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Optical Properties of the Subcutaneous Adipose Tissue in the Spectral Range 400–2500 nm

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Abstract—The optical characteristics of subcutaneous adipose tissue are studied in the wavelength range 400–2500 nm. The experiments are conducted in vitro using a Cary 2415 spectrophotometer. Based on the measured diffuse reflectance and total transmittance spectra, the spectra of the absorption and transport scattering coefficients are calculated in terms of the inverse adding–doubling method. © *2005 Pleiades Publishing, Inc.*

INTRODUCTION

Knowledge of the optical characteristics of biological tissues is crucial in the development of mathematical models adequately describing the propagation of light in biological tissues. This is of great importance in the development of novel optical methods used in various applications in biology and medicine for photodynamic and photothermal destruction of cells and tissues, as well as in the development of new approaches in photodynamic therapy, optical tomography, optical biopsy, etc. [1–4]. Despite the fact that numerous investigations have been devoted to determination of the optical parameters of biological tissues, the optical properties of many tissues have not been studied in a wide wavelength range. However, the analysis of the absorption spectra of biological tissues in the visible and near-IR ranges is of fundamental importance in the development of methods for the optical diagnostics and photodynamic and photothermal therapy of various diseases. In addition, the behavior of the scattering characteristics of biological tissues in the range of absorption bands also remains insufficiently studied. In particular, the nonmonotonic spectral behavior of the scattering properties (as compared to their classical monotonic dependences) is frequently interpreted as a calculational or experimental error [5]. At the same time, the dosimetry of optical radiation in laser therapy is virtually impossible without taking into account the scattering characteristics of biological tissues. By virtue of this, the analysis of light scattering in the range of absorption bands proves to be very important for optics of biological tissues.

The problem of the treatment of obesity and cellulite remains important in cosmetology despite the wide use of modern drugs and surgical techniques [6, 7]. One of the methods of treatment of obesity and cellulite consists of exposing the adipose tissue to laser radiation in the visible or near-IR spectral range [8, 9]. However, despite numerous investigations in the field of optics of biological tissues, the optical parameters of subcutaneous adipose tissue in a wide spectral range remain poorly studied. At the same time, a knowledge of these parameters is essential for the sectional dosimetry of laser radiation used in the treatment of obesity and cellulite. In addition, the study of the optical characteristics of the adipose tissue in a wide spectral range is of interest for other areas of modern medicine, in particular, in dermatology for photodynamic therapy of subcutaneous neoplasms, including malignant tumors [4], and, taking into account the similarity between the structural and morphological properties of various fatty tissues, in oncology for diagnostics and treatment of breast cancer [10].

The goal of this paper is to determine the scattering and absorption characteristics of subcutaneous adipose tissue in the wavelength range from 400 to 2500 nm.

MATERIALS AND METHODS

As material for in vitro studies, we used ten samples of subcutaneous adipose tissue obtained from ten laboratory rats, which were six months in age and 220–250 g in weight. Before the experiments, the rats were kept under the same illumination (14 h per day and 10 h per night) and feeding conditions. Five samples were taken post-mortem from the dorsal skin area and another five were cut from the abdominal skin area of the animals. Immediately after autopsy, the samples were placed into a 0.9% NaCl solution and stored at room temperature (about 20°C) for 2–3 h prior to measurements. The area of the samples was in the range 200–250 mm^2. To measure the thickness of the samples, they were placed between two cover glasses; the measurements were performed with a micrometer at several points of each sample. The accuracy of a single measurement was within ±50 μm. The values obtained were averaged. The thickness of the experimental samples varied from 1 to 2 mm and amounted to 1.5 ± 0.46 mm on the average.

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The optical properties of the subcutaneous adipose tissue were studied in the spectral range 400–2500 nm on a Cary 2415 spectrophotometer (Varian, Australia) with an integrating sphere. This instrument is a two-channel diffraction monochromator with a built-in system of control and signal recording. As a radiation source, a halogen incandescent lamp was employed. The cross section of the light beam incident on the sample was 5×5 mm; the scanning rate was 2 nm/s.

For processing the experimental data and determining the optical parameters of the subcutaneous adipose tissue, we employed the inverse adding–doubling method [11], widely used in optics of biological tissues for data processing in integrating sphere spectrophotometry [5, 12–17]. This method allows one to determine the absorption coefficient (μ_a) and the transport scattering coefficient ($\mu_s' = \mu_s(1 - g)$ of a biological tissue using the diffuse reflectance and the total transmittance. Here, μ_s is the scattering coefficient and g is the anisotropy scattering factor. In calculations, the value of the latter parameter is fixed. In this study, g was assumed equal to 0.9 since this value is the most typical for the majority of biological tissues in the visible and near-IR spectral ranges [4].

The wide use of the inverse adding–doubling method for spectrophotometric data processing is related to the fact that this method does not depend on the ratio between the absorption and the scattering characteristics of biological tissues [11]. This is the main advantage of the method in comparison with other techniques used for solving the radiative transfer equation in strongly scattering media, such as, e.g., the diffusion approximation of the radiative transfer equation [18–20], fairly frequently used in optics of biological tissues, or the Kubelka–Munk method [21–23], which both require the satisfaction of the condition $\mu_a/\mu_s \ll 1$ as one of the basic criteria of their applicability [1–4]. This particular feature of the inverse adding–doubling method becomes fundamentally important in the case of determination of the optical properties of tissues within strong absorption bands, when the absorption and scattering coefficients become comparable in value. The main restriction of the method is associated with possible losses of scattered radiation through lateral surfaces of the sample [24], which is possible when the size of the sample is small in comparison with the size of the incident beam or when the absorption and scattering coefficients of the biological tissue are comparatively small. Neglect of lateral losses (if they occur) leads to an overestimation of the absorption coefficient [24]. For the correct application of the inverse adding–doubling method, it is necessary that the distance from the edge of the light spot from the probing light beam to the nearest edge of the sample be greater than the transport mean free path of photons, which is determined as $1/(\mu_a + \mu_s')$ [1].

The optical parameters were calculated separately for each spectral point. The algorithm used involves the following steps:

(1) Setting of the initial values of μ_a and μ_s'. The analytical expressions for these quantities are presented in [17] with reference to [11].

(2) Calculation of the diffuse reflectance and total based on the initial values of μ_a and μ_s' by the adding–doubling method [25].

(3) Comparison of the calculated and experimentally measured values of these parameters.

(4) Performance of the iteration procedure up to coincidence between the calculated and the measured data within a specified accuracy.

As an iteration procedure, we employed the Nelder–Mead simplex method, described in detail in [26]. As a criterion of the completion of this procedure, we used the condition

$$\left|R_d^{\exp} - R_d^{\text{calc}}\right|/R_d^{\exp} + \left|T_t^{\exp} - T_t^{\text{calc}}\right|/T_t^{\exp} < 0.001,$$

where $R_d^{\exp}$, R_d^{calc}, $T_t^{\exp}$, and T_t^{calc} are, respectively, the experimentally measured and calculated diffuse reflectance and total transmittance.

RESULTS AND DISCUSSION

Figure 1 shows the typical spectra of the total transmittance and diffuse reflectance of a sample of the subcutaneous adipose tissue measured on the Cary 2415 spectrophotometer in the range 400–2500 nm. The thickness of the sample is 1.4 ± 0.1 mm. The shape of the spectra in the visible wavelength range is determined by the absorption bands of blood hemoglobin localized in the capillary vessels of the adipose tissue and by the spectral dependence of the scattering coefficient. In the IR spectral range, the shape of the reflection and transmission spectra is determined by the absorption bands of water, lipids, and proteins of the tissue matrix. In both the visible and the IR spectral ranges, the role of the main scatterers is played by spherical droplets of lipids (mainly, triglycerides), which are fairly uniformly distributed within the adipose tissue.

Structurally, the subcutaneous adipose tissue consists of aggregates of fat cells, adipocytes, which contain lipids in the form of isolated triglyceride droplets. Each single adipocyte consists of fat (lipids) to the extent of 95 vol %. The size of the adipocytes is in the range from 15 to 250 μm [27]. In the intercellular space, there are blood capillaries (arterial and venous branches), closely surrounding each adipose cell. In addition, there are reticular fibers in the space between the cells, which envelop the blood capillaries [28].

In both the visible and the IR spectral ranges, the shape of the total transmittance spectrum correlates rather well with the shape of the diffuse reflectance

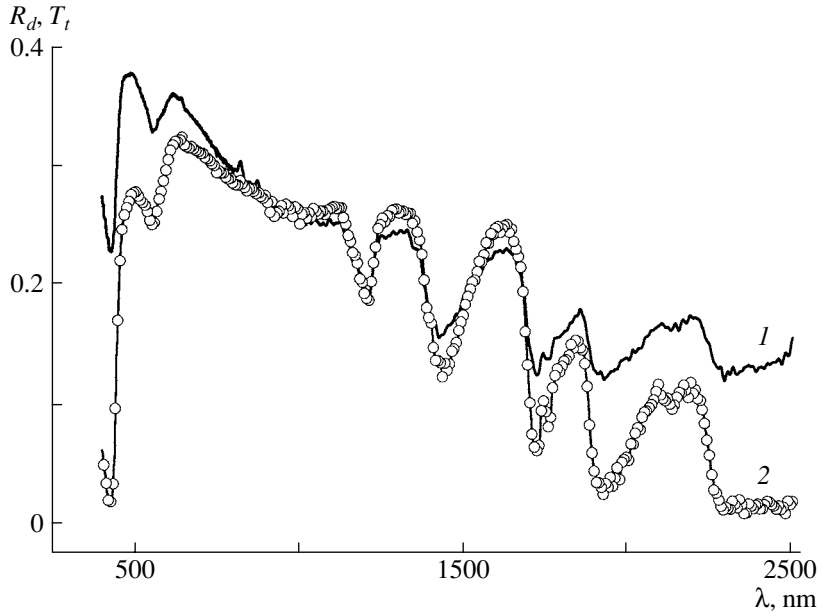

Fig. 1. Spectra of the (*1*) diffuse reflectance (R_d) and (*2*) total transmittance (T_t) of a sample of subcutaneous adipose tissue. The thickness of the sample is 1.4 mm.

spectrum; i.e., the diffuse reflectance and the total transmittance of the biological tissue simultaneously decrease with increasing wavelength, showing sharp minima in the range of the absorption bands of hemoglobin, water, and lipids.

At present, the absorption spectra of hemoglobin and water have been well studied. In the visible wavelength range, hemoglobin (in the deoxygenated state) is characterized by two absorption bands, with their maxima being located at 425 and 555 nm. In the oxygenated state, hemoglobin has three absorption bands, whose maxima are located at 415, 540, and 575 nm [29]; the absorption of water in this range is negligibly small [30]. In the IR spectral range, the main chromophores are water, whose absorption bands are located at 1197, 1450, and 1930 nm [31, 32], and lipids, whose basic absorption bands lie at 1212 nm [33] and 1730 and 1750 nm [34]. It is clearly seen from Fig. 1 that the minima corresponding to the absorption bands of water, hemoglobin, and lipids are observed both in the total transmittance spectrum of the sample of subcutaneous adipose tissue and in its diffuse reflectance spectrum.

Figures 2 and 3 show the spectra of the absorption and transport scattering coefficients calculated by the inverse adding–doubling method from the experimentally measured diffuse reflectance and total transmittance. An analysis of the absorption and scattering spectra presented shows that the inverse adding–doubling method can indeed be applied in determining the optical parameters of these samples of the adipose tissue. The maximum value of the transport free path length of photons calculated by the formula $1/(\mu_a + \mu_s')$ and observed at a wavelength of 1287 nm amounts to 0.7 mm. Taking into account the cross section of the probing beam incident on the sample of the biological tissue (5×5 mm), we obtain that the minimal size of the sample should be no less than 7 mm, which is fulfilled for the smallest of all the samples studied (whose sizes are 13×15 mm).

Figure 2 shows the absorption spectrum of the adipose tissue in the spectral range from 400 to 2500 nm. The vertical lines indicate the standard deviation (*SD*) calculated by the formula

$$SD = \sqrt{\frac{\sum_{i=1}^{N} (\bar{\mu}_a - \mu_{ai})^2}{N(N-1)}},$$

where $N = 10$ is the number of samples under measurement, μ_{ai} is the absorption coefficient of the *i*th sample of biological tissue, and $\bar{\mu}_a$ is the average absorption coefficient at each spectral point calculated by the formula $\sum_{i=1}^{N} \mu_{ai}/N$. In the spectrum, absorption bands of blood deoxyhemoglobin (425 and 555 nm), water (1424 and 1927 nm), and lipids (1724 and 1759 nm) are clearly seen.

The absorption band at 1210 nm is a combination of the absorption bands of water and lipids located at 1197 and 1212 nm, respectively. The shifts of water bands from 1450 to 1424 nm and from 1930 to 1927 nm are associated with changes in the electronic–vibrational structure of water molecules as a result of their binding to proteins of the tissue matrix and structural components of cell membranes [34]. It is likely that the shifts

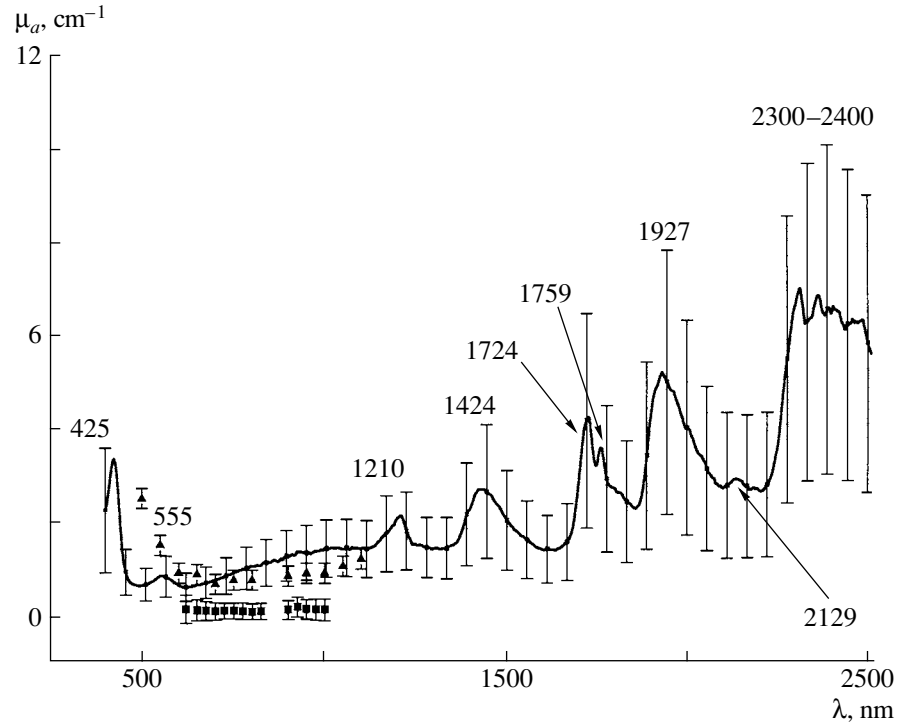

Fig. 2. Spectral dependence of the absorption coefficient μ_a of the subcutaneous adipose tissue calculated from the experimental data by the inverse adding–doubling method. The vertical lines indicate the standard deviation. The triangles and squares show data obtained in [10] and [36], respectively. The arrows and numerals indicate the absorption band maxima (see the text).

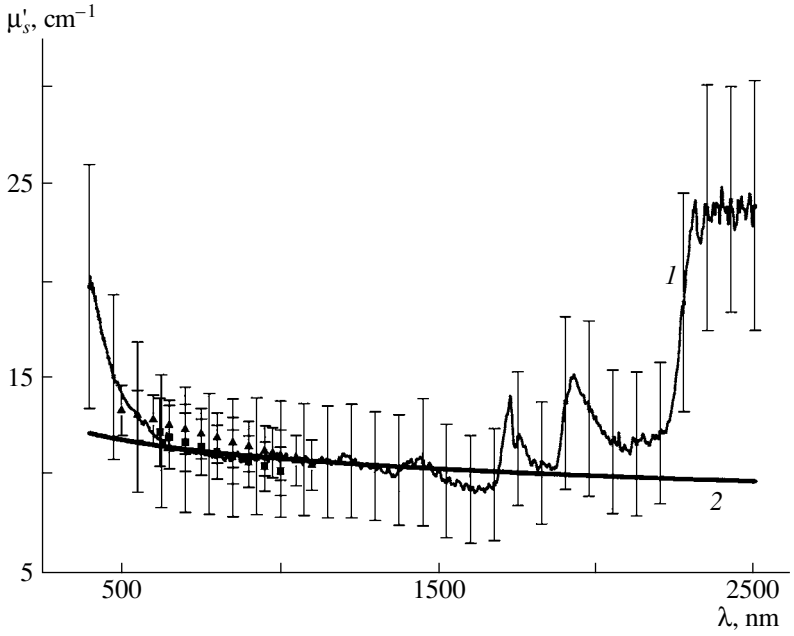

Fig. 3. Spectral dependence of the transport scattering coefficient μ'_s of the subcutaneous adipose tissue (*1*) calculated from the experimental data by the inverse adding–doubling method and (*2*) approximated by the power function $\mu'_s = 25.51\lambda^{-0.12}$. The vertical lines indicate the standard deviation. The triangles and squares show data obtained in [10] and [36], respectively.

of lipid absorption bands from 1730 to 1724 nm and from 1750 to 1759 nm are also associated with intermolecular interactions between different components of the biological tissue. In the range 2300–2400 nm, numerous absorption bands of tissue matrix proteins are observed [35]. In our opinion, the low-intensity

absorption band located at 2129 nm can also be associated with the absorption of tissue matrix proteins. The increase in the standard deviation of the absorption coefficient observed in the range of the absorption bands indicates that the filling of the blood vessels and the water content in different samples of biological tissue are different. It should be noted that, in measurements of the absorption spectrum of the adipose tissue in vivo, the absorption bands of oxyhemoglobin of blood at 415, 540, and 575 nm are expected to appear instead of the deoxyhemoglobin bands at 425 and 555 nm.

The symbols in Fig. 2 show experimental data presented in the literature [10, 36]. The triangles denote data obtained in [10], while the squares show results of [36]. Comparison of these data with our results demonstrates fairly good agreement between them, which is especially good for the data from [10]. Somewhat worse agreement is observed between our data and those reported in [36], in which the optical characteristics of human subcutaneous adipose tissue were studied. At the same time, this difference cannot be considered as significant since the data of [36] were obtained for only one sample.

Figure 3 presents the spectral dependence of the subcutaneous adipose tissue. This dependence was obtained by averaging the spectra of the transport scattering coefficient for the ten samples of adipose tissue. The vertical lines indicate the values of the standard deviation of the scattering characteristics of the adipose tissue obtained in the measurements. It is clearly seen that, in the range 400–1600 nm, the transport scattering coefficient decreases fairly smoothly with increasing wavelength, which, on the whole, corresponds to the general spectral behavior of the scattering characteristics of biological tissues [4, 37–39]. However, beginning from 1600 nm, the transport scattering coefficient increases with increasing wavelength and the shape of the spectrum is distorted; i.e., it deviates from a monotonic dependence in the range of the absorption bands. The symbols in Fig. 3 show experimental data on the scattering characteristics of the adipose tissue presented in the literature [10, 36]. The triangles denote data obtained in [10], while the squares show results of [36]. It is seen from Fig. 3 that our data agree well with data in the literature.

In the range 600–1400 nm, the spectral dependence of the transport scattering coefficient can be approximated to a good accuracy by a power function of the form $\mu_s'(\lambda) = a\lambda^{-w}$ [37–39]. Here, the parameter a is determined by the concentration of scattering centers of the biological tissue and by the ratio of the refractive indices of the scatterers and the surrounding medium, while the parameter w (the wavelength exponent) characterizes the average size of the scatterers and determines the spectral behavior of the transport scattering coefficient [40–43]. In Fig. 3, the spectrum of the transport scattering coefficient is approximated by the function $\mu_s'(\lambda) = 25.51\lambda^{-0.12}$, where λ is the wavelength in nanometers. It is seen from this figure that, in the spectral range 600–1400 nm, this function approximates well the experimental data, whereas, in the ranges 400–600 and 1400–2500 nm, the approximating function differs considerably from the experimental data. Typical values of the wavelength exponent obtained for tissues of the aorta, skin, sclera, dura mater, etc., lie between 1 and 2 [5, 38, 39, 43–48]. The size of scatterers is in the range from 0.1 to 1 μm. We found that the wavelength exponent of the adipose tissue samples under study is equal to 0.12, indicating that the size of isolated lipid droplets in the tissue, which act as light scatterers, is rather large, varying from 10 to 200 μm [27, 28]. It seems that aggregates of fat cells can also act as scatterers, which will additionally reduce the value of the wavelength exponent.

The deviation of the spectrum of the transport scattering coefficient from a monotonic dependence is explained by the fact that, in the range of absorption bands, the influence of the imaginary part of the complex refractive index of scattering centers, adipose cells, increases. According to the Mie theory [49], the intensity of scattered radiation is mainly determined by the complex refractive index of scatterers of biological tissue and an increase in the imaginary part of the complex refractive index in the range of absorption bands leads to an increase in the scattering cross section and, naturally, to growth of the transport scattering coefficient in this spectral range. Apart from this, an increase in the imaginary part of the complex refractive index causes a considerable decrease in the scattering anisotropy factor g, which, along with the scattering coefficient μ_s, forms the spectrum of the transport scattering coefficient $\mu_s' = \mu_s(1 - g)$. It was shown experimentally in [50, 51] that, in the range of the water absorption bands at 1450 and 1930 nm, the scattering anisotropy factor considerably decreases, which inevitably leads to an increase in the transport scattering coefficient and the appearance of bands in its spectrum. In this case, the decrease in the scattering anisotropy factor in the range of absorption bands is proportional to the intensity of these bands. The data shown in Fig. 3 agree well with the above inference. In the range 600–1400 nm, either the absorption of the adipose tissue is insignificant or the intensity of the absorption bands is comparatively low (Fig. 2). Accordingly, the formation of the scattering spectrum in this range is determined only by the real part of the complex refractive index and the spectrum of the transport scattering coefficient decreases fairly monotonically with increasing wavelength. In the range 400–600 nm, a considerable contribution to the formation of the scattering spectrum is made by erythrocytes, which, in addition to strong absorption, possess substantial scattering properties [52], which leads to an increase in the transport scattering coefficient in this spectral range. In the range 1400–2500 nm, comparatively intense absorption bands of water and adipo-

cyte lipids, as well as of proteins of the tissue matrix, are observed in the absorption spectrum of the adipose tissue (Fig. 2). In the presence of strong absorption bands, both the real and the imaginary parts of the complex refractive index of the scattering centers of biological tissue contribute to the formation of the scattering spectrum of the tissue. As a result, the transport scattering coefficient increases in this spectral range and rather strong peaks appear in its spectrum in the range of the absorption bands.

The shift of band maxima in the spectrum of the transport scattering coefficient of the adipose tissue with respect to the absorption bands of water, lipids, and hemoglobin is associated with the effect of the anomalous dispersion of the real part of the complex refractive index in the range of the absorption bands.

CONCLUSIONS

A knowledge of the optical characteristics of adipose tissue in a wide spectral range is necessary for the development of various treatment methods in cosmetology and in photodynamic therapy of dermatological and oncological diseases. In this paper, we studied experimentally the optical parameters of the subcutaneous adipose tissue and compared them with data in the literature. The experiments were conducted in vitro with a Cary 2415 spectrophotometer in the spectral range 400–2500 nm. Based on the measured diffuse reflectance and total transmittance spectra, the spectra of the absorption and transport scattering coefficients were calculated with the help of the inverse adding–doubling method. The results obtained can be used in development of new methods and optimization of existing ones for photodynamic therapy of dermatological and oncological diseases.

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