Obesity has only relatively recently been acknowledged as a chronic disease. The capacity to readily accumulate fat began as an evolutionary adaptive trait; however, this has become a liability in many countries with wide availability of food supplies and more sedentary lifestyles. The prevalence of obesity has increased during the past several decades to the point where the World Health Organization has declared the global obesity epidemic a worldwide public health crisis.2

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The morbidity and mortality associated with obesity are now well documented; however, it is also apparent that there is great heterogeneity on the health risks associated with obesity. This critical concept has underscored the need for more accurate phenotyping of adipose depots among individuals to determine health risks. Although phenotyping adiposity may eventually be aided with a panel of circulating biomarkers, measurement and imaging of adiposity have been the most predictive of morbidities thus far. To maximize the potential of adipose tissue imaging, there is a need for even further refinement of adipose tissue imaging techniques.

The purpose of this review is to provide a brief overview of methods used to measure and image body fat. Other reviews in this miniseries will address differences in adipose depots and health implications in detail.3,4

Measurements of Weight and Adiposity

Insurance companies were among the first to document the risks associated with obesity. When platform scales became widely available in the second half of the 19th century, body weight data became accessible to insurance companies. Louis Dublin (1882–1969), a statistician and vice president of the Metropolitan Life Insurance Company, led studies demonstrating associations among weight, comorbidities, and mortality.5 Based on these data, ideal body weights for age and height were reported. To more clearly represent the interdependence of weight and height, astronomer and mathematician Alphonse Quetelet developed the Quetelet Index or body mass index (BMI; weight, kg/height [m²]).6 This index is still used widely to classify subjects as overweight (BMI>25) or obese (BMI>30).

However, BMI is not always a measure of fatness and individuals with high muscle mass may be incorrectly classified as obese—defined as excess body fat that has accumulated to the extent that it may have a negative effect on health, leading to reduced life expectancy and increased health problems.7

Waist circumference and the waist/hip ratio are other measures that may correlate better with body fatness and be more predictive of adverse metabolic effects because of obesity, as well as cardiovascular complications. For example, in the INTER-HEART study, a global case-control study designed to identify risk factors associated with acute myocardial infarction,8 with >27,000 participants from 52 countries, BMI showed only a modest association with myocardial infarction between the top and bottom quintiles that was not significant after adjustment for other cardiovascular risk factors. In contrast, waist/hip ratio and waist circumference were strongly associated with myocardial infarction, even after adjustment for other risk factors. This study indicates that regional distribution of adiposity may be critical in determining the cardiovascular risk associated with obesity.

Other measures of fatness include skinfold thickness using calipers at specific body sites (ie, trunk, thighs, shoulder blade, buttocks, and supraclavicular area) or with a dedicated skinfold caliper. Skinfolds may be used to estimate total body fat mass (TBFM), total body water (TBW), and extracellular water (ECW). However, these techniques are not easy to perform in the clinic setting and the accuracy of estimates can be influenced by an individual’s skinfold thickness.11

Other techniques used to measure and image body fat include impedance techniques (eg, bioelectrical impedance analysis, BIA), dual-energy X-ray absorptiometry (DXA), and magnetic resonance imaging (MRI). These methods can be used to determine the mass and density of fat and muscle, and the results can be used to calculate body fat percentage (BF%). Although these methods are time-consuming and expensive, they are considered gold standard techniques in research settings.

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Abstract—Obesity is a worldwide epidemic and is associated with multiple comorbidities. The mechanisms underlying the relationship between obesity and adverse health outcomes remain poorly understood. This may be because of several factors including the crude measures used to estimate adiposity, the striking heterogeneity between adipose tissue depots, and the influence of fat accumulation in multiple organs. To advance our understanding of fat stores and associated comorbidities in humans, it will be necessary to image adiposity throughout the body and ultimately also assess its functionality. Large clinical studies are demonstrating the prognostic importance of adipose tissue imaging. Newer techniques capable of imaging fat metabolism and other functions of adipose tissue may provide additional prognostic use and may be useful in guiding therapeutic interventions. (Arterioscler Thromb Vasc Biol. 2014;34:2217-2223.)

Key Words: abdominal fat ■ adipose tissue ■ brown fat ■ inflammation ■ obesity ■ white fat
Fat Imaging Modalities

Dual-Energy X-Ray Absorptiometry

Dual-energy x-ray absorptiometry (DXA) scanning uses low-level x-rays that pass through different types of tissues at different rates providing estimates of fat mass, fat-free mass, and bone density. This method is used widely to measure bone density and can also accurately measure fat mass (Table). A limitation of most DXA scanners is the capacity to image extremely obese persons. However, half-body scans have been shown to provide an accurate body compositional analysis (Figure 1). In this study, total fat mass, nonbone lean mass, or percent fat was comparable for the whole-body scans, left, and right side scans (>97% within individuals and >99.9% for the group). This study demonstrated that with new generation DXA, accurate, reproducible fat mass measurements can be obtained.

DXA is relatively simple to perform, less expensive, and more accessible than MRI or computed tomography (CT). Radiation exposure is much less than CT. Although subcutaneous and visceral fat cannot be clearly separated by DXA, abdominal fat mass determined by DXA correlates well with visceral fat as determined by other methods such as CT and MRI. The added prognostic information obtained by abdominal DXA scanning compared with simple waist circumference measurement is controversial. In a study of postmenopausal women, DXA-derived abdominal fat mass and waist circumference were found to be of equal use in predicting dyslipidemia.

Computed Tomography

X-ray CT uses x-rays that are computer processed to produce tomographic images of specific areas of a scanned object. From a large series of cross-sectional images taken around a single axis of rotation, a 3-dimensional image of the area of interest is created. This technology is now used widely for imaging in the medical field (Table). Approximately 72 million scans were performed in the United States in 2007.

CT has been shown to be an excellent method for measuring regional adiposity. CT has been used to provide ethnicity- and sex-specific visceral abdominal adipose tissue (VAT) and subcutaneous adipose tissue prediction equations, derived from a large triethnic sample. These equations will be useful in future studies of mechanisms of cardiometabolic disease across ethnicities in countries where imaging data are not available.

CT can also detect calcified and noncalcified atherosclerotic plaques in the vasculature, including the coronary arteries, so correlations can be made between volume of adipose tissue deposits and vascular disease. As part of the Framingham Heart Study, 1155 participants without known cardiovascular disease underwent imaging with multidetector CT for quantification of VAT, intrathoracic fat, pericardial fat, and for quantification of aortic and coronary artery calcification. In this study, pericardial fat volume was defined as any adipose tissue located within the pericardium. Common cardiac risk factors, BMI, and waist circumference were also measured. Investigators found that both pericardial and intrathoracic fat correlated with waist circumference, BMI, VAT, hypertension, metabolic syndrome, diabetes mellitus, and dyslipidemia. These results strongly supported the adverse relationship among obesity, metabolic abnormalities, and vascular disease. Of particular interest, however, was the finding that pericardial fat, but not intrathoracic fat, is a strong predictor of the presence of coronary artery disease by CT angiography. Patients with coronary artery disease had greater visceral fat thickness and a higher waist/hip ratio compared with those without coronary disease, whereas preperitoneal fat thickness, subcutaneous fat thickness and abdominal fat index were not correlated with coronary artery disease. However, after adjusting for traditional cardiovascular risk factors, only waist/hip ratio remained associated with coronary artery disease. Therefore, this study indicated that abdominal sonography measurement of fat indices was not superior to anthropometric measurements for prediction of coronary artery disease. Improvements in ultrasound devices and software designed specifically for the purpose of assessing fat composition may enhance the clinical use of ultrasound.

Transthoracic echocardiography, routinely used to analyze cardiac function, may be useful in measuring epicardial fat thickness overlying the right ventricle (Figure 3). Human epicardial fat may be particularly relevant as it has been shown to be a source of local inflammatory mediators and could therefore promote atherosclerosis in the adjacent epicardial coronary arteries. Epicardial adipose tissue detected by transthoracic echocardiography has shown a strong correlation with anthropometric and other imaging measurements of visceral adipose tissue. Therefore, transthoracic echocardiography may represent a relatively easy and reliable imaging method for visceral adipose tissue prediction. As with other ultrasound techniques, some studies have found poor reproducibility with echocardiogram measurements of epicardial fat thickness.

Ultrasound

Ultrasound has been used widely as an effective technique to assess body fat composition for decades. Using a portable imaging device, this technique is capable of making rapid estimates of fat in specific regions (Figure 2). Ultrasound is an attractive tool to evaluate obesity when other methods are not accessible (Table). The major limitation of ultrasound is lack of standardization for measurements, so accuracy is dependent on operator proficiency. Abdominal fat ultrasonography has been compared with anthropometric measurements of central obesity for prediction of the presence of coronary artery disease by CT angiography. Patients with coronary artery disease had greater visceral fat thickness and a higher waist/hip ratio compared with those without coronary disease, whereas preperitoneal fat thickness, subcutaneous fat thickness and abdominal fat index were not correlated with coronary artery disease. However, after adjusting for traditional cardiovascular risk factors, only waist/hip ratio remained associated with coronary artery disease. Therefore, this study indicated that abdominal sonography measurement of fat indices was not superior to anthropometric measurements for prediction of coronary artery disease. Improvements in ultrasound devices and software designed specifically for the purpose of assessing fat composition may enhance the clinical use of ultrasound.

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fat, was associated with coronary artery calcification, even after adjustment for multiple variables and VAT. Similarly, intrathoracic, but not pericardial fat, was associated with calcification of the abdominal aorta. This intriguing finding suggested that fat depots surrounding vascular structures may exert local toxic effects that promote vascular disease, in addition to the adverse metabolic effects of generalized obesity. This study demonstrates the added information that could potentially be obtained with adipose tissue imaging by CT or other modalities. Epicardial fat is the adipose tissue accumulated between the visceral pericardium and the myocardium and may be particularly relevant given its direct contact with coronary arteries. Preclinical studies have supported a complex role of brown, beige, and white perivascular adipose tissue on a variety of vascular phenotypes. Diseases of the aorta are not uncommon, contributing to ≈16,000 deaths per year in the United States. As part of the Framingham Heart Study, 3001 male and female participants underwent imaging with CT for quantification of aortic dimensions and periaortic fat. Investigators found that periaortic fat volumes were directly associated with thoracic and abdominal dimensions. This association was independent of other cardiac risk factors, including BMI and visceral adipose tissue volume. This study thus strongly supports a local effect of periaortic adipose tissue in aortic remodeling.

As CT imaging becomes increasingly practical and widespread, it must be kept in mind that the radiation doses are much higher than conventional x-ray. This can lead to significant radiation exposure, especially in subjects who receive repeated scans. Ionizing radiation can cause DNA mutations linked to induction of cancer. It is estimated that 1.5% to 2.0% of all cancers in the United States may be attributable to radiation from CT studies.

Magnetic Resonance Imaging

Anatomic imaging of different fat depots can also be accomplished with MRI and without the ionizing radiation required for CT (Table). As with CT, MRI imaging protocols provide not only detailed images of fat depots but can also characterize disease processes in other organs that may be associated with different types of obesity. This is important because the association of obesity with vascular disease is complex with clear heterogeneity related to different types of adiposity. Imaging techniques that can simultaneously quantify vascular disease processes (or complications of disease processes) and accurately measure adipose depots may provide unique insight...
into associations between different patterns of adiposity and disease. For example, a recent study demonstrated that body fat distribution was a risk factor for cerebrovascular disease. In a study of patients with MRI evidence of hyperacute ischemic stroke, MRI-based volumetric analysis of subcutaneous and visceral fat distribution was performed and then correlated with white matter lesion load by MRI and also total atherosclerotic plaque volume by CT angiography. These investigators found that neither total abdominal fat volume nor subcutaneous fat volume correlated with white matter lesion load by MRI and also total atherosclerotic plaque volume. However, when the specific volume of visceral adipose tissue was determined, there was a direct correlation between a greater percentage of visceral adipose tissue and both white matter lesion load and total atherosclerotic plaque volume. Thus, even at sites distant from the disease process, this study suggests that visceral adiposity is a risk factor for cerebrovascular disease.

MRI may also be useful for analyzing intrahepatic fat or hepatic steatosis that occurs in dysmetabolic syndromes. Hepatic steatosis may be a risk factor/mediator for cardiovascular risk associated with obesity. Liver biopsy is an invasive procedure and has sampling error. MRI can decompose the liver signal into fat and water components and measure liver fat more directly than ultrasound or CT. Figure 5 shows an example of hepatic imaging in a subject with hepatic steatosis and severe dyslipidemia before and after treatment of the dyslipidemia with plasmapheresis and multidrug therapy.

Other organs may also be clinically useful to image for fat content. Skeletal muscle plays a key role in insulin resistance. Both intramyocellular and extramyocellular lipid content increase with obesity. Skeletal muscle triglyceride content, as determined by 1H-nuclear magnetic resonance spectroscopy has been inversely correlated with insulin sensitivity in obese adolescents. Myocardial fat may also be useful to image although differentiating normal from pathological fat infiltration by CT or MRI is often difficult.

**Figure 2.** BodyMetrix ultrasound image of thigh. The top line indicates the subcutaneous fat-muscle interface (average thickness of 3.69 mm). The bottom line indicates the muscle–bone boundary. The muscle thickness ranges from 32.0 to 46.6 mm. The white layer in the center is the boundary of the rectus femoris and vastus intermedius. Reproduced from Wagner with permission of the publisher. Copyright © 2013 Dale R. Wagner.

**Figure 3.** Measurement of epicardial adipose tissue measured in parasternal long-axis view of transthoracic echocardiography. AO indicates aorta; EAT, epicardial adipose tissue; LA, left atrium; LV, left ventricle; and RV, right ventricle. Reproduced from Chao et al with permission of the publisher. Copyright © 2013 Chao et al.

**Figure 4.** Measurement of visceral adipose tissue and subcutaneous adipose tissue from computed tomographic image at L4. Adipose tissue is dark gray, white line shows delineation of visceral compartment, white arrow indicates depth of subcutaneous compartment. Reproduced from Eastwood et al with permission of the publisher. Copyright © 2013 Eastwood et al.

**Figure 5.** Serial liver MRI scans to demonstrate changes in liver fat during therapy. Serial proton density fat-fraction (PDFF) maps obtained with complex-based MRI were acquired in a 41-year-old man with hypertriglyceridemia and insulin resistance undergoing plasmapheresis and multidrug therapy to lower serum triglycerides. Follow-up MRI demonstrates reduction in hepatic PDFF (from 33% to 33%). Reproduced from Reeder et al with permission of the publisher. Copyright © 2011 Wiley-Liss, Inc.
Pathological conditions associated with myocardial fat include arrhythmic right ventricular dysplasia, healed myocardial infarction, lipoma, tuberous sclerosis, and dilated cardiomyopathy. In patients with lipodystrophy, magnetic resonance spectroscopy has demonstrated cardiac steatosis associated with severe concentric left ventricular hypertrophy. Fatty pancreas has also been described recently by MRI. In 1 study, 16.1% of a community cohort met criteria for above normal levels of fat in the pancreas. Fatty pancreas was associated with central obesity, hypertriglyceridemia, and insulin resistance.

**Positron Emission Tomography**

Although CT and MRI have been extremely informative in quantifying fat in depots and within organs, the analysis is largely limited to fat volumes. These techniques have reinforced the importance of anatomic, regional, fat heterogeneity; however, it remains unclear why certain regions of fat are more harmful than others. In vivo imaging techniques that can provide metabolic information specific to a particular fat storage site may add significantly to our understanding of adipose tissue in living humans (Table).

Positron emission tomography (PET) detects γ-ray emissions from a molecule labeled with a positron-emitting tracer. Images are then constructed revealing a 3-dimensional representation of tracer activity at a particular site of interest. A widely used tracer is the glucose analog, 2-fluorodeoxy-β-glucose (2FDG). Concentrations of this tracer reflect tissue glucose uptake, serving as a surrogate for tissue metabolic activity. PET may be useful in phenotyping fat and has been used to track the heightened metabolic activity of brown adipose tissue (Figure 6). The in vivo identification of brown adipose tissue could be useful to monitor future therapies designed to promote transformation of white to more metabolically active brown fat. Recently, MRI has also been shown to reliably identify brown adipose tissue that was confirmed histologically.

Regional differences in FDG activity have also been demonstrated among white adipose tissue depots. In a study of nonobese humans, adipose tissue volume was calculated with MRI and DXA, and dynamic PET-FDG imaging was performed at baseline and after infusion of insulin. Visceral adipose tissue had higher rates of glucose uptake compared with subcutaneous adipose tissue, and glucose uptake correlated with systemic insulin sensitivity. Interestingly, VAT mass correlated negatively with glucose uptake and insulin sensitivity, whereas no correlation was observed between subcutaneous fat mass and insulin sensitivity. These findings indicate that regional differences exist between adipose depots related to insulin sensitivity and may shed light on the metabolically obese but normal-weight phenotype. FDG-PET imaging has also been performed in obese subjects. In this study, FDG uptake was higher in VAT compared with subcutaneous adipose tissue. Based on additional studies performed with stromal vascular cells from diet-induced obese mice, the authors speculated that the increased FDG uptake could be mediated by cells in the stromal vascular fraction of visceral adipose tissue.

PET and other nuclear imaging techniques may also be useful in the future to image blood flow and lymphatic transport in fat depots. The biological effect of an adipokine secreted from a fat depot may vary depending on whether it is transported via the lymphatic system or capillaries. It has been demonstrated, for example, that partitioning of adipokines to lymphatics or capillaries is a function of their molecular size. Lymphatic drainage may also be impaired in adipose depots in obese subjects and this could affect local inflammatory response if there is impaired clearance of inflammatory cytokines such as tumor necrosis factor-α.

**In Vivo Optical Imaging (Animals)**

A simpler method to follow glucose utilization in smaller animal models used Cerenkov luminescence imaging. Cerenkov luminescence imaging uses luminescence generated from the β− and β+ decay of radionuclides such as 18F in the medium. It has been shown to identify brown adipose tissue activity in a mouse model (Figure 7). This method is cheaper and more feasible to use than PET imaging and may be more practical for research laboratories (Table).

**Molecular Imaging (Animals)**

As interest in monitoring other molecules involved in adipose tissue phenotypes evolves, other imaging methods to track molecular activity of fat may be useful (Table). In a recent study, a novel peptide was identified that selectively binds to the vascular endothelium of brown adipose tissue, even in the absence of sympathetic nervous system stimulation.
This peptide probe was used to identify brown adipose tissue depots in mice in conjunction with whole-body near-infrared fluorescence imaging (Figure 8).

What Can Adipose Tissue Imaging Tell Us About Patients and Their Diseases?

The use of imaging adipose tissue will need to be studied in future clinical trials. Whether imaging will add additional prognostic information to relatively simple anthropometric measures, lipids, and serum biomarkers remains to be proven. One population that may benefit from additional adipose phenotyping is patients with type 2 diabetes mellitus. Although diabetes mellitus is considered a coronary disease equivalent, it has become clear that there is heterogeneity in the type 2 diabetic population with regard to cardiovascular risk, with apparent recent lowering of CV risk in the population as a whole. However, the burden of comorbidities remains high and identification of high-risk phenotypes will become increasingly important. There are many different drugs available to treat diabetes mellitus, some of which may be advantageous to a particular subgroup of diabetes mellitus with abundance of inflammatory visceral fat and high vascular risk. Those patients at high vascular risk may be treated differently than patients at low vascular risk. It may also be that specific therapies will be tailored to the particular organ involved. In 1 study of metabolic dysfunction, increased adiposity produced adverse metabolic effects that only correlated with the degree of intrahepatic triglyceride content. Thus, therapies targeting hepatic steatosis may be sufficient to treat metabolic derangements in certain patient subgroups. In addition to mediating metabolic derangements, hepatic steatosis may progress to steatohepatitis and fibrosis, which is a risk factor for cirrhosis and liver cancer. Thus, noninvasive monitoring of hepatic steatosis may facilitate therapeutic interventions to prevent progression to more advanced liver disease. For example, the drug colestevlam was tested for reduction of liver fat, quantified by MRI, in patients with nonalcoholic steatohepatitis. A parallel study compared MRI-estimated proton density fat fraction (MRI-PDF), which allows fat mapping of the entire liver to MR spectroscopy-proton density fat fraction, which provides a biochemical measure of liver fat in small regions of interest. Investigators found that MRI-proton density fat fraction correlated well with MR spectroscopy-proton density fat fraction and was more sensitive than histology-determined steatosis grade in quantifying changes in liver fat content. Thus, hepatic MRI can be used to follow changes in liver fat. As knowledge of the pathophysiology of adipose tissue continues to grow, personalized, targeted therapeutic approaches may be most effective for preventing comorbidities and these approaches will be aided by adipose tissue imaging. These hypotheses will require validation in clinical trials.

Conclusions

Basic and preclinical studies have now clearly demonstrated marked heterogeneity between adipocytes and different fat depots. In parallel efforts, clinical and epidemiological studies have confirmed that different distributions of adiposity lead to different clinical outcomes. Thus, imaging of fat will likely become increasingly relevant for risk stratification and guiding future therapeutic interventions.

Sources of Funding

This work was supported by the National Institutes of Health (HL073150 to D.T. Eitzman).

Disclosures

None.

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Imaging Body Fat: Techniques and Cardiometabolic Implications
H. Wang, Y.E. Chen and Daniel T. Eitzman

Arterioscler Thromb Vasc Biol. 2014;34:2217-2223; originally published online August 21, 2014;
doi: 10.1161/ATVBAHA.114.303036

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2014 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

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