Chapter 2

Characterization and Differentiation of Adipose Tissue by Spectroscopic and Spectral Imaging Techniques

Fatma Küçük Baloğlu and Feride Severcan

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.75156

Abstract

Adipose tissue is a metabolically active endocrine organ having a distribution in a variety of locations in whole body; therefore, it is crucial to understand the adipocyte metabolism in health and disease. Spectroscopic techniques such as Fourier transform infrared (FTIR), Raman, nuclear magnetic resonance (NMR) are widely used to characterize biological systems by monitoring cellular molecules such as lipids, carbohydrates, and proteins. Obesity or insulin resistance-induced molecular alterations in adipose tissue can be detected using these techniques. Spectral imaging of adipose tissue provides high-quality information involving molecular compositional, structural, and functional alterations for characterization and differentiation of adipocytes (brown, white) in different adipose tissue regions (visceral, subcutaneous, etc.). In this chapter, applications of spectroscopic and spectral imaging techniques for characterization and differentiation of various adipose tissues will be discussed, which will shed light to better understand adipose tissue metabolism and provide new insight into diagnosis and treatment of some metabolic diseases such as obesity.

Keywords: adipose tissue, characterization, differentiation, spectroscopy, spectral imaging, infrared (IR), Raman, NMR, MRI, adipocyte, brown adipocyte, white adipocyte, visceral adipose tissue, subcutaneous adipose tissue, obesity, diabetes

1. Introduction

Adipose tissue is a complicated, crucial, and highly active metabolic and endocrine organ. Adipocytes are the cells that primarily constitute adipose tissue. Besides adipocytes, adipose tissue also includes the stromal vascular fraction (SVF) of cells including fibroblasts, vascular endothelial cells, and a variety of immune cells such as macrophages. Adipose tissue is the primary storage location for excess energy but it may also be defined as an endocrine organ. Adipose tissue

not only replies to afferent signals from endocrine system and the central nervous system but also releases many components having crucial endocrine functions. These components involve leptin, adiponectin, tumor necrosis factor alpha (TNFα), interleukin 6 (IL-6), plasminogen activator inhibitor-1, proteins of the renin-angiotensin system, and resistin [1]. They are responsible for controlling of immune system, thermogenesis, and also neuroendocrine function [2].

Adipose cell, also called adipocyte or fat cell, is basically a connective tissue cell specialized in synthesize and storage large amounts of fat. Adipocytes are complex cells composing a number of signals including cytokines, hormones, and growth factors. These components can affect the neighboring cells and target tissues associated with energy metabolism, physiologic, and pathologic processes [3]. There are two types of adipocytes: white adipocytes contain large lipid droplets, a small amount of cytoplasm, and decentralized nucleus; while brown adipocytes contain lipid droplets of varied size, a large amount of cytoplasm, a lot of mitochondria, and round, centralized nucleus [4]. White adipocytes are globular cells whose changeable size principally depends on the size of the single lipid droplet accumulated within them. These lipid droplets are composed of triglycerides (TGs) and they take up more than 90% of the cell volume. White adipocytes account for storing of energetic molecules to provide energy to the cells between the meals. Brown adipocytes also include triglycerides as multiple small vacuoles; they have generally polygonal shape with a variable diameter. Mitochondria are the most characteristic organelles of brown adipocytes. The color of brown adipocytes proceeds from its high mitochondrial density and high vascularization [5, 6].

The adipose tissues are classified based on their colors because there are alterations in histological composition between white adipose tissue (WAT) and brown adipose tissue (BAT) [4]. Amounts of these tissues in the body show variation with respect to age, gender, strain, and environmental elements. The conventional function of WAT is to supply a long-term energy fuel stock which can be mobilized during lack of food by releasing of fatty acids for oxidation in related organs [7]. Moreover, WAT secretes a range of fundamental components affecting the metabolism of the whole system like leptin that can affect especially the eating behavior [8]. Leptin is a hormone of long-term regulation of energy balance, suppressing food intake and then inducing weight loss. BAT and WAT differ from each other in many respects. BAT has a completely different role than WAT, because it is responsible for thermogenesis. Since BAT requires more oxygen, it has more capillaries than WAT. Nerve supply is also more intense in BAT than in WAT. On the other hand, BAT can pass the energy gained from nourishment to energy [9, 10]. Uncoupling protein 1 (UCP1) that is specifically expressed in BAT is responsible for this conversion of the energy that is not utilized in oxidative metabolism [11]. Noradrenaline is capable to activate the beta-3-adrenoceptors that promotes brown adipocytes to form heat. The brown adipocytes can lose most of their brown characteristics, when they are not stimulated adrenergically, this process cause transdifferentiation of brown adipocytes into white adipocytes [4, 12]. This morphological transformation is reversible and it occurs with UCP-1 gene inhibition and leptin gene activation [13]. BAT phenotype in adipose tissues is so crucial in rodents for the inhibition of various metabolic diseases such as obesity and diabetes [4].

Adipose tissue is approved as a crucial, complicated, and metabolically active endocrine organ having a distribution in a variety of locations in whole body differently from the other

organs [1, 14]. The murine adipose organ composed of two main subcutaneous storages (anterior and posterior) and several visceral storages (mediastinal, omental, mesenteric, perirenal, retroperitoneal, perigonadal, and perivesical) [6]. The locational fat distribution is more critical than whole fat content in the body in the matter of obesity-linked metabolic diseases [15]. It has been reported that more than 80% of total body fat is constituted from subcutaneous adipose tissue (SCAT) in the body and approximately 10–20% from visceral adipose tissue (VAT) in adults [16]. SCAT consists of two different anatomical layers where superficial and deep layers are separated by the fascia superficialis (Scarpa's facia). Subcutaneous fat depots represent 80% of the whole fat mass in normal weight subjects. VAT is an intraperitoneal adipose tissue and it primarily consists of the omental and mesenteric fat depots [17]. SCAT and VAT differ from each other in respect to the type of adipocytes, lipolysis process, endocrine functions and their reaction to insulin and other hormones [18]. VAT has a crucial role in the expression of inflammatory cytokines and secretion of various hormones causing the metabolic effects of obesity since it possesses a special position near portal vein [15, 18–21]. VAT is also different from the SCAT due to the fact that visceral adipocytes have more lipolytic activity metabolically and they are more active than subcutaneous adipocytes [22, 23]. VAT involves greater number of large adipocytes contrary to SCAT, which contains the small adipocytes. These small adipocytes are more insulin-sensitive and more prone to free fatty acid (FFA) and triglyceride (TG) uptake in order to avoid their storage in non-adipose tissue [24, 25]. On the other hand, VAT may have a role in lipolysis of central SCAT causing the release of peripheral FFA [20]. VAT is more sensitive to the catecholamine-induced lipolysis and less sensitive to the antilipolysis action of insulin; therefore, VAT possesses a higher glucose uptake upon insulin stimulation; thus, it becomes more insulin resistant than SCAT [16, 26, 27]. VAT has more vascular structure which is rich in blood supply and more nerve cells than SCAT and they also differ in capacity to produce and secrete adipokines [18].

Obesity results from a chronic imbalance between the level of energy intake and consumption causing extreme weight gain. Obesity constitutively results in storage of triglycerides in different adipose tissues [28]. The increase in fat mass results in the greater adipocyte size (hypertrophy) and increased numbers of adipocytes (hyperplasia). The hypertrophy, hyperplasia, or both of them occur in return for energy imbalance that may alter the location of the adipose tissue [29]. The VAT deposition occurs only if SCAT capacity has been reached to the maximum in early stage of obesity [30]. These changes related to adipocyte hypertrophy could be the first steps toward adipocyte dysfunction. Obesity can be defined as the expansion of VAT and SCAT mass in the body, causing alterations in cellular biology, that is, disturbed glucose and lipid metabolism. Though, abdominal obesity is determined by the storage of both VAT and SCAT in the body, VAT is considered as having more critical role in the metabolism of obesity [20].

Recent studies point to the importance of adipose tissue in diagnosis and treatment of obesity and obesity-related diseases. In current chapter, after mentioning briefly about adipose tissue, the applications of mainly infrared (mid and near), Raman, and nuclear magnetic resonance spectroscopic and microspectroscopic techniques in characterization and differentiation of adipose tissues will be discussed in detail. The other spectroscopic techniques such as circular dichroism, electron spin resonance and fluorescence spectroscopy were not included, because their application to adipose tissues are very limited or none so far.

2. Characterization and differentiation of adipose tissue by vibrational spectroscopic and spectral imaging techniques

2.1. Basis of vibrational spectroscopy

Spectroscopy is the study of the interaction of electromagnetic radiation in its all forms with matter which gives a data called spectrum. "A spectrum is a plot of the intensity of energy detected versus the wavelength, wavenumber or frequency of the energy" [31]. Electromagnetic radiation is the main source of energy used for spectroscopic studies. These spectroscopic studies include irradiation of a sample with several forms of electromagnetic radiation. Monitoring of the spectral parameters derived from absorption, emission, or scattering of the electromagnetic radiation as a result of interaction with matter provides information about the atomic and molecular design of samples [32].

The electromagnetic radiations covering a very wide range of wavelengths have been utilized by several techniques that are used in both biology and medicine. Among these techniques, infrared (IR) spectroscopy has a potential as a powerful tool for structural characterization of molecules and it was accepted as a crucial tool for understanding the structure of biomolecules. Besides IR spectroscopy, another vibrational spectroscopic method that is in current use includes Raman spectroscopy. The main principle based on these physical techniques is the transitions between quantized vibrational energy states of molecules as a result of absorption of electromagnetic energy. In IR spectroscopy, the incident electromagnetic radiation matches the difference between the transition energy levels and this process occurs with high probability. This energy is in the 14,000–4000 cm^{-1} range for the near infrared (NIR), and in the 4000–400 cm^{-1} range for mid-infrared spectroscopy [33].

In addition to absorbance of light, the sample molecules can also scatter light and this can be detected at 90° to the propagation direction of the incident beam. This phenomenon known as Raman scattering is the basis of Raman spectroscopy. Although incident and scattered energy are in the visible range of electromagnetic radiation, the transition between the vibrational energy levels is induced. This is a low-probability event, since the incident energy does not match the energy differences between the vibrational energy levels.

Since each molecule will have its own unique vibrational characteristics, each molecule possesses a unique infrared or Raman spectrum. This fact makes vibrational spectroscopy a golden tool in the characterization of molecular structure. Some properties of vibrational band, such as its position, intensity/area, and width, can be utilized for monitoring a certain functional group or molecule in different conditions. These conditions can be metabolic conditions, alterations in the environmental factors, or genetic modifications in the organisms which are able to alter the molecular composition, concentration, structure, and function of biomolecules. Since these alterations can affect the vibrational transitions which are directly reflected in the vibrational spectral bands and therefore, they can be evaluated by using vibrational spectroscopy techniques. Vibrational spectroscopy obtains qualitative and quantitative information as a rapid, accurate, cost-effective, and operator-independent technique for identification of the spectral differences arising from pathological or environmental conditions [33].

Fourier transform infrared (FTIR) and Raman spectroscopy are complementary techniques for the study of molecular vibrations and structure. The combination with a microscope results in an analytical method known as microspectroscopy that allows spatially resolved investigation of the biochemical compounds of biological samples. The high spatial resolution makes it possible to study areas down to approximately 10 × 10 μm with FT-IR microspectroscopy and approximately 1 × 1 μm with Raman microspectroscopy [34]. As the most common techniques of vibrational microspectroscopy, infrared and Raman microspectroscopy allow determination of the inherent vibrational spectra of the biochemical components of a cell [35]. Hence, they allow visualization of cellular composition based on their chemical properties and provide metabolic clues in disease diagnosis and therapy. Therefore, these techniques have been approved widely in the field of biology and medicine because of their fast and non-invasive nature [36].

Application of spectroscopic and spectral imaging techniques has been gaining importance in discrimination and characterization of different tissue types such as adipose tissue. Research in this field has focused on the characterization of differentiation of adipose tissue by investigation of molecular composition, structure, and distribution of adipocytes to better understanding of roles of different adipocytes in metabolism in healthy and disease states such as obesity and type II diabetes. Another application of this field is on obesity and diabetes-induced alterations in adipose tissue in order to determine spectral parameters that can be used in the generation of new and rapid methods for diagnosis and treatment of these diseases in the future. In the current chapter, various applications of spectroscopic and spectral imaging techniques in the evaluation of different adipose tissue types will be presented.

2.2. Applications of vibrational spectroscopic and imaging techniques on adipose tissue

Biological samples contain biochemical substances such as carbohydrates, proteins, lipids, and nucleic acids, and these biochemical molecules have their unique vibrational fingerprints individually. In biological systems, the infrared spectrum is complex and the sum of contributions coming from proteins, lipids, nucleic acids, and other chemical species present in the cells [37, 38]. **Figure 1** shows the representative spectra of adipose tissue of control and obese mice in the 4000–650 cm^{-1} region and the main bands are labeled in the figure, and band assignments are given in integrated table.

FTIR spectroscopy, as a sensitive technique detecting the alterations in the functional groups of biological tissue constituents, is capable to differentiate the spectra of healthy and diseased biological samples [45–48]. These disease conditions induce significant changes in molecular content, concentration, structure, and dynamics in tissues, cells, body fluids, and membranes. These alterations can be detected rapidly and sensitively without using external agents by attenuated total reflectance-fourier transform infrared (ATR-FTIR) spectroscopy [49, 50]. Sen et al. [51] aimed to investigate the effects of obesity on macromolecular alterations in order to characterize berlin fat mice (BFMI) lines according to the macromolecular alterations within SCAT and VAT by using ATR-FTIR spectroscopy. For this purpose, detailed spectral analysis was performed to characterize lipid and glycogen content of adipose tissues of mouse models

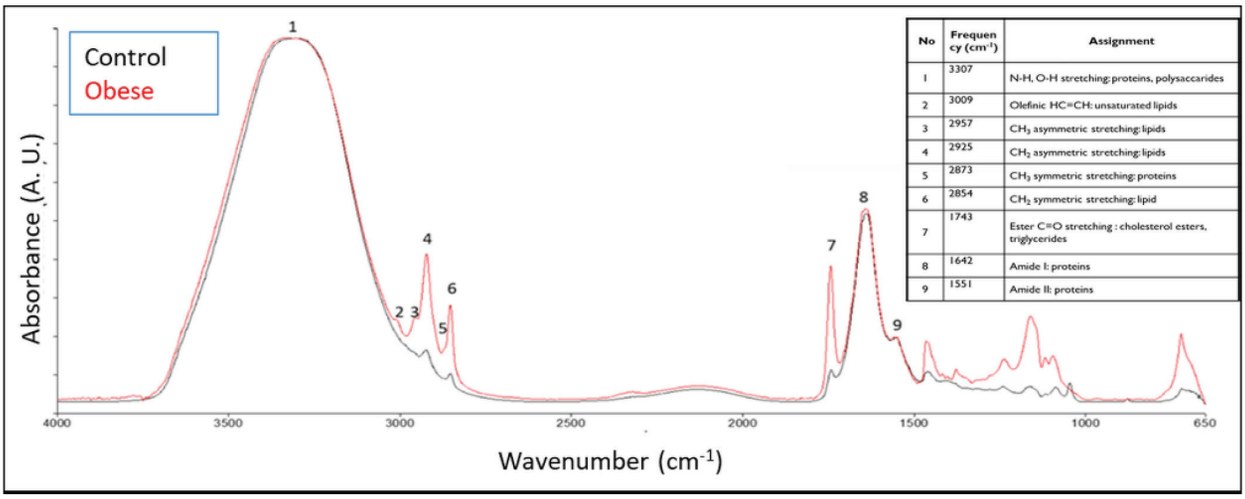

Figure 1. The representative ATR-FTIR spectra of adipose tissue of control and obese mice in 4000–650 cm^{-1} region. The spectra are normalized with respect to amide a band located around 3300 cm^{-1}. General band assignment of FTIR spectrum of adipose tissue based on literature was given in box [39–44].

of spontaneous obesity. This study revealed that there was a loss of unsaturation in BFMI860 and 861 lines in SCAT with a decrease in the hydrocarbon chain length of lipids suggesting an increased lipid peroxidation. In addition, there was an increase in saturated lipid and triglyceride content in all tissues of BFMI lines. These results demonstrated that SCAT also indicated considerable obesity-induced alterations. In conclusion, these results revealed alterations in lipid structure and content of BFMI lines, which may arise from different insulin-sensitivity levels of the lines. These obesity-induced alterations revealed by FT-IR spectroscopy in lipids of the lines, such as acyl chain length and degree of unsaturation, may be a consequence of the alterations in insulin sensitivity levels. Another important result of this study is that SCAT and VAT indicated significant obesity-induced alterations and both of them take a role in lipid metabolism in obesity. Furthermore, the current study clearly revealed the characterization power of ATR-FTIR spectroscopy in the precise determination of spectral variations in different adipose tissue components of mouse models of juvenile obesity without a high-fat diet induction.

Since each sample has a characteristic composition of molecules, IR spectroscopy provides a phenotypic fingerprint. This fact makes FTIR spectroscopy as a complementary technique for genomic approaches to detect unique genetic variants among individuals. In a recent study, ATR-FTIR spectroscopy was combined with a genetic approach to identify genetic differences responsible for phenotypic alterations in adipose tissue. The phenotypic characterization obtained by ATR-FTIR spectroscopic data was used to identify novel chromosomal regions contributing to distinct features of high-fat diet-induced obesity. The analytical technique of ATR-FTIR spectroscopy accompanied with quantitative trait loci (QTL) analysis was introduced as a novel phenotyping method that enables to characterize the macromolecular composition of adipose tissue. By performing this method, the alterations between the BXD RI strains with respect to the trait of interest reflecting genetic variation were obtained and two genomic regions that may have a function in obesity-induced tissue dysfunction were revealed [52].

Chemometrics can be defined as the application of statistical and mathematical calculations to obtain the information from the vibrational spectra [53]. Since vibrational spectra have many

molecular-based specific spectral peaks [54]. Multivariate statistics is convenient in spectral analysis because it improves the simultaneous inference of multiple spectral intensities, which enhances the precision and predictive ability of the analysis [55]. When coupled with appropriate multivariate statistical methods, FTIR spectroscopy can be a discriminatory technique with specific spectral markers [56]. Different multivariate analysis methods coupled with FTIR spectroscopy were successfully applied to the diagnosis of several diseases, such as obesity, diabetes, cancer, Alzheimer's disease [46, 47, 57–59]. In an obesity study, triglyceride band located at 1770–1720 cm^{-1} spectral region was proposed as a more sensitive obesity-related biomarker using the diagnostic potential of FTIR spectroscopy coupled with multivariate analysis in SCAT and VAT [57]. Principal component analysis (PCA) firstly was performed to examine the possible clustering of samples and to determine IR spectral bands that can differentiate the control and obese adipose tissue samples. The PCA results showed that the most dramatic difference in loading plots of control and obese groups was obtained from the 1770–1720 cm^{-1} region which belongs to triglyceride band as shown in the **Figure 2A** and **B**. This result implied that the triglyceride region has a significant contribution in the discrimination of the control and obese groups for both types

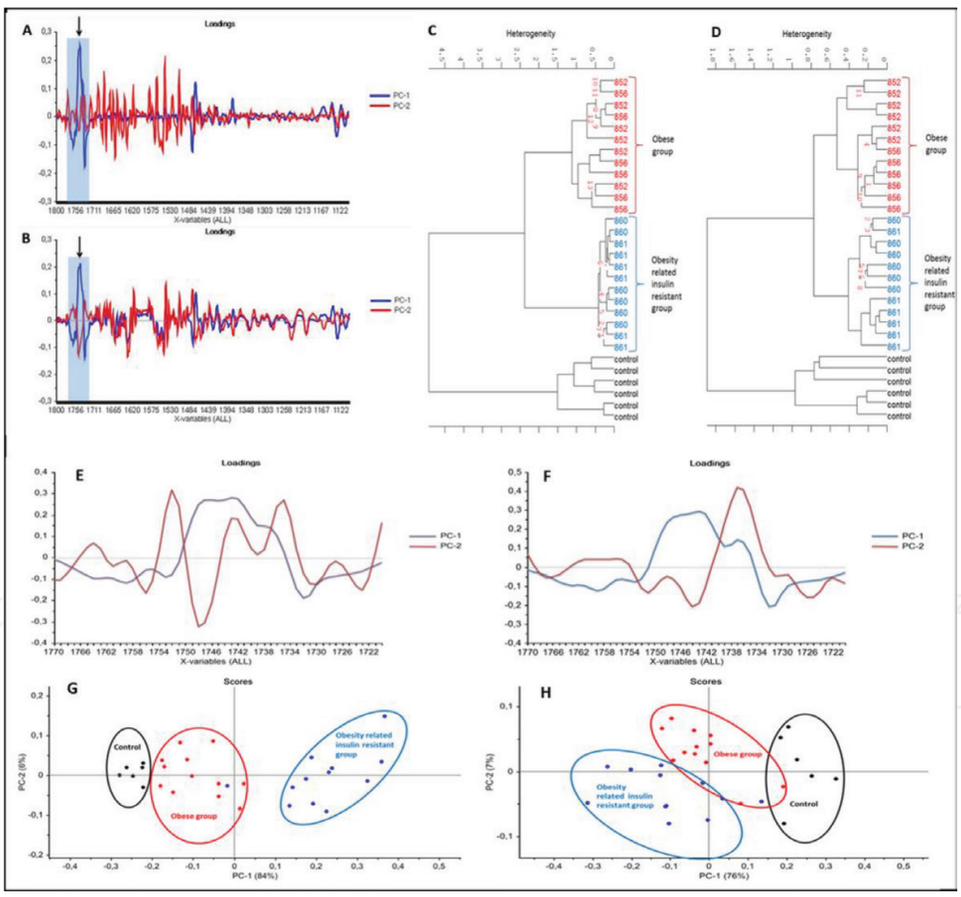

Figure 2. PCA loading plots for VAT (A) and SCAT (B) of control and obese groups in the 1800–1000 cm^{-1} spectral region. The triglyceride region is shown by arrow. Hierarchical clustering of control, obese, and obesity-related insulin resistant groups in SCAT (C) and VAT (D) in the 1770–1720 cm^{-1} spectral region. Control group (black), obese group (BFMI 852–856) (red), obesity-related insulin resistant group (BFMI 860–861) (blue). PCA loading plots of control, obese, and obesity-related insulin-resistant groups for SCAT (E) and VAT (F) samples in the 1770–1720 cm^{-1} spectral region. PC1 versus PC2 scores plot of the second derivative vector normalized spectra in the same range of SCAT (G) and VAT (H). Control group (black), obese group (BFMI 852–856) (red), obesity-related insulin-resistant group (BFMI 860–861) (blue) (Reproduced from [57], with permission from John Wiley and Sons).

of adipose tissues. Consistently, in hierarchical cluster analysis (HCA) results, a successful differentiation between the control, obese, and obesity-related insulin resistant groups was obtained in the triglyceride region with 100% sensitivity and specificity values as demonstrated in **Figure 2C** and **D**. In addition, PCA loading plots indicated a high difference between PC1 and PC2 which strongly indicates a successful differentiation in this region (**Figure 2E** and **F**). The PC1 versus PC2 scores plot for the triglyceride spectral range, as shown in **Figure 2G** and **H**, also supported the existence of a successful discrimination between the control, obese, and obesity-related insulin-resistant groups in both types of adipose tissues. Another finding is reported that the effects of obesity on the VAT highly correlate with the SCAT. Based on these results, this diagnostic technique can be transferred to medical research in the field of obesity. Since the SCAT is more accessible than the VAT for medical interventions, SCAT can be used in biopsies and bariatric operations preferably. The discriminatory power of FTIR spectroscopy coupled with multivariate analysis in diagnosis of obesity can be easily examined in human studies and this combined technique will shed light on the internal diagnosis of obesity in medical research [57].

FTIR microspectroscopy enables to acquire visible images of the investigated tissue whose each pixel consists of a spectrum arising from vibrational fingerprints. FTIR spectroscopic imaging is a label-free and nondestructive technique that quantifies the distribution of biologically relevant components in samples, concurrently revealing biochemical composition and morphology. FTIR imaging permits detecting the inherent vibrational mid-IR spectra of the biochemical constituents of cells and characterization of localized biochemical changes. The representative FTIR spectral maps obtained from control and obese groups of VAT samples are presented in **Figure 3**. Kucuk Baloglu et al. [60] aimed to characterize and compare VAT and SCAT according to biomolecular content and identify the possible transdifferentiation from brown to white adipocytes by using FTIR microspectroscopy and uncoupling protein 1 (UCP1) immunohistological staining in VAT and SCAT of spontaneously obese mice. In obese groups, a significant increase in the lipid/protein ratio, accompanied with a decrease

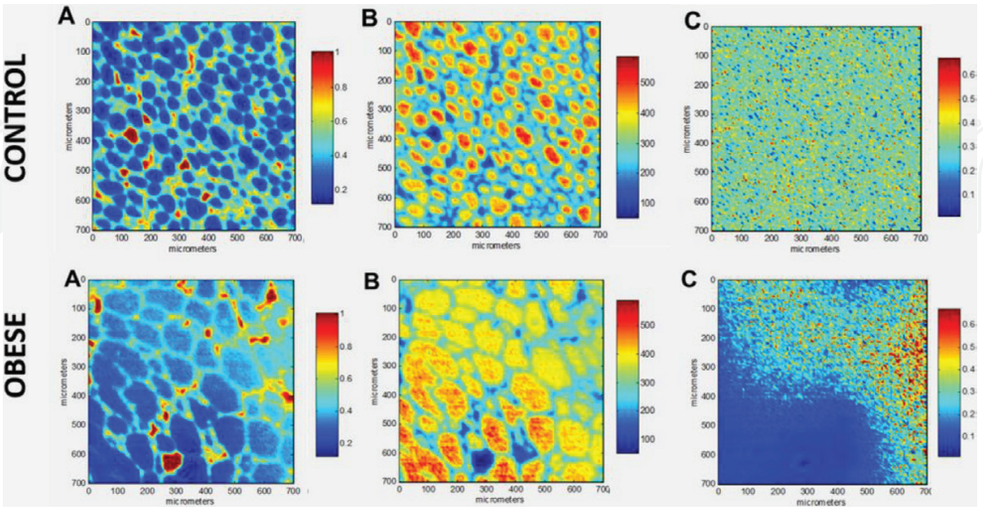

Figure 3. Representative FTIR spectral maps of VAT samples of control and obese mice. The absorbance in the spectral maps was represented in color-coded images, where low absorption was represented in blue and high absorption was represented in red color. [(A) amide I, (B) lipid to protein ratio, (C) unsaturation ratio].

http://dx.doi.org/10.5772/intechopen.75156

of UCP1 protein content was obtained which might be arising from transdifferentiation of brown adipocytes to white adipocytes. In addition, when compared to control group, obese groups indicated a decreased unsaturation ratio, qualitatively longer hydrocarbon acyl chain length of lipids and increased amount of triglycerides revealed by FTIR microspectroscopy in both types of adipose tissues. Another finding indicated that SCAT was more prone to obesity-induced structural changes than VAT, which could originate from it, possessing a lower amount of brown adipose tissue. The current study clearly revealed the power of FTIR microspectroscopy in the precise determination of obesity-induced structural and functional changes in SCAT and VAT.

Aboualizadeh et al. [61] used FTIR microspectroscopy to characterize BAT and subcutaneous-WAT (s-WAT) derived from mice exposed to 30°C (thermoneutral condition), 24°C (room temperature), and 10°C (cold exposed). As seen from **Figure 4**, bright-field images of 10°C BAT (i) and s-WAT (ii) and some tissue regions with different morphological appearances are highlighted (red boxes). PCA results from the spectra that were derived from FTIR microspectroscopy facilitate the identification of spectra and enable to determine which peaks contribute the most in distinguishing the spectra. The loading plot from PCA shows that three positive bands attributed to proteins (3290, 1654, and 1544 cm^{-1}) and three negative bands attributed to C=O ester in phospholipids (1745 cm^{-1}), and symmetric and asymmetric stretching of CH_2 (2923, 2854 cm^{-1}). Scores in red color were attributed to the regions similar to the superimposed red boxes in **Figure 4B** and the green scores were attributed to the regions similar to the

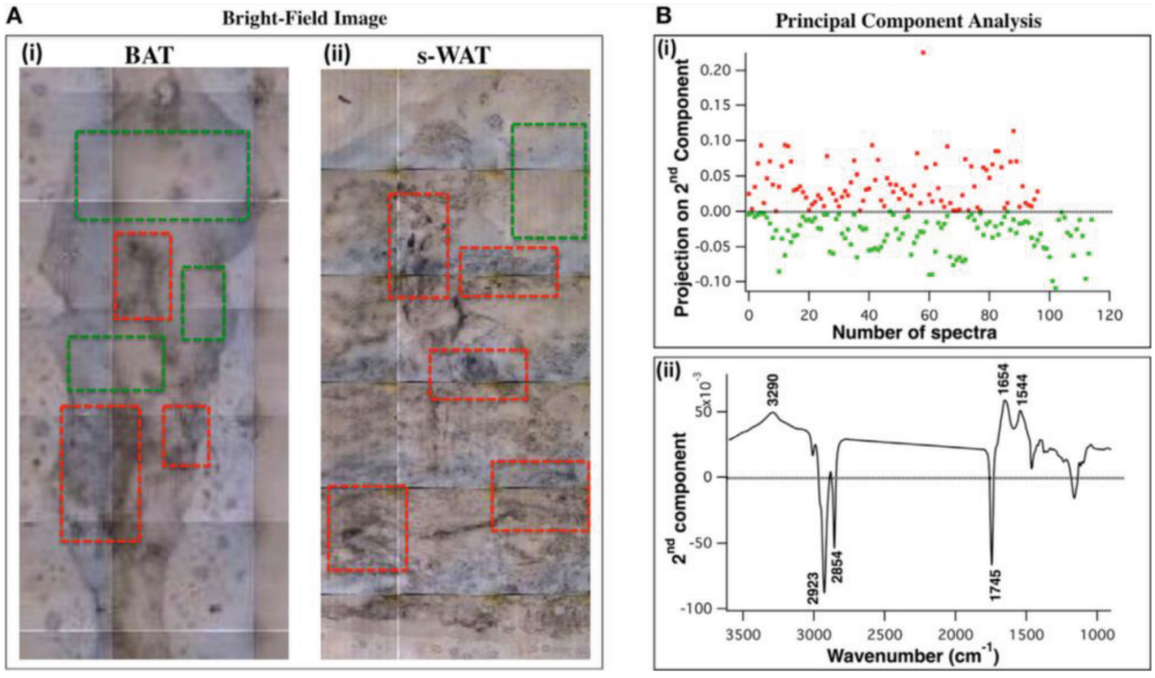

Figure 4. (A) Bright-field image of a cold-exposed BAT [(A), i] and s-WAT [(A), ii] are shown red boxes show the representative regions with different morphological appearances within tissue section and green boxes show more homogeneous regions within tissue. (B) Projection of spectra on the second component (scores) from PCA [(B), (i)] and associated loading plot [(B), (ii)] with the greatest varying wavenumbers are shown. Along PC2, positive scores are shown in red and negative scores are shown in green [(B)] (Reproduced from [61], with permission from Frontiers).

green boxes in **Figure 4B**. Spectral maps were generated by integrating over different spectral regions including signals of carbohydrates, proteins, and lipids. According the results, for 30, 24, and 10°C BAT and s-WAT showed gradual increases in protein to lipid ratio, as going from 30 to 24 to 10°C groups. Protein to lipid ratio was significantly higher in cold-exposed BAT and s-WAT compared to 24, and 30°C tissues. In addition, olefinic to lipid ratio indicated an increase in a progressive manner from 30 to 24 to 10°C groups. Cold-exposed tissues (10°C) showed a significantly higher level of olefinic to lipid ratio in BAT and s-WAT. FTIR microspectroscopy suggested that cold-exposed tissues AT had greater unsaturated lipid content than the warmer temperatures, and 1H nuclear magnetic resonance (NMR) studies validated these results as a complementary method which will be discussed in the following sections.

The near-infrared radiation (NIR) window, also known as the "therapeutic window," is the range of wavelengths that has the maximum depth of penetration in tissue. Since NIR is minimally absorbed by water and hemoglobin, making it a golden tool for medical applications, including functional analysis of biological tissues, as well as an analytical tool for diagnosing diseases [62]. Since BAT has abundant capillaries and mitochondria compared with WAT, near-infrared time-resolved spectroscopy (NIRTRS) is able to detect and evaluate the activation of BAT. When NIRTRS was used to assess the optical characteristics of the supraclavicular (SCV) BAT, it evaluated BAT density as a simple and noninvasive method by measuring the markers of tissue hemoglobin concentration and mitochondrial density [63, 64]. Moreover, NIRS as a noninvasive technique was combined with infrared thermography (IRT) to identify and monitor thermogenesis related to human BAT in adults with different BMI. In this study, lean and overweight subjects showed a consistent and highly localized increase in local temperature within the supraclavicular (SCV) region induced by a glucose ingestion followed by a cold stimulus in thermoneutral conditions (20°C). During OGTT and after cold stimulation, skin temperature was consistently higher in lean subjects compared to obese ones [65].

Raman spectroscopy is also widely used in biological applications because of its obvious advantages such as, being not influenced by water bands, high spatial resolution, high sensitivity to low-frequency vibrations, having less sample preparation steps [33]. Raman spectroscopy is especially suitable to *in vivo* measurements because the powers and excitation wavelengths used are non-destructive to biological tissues [66]. Raman spectroscopy is a proper method to monitor adipose tissue because this method is particularly sensitive to adipose tissue and lipids that exhibit large Raman scattering cross sections in comparison to other biological molecules. In a recent study, it has been demonstrated that Raman spectroscopy enables to detect WAT inflammation with high sensitivities and specificities in both mouse models of obesity and human tissues [67]. Beattie et al. [68] used Raman spectroscopy coupled with multivariate analysis to classify adipose tissue from four different species (chicken, beef, lamb, and pork). They reported that Raman spectroscopy with multivariate and neural network analytical methods allows to classify different species of an adipose tissue sample with higher than 99% accuracy. It is known that FT-IR and Raman spectroscopy applications have been used to examine different components in fats and oils including, among others, determination of trans-unsaturation [69, 70]. Olsen et al. [71] revealed that FTIR and Raman spectroscopy can be used as rapid, nondestructive method to determine omega-6 and omega-3 fatty acids in melted adipose tissue samples of porks. They also achieved to measure polyunsaturated, monounsaturated, and saturated fatty acids quantitatively in pork adipose tissue with nondestructive Raman spectroscopy [72].

3. Characterization and differentiation of adipose tissue by nuclear magnetic resonance spectroscopic and spectral imaging techniques

3.1. Basis of nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) is a spectroscopic technique that is used to monitor transitions between the energy levels due to nuclear-spin reorientation in an applied magnetic field. The incident energy which matches these energy differences is in the radiowave energy range. NMR spectroscopy and magnetic resonance imaging (MRI) can be used to examine a wide range of biological processes in systems from a single cell to a sample of tissue. One of the greatest advantages of NMR techniques is noninvasiveness. Hence, both biochemical (spectroscopy) and spatial information (imaging) can be obtained without destroying the sample. Another advantage of NMR methods is the lack of ionizing radiation for both imaging and spectroscopy. Most of non-NMR-based techniques use ionizing radiation in different forms for imaging or for in vivo studies. These two important advantages make NMR methods more useful for in vivo studies. Moreover, NMR spectroscopy and MRI can be combined to obtain metabolic, physiological, and anatomical data in a single experiment [73]. Other advantages of NMR can be listed as follows: It provides to study biological systems in their native aqueous environment; the NMR signals are sensitive to environment; the theory behind of it is well understood and therefore the relationship between spectral parameters and the information of interest (such as, concentration, structure, and dynamics) is defined well [74]. The main disadvantage of NMR spectroscopy is the lack of sensitivity due to very small population differences between the energy levels.

3.2. Applications of nuclear magnetic resonance spectroscopic and imaging techniques on adipose tissue

NMR spectroscopy, also known as magnetic resonance spectroscopy (MRS), is a non-invasive, ionizing-radiation-free analytical technique that that can be used to complement the more common MRI in the characterization of tissues in pathological conditions. Since MRI is able to distinguish between fat and water protons as regards their different magnetic resonance properties, adipose tissue samples can be characterized by this special contrast which is a phenomenon known as chemical shift. The noninvasive MRS and MRI techniques with various signal contrasts and underlying mechanisms allow to differentiate BAT from WAT morphologically and to assess BAT functionally because of its ability to enhance energy consumption through increased thermogenesis [75, 76]. Morphological distinction between BAT and WAT was obtained in rat by Osculati et al. [77] and they reported that there was a higher water content in BAT using 1H spectroscopy and suggested differences in fat-to-water ratio between BAT and WAT contributes to differences in signal intensities in MRI data. In addition, dimensions of BAT deposits can be determined by a combination of MRI and morphometry, and also MRI enables to differentiate areas of BAT responsive to acute adrenergic stimulation by giving information on the thermogenetically active tissue in vivo [78]. Verma et al. [79] characterized the biophysical properties of BAT and WAT by using quantitative translational diffusion measurements by high-resolution diffusion NMR spectroscopy to study the apparent diffusion coefficient (ADC) of fat molecules in rat BAT and WAT samples. The ADC of fat in BAT and WAT from chow diet rats was compared to high-fat diet rats to determine how the diffusion properties change based on obesity-related parameters such as lipid droplet size,

fatty acid chain length, and saturation. They reported that feeding with high-fat diet causes increased saturation, increased chain lengths, and reduced ADC of fat in WAT. Since, diffusion of fat was limited in BAT because of the presence of small lipid droplets, the ADC of fat was lower in BAT compared to WAT in rats fed both chow and high-fat diets. These findings indicated that in vivo diffusion could be a potential way for better characterization of BAT and WAT with metabolic alterations in both lean and obese sample. In both preclinical and clinical metabolic research, methods for in vivo investigation of adipose tissue would be invaluable for evaluating of metabolic diseases such as obesity. Branca et al. [80] proposed an in vivo NMR spectroscopic method to evaluate the effects of diet on fatty acid composition of the predominant chemical components of adipocytes in mice. The high resolution and sensitivity of the this method may be useful for the rapid detection of small changes in the composition of fatty acids in response to diet, exercise, and fat-metabolic diseases. Strobel et al. [81] achieved in vivo measurement of lipid composition in very small voxels (1.5 × 1.5 × 1.5 mm) in adipose tissue by using proton magnetic resonance spectroscopy (^{1}H-MRS) method in mice. This method uses localized point-resolved spectroscopy to collect ^{1} H spectra from voxels in intra-abdominal WAT and BAT depots for the characterization and differentiation of adipose tissue in rodent models of disease. This potential tool enables to study lipid metabolism in small animal models of disease during the initiation, progression, and manifestation of obesity-related disorders in vivo. In another MRI study, VAT and SCAT volume were measured and it was reported that children with an muscle fat content (MFC) ≥5%, compared with children with an MFC <5%, had a higher BMI and a higher VAT. On the other hand, there was no significant difference in SCAT, SCAT/VAT ratio [82].

4. Conclusion

Although spectroscopic techniques are in competition with electrochemistry for analytes detection in clinical chemistry, their some specific properties as in vivo analysis for metabolic studies and continuous monitoring, analysis of samples without reagents, detection of lower concentrations of biological components, capacity of diagnosis of diseases at very early stage, made the spectroscopic techniques state-of-the-art technology. Therefore, these techniques have taken part in the field of biomedicine especially in disease diagnosis and treatment-oriented monitoring in clinical investigations. The requirement of a rapid and operator-independent diagnostic method to characterize and differentiate cells and tissues is increasing with the expansion knowledge about metabolic diseases. With this diagnostic approach, they can be coupled with multivariate analysis to distinguish between normal and abnormal conditions in biological systems with a very high specificity and sensitivity.

In recent years, the evaluation of BAT using spectroscopic techniques gained momentum after BAT have been proposed as a potential therapeutic target for obesity and related metabolic diseases. Since targeting BAT, thermogenesis and monitoring BAT metabolism have a possible therapeutic potential for these metabolic diseases, the spectroscopic techniques focused on adipose tissue for medical investigations. Recent innovations in spectroscopy and microspectroscopy contributed to evaluation, characterization, and differentiation of the adipose

tissue. With this continuously evolving spectroscopic approach, these pioneer studies can be transferred to medical applications and they will shed light on the diagnosis and treatment of obesity and related metabolic diseases.

Conflicts of interest

The authors report no financial conflicts of interest. The authors are only responsible for the content and writing of this chapter.

Author details

Fatma Küçük Baloğlu[1] and Feride Severcan[2,3]*

*Address all correspondence to: feride.severcan@altinbas.edu.tr

1 Department of Biology, Giresun University, Giresun, Turkey

2 Department of Biophysics, Faculty of Medicine, Altınbaş University, İstanbul, Turkey

3 Department of Biological Sciences, Middle East Technical University, Ankara, Turkey

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