FOCUS PAPER

Body Composition Techniques

Considerations for Early Phase Clinical Research for Obesity, Type 2 Diabetes, and NAFLD/NASH

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Key Focus Points

- Determination of body fat and its distribution is key for the development of clinical interventions for metabolic diseases.
- Increased level of abdominal fat, or visceral adipose tissue (VAT), is associated with a higher risk of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH).
- BMI and other anthropometric methods, such as waist circumference measurement and waist-hip ratio, do not reflect the level and distribution of body fat. Some bio-physical methodologies, such as hydrodensitometry and bioelectrical impedance, have some of the same limitations as BMI to determine body fat distribution.
- Imaging techniques to define body composition are superior to traditional approaches to define body fat distribution. Ultrasound (US), computed tomography (CT), dual-energy X-ray absorptiometry (DXA), magnetic resonance imaging (MRI), are now commonly used in clinical research. Of these, MRI can provide the most accurate and high-resolution measure of body composition.

Introduction

Obesity, defined as a body mass index (BMI) equal or superior to 30 kg/m^2, is a worldwide epidemic. According to the Centers for Disease Control and Prevention (CDC), 42.4% of adults and 19.3% of children and adolescents in the U.S. are obese (2017-2018).[1] Several epidemiologic studies have shown the association between obesity and an increase in all-cause mortality.[2] According to some estimates, high BMI is the second root cause of deaths and disability in the U.S. after tobacco.[3] The impact of obesity on morbidity and mortality can be attributed to the links between excess body fat and an increased risk for many health conditions including type 2 diabetes (T2DM), cardiovascular disease (CV), stroke, arthritis, metabolic syndrome, nonalcoholic fatty liver disease (NAFLD), and nonalcoholic steatohepatitis (NASH).[4-6]

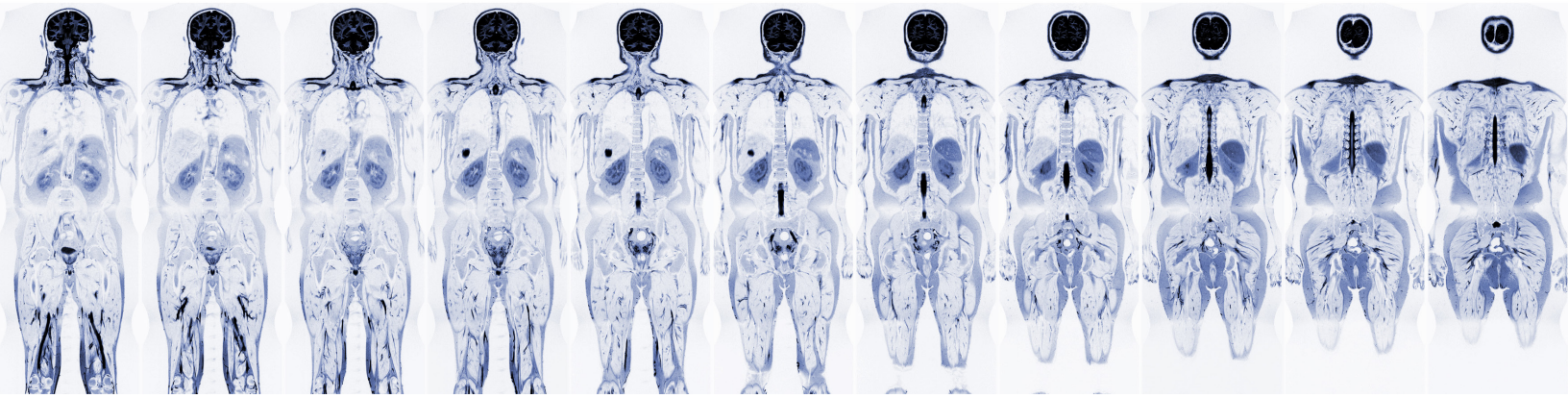
Evidence shows that not all excess fat contributes to disease risk in the same way. Studies have shown that abdominal fat (visceral adipose tissue; VAT) is more dangerous than subcutaneous fat because visceral fat cells release proteins that contribute to inflammation, atherosclerosis, dyslipidemia, and hypertension. Consequently, VAT is more strongly associated with T2DM than other manifestations of obesity.[7-9] Similarly, understanding fat-related diseases and their mechanisms of action requires ever more detailed fat distribution measurements going from the whole body to body sections, to organs and tissues, and ultimately, the cellular level.

The successful development of health interventions aimed at reducing the health impact of obesity requires the use of the right body composition determination technologies. Anthropometric approaches are appropriate for quick screening of subjects, but the development of high-quality clinical data increasingly relies on accurate, safe, and non-invasive imaging techniques.

This article will discuss:

Body Composition (BC) Models

When discussing body composition, it is useful to think of the human body as composed of different "compartments."[10] We will briefly discuss the differences in these models as they introduce concepts useful for the understanding of the different body composition measurement methodologies. We will follow up with a discussion of the most relevant body composition technologies from the clinical trial perspective and highlight their value in the development of disease interventions.



One-compartment (1C) model

In the simplest approach, the body can be considered as one unit. When this model is used, the clinician will draw inferences solely from the person's weight, height, and other anthropometric measures and health risks. The most relevant method in this category is the body mass index (BMI).[11]

Two-compartment (2C) model

In the two-compartment model (2C) the body weight is divided into fat mass (FM) and fat-free mass (FFM). The anhydrous FM is assumed to have a density of 0.9007 g/cm^3, whereas the FFM is assumed to have a density of 1.1000 g/cm^3 and water content of 73.72%. Hydro densitometry (HD), and air displacement plethysmography (ADP) are based on 2C model.[12]

Three-compartment (3C) model

The three-compartment (3C) model of body composition includes a third component where the FFM is divided into lean tissue mass (LTM) and bone mineral content (BMC). In the 3C model, the FFM is divided into total body water (TBW) and the remaining solids (fat-free dry mass; FFDM). The 3C model has shown better results over the 2C model but must be used with caution in patients with depleted body protein or bone mineral mass, as the estimated values for density, and thus, the final estimate of body FM will not be accurate. The dual-energy X-ray absorptiometry (DXA; formerly DEXA) method is based on a 3C BC model.[12]

Four-compartment (4C) model

The 4C model of BC is obtained by combining many methods to partition body mass into fat, mineral, TBM and protein (residual) and thus, removes the need to make assumptions about the relative proportion of these constituents in the body. The 4C model controls for biological variability and it is therefore theoretically more valid than the 3C model. The 4C method is, however, often limited in clinical settings and large studies, in view of the time, cost and equipment needed for the multiple measurements.[12]

Multicompartment models

Atomic models of body composition require the direct analysis of the major elements of the body. Neutron activation analysis (NAA) can be used to measure the total body content of elements (calcium, sodium, chloride, phosphorus, nitrogen, hydrogen, oxygen, and carbon). Although the multicompartment models provide accurate measures of body composition, for validating other methods, the lack of appropriate facilities, the high expense and the exposure to radiation limit their regular use.

Body mass index (BMI) and other anthropometric-based techniques

BMI, the most used metric in this class, is defined as a person's weight in kilograms divided by the square of height in meters.[13] Although BMI is useful as a screening tool it does not diagnose body fatness or the health of an individual. To determine if a specific BMI is a health risk for the individual, the healthcare provider will compare against actuarial tables and perform further assessments. Such assessments include, evaluations of diet, physical activity, and family history, among others. Some of the known limitations of BMI are:

- For a given BMI, women tend to have more body fat than men.
- For a given BMI, Blacks have less body fat than do Whites, and Asians have more body fat than do Whites.[14-16]
- At the same BMI, older people, on average, tend to have more body fat than younger adults.

Hydro densitometry

Hydro densitometry (HD), or under water weighing was considered the gold standard for the determination of body fat before the arrival of body imaging techniques. This technique is based on the principle whereby the volume of a body is equal to the volume of liquid displaced by it. A correction is made for the buoyancy of the air in the lungs and other body spaces. In this manner, body weight (BW) is measured in the air and water to determine body density (Db). Body fat (BF) is determined with either of the following equations:

$$BF = (4.57/Db - 4.142) \times 100$$[17]

$$BF = (4.95/Db - 4.5) \times 100$$[18]

Whole-body air displacement

Whole-body air displacement plethysmography (ADP) uses the same basic principles as HD, but ADP is based on the displacement of air instead of water.[19] ADP is quick, comfortable, and non-invasive. However, ADP's accuracy drops at extreme of body fat composition as determined by DXA.[20]

Bioimpedance (BIA)

Bioimpedance analysis (BIA) is a commonly used, non-invasive, low-cost method to determine body fat content. Bioimpedance or biological impedance refers to the property of biological tissues to impede or resist an alternating electrical current. In BIA, the body is modeled as five cylindrical compartments; the trunk and the four limbs, while fat is an insulator. The impedance is assumed to be proportional to the height and inversely proportional to the cross-sectional area of each compartment. A weak electric current is made to flow between to electrodes, typically on either hand or one hand and one foot. Most body water is stored in muscle. Therefore, if a person is more muscular there is a high chance that the person will also have more body water, which leads to lower impedance. Impedance is then used to estimate total body water (TBW), which can be used to estimate fat-free body mass and, by difference with body weight, body fat.[21]

Although inexpensive, BIA is not as accurate as other methods, especially DXA and MRI.[21] Variations in limb length recent physical activity, nutrition, body temperature and hydration, blood chemistry, ovulation and electrode placement are potential sources of error; BIA cannot be used to determine VAT.[22]

Body composition through imaging techniques

Ultrasound (US)

US techniques use echo reflections to generate a two-dimensional image of a localized area of the body. In these gray-scale images, white indicates a strong ultrasound reflection while black indicates no echo. US techniques have been used to show skin-subcutaneous fat boundaries as well as fat-muscle and muscle-bone interfaces.[23] US methods are considered accurate and reproducible for the analysis of abdominal fat.[24]

Computed tomography (CT)

CT uses computer processing of X-ray data of the body to produce a high-resolution, three-dimensional image. The differences in X-ray attenuation by different body fat and lean tissue are used in CT to calculate differences in composition and location in the body. CT has been used to determine fat in liver and skeletal muscle.[25,26] Although in principle CT could be used to estimate organ and body part volumes, in practice CT is used to analyze two-dimensional slices of the body. This limitation is due in part to the need to minimize exposure of the subject to ionizing radiation (X-rays). This is particularly relevant in clinical trials where healthy volunteers are involved.

Dual-energy X-ray absorptiometry

Use of DXA yields body fat percentage, body composition, and bone mineral density. DXA is based on the use of two low energy X-ray beams. The attenuation of X-rays as they pass through the body is dependent on the thickness of the tissue and the tissue's attenuation coefficient, which dependents on the X-ray energy. Comparing the attenuation for each of the two X-ray energies, DXA provides a detailed image of the body.[27] DXA is the most widely used method to determine bone density where it is considered the gold standard. DXA can also be used to measure total body composition, fat content and distribution.[28] The capabilities of DXA to determine VAT are being improved using improved computer algorithms. On the other hand, limitations on the weight and height of the subject that can be accommodated in the DXA scanner can be a limitation.

DXA is more accurate than body density-based methods for estimating total body fat.[29] A potential source of error is that the DXA analysis assumes a constant hydration of lean soft tissue.

Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI) technologies allow for the precise measurement of body fat and other soft tissues such as muscle using the magnetic properties of chemical elements.[30,31] Quantitative fat water imaging MRI, a commonly used imaging method, has been used to generate precise measurements of lean tissue and body fat. This approach is based on the different magnetic resonance frequencies of protons in fat and water; these differences are used for separating the two signals into a fat image and a water image.

Importantly, a magnetic resonance image on its own is not calibrated to be quantitative. Two MRI techniques that successfully address this limitation are proton density fat fraction (PDFF) measuring the fraction of fat in MR-visible soft tissue and fat-referenced MRI.[32,33]

In the other hand, large subject size, and claustrophobia can be a limitation.

Comparison of Capabilities

The following table summarizes the capabilities of different techniques of body composition analysis.

	ADP	BIA	CT	DXA	MRI	US
Total Fat	Yes	Yes	Yes	Yes	Yes	No
Total Lean Tissue	Yes	Yes	Yes	Yes	Yes	Approx
VAT	No	No	Yes	Approx	Yes	Approx
Muscle Volume	No	No	Yes	No	Yes	No
Diffuse Fat Inflitration	No	No	Yes	No	Yes	Approx
Ionizing Radiation	No	No	Yes	Yes	No	No

Determination of Liver Fat

Diagnosis, management, and development of novel interventions for nonalcoholic liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) depend on the accurate and reproducible determination of liver fat. In this section, we will discuss the application of imaging technologies in the context of NAFLD/NASH.

Ultrasound

Healthy liver tissue is as echogenic as adjacent organs such as the spleen and kidneys. However, when there is an abnormal retention of fat in the liver (steatosis), the organ appears brighter in the ultrasound image due to increased scatter of the ultrasound beam by fat droplets. Similarly, liver fat weakens the ultrasound beam, resulting in blurry imaging of liver structures such as intrahepatic vessels and bile ducts.[34]

Ultrasound can be used to both diagnose and grade the degree of liver steatosis. Liver brightness on the ultrasound image is compared to that of the kidney or spleen which work as internal standards. However, ultrasound techniques are relatively insensitive to the detection of mild steatosis and may not perform adequately if there is another underlying liver disease.

Ultrasound is a safe, widely available, and patient friendly imaging modality. The associated cost of ultrasound is low compared to other imaging modalities. On the other hand, there are some limitations of the technology including (a) overestimation of steatosis in heavy set subjects, and (b) confounding of the ultrasound image by inflammation, fibrosis, and other features of chronic hepatic disease.[35] In addition, the quality of the ultrasound diagnosis is strongly dependent of the operator skills, calibration of the instrument, and manufacturer of the machine. Given these performance characteristics, ultrasound produces qualitative classifications of steatosis that are hard to compare between subjects and clinical sites.

Computed tomography

CT images are created from detection of X-rays traversing tissues. Weakening of the X-ray as it passes through the body is a key parameter used to define the brightness of the tissue in the CT image. In this manner, dense tissues will attenuate the X-ray beam the most and result in a brighter rendition on the image. A healthy liver will appear brighter than the spleen in a CT scan. As fat content in the liver increases its corresponding image will become darker.[36]

CT provides relatively fast data acquisition and quantitative results. On the other hand, similarly to ultrasound, CT is relatively insensitive in cases of mild steatosis. CT liver images can also be confounded by other factors such as concentration of iron, glycogen, and hematocrit.[37] Furthermore, there is a strong dependence on scanner-specific calibration which depends on the instrument manufacturer and the underlying calibration algorithms.[38] CT is not usually recommended as the primary modality to measure liver fat given its lack of sensitivity for mild steatosis and the need for exposure of the subjects to ionizing radiation (X-rays).

Magnetic resonance imaging

Current MRI imaging technologies are considered the gold standard for the measurement of liver steatosis. In contrast with CT and US, MRI can directly measure and distinguish the signal from water versus triglycerides. Current MRI algorithms can quantitate livers fat. This methodology is commonly referred as MRI proton density fat fraction imaging or MRI-PDFF for short.

Data from NAFLD studies using MRI-PDFF show that the findings are highly reproducible across scanners.[39] There is also high correlation between MRI-PDFF liver fat measurements and biochemical determination of triglycerides.[40] Importantly, MRI-PDFF accurately classifies using histology as a gold standard, and the change in PDFF accurately classifies change in steatosis over time.[41]

The power of MRI-PDFF has been used to evaluate potential liver fat-reducing therapeutic candidates. For example, Loomba et al. reported that obeticholic acid (OCA) was better than placebo in reducing liver fat. Data from this multicenter trial showed the association between a 30% decline in MRI-PDFF relative to baseline and histologic response in NASH.[42] In another example, Beysen et al. explored the therapeutic potential of drug candidate FT-4101, a fatty acid synthase (FASN) inhibitor, on hepatic steatosis in patients with NAFLD. The authors used MRI-PDFF to measure the impact of the drug on liver fat.[43]

Conclusions

Identifying a CRO partner with deep expertise in protocol development and advanced methods for body fat and distribution is critical for obesity clinical research, and often a key element in clinical trials for type two diabetes, NAFLD, NASH, and related metabolic diseases. Drug and device development companies with preclinical and early phase assets should consider the following in choosing a CRO partner for the design and conduct of early phase clinical trials:

- Scientific expertise in body composition techniques
- Clinical expertise in using body composition determination modalities
- Clinical expertise in the interpretation of body composition data

Clinical trials are likely to need different body composition techniques at distinct stages of the trial. For example, clinicians will use anthropometric approaches like BMI for rapid, high-volume screening of subjects. On the other hand, clinical evaluation of drug candidates and other health interventions requires accurate, reproducible body composition modalities that provide data on excess fat distribution and its change over time. This is particularly true when data from multiple clinical sites are generated and the need for data consistency is paramount. Therefore, partnering with a clinical organization with deep expertise in the science and execution of body composition determination is crucial for the success of obesity and NAFLD/NASH clinical research.

Partnering with ProSciento

ProSciento is the leading specialized CRO exclusively focused on diabetes, obesity and NASH. ProSciento provides full-service clinical development services for multinational, early development clinical trial programs for biopharma companies worldwide. When partnering with ProSciento, all client interactions are with a ProSciento team of experts who are focused on tailoring services to meet individualized sponsor-specific programs.

Contact us at bd@prosciento.com to discuss your drug or device clinical development program.

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