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Diagnostic imaging in metabolic disorders. From traditional to modern methods

Diagnostyka obrazowa w zaburzeniach metabolicznych. Od metod tradycyjnych do nowoczesnych

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S u m m a r y

The prevalence of metabolic disorders, first of all of obesity with its combination of comorbidities consisting metabolic syndrome is increasing at a very high rate, affecting millions individuals worldwide. Cardiovascular complications associated with metabolic disorders contribute to high rates of morbidity and mortality. For these reasons there is a strong need for precise and sensitive tools for early diagnose and control a natural course of metabolic disturbances. In this article traditional and modern methods of visualizing metabolic processes with their signs and symptoms in physiologic and pathologic conditions are briefly discussed. Ultrasonography, radiology, computed tomography, positron emission tomography, single-photon emission computed tomography, magnetic resonance imaging, and other techniques with their modalities are used in experimental medicine and, in large extend in clinical practice. With these methods not only body composition or ectopic fat accumulation may be recognize. Also β-cell function, vessel reconstruction and visualization, cardiac function, atherosclerotic plaques distribution, their structure and even estimation of risk of rupture can be made. Many fundamental questions regarding the etiology and natural course of the metabolic disorders are still unanswered. New technologies may be helpful for better understanding of their nature and impact on cardiovascular diseases – currently the greatest cause of mortality.

Key words: metabolic syndrome, obesity, insulin resistance, diagnostic imagine

S t r e s z c z e n i e

Częstość występowania zaburzeń metabolicznych, zwłaszcza otyłości i powiązanych z nią zaburzeń wchodzących w skład zespołu metabolicznego, wykazuje stałą tendencję wzrostową i obecnie dotyka już milionów ludzi na świecie. Stan taki wymaga posiadania odpowiednio precyzyjnych i czułych narzędzi do rozpoznawania i śledzenia naturalnej historii zaburzeń metabolicznych. W artykule krótko przedstawione są tradycyjne i nowoczesne metody obrazowania procesów metabolicznych i ich objawów w warunkach fizjologicznych i patologicznych. Techniki takie jak ultrasonografia, radiologia, tomografia komputerowa, pozytronowa tomografia emisyjna, tomografia emisyjna pojedynczych protonów, rezonans magnetyczny wraz z ich modyfikacjami znajdują obecnie zastosowanie zarówno w badaniach eksperymentalnych, jak i w coraz większym zakresie w praktyce klinicznej. Pozwalają one nie tylko np. na badanie składu ciała, w tym na ocenę tłuszczu zlokalizowanego ektopowo, ale także na wgląd w czynność komórek β trzustki, obrazowanie serca i naczyń, rozprzestrzenienie blaszek miażdżycowych, dokładną ocenę ich budowy, a nawet określenie ryzyka pęknięcia. Wiele pytań dotyczących etiologii i przebiegu zaburzeń metabolicznych wciąż pozostaje bez odpowiedzi. Nowe technologie mogą przyczynić się do lepszego poznania natury tych zaburzeń i związanych z nimi zagrożeń, w szczególności dotyczących układu sercowo-naczyniowego, którego choroby są obecnie główną przyczyną zgonów.

Słowa kluczowe: zespół metaboliczny, otyłość, insulinooporność, diagnostyka obrazowa

For ages, physicians have been strived to observe structures and functions of the human body. The great discoveries: of X-rays (in 1895) or ultrasounds (first technological application in 1917) have made possible to use these phenomena in medicine. At present, modern diagnostic departments use ultrasonography (US), radiology, computed tomography (CT), positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), and other techniques for the diagnosis and control of therapy of a range of diseases. In numerous biomedical research centers efforts are made in attempt to look inside the living cells and to observe life at its molecular level. So called molecular imaging is the

non-invasive technique to visualize cellular processes at a molecular or genetic level. It includes the imaging of endogenous molecules, use of activable agents that sense specific cellular processes, use of labeled particles to follow particular metabolic pathways and exploit of genetic engineering to express specific protein products.

Naturally, these techniques lend themselves to all medical fields. However, given that metabolic disorders has emerged as a growing public health problem worldwide that reached epidemic proportion use of diagnostic imaging in patients with suspected or establish metabolic problems seems to be of crucial significance. Metabolism is the complex set of chemical reactions that organism uses to maintain life, including energy production and utilization. Food, made up of proteins, carbohydrates, and fats is a fuel, that is used right away, or is transferred into energy stored in body tissues. This energy is afterwards utilize for everyday activity. A metabolic disorder occurs when abnormal chemical reactions disrupt this process.

Starting point to look into metabolic disorders may be the metabolic syndrome (MS), for the first time described by Reaven in 1993 (1). Now function different definitions of this syndrome, but according to all of them MS integrates a group of abnormalities that enhance the risk of cardiovascular diseases. Central obesity, hyperinsulinemia, dyslipidemia and hypertension are regarded as an elements of the metabolic syndrome (2).

The main problem of MS is obesity. There are various methods for measurement of obesity, of which body mass index (BMI) and waist-to-hip ratio (WHR) are probably most popular. However, also some techniques to visualize body fat were developed. One of them is dual-energy X-ray absorptiometry (DEXA). It is a valuable method, that allows to measure simply and accurately both total and regional fat with marginal exposure to radiation. In order to perform measurement region of interest (ROI) program according to specific computer software related to device (densitometer) should be used (3). In author's clinic abdominal fat is estimated in region between the upper part of the pelvis with the upper margin 96 mm superior to the lower part of this region. The lateral part of this region is defined by the lateral part of the thorax. The upper part of the gynoid fat region is defined by the superior part of trochanter major, with the lower margin 96 mm inferior to the upper part of the trochanter major. The lateral part of this region is defined by the subcutaneous tissue on the hip, which can be visualized using the Image Values option (fig. 1). There are also other techniques that allow to measure of adiposity including computed tomography, total body water or total body potassium estimations, but they have important limitations: exposure to ionizing radiations, relatively high cost or methodological complexity.

The phenomenon related directly to general obesity is ectopic lipid accumulation, i.e. in organs other than white adipose tissue, such as liver, muscles or heart. At present, magnetic resonance (MRI) is thought to be the most suitable method to visualize lipids content in

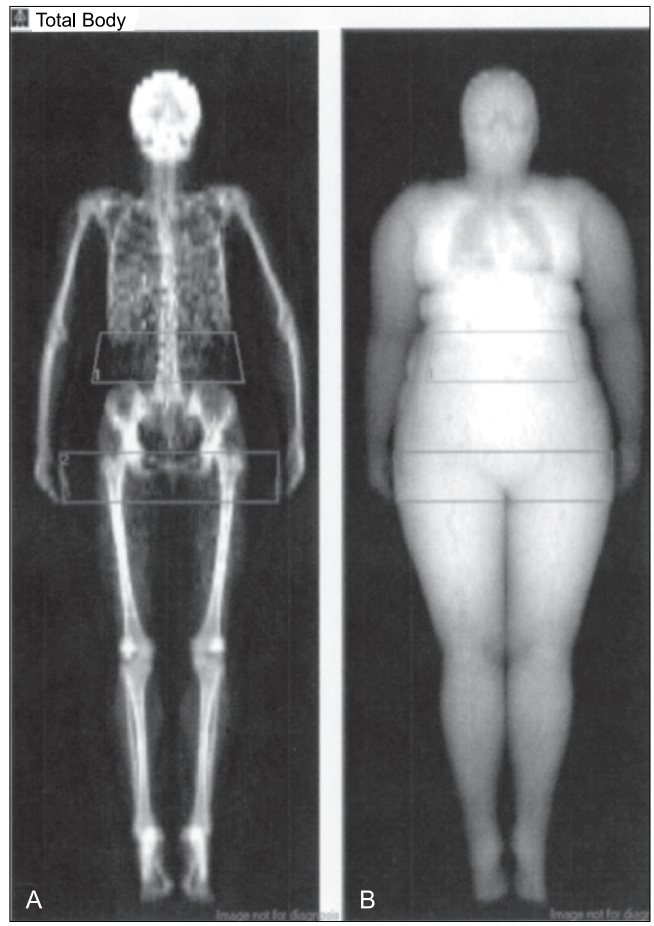
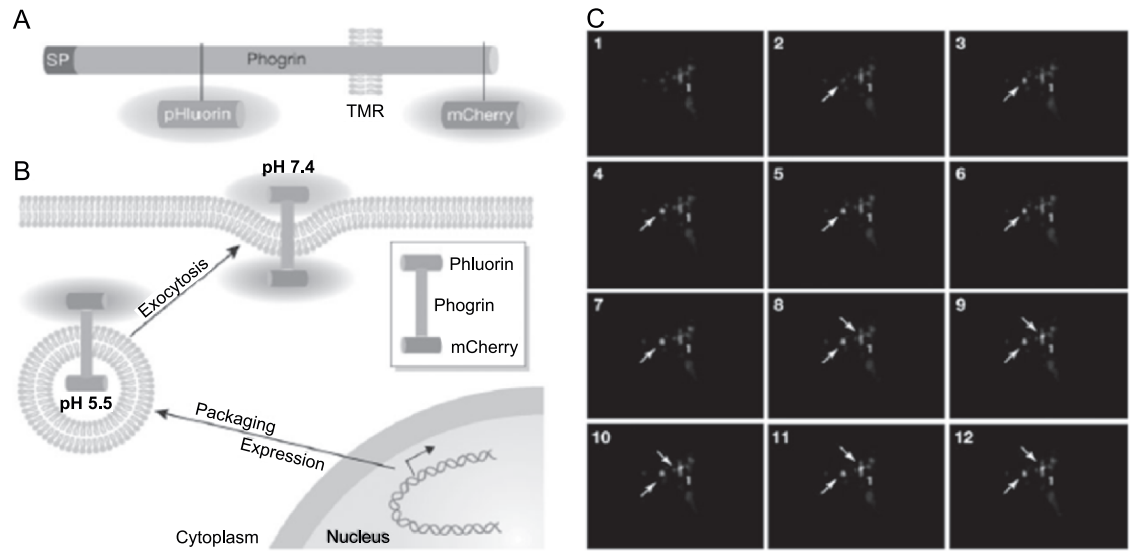


Fig. 1. Example of the regions of interest (ROI) delimiting abdominal (A) and gynoid (B) fat in one of woman examined in author's clinic.

peripheral tissues. In this technique, aided with 1-diamentinal magnetic resonance spectroscopy (MRS) measurement of total body fat mass with subsequent three-dimensional reconstruction and quantification of various fat depots is performed. Still present limitations of this method result from difficulties in precise determining the extend of saturated and unsaturated lipids within a tissue compartment, what is very important for cardiovascular risk prediction. To overcome these limitations use of spatially resolved MRS techniques were recently proposed (4-6). MRS is an analytical technique complement the MRI in the characterization of tissues. MRI uses the information to create 2-dimensional images of the structure, while MRS uses proton signals to determine the relative concentrations of target metabolites (7).

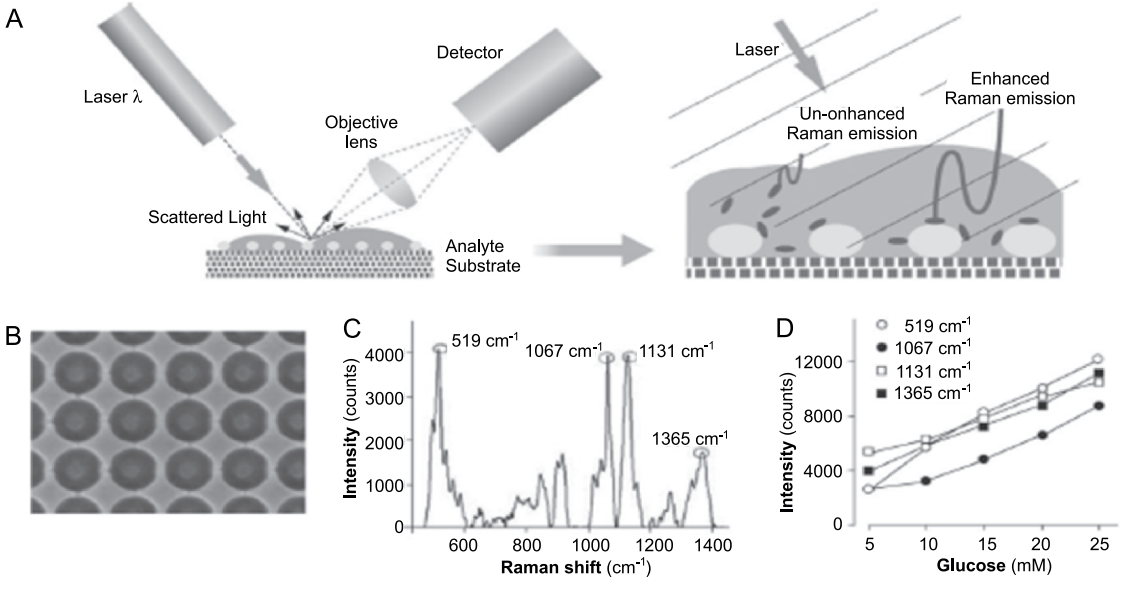
Another, very important components of metabolic syndrome, associated with obesity, especially in its abdominal form is insulin resistance with subsequent hyperinsulinemia. Traditionally, in everyday clinical practice insulin levels can be measured by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). Insulin resistance is usually estimated as the result of various formulas of insulin and glucose calculations (e.g. HOMA index). Glucose metabolic clamp is still regarded as the gold standard for insulin sensitivity measurement. However, modern

diagnostic imaging currently makes possible to visualize process of insulin secretion from pancreatic β-cells. It was demonstrated in rodents, that optical sensor of exocytosis can be implemented into animal β-cell. Then, insulin granulate exocytosis now can be observed intravital in fluorescent microscopic imaging (fig. 2) (8). Other method, namely surface-enhanced Raman spectroscopic (SERS) was recently developed to identify and visualize glucose and insulin molecules. Briefly, SERS tag (organic molecule immobilized on metallic nanoparticle) serves to label and track an analyte such as insulin or glucose (fig. 3) (9). This method is characterize



A. The sensor based on a chimeric fusion protein that consists of a secretory granule resident protein, phogrin and two fluorescent proteins: a highly pH-sensitive pHluorin inside the secretory granules and a red mCherry.
B. Schematic showing the design strategy of the optical sensor. pHluorin is inside the acidic lumen and remains non-fluorescent at resting state. Upon exocytosis, pHluorin faces the extracellular fluid at neutral pH, and becomes highly fluorescent, while mCherry remains in the cytosol during the process, and serves as a label for granule tracking and a standard for ratiometric quantification (adapted from Lu et al, 2009).
C. A time-lapse showing detected exocytosis events in insulin-secreting cells. Arrows indicato exocytosed insulin granules. SP, signal peptide; TMR, transmembrane region. The images are unpublished observations by Gustavsson.

Fig. 2. An optical sensor for visualizing insulin granulate exocytosis (10).



A. Basic concept of SERS. Raman scattering occurs at very low intensities in solution phase when molecules (oval-shaped grains) are distant from the metallic nanoparticles (yellow spheres). When the molecule is close to the nanosurface, upon laser excitation, the intensity is enhanced by the interaction of the molecule with the surface electrons.
B. Scanning EM image of silver and gold bimetallic SERS substrate by deep UV lithography on silicon wafers designed for glucose sensing.
C. Raman spectrum of glucose. Glucose sensing using surface functionalized bimetallic SERS substrate shown in 5B in SERS mode. The peaks indicate different vibrational levels in the molecules. Note that the narrowness of the peaks allows structurally similar multiple analytes to be detected simultaneously.
D. Glucose quantification by SERS-based glucose sensing. Areas-under-curves of the vibrational bands of glucose Raman spectrum at 519, 1067, 1131 and 1365 cm_1 are plotted against glucose concentrations.

Fig. 3. Basic concept and applications of surface-enhanced Raman spectroscopy (10).

by lack of toxicity, so theoretically the whole SERS active substrate could be implanted in living organism to obtain quantitative sensing of detected substance upon laser excitation (11). The promise of SERS lies on the detection of glucose, insulin or other protein hormones using SERS-active nanoparticles attached to a fibre optic sensor. It is believed, that the fibre can be readily configured for in vivo applications to allow studies in living animals in the near future (12).

Other important process contributing to impaired insulin secretion and in consequence to diabetes mellitus development is reduction in β-cell mass. Hence, it is worthy to quantify β-cell mass *in vivo* and to correlate it with glucose homeostasis in the course of diabetes development. Also evaluating of potential of drugs in preserving β-cell mass and function requires sensitive method to imaging of pancreatic cell mass. Unfortunately, by now very limited success in this filed has been achieved using MRI method in experiments in rodents (13). This diagnostic failure is mainly due to lack of sufficient difference in proton density between β-cells and surrounding tissue. Owing to that fact new approaches to visualize and quantificate of β-cells mass in vivo are proposed. One of them is genetic introduction of complementary DNA encoding proteins that bind to MRI-, PET- or SPECT-compatible probes or may together with iron form particles detectable in magnetic resonance (14). This method yet cannot be used in clinical practice but in experimental conditions provide models to evaluate compounds for their pleiotropic properties in relation to β-cells.

Currently, there are techniques, that allow visualization of pancreatic blood flow and vascularization. Data obtained with these methods may be useful for estimation of isles cells function during disease or after transplantation. Method that allows observation of blood flow in pancreas is dynamic contrast enhanced MRI with use of Gadolinium containing agents (15). After *i.v.* injection of contrast, area under curve of the contrast agent kinetics is analyze.

Not only blood flow, but also glucose, glycogen and their derivates can be detected in pancreas and other tissues by MRI. In this case specific paramagnetic lanthanide complex, which generate a chemical exchange saturation transfer (CEST) effect should be used (16). In fact, at present this technique is suitable only for measurements *ex vivo*, because of strong magnetic field heterogeneity while examinations (17). Nowadays, efforts are made on further improvement of these methods in order to make possible to measure not only the ensemble of all estimated parameters but also their individual components. For example, one of these new methods relay on use of frequency-shifting contrast agents to change the resonance frequency of the water instead of changing the T1 or T2 relaxation times (18).

Metabolic disturbances related to metabolic syndrome lead to progression of cardiac and vascular dysfunction which are the greatest cause of mortality. For this reason, development of noninvasive techniques for assessment of cardiac function and metabolism is extremely important. Traditionally, information on the coronary blood flow and heart structure and function can be obtained noninvasively with use of transthoracic Doppler echocardiography (19). New echocardiography techniques encompass tissue Doppler imaging, strain and strain rate. These methods enable detection of early systolic and diastolic dysfunctions in patients with metabolic disturbances. Tissue Doppler imaging is an echocardiographic technique that uses Doppler principles to measure the velocity of myocardial motion. This method allows to evaluate longitudinal, radial and circumferential heart function, and in this way to elucidate subclinical myocardial dysfunctions. Strain is the method related to fiber shortening (20). Strain rate indicates speed of fiber shortening, which is a measure of contractility (21). As traditional echocardiography measurements are load-dependent and therefore their reliability in obesity may be questionable – new methods looks like very promising. For example, reduced systolic and diastolic function in overweight, and in other study in obese persons were demonstrated with use of strain and strain rate techniques (22, 23). More recently, improvement in cardiac function in obese subjects after a training program was proved with use of the same methods (24).

Another important issue in the field of imaging of cardiac function in metabolic disturbances is estimation of left atrium size and contractility. Using traditional echocardiography enlargement of left atrium in obese persons even without any cardiovascular disease was found (25). On the other hand, it was demonstrated, that enlargement of left atrium is linked to increased risk of mortality (25). Using strain and strain rate methods reduced left and right atrial function in obese non-hypertensive children were found (26).

Regarding estimating of metabolic processes in the heart on cellular and molecular level new methods in this field were also developed. MRS using carbon ^{13}C is used for these purposes for about 30 years. Recently, a new technique – DNP-MR, which combines the solid-state method of dynamic nuclear polarization (DNP) with rapid dissolution procedure to produce stable injectable solutions was introduced. In this method, hyperpolarized MR – metabolism of (1-^{13}C)pyruvate is estimate (27). It seems, that use of this technique may be very helpful in the near future in understanding of lipid changes, that occur during development of obesity and other metabolic disorders.

Disturbances grouped in metabolic syndrome increase a risk for heart disease and stroke. The direct cause of these potentially fatal incidents is atherosclerotic rupture in coronary or carotid artery. From this point of view non-invasive or instrumental imaging technique that allows to investigate morphological features and biological characteristics of the atherosclerotic plaque is substantially important. Such techniques include ultrasound, magnetic resonance imaging, computed tomography, and nuclear imaging. In modern clinical practice vessel

reconstruction in US imaging is one of the most validated. In particular, measuring of the intima-media thickness (IMT), as the early marker of atherosclerosis, and indicator of hypertension risk is frequently made. Increase in IMT is considered as the predictor of cardiovascular events and is associated with cardiovascular and total mortality risk (28). Despite of widespread use of IMT it provides only limited information about atherosclerotic process, especially when it occurs distally in arteries. With modality in form of Multigate Doppler system real-time processing of the echo signals produced along artery is possible (29). Recently, a new technique named integrated backscatter (IB) analysis was introduced (30). With this technique different plaque components may be distinguished, on the basis of their specific spectral content. **Then, integrated backscatter ultrasonography (IB-US) allows in noninvasive manner evaluate the tissue substructure, in this composition of atherosclerotic plaques. High sensitivity and specificity of this method for detecting thrombi, lipid pools and fibrous tissue, reaches 80-85% and 78-91%, respectively** (31). **Another new method of visualizing atherosclerotic plaque is contrast-enhanced ultrasonography (CE-US)** (32). This method relies on detection of signals produced by microtubules containing gas, that are inserted into the sites of disease. CE-US method is particularity useful for detection of neovascularisation of plaque, what is considered to be associated with its instability and vulnerability (33). Also, detection of inflammation process within plaque with this method is possible (34). Recent developments in MRI technique allow to imaging of atherosclerotic plaques with resolution of 300 μm (35). Moreover, with multispectral imaging identification of plaque components (lipid core, fibrous cap, calcification, haemorrhage and thrombus) is possible (36). More sophisticated modalities of MRI, e.g. magnetization-prepared 3-dimensional rapid acquisition gradient echo (MP-RAGE) or slab-selective phase-sensitive inversion-recovery (SPI) are suitable for direct imaging of thrombus or intraplaque hemorrhage (37, 38).

Ionizing imaging techniques include computed tomography (CT) and positron emission tomography (PET). Specific methods appropriate to asses atherosclerotic plaque are electron-beam CT and multiple detector CT (MDCT). The first method uses tungsten rings to generate X-ray images at 3-mm slice thickness and is used to calculate coronary artery calcium score for the assessment of cardiovascular risk (39, 40). In the second, with one continuously rotating X-ray source 0.5 mm slices can be observed. Intravenous contrast is used to perform coronary angiographic (CTA) imaging in order to obtain information on atherosclerotic plaques in the coronary arterial wall (39). Also contrast-enhanced MDCT was recently introduced to imaging of plaque morphology (40).

PET and single photon emission CT (SPECT) are able to evaluate tissue metabolic activity by means of positron emitting isotopes, typically bind do glucose (i.e. 18-fluorodeoxyglucose – 18FDG) injected into the subject (42). 18FDG signal was found to be associated with levels of inflammatory biomarkers and to factors of the metabolic syndrome (43). **SPECT ligands have been used to investigate processes of atherosclerosis progression and plaque rupture** (44). Hence, PET and SPECT are considered as important tools for identifying inflammatory vasculitides, and for monitoring patients with inflammatory diseases. In clinical conditions CT may be combined with PET (PET-CT) to detect atheromas *in vivo* (45) or to apprise the effectiveness of statin therapy in reducing the level of inflammation (46). Another interesting application for PET-CT, usually with use of 18FDG is imaging of brown adipose tissue (BAT). However, evaluation of brown tissue may be difficult, as in adults BAT in limited amount and because with this method only metabolically active tissue may be visualized. Evaluating tissue may be recognize as BAT when its layer is > 4 mm, its density is similar to white tissue in CT scan (-250 do -50 jH) and SUV i. e. activity/ml in ROI/isotope dose (MBq/g body weight) ^{18}F-FDP is at least 2 g/mL (47).

Another condition strongly associated with metabolic syndrome is non-alcoholic fatty liver disease (NAFLD). It is a chronic liver disease, considered as the hepatic manifestation of metabolic syndrome. Obesity, type 2 diabetes mellitus or/and dyslipidemia are usually seen in patients with liver steatosis. NAFLD is an important public health problem and is the main cause of chronic liver diseases in development countries (48). Obese patients with liver steatosis have 3-fold higher prevalence of prediabetes and type 2 diabetes mellitus than healthy individuals (49). NAFLD is diagnose according to criteria of American Association for the Study of Liver Diseases by ultrasonographic detection of ectopic fat accumulation in liver in the absence of other identifiable causes of liver steatosis, in particular in the absence of excessive alcohol consumption, use of steatogenic medication or hereditary disorders (50). Then, ultrasound visualization of ectopic fat accumulation in the liver is the first line screening of NAFLD detection. Steatosis on a liver ultrasound appears as hyperechogenic when compared with the spleen or kidney. Figure 4 presents an example of fatty liver in US image.

Metabolic disturbances are strongly associated with polycystic ovary syndrome (PCOS). Approximately 50% of the women with PCOS characterize by overweight or obesity (51). Abdominal type of obesity that is dominating in this syndrome leads to insulin resistance in patients and is a well-recognized risk factor for further metabolic and hormonal disturbances. The main signs and symptoms of PCOS vary, but one of the important features are polycystic ovaries, usually recognized by ultrasound imaging. Ovaries in US are defined as polycystic when they included either 10 or more follicles measuring 2-9 mm in diameter or their volume was greater than 10 cm^3 (fig. 5).

Metabolic disturbances, especially integrated as the metabolic syndrome are one of the most rapidly rising

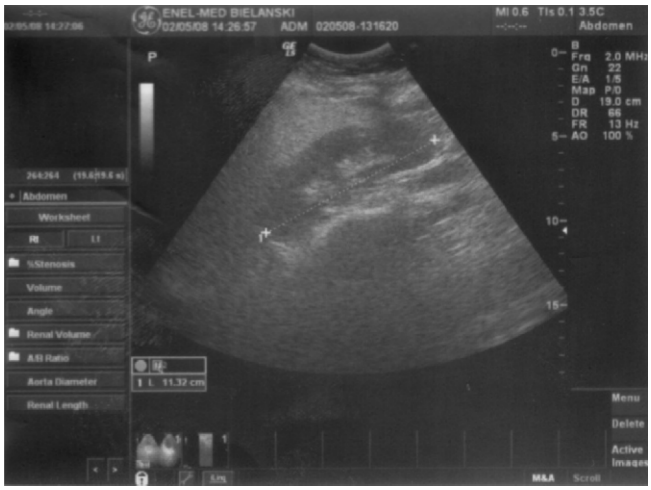


Fig. 4. Example of ultrasound imaging of liver steatosis in one of subjects examined in author's clinic.

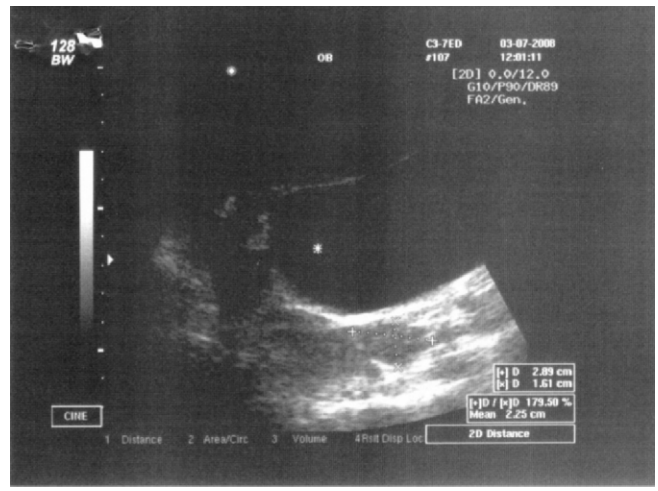


Fig. 5. Example of ultrasound imaging of polycystic ovary in one of patients examining in the author's clinic.

medical problems for almost all the developed countries and many developing nations. This is the reason, why intense efforts from biomedical and clinical scientists are made in order to elucidate their etiology and natural course. These efforts include development of suitable imaging technologies to visualize lipid composition and distribution, insulin secretion, β-cell mass and functions *in vivo*, heart structure and function or atherosclerotic plaque structure and distribution in arteries. New technologies, despite limitations seems to be increasingly helpful tools for diagnosis of metabolic disorders such as obesity, diabetes or atherosclerosis, for our better understanding of their complexity, and also for drug development and assessment of treatment efficacy.

BIBLIOGRAPHY

1. Reaven GM: Role of insulin resistance in human disease (syndrome X): an expanded definition. Annu Rev Med 1993; 44: 121-131.
2. Björntrop P: Metabolic implications of body fat distribution. Diab Care 1991; 14: 1132-1143.
3. Rissanen P, Hamalainen P, Vanninen E et al.: Relationship of metabolic variables to abdominal adiposity measured by different anthropometric measurements and dual-energy X-ray absorptiometry in obese middle-aged women. Int J Obes Relat Metab Disord 1997; 21: 367-371.
4. Springer F, Machann J, Claussen CD et al.: Liver fat content determined by magnetic resonance imaging and spectroscopy. World J Gastroenterol 2010; 16: 1560-1566.
5. Hammer S, Snel M, Lamb HJ et al.: Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. J Am Coll Cardiol 2008; 52: 1006-1012.
6. Vessby B, Gustafsson IB, Tengblad S et al.: Desaturation and elongation of fatty acids and insulin action. Ann N Y Acad Sci 2002; 967: 183-195.
7. Velan SS, Said N, Durst C et al.: Distinct patterns of fat metabolism in skeletal muscle of normal-weight, overweight, and obese humans. Am J Physiol Regul Integr Comp Physiol 2008; 295: R1060-1065.
8. Lu J, Gustavsson N, Li Q et al.: Generation of transgenic mice for in vivo detection of insulin-containing granule exocytosis and quantification of insulin secretion. J Innov Opt Health Sci 2009; 2: 397-405.
9. Faulds K, Barbagallo RP, Keer JT et al.: SERS as a more sensitive technique for the detection of labeled oligonucleotides compared to fluorescence. Analyst 2004; 129: 567-568.
10. Han W, Chuang K-H, Chang Y-T et al.: Imaging metabolic syndrome. EMBO Mol Med 2010; 2: 196-200.
11. Stuart DA, Yuen JM, Shah N et al.: In vivo glucose measurement by surface-enhanced Raman spectroscopy. Anal Chem 2006; 78: 7211-7215.
12. Shi C, Zhang Y, Gu C et al.: Molecular fiber sensor based on surface enhanced Raman scattering (SERS). J Nanosci Nanotechnol 2009; 9: 2234-2246.
13. Zheng Q, Dai H, Merritt ME et al.: A new class of macrocyclic lanthanide complexes for cell labeling and magnetic resonance imaging applications. J Am Chem Soc 2005; 127: 16178-16188.
14. Cohen B, Ziv K, Plaks V et al.: MRI detection of transcriptional regulation of gene expression in transgenic mice. Nat Med 2007; 13: 498-503.
15. Coenegrachts K, Van Steenbergen W, De Keyzer F et al.: Dynamic contrast-enhanced MRI of the pancreas: initial results in healthy volunteers and patients with chronic pancreatitis. J Magn Reson Imaging 2004; 20: 990-997.
16. Zhang S, Trokowski R, Sherry AD: A paramagnetic CEST agent for imaging glucose by MRI. J Am Chem Soc 2003; 125: 13511-13516.
17. Antkowiak PF, Tersey SA, Carter JD et al.: Nonivassive assessment of pancreatic beta-cell function *in vivo* with manganese-enhanced magnetic resonance imaging. J Am Physiol Endocrinol Metab 2009; 296: E573-578.
18. Zabow G, Dodd S, Moreland J, Koretsky A: Micro-engineered local field control for high sensitivity multispectral MRI. Nature 2008; 453: 1058-1063.
19. Takeuchi M, Lodato JA, Furlong KT et al.: Feasibility of measuring coronary flow velocity and reserve in the left anterior descending coronary artery by transthoracic Doppler echocardiography in a relatively obese American population. Echocardiography 2005; 22: 225-232.
20. Pavlopoulos H, Nihoyannopoulos P: Strain and strain rate deformation parameters: from tissue Doppler to 2D speckle tracking. Int J Cardiovasc Imaging 2008; 24: 479-491.
21. Heimdal A, Stoylen A, Torp H, Skjaerpe T: Real-time strain rate imaging of the left ventricle by ultrasound. J Am Soc Echocardiogr 1998; 11: 1013-1039.

22. Wong C, O'Moore-Sullivan T, Leano R et al.: Alterations of left ventricular myocardial characteristics associated with obesity. Circulation 2004; 110: 3081-3087.
23. Lorch S, Sharkey A: Myocardial velocity, strain, and strain rate abnormalities in healthy obese children. J Cardiometab Syndr 2007; 2: 30-34.
24. Ingul CB, Tjonna AE, Stolen TO et al.: Impaired cardiac function among obese adolescents: effect of aerobic interval training. Arch Pediatr Adolesc Med 2010; 164: 852-859.
25. Benjamin E, D'Agostino R, Belanger A et al.: Left atrial size and the risk of stroke and death. The Framingham Heart Study. Circulation 1995; 92: 835-841.
26. Hirschler V, Acebo H, Fernandez G et al.: Influence of obesity and insulin resistance on left atrial size in children. Pediatr Diabetes 2006; 7: 39-44.
27. Chen AP, Albers MJ, Cunningham CH et al.: Hyperpolarized C-13 spectroscopic imaging of the TRAMP mouse at 3T-initial experience. Magn Reson Med 2007; 58: 1099-1106.
28. Bots ML, Hoes AW, Koudstaal PJ et al.: Common carotid intima--media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96: 1432-1437.
29. Tortoli P, Bambi G, Guidi F, Muchada R: Toward a better quantitative measurement of aortic flow. Ultrasound Med Biol 2002; 28: 249-257.
30. Urbani MP, Picano E, Parenti G et al.: *In vivo* radiofrequency--based ultrasonic tissue characterization of the atherosclerotic plaque. Stroke 1993; 24: 1507-1512.
31. Kawasaki M, Takatsu H, Noda T et al.: Noninvasive quantitative tissue characterization and two-dimensional color-coded map of human atherosclerotic lesions using ultrasound integrated backscatter: comparison between histology and integrated backscatter images. J Am Coll Cardiol 2001; 38: 486-492.
32. Lindner JR: Molecular imaging of cardiovascular disease with contrast-enhanced ultrasonography. Nat Rev Cardiol 2009; 6: 475-481.
33. Feinstein SB: The powerful microbubble: from bench to bedside, from intravascular indicator to therapeutic delivery system, and beyond. Am J Physiol Heart Circ Physiol 2004; 287: H450-457.
34. Owen DR, Shalhoub J, Miller S et al.: Inflammation within carotid atherosclerotic plaque: assessment with late-phase contrast-enhanced US. Radiology 2010; 255: 638-644.
35. Hayes CE, Mathis CM, Yuan C: Surface coil phased arrays for high-resolution imaging of the carotid arteries. J Magn Reson Imaging 1996; 6: 109-112.
36. Yuan C, Mitsumori LM, Ferguson MS et al.: *In vivo* accuracy of multispectral magnetic resonance imaging for identifying lipid--rich necrotic cores and intraplaque hemorrhage in advanced human carotid plaques. Circulation 2001; 104: 2051-2056.
37. Moody AR, Murphy RE, Morgan PS et al.: Characterization of complicated carotid plaque with magnetic resonance direct thrombus imaging in patients with cerebral ischemia. Circulation 2003; 107: 3047-3052.
38. Wang J, Ferguson MS, Balu N et al.: Improved carotid intraplaque hemorrhage imaging using a slab-selective phase-sensitive inversion-recovery (SPI) sequence. Magn Reson Med 2010; 64: 1332-1340.
39. Detrano R, Guerci AD, Carr JJ et al.: Coronary calcium as a predictor of coronary events in four racial or ethnic groups. N Engl J Med 2008; 358: 1336-1345.
40. Motoyama S, Sarai M, Harigaya H et al.: Computed tomographic angiography characteristics of atherosclerotic plaques subsequently resulting in acute coronary syndrome. J Am Coll Cardiol 2009; 54: 49-57.
41. Komatsu S, Hirayama A, Omori Y et al.: Detection of coronary plaque by computed tomography with a novel plaque analysis system, "Plaque Map", and comparison with intravascular ultrasound and angioscopy. Circ J 2005; 69: 72-77.
42. Rudd JH, Warburton EA, Fryer TD et al.: Imaging atherosclerotic plaque inflammation with (18F)-fluorodeoxyglucose positron emission tomography. Circulation 2002; 105: 2708-2711.
43. Tahara N, Kai H, Yamagishi S et al.: Vascular inflammation evaluated by (18F)-fluorodeoxyglucose positron emission tomography is associated with the metabolic syndrome. J Am Coll Cardiol 2007; 49: 1533-1539.
44. Choudhary S, Higgins CL, Chen IY et al.: Quantitation and localization of matrix metalloproteinases and their inhibitors in human carotid endarterectomy tissues. Arterioscler Thromb Vasc Biol 2006; 26: 2351-2358.
45. Rudd JH, Warburton EA, Fryer TD et al.: Imaging atherosclerotic plaque inflammation with (18F)-fluorodeoxyglucose positron emission tomography. Circulation 2002; 105: 2708-2711.
46. Rudd JH, Warburton EA, Fryer TD et al.: Imaging atherosclerotic plaque inflammation with (18F)-fluorodeoxyglucose positron emission tomography. Circulation 2002; 105: 2708-2711.
47. Cypess AM, Lehman S, Williams G et al.: Identification and importance of brown adipose tissue in adult humans. N Engl J Med 2009; 360: 1509-1517.
48. Targher G, Byrne CD: Nonalcoholic Fatty Liver Disease: A Novel Cardiometabolic Risk Factor for Type 2 Diabetes and Its Complications. J Clin Endocrinol Metab 2013; 98: 483-495.
49. Ortiz-Lopez C, Lomonaco R, Orsak B et al.: Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). Diabetes Care 2012; 35: 873-878.
50. Chalasani N, American Gastroenterological Association, American Association for the Study of Liver Diseases et al.: The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. Gastroenterology 2012; 142: 1592-1509.
50. Gambineri A, Pelusi C, Vicennati V et al.: Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord 2002; 26: 883-896.

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