring system and calculated as the mean value of two measurements.

2.3 Measurements of tissue optical characteristics

Tissue optical characteristics were measured for 1 min at 23–27°C using NIR_{TRS} (TRS-20; Hamamatsu Photonics K.K., Hamamatsu, Japan). The probes were placed on the skin of the supraclavicular, deltoid, and abdominal regions at the same point of the ultrasound measurements, and participants were required to remain in a sitting position during the measurements, as previously described^{16, 18}. Compared with visible light wavelengths, NIR wavelengths (700–3 000 nm) show less scattering and, consequently, better penetration into biological tissue. However, light absorption by water limits tissue penetration above 900 nm wavelength; thus, the 650 to 900 nm range is suitable for measurements^{19,20}. Accordingly, we used NIR wavelengths of 760, 800, and 830 nm to evaluate Hb concentrations. The optode separation for NIR_{TRS} was 3 cm for the supraclavicular and deltoid measurements, and 2 cm for the abdominal measurements^{21,22}.

The tissue was illuminated using a 200 μm core diameter optical fiber by the pulsed light generated from ps light pulses, with 100 ps full width at half-maximum, a 5-MHz repetition rate, and an average power of 80- μW for each wavelength. The emitted photons penetrated the tissue and were reflected to a 2 or 3 mm diameter optical bundle fiber, through which they were sent to a photomultiplier tube for single-photon detection and a signal processing circuit for time-resolved measurement. Using the nonlinear least-squares method, the digitized temporal profile data from an *in vitro* sample or tissue was fitted with a theoretical temporal profile, derived from the analytical solution of the photon diffusion theory with a semi-infinite homogeneous reflectance model. After convolution with the instrumental response function, so the time response of the instrument could be compensated, the absorption coefficient values and μ_s' values at 760, 800, and 830 nm were obtained using the least-squares fitting method. In sequence, the absolute [total-Hb] was calculated as the sum of oxy-hemoglobin and deoxy-hemoglobin concentrations^{19,20}. The NIR_{TRS} system collected data every 10 s. The variation coefficient for repeated measurements of the [total-Hb] and μ_s' were 4.9% and 6.2%, respectively²³.

2.4 Data and statistical analysis

As the optode separation in the abdominal region was 2.0 cm, data for the thickness of subcutaneous adipose tissue in the abdomen smaller than 1.0 cm were excluded to avoid a signal contamination from the underlining muscle. Thus, 36 data samples remained to be analyzed. The [total-Hb] is known to be influenced by the underlining subcutaneous adipose tissue thickness^{24,25}; in the supraclavicular and deltoid regions, this parameter is adjusted by the thickness of the subcutaneous adipose tissue²⁶. Data are expressed as medians [the first quartile, the third quartile] or mean \pm standard

deviation (SD). All analyses were performed using SPSS (IBM SPSS Statistics 25, IBM Japan, Tokyo, Japan) and considered statistically significant for $P < 0.05$.

3. RESULTS

3.1 Subjects characteristics

The subjects included in the data analysis were healthy: 16 men and 20 women in winter. Table 1 shows a detailed anthropometric information of the included subjects.

Table 1. Anthropometric information of the subjects.

	Summer (n = 36)	Winter (n = 36)
Age (year)	44.0 [37.3, 47.8]	44.5 [37.5, 48.0] *
Body height (cm)	165.8 [161.0, 174.2]	165.8 [161.2, 174.0]
Body weight (kg)	62.4 [54.5, 72.1]	61.9 [54.6, 72.9]
BMI (kg/m ²)	22.6 [20.7, 24.3]	22.3 [21.0, 24.2]
%BF (%)	28.0 [22.0, 30.1]	28.3 [22.4, 30.2] *
VFA (cm ²)	43.0 [28.0, 62.0]	44.0 [30.3, 65.8]
Thickness of the subcutaneous adipose tissue in the supraclavicular region (cm)	0.18 [0.15, 0.19]	0.19 [0.17, 0.21] *
Thickness of the subcutaneous adipose tissue in the deltoid region (cm)	0.85 [0.73, 0.93]	0.85 [0.71, 0.97]
Thickness of the subcutaneous adipose tissue in the abdominal region (cm)	1.91 [1.49, 2.39]	1.71 [1.31, 2.19] *
BAT-d (μM)	53.7 [44.4, 69.1]	60.7 [48.9, 74.7] *
BAT-d as adjusted by a subcutaneous adipose tissue thickness (μM)	55.1 [47.2, 71.1]	63.2 [51.8, 76.8] *

Values are expressed as medians [the first quartile, the third quartile]. * $P < 0.05$ summer *versus* winter. BMI: body mass index; %BF: body fat percentage; VFA: visceral fat area; BAT-d: brown adipose tissue density

3.2 Optical characteristics and Hb concentrations in the supraclavicular, deltoid, and abdominal regions

The data on μ_s' are plotted as a function of [total-Hb] in Fig. 1 (A) (Deltoid ($\mu_s' = 9.6$ [9.1, 10.4] cm⁻¹, [total-Hb] = 114.9 [107.0, 127.7] μM); abdominal ($\mu_s' = 9.0$ [7.9, 10.1] cm⁻¹, [total-Hb] = 11.2 [8.0, 16.0] μM); and supraclavicular ($\mu_s' = 7.9$ [7.2, 8.7] cm⁻¹, [total-Hb] = 60.7 [48.9, 74.7] μM)). Some data are overlapped between groups of muscle and BAT in winter (Fig. 1 (B) presents the results regarding data collected during summer: deltoid ($\mu_s' = 9.8$ [9.2, 10.3] cm⁻¹, [total-Hb] = 124.6 [109.1, 144.9] μM); abdominal ($\mu_s' = 8.9$ [8.2, 9.8] cm⁻¹, [total-Hb] = 11.8 [8.2, 14.4] μM); and supraclavicular ($\mu_s' = 7.9$ [7.3, 8.9] cm⁻¹, [total-Hb] = 53.7 [44.4, 69.1] μM)).

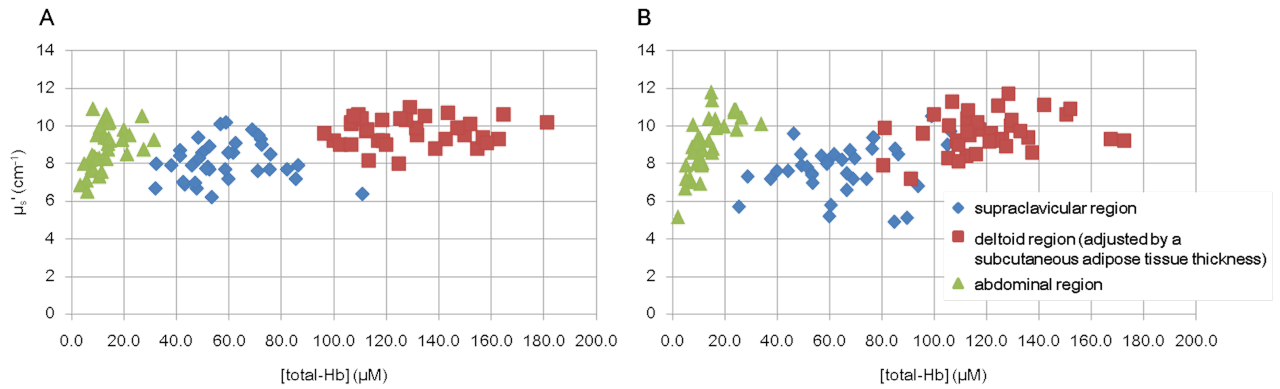


Figure 1. Optical characteristics and hemoglobin (Hb) concentrations in the supraclavicular, deltoid, and abdominal regions in summer (A) and winter (B).

4. DISCUSSION

We successfully distinguished tissue characteristics among BAT located in the supraclavicular region, WAT in the abdomen, and deltoid muscle using optical characteristics (μ_s') and [total-Hb] in humans. However, there are some overlaps between groups of muscles and BAT in the [total-Hb] - μ_s' relationship. It is reported that BAT is activated by acute cold exposure and increases by chronic cold exposure in winter, and this was confirmed by the majority of the ^{18}F FDG-PET/CT^{1,27,28} and NIR_{TRS} studies^{18,29}. The transition of BAT characteristics such as an increase in capillarity or [total-Hb], presumably owing to an elevated energy demand in winter, may be the cause of the overlaps of data on the two groups.

Muscle tissue showed the highest μ_s' and [total-Hb], presumably owing to an abundant vascular bed and mitochondria. BAT showed a significantly lower μ_s' and higher [total-Hb] than WAT. There was a strong correlation ($r = 0.99$) between μ_s' determined by NIR at a wavelength of 780 nm and the *in vitro* mitochondrial concentration in a homogeneous tissue mixture³⁰. A human study also reported that μ_s' measured by NIR_{TRS} was positively correlated with the BAT parameter estimated by FDG-PET/CT, specifically in the supraclavicular region potentially containing BAT deposits²³. However, a later longitudinal study reported that μ_s' is less sensitive as BAT markers. WAT *in vitro* showed a wide range of μ_s' (around 7.0 ~ 20) in the literature^{31,32} with a 4-cm optode separation. The *in vivo* values of μ_s' obtained in the current study (9.0 on average) are lower values compared to the reported data.

Recently, results have indicated that WAT has a potential to obtain healthier metabolic characteristics similar to BAT, namely beigeing³³. The inverse transition can be possible, where BAT fails to receive adequate stimuli such as cold exposure and β_3 -receptor stimulation³⁴. Several methods have been proposed to monitor visualization of BAT characteristics using contrast agents³⁵, beigeing of the white adipocyte³¹, or UCP-1 modification^{36,37}, but noninvasive *in vivo* human monitoring methods are lacking. Thus, the current [total-Hb] - μ_s' relationship can be used to distinguish BAT from other tissues and to monitor a transition between WAT and BAT.

5. CONCLUSIONS

The results of this study indicate that the relationships between [total-Hb] and μ_s' show region-specific characteristics. We conclude that using [total-Hb] - μ_s' relationship determined by NIR_{TRS} is a useful strategy to distinguish BAT from other tissues in a simple, rapid, and non-invasive manner.

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