Advances in Body Composition: Applications for NASH Clinical Trials

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Introduction

As obesity rates have risen worldwide, rates of liver disorders such as non-alcoholic fatty liver disease (NAFLD) have also increased. The spectrum of fatty liver disease ranges from NAFLD to more severe liver diseases including non-alcoholic steatohepatitis (NASH) and liver cirrhosis. In the United States and globally, NAFLD is present in approximately 25% of the population. Of those cases, NASH, a more severe form of NAFLD, is present in nearly 70% (Fingas et al. 2016). Many patients with NASH develop liver cirrhosis or cancer (i.e. hepatic carcinoma (HCC)), both of which have relatively poor clinical outcomes with limited therapeutic options. Some suggest that development of NAFLD and NASH may become the most common underlying risk factor for HCC globally (Fingas et al. 2016, Ekstedt et al. 2017). Furthermore, NASH is second only to Hepatitis C as the most common indicator for liver transplants in the US. Finally, strong evidence exists that NAFLD is also associated with cardiovascular disease mortality, the number one cause of death of people across the globe (Targher et al. 2005, Targher et al. 2010, Younossi et al. 2016).

"MRI and other imaging modalities are rapidly gaining ground as the new norm for NAFLD/NASH diagnosis."

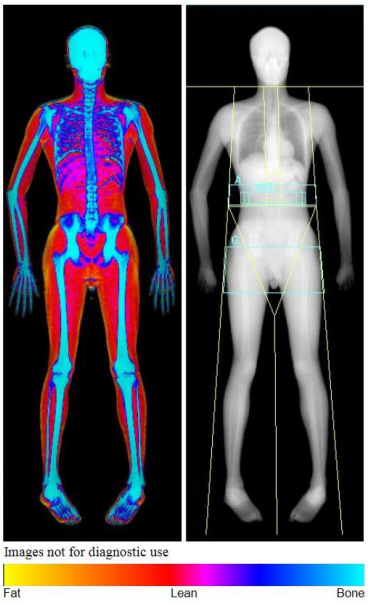
As we currently understand the disease, the main risk factors for development of NAFLD are related to a sedentary lifestyle with excess calorie intake, particularly intake of excess fructose (Softic et al. 2016), although gender, age, and insulin resistance also appear to play a role (Arab et al. 2018). There are currently no approved pharmaceutical agents on the market for the treatment of NAFLD; nevertheless, lifestyle changes such as diet and exercise modification have shown some efficacy and many NAFLD/NASH treatments are currently in development (Arab et al. 2018).

To date, the gold standard for diagnosing NAFLD/NASH has been an invasive percutaneous liver biopsy, this approach, however, presents certain drawbacks. Liver biopsy results can be highly variable depending on the specific location that the sample is drawn since only a very small area of the liver (typically 1:50,000 of the total liver) is acquired. Furthermore, the hepatic biopsy process increases the risk of patient morbidity and mortality. For these reasons, non-invasive means of diagnosis, including assessment via Magnetic Resonance Imaging (MRI) and other imaging modalities, are rapidly gaining ground as the new norm for NAFLD and NASH diagnoses. BioTel Research has experience in all imaging modalities including MRI to measure Proton Density Fat Fraction (PDFF), Magnetic Resonance Elastography (MRE) to determine liver stiffness, and dual energy x-ray absorptiometry (DXA) to assess fat to lean body composition. BioTel Research partner AMRA Medical (AMRA) has introduced a new technology that allows for whole body composition analysis in addition to liver metrics using a rapid 6-minute MRI scan. The exclusive alliance between BioTel Research and AMRA can bring advanced liver imaging and automated body composition assessments to your clinical trials.

Current Imaging Methods for Measuring Liver Health

Dual Energy X-ray Absorptiometry (DXA)

DXA is a well-established and widely-available technique typically used to measure lean body mass, fat mass, and bone mineral density *(Figure 1*). DXA is often used to assess fracture risk due to age or in response to medication. Although inexpensive, safe, and readily available, the information DXA yields is only peripherally related to liver function. DXA cannot currently be used to measure fatty infiltration of the liver, which is typically a primary efficacy measurement in NAFLD/NASH clinical trials. Furthermore, DXA is performed in two-dimensions resulting in inaccurate volumetric estimates and can be highly variable due to the individual placement of anatomic cut-lines by different reviewers analyzing the data.



Body Composition Results

Region	Fat Mass (g)	Lean + BMC (g)	Total Mass (g)	% Fat	%Fat Percentile YN	%Fat Percentile AM
L Arm	525	2657	3182	16.5		23
R Arm	585	2940	3525	16.6		21
Trunk	4166	21961	26127	15.9		19
L Leg	1931	8391	10322	18.7		15
R Leg	2012	8433	10445	19.3		16
Subtotal	9219	44382	53601	17.2		18
Head	1057	3636	4693	22.5		
Total	**10276**	**48018**	**58294**	**17.6**		**20**
Android (A)	589	2722	3310	17.8		
Gynoid (G)	1975	7474	9449	20.9		

Adipose Indices

Measure	Result	Percentile YN	Percentile AM
Total Body % Fat	**17.6**		**20**
Fat Mass/Height² (kg/m²)	3.42		12
Android/Gynoid Ratio	0.85		
% Fat Trunk/% Fat Legs	0.84		47
Trunk/Limb Fat Mass Ratio	0.82		36
Est. VAT Mass (g)	209		
Est. VAT Volume (cm³)	226		
Est. VAT Area (cm²)	43.4		

Figure 1: Example of a whole-body DXA image and associated analysis. The color spectrum indicates the level of fat (yellow) to lean body tissue (red/pink) to bone (blue). Relevant cut lines separating the anatomy are indicated in the right panel. A typical DXA analysis is shown in the associated tables.

Despite these shortcomings, DXA analysis may prove useful for measuring off target effects in NAFLD/NASH clinical trials and thereby mitigate some risks associated with novel drug delivery. Overall, while not an ideal imaging modality for fatty liver assessment, DXA is widely available and central review of DXA images by BioTel Research's expert radiologists improves the accuracy and consistency of body composition data for clinical trials.

Ultrasound

Ultrasound utilizes high frequency sound waves to generate images of internal organs, bones, and soft tissues (*Figure 2).* Ultrasound is commonly used in the clinical setting to assess tendon injuries or fetal health during pregnancy, for example. Ultrasound does not require ionizing radiation, nor does it require oral or injectable contrast, making ultrasound a very safe and readily available imaging method. Although ultrasound has excellent sensitivity and specificity for detection of severe steatosis, it is less useful for detection of lower amounts of fatty infiltration (Ekstedt et al. 2017) and is highly influenced by the sonographers skill set; a slight turn of the wrist or adjustment to the ultrasound probe makes a sizable difference in the image that is collected. Furthermore, the image quality of ultrasound is inferior to that of other imaging techniques such as MRI that are used to assess liver health. In addition to the low resolvability of the images, the dependence upon the individual sonographer's skill set makes ultrasound a less desirable modality for clinical trials that require data to be collected from many different sites, particularly in a global setting.

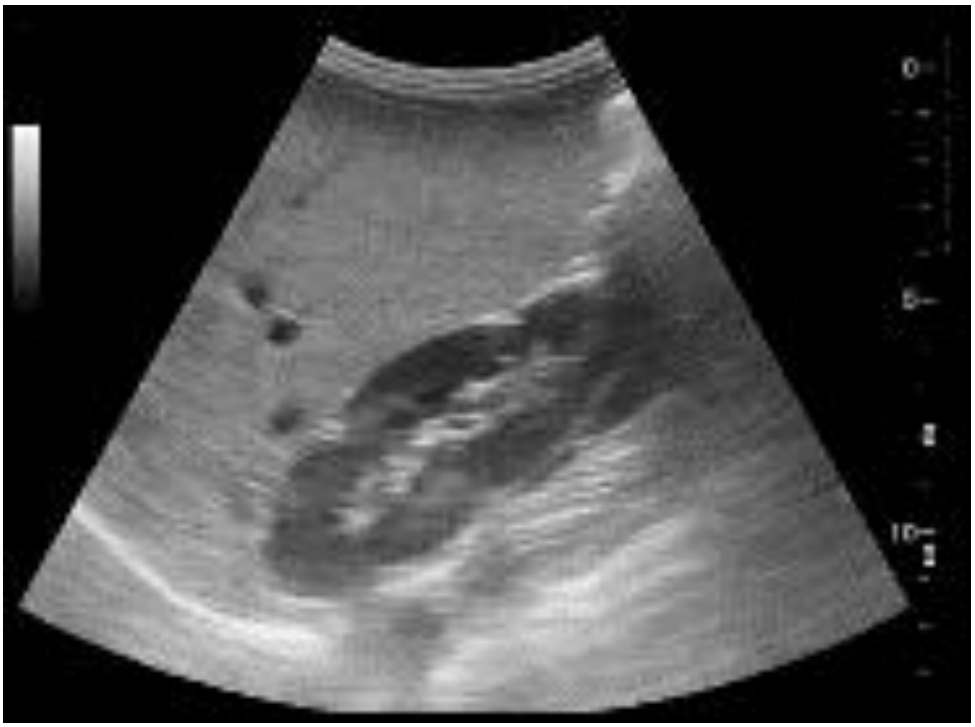


Figure 2: Example of an abdominal ultrasound showing the liver.
Photo: https://openi.nlm.nih.gov/index.php (License: CC BY 2.0; image cropped)

Computed Tomography (CT)

CT allows for the creation of detailed images of internal organs, bones, soft tissues, and blood vessels. It is readily available, reasonably affordable, fast, accurate, and less sensitive to motion artifact than MRI techniques. Although CT can yield hepatic fat fraction (HFF), which is typically the primary efficacy measurement in NAFLD/ NASH clinical trials (*Figure 3)*, it is typically not as accurate as proton density fat fraction (PDFF) measurements generated from MRI, particularly for detection of lower fat fraction amounts (Kramer et al. 2017). The main drawback to the use of CT for NAFLD/NASH clinical trials, however, is the large dose of ionizing radiation that patients receive during the scanning, making it impractical for trials enrolling healthy participants. If a patient is receiving CT for another reason such as oncology assessments, already acquired CT images may be used for the assessment of fatty infiltration of the liver; but overall, CT is rarely used for NAFLD/NASH assessments.

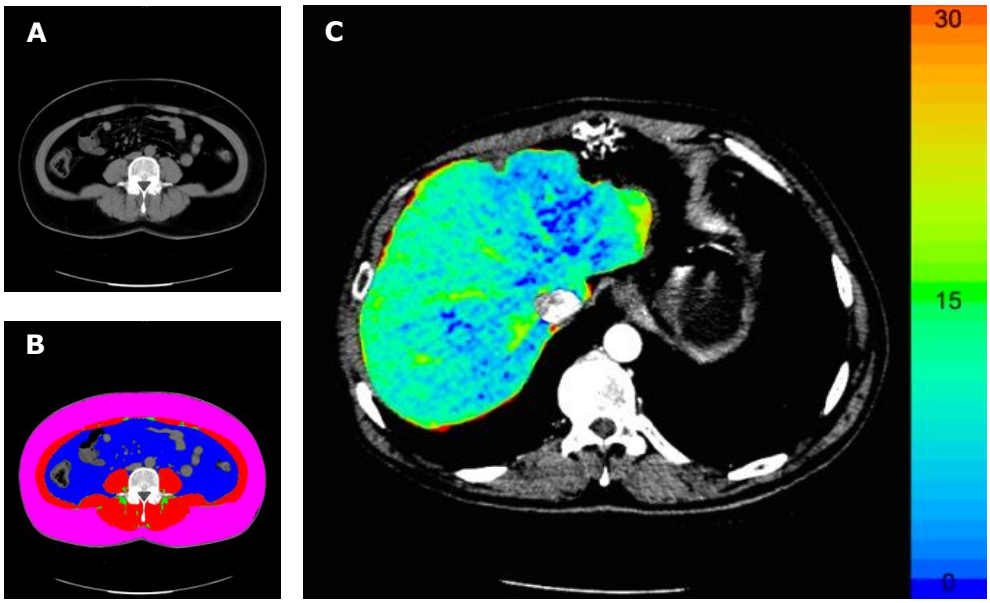


Figure 3: ***A)*** *Contrast enhanced abdominal CT. Spine/vertebral body is shown in white. Darker areas in this image are indicative of fat and muscle.* ***B)*** *The same image as in panel A, but segmented and pseudocolored to demonstrate areas of subcutaneous fat (pink), visceral adipose tissue (VAT-blue), muscle (red), and intra/inter muscular fat (green).* ***C)*** *A hepatic fat fraction map calculated from a CT image of the abdomen. Liver is shown to the left with a heat map indicative of the percent of fat on the right. Hepatic fat ranges in this image from close to zero (blue) to a high of 30% (orange).*

Magnetic Resonance Imaging (MRI)

MRI utilizes a powerful magnetic field and radio frequency pulses to align hydrogen atoms within the body to produce detailed images of soft tissues. MRI is a very versatile technique often used to measure lesions for oncology, organs of the chest, abdomen, and pelvis, as well as lymph nodes, blood vessels, liver, and brain. MRI has many advantages over other imaging techniques that may be utilized for assessing liver health. For instance, MRI produces high quality images that allow for the differentiation of abnormal tissue from normal tissue without any radiation exposure to the patient. Furthermore, true three-dimensional measurements are possible with MRI that are not feasible with many other imaging modalities. While it is readily available and advantageous for many reasons, MRI can be challenging for patients with claustrophobia or who are unable to fit inside the machine.

In addition to tumor evaluation or organ size measurements, there is increasing availability of new MRI techniques relevant to liver studies including determination of PDFF and MRE on traditional MRI scanners (*Figure 4)*. These methods allow for non-invasive quantification of liver steatosis and fibrosis which are often primary efficacy measurements in NAFLD/NASH clinical trials. Although adoption of MRE technology is ever-increasing, for clinical trials it can be challenging to find enough sites that offer MRE imaging to accommodate the target population. Despite these challenges, the advantages of MRI over other available imaging modalities make it the gold standard for imaging in NAFLD/NASH clinical trials.

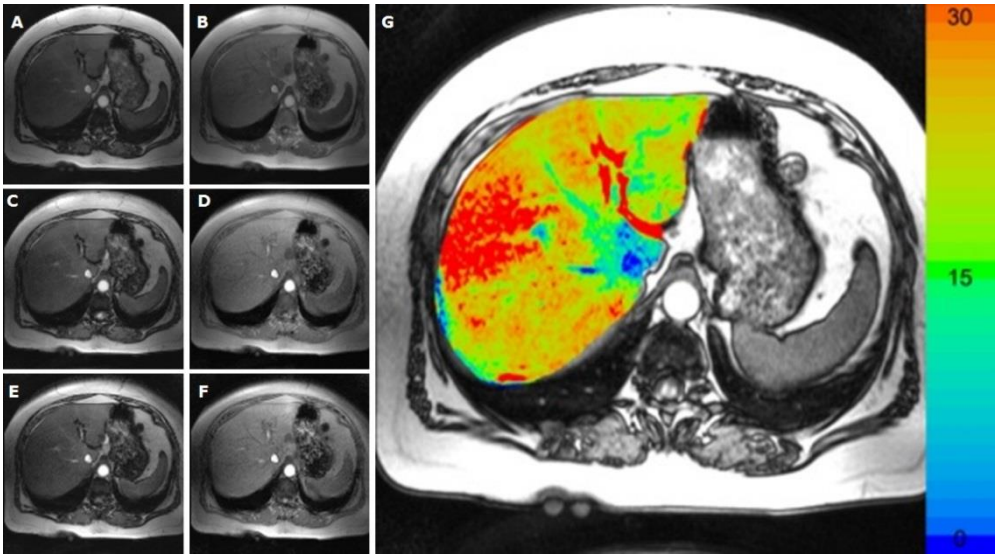


Figure 4: Liver MRI example to calculate PDFF. ***A-F)*** *Six images showing out-of-phase and in-phase MRIs of the liver which are utilized to calculate PDFF.* ***G)*** *Color coded PDFF of liver demonstrates differing regions of fat fraction. Heat map indicates percent fat shown in color-coded map on far right.*

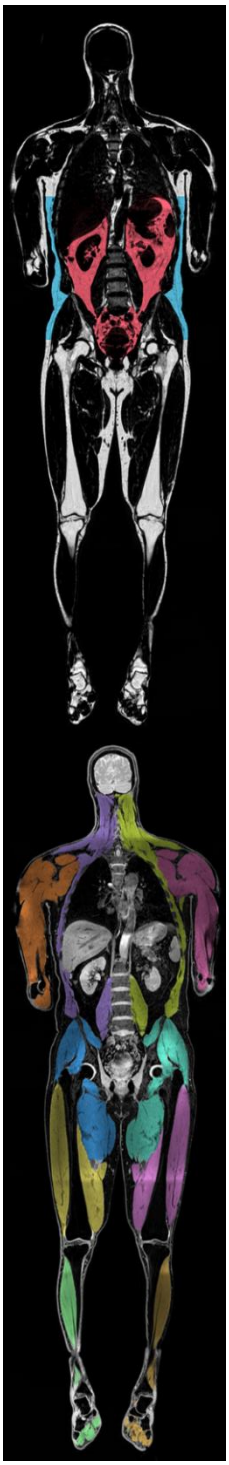


Figure 5: AMRA whole body profiling. Abdominal (visceral and subcutaneous abdominal fat) are shown in the upper panel and different muscle groups are color coded in the lower panel.

NAFLD disease progression is characterized by increased fat content in the liver followed by enhanced fibrosis as the liver becomes more cirrhotic. Fibrosis has been shown to be well correlated with non-invasive MRE imaging assessments.

While MRI measurement of liver fat or MRE measurement of stiffness provide valuable information in NAFLD/NASH clinical trials, MRI can also provide valuable insights regarding adipose (fat) and muscle tissue beyond the liver. In the past, adipose and muscle tissue volumes were typically assessed on MRI through manual segmentation, a time-consuming method that increased the chance of variability. For this reason, whole-body composition analyses have not been routinely performed in the clinical setting or in clinical trials. In response to this need, AMRA has developed AMRA® Profiler Research that addresses the need for a reliable, fast, automated method to assess whole-body composition.

AMRA's Body Composition Profiling

Utilizing a rapid, highly standardized Dixon MRI sequence to acquire water and fat images of the entire body in just 6 minutes, AMRA's analysis platform is unquestionably leading the field towards a more comprehensive understanding of body composition *(Figure 5)*. In addition to PDFF, AMRA's unique algorithms measure several biomarkers that are important when assessing liver health including: intramuscular fat (IMAT) or muscle fat infiltration, abdominal subcutaneous adipose tissue (ASAT), visceral adipose tissue (VAT), total adipose tissue volume (TAT), and individual muscle group volume assessments- in particular thigh muscle volume and total lean tissue volume (TLT). Overall, understanding body composition may help predict an individual's predisposition towards particular diseases and yield more comprehensive, patient-centric treatment.

Not surprisingly, obesity is a primary risk factor in the development of NASH, and high VAT volume has been shown to correlate with development and progression of the disease (Yu et al. 2015). Historically, obesity has been assessed in many ways including circumferential waist and traditional body mass index (BMI) measurements. BMI, which utilizes both height and weight in its calculation, has long been considered the primary criterion in obesity determination. However, in more recent years, studies have shown that across subjects with the same BMI, VAT measurements (as well as other body composition indices) often differ significantly. For example, in 6 male subjects with identical BMI scores, the VAT assessed by AMRA from MRI varied by nearly *ten-fold*, from 0.7 liters to 6.8 liters *(Figure 6)*. Anthropometric measurements, such as BMI, waist circumference, or body weight, can only categorize individuals by similar body composition. Moreover, the use of broad and discrete qualitative categories like *obese*, or simply *high* or *low liver fat* to

Figure 6: Six male subjects with identical BMI of 21, but with vastly different VAT measurements and therefore, different metabolic risks.

describe the individual should be questioned. The difference in VAT measurements in patients with the same BMI underscores the importance of developing a more holistic approach to body composition analyses. AMRA's multivariable description of body composition more accurately characterizes the individual patient. This may allow for a better understanding of pre-disposition for particular diseases.

An understanding of body composition is of particular relevance to NAFLD/NASH clinical trials, where this information can aid pharmaceutical companies to establish more precise, multivariate inclusion and/or exclusion criteria. AMRA's analysis platform allows for quick and accurate determination of liver fat levels which is a particularly relevant inclusion criterion for NAFLD/NASH trials. While inclusion criteria for NAFLD/NASH clinical trials often require invasive liver biopsy, it is conceivable that future trials will employ more non-invasive imaging assessments, such as MRI instead. MRI inclusion would allow stratification of trial participants by body composition biomarkers, such as visceral or intramuscular fat levels. Stratification by body composition parameters could serve either to decrease the variability of patients enrolled or to inflate the range of subjects in order to more accurately model a drug's effect on a typical population. Inclusion/exclusion criteria that consider whole body factors could prove key to a clinical trial's success.

Potential NAFLD/NASH therapeutics likely have effects that span across the body and may be difficult to measure using traditional assessments. While a promising therapy may decrease liver fat, AMRA's precise algorithms can help detect both beneficial and adverse unexpected drug-related effects within the whole body. Examples of responses to drug treatment might include favorable outcomes such as decreases in subcutaneous fat, or adverse effects such as increases in visceral fat or decreases in muscle mass (sarcopenia). More subtle changes associated with sarcopenia, such as an increase in intramuscular fat at the expense of (i.e. decrease in) lean muscle mass, can also be derived using AMRA's unique platform. Early detection of unintended changes in adipose and muscle tissue may aid in a more accurate risk assessment, particularly during early drug development.

AMRA's whole body composition analysis allows for acquisition of multiple pieces of information which are tied into a single assessment profiler that together may aid in the understanding of a drug's efficacy, as well as disease processes. Increased risk of type II diabetes, as well as coronary heart disease, are often associated with development of NAFLD and NASH (Targher et al. 2005, Targher et al. 2010, Ekstedt et al. 2017). In fact, coronary heart disease is one of the leading causes of mortality in NASH patients (Targher et al. 2005, Targher et al. 2010). Furthermore, the development of sarcopenia can also predict whether patients are likely to progress from NASH on to liver cirrhosis (Bhanji et al. 2017, Praktiknjo et al. 2017). Most adipose tissue compartments in the body are

correlated with general adiposity, which in turn is associated with increased disease risks (Jensen et al. 2014), leading many of them to be separately linked to disease progression. But, more importantly, it has been shown that disease risks tend to be related to specific patterns of, or imbalances in, fat accumulation (Therkelsen et al. 2013, Neeland et al. 2015, Lee et al. 2016). This stresses the need to measure, and simultaneously investigate, several adipose tissue compartments to understand diseases previously linked to any kind of adiposity. A more complete description of a patient's fat distributions may allow for a more accurate prediction of a patient's propensity to develop co-morbidities during a trial, which may lead to better patient outcomes and generation of more effective therapeutics. Investigators no longer need to rely on liver fat alone as an indicator of NAFLD disease progression or treatment efficacy, as AMRA's simultaneous calculation of numerous variables yields multiple body composition measurements from a single MRI scan. These multiple variables quantified using AMRA® Profiler Research can be combined in a star-shaped diagram *(Figure 7)*, which highlights the individual's fat distribution and yields an indication of the individual's propensity for development of specific diseases.

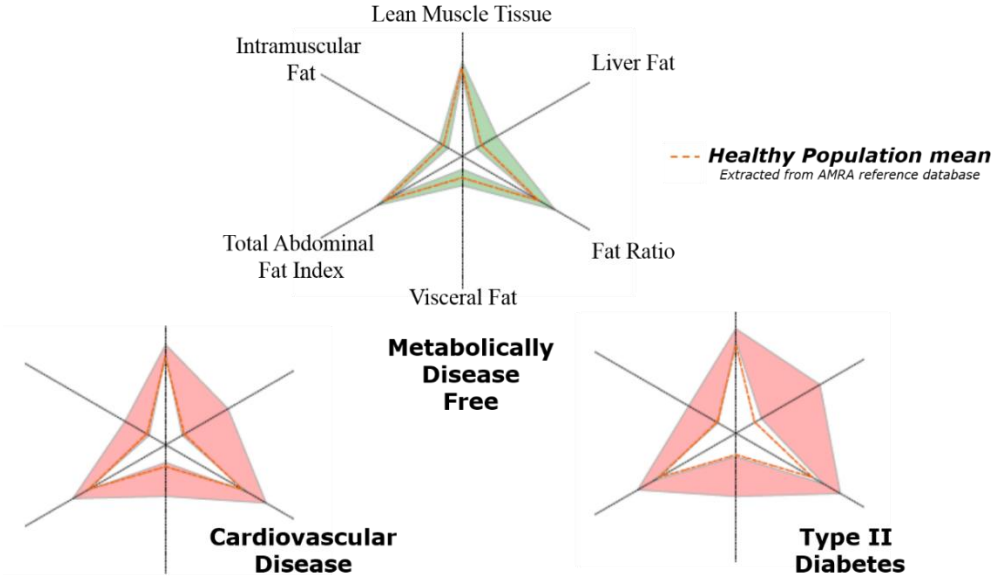


Figure 7: AMRA's Body Composition Profile (BCP) combines multiple assessments into an easy to understand, star-shaped diagram, which demonstrates an individual's propensity for development of particular disease. Above, the figure in green shows metabolically disease free individuals with the 25th to the 75th percentiles for each variable of the assessment labeled on the individual axes. The two pink figures show corresponding ranges for individuals with cardiovascular disease or Type II diabetes from those enrolled in UK Biobank imaging cohort.

The traditional gold standard for HFF measurements has been the invasive liver biopsy. However, in more recent years, development of non-invasive imaging techniques has led to the adoption of MRI as a highly-valued tool for HFF

derivation. Other imaging techniques that might be utilized for NAFLD and NASH clinical trials include DXA, ultrasound, CT, and MRE analysis. Of these tools, the most popular for evaluation of liver fat is MRI. Development of an automated, algorithmic based MRI analysis provides additional body composition information relating to adipose and muscle tissue volumes generating added value to your clinical trial results.

Although calculation of HFF from MRI is still considered a primary efficacy measure for many NAFLD or NASH clinical trials, adoption of a more holistic, whole body approach is at the forefront of research into this complex disease enabling identification of NAFLD sub-phenotypes. A more in-depth understanding of combined body composition metrics, such as accumulation of VAT or changes in lean muscle mass and IMAT, may be used to predict associated NAFLD risks such as type II diabetes, coronary heart disease, or sarcopenia.

"The partnership between AMRA and BioTel Research is a win-win for NAFLD/NASH clinical trials..."

Conclusions

Your clinical trials require the expertise that the BioTel Research/AMRA partnership brings to the table. The partnership between AMRA and BioTel Research is a win-win for NAFLD/NASH clinical trials by combining the technical prowess, advanced imaging, and automation capabilities of an international digital health company with the strengths of an established, reputable imaging core lab that has superior site and project management teams.

As a leader in hepatic fat imaging and quantitation, BioTel Research will strengthen your trial with its unparalleled Phase I to Phase IV imaging solutions, leveraging a flexible operations team that specializes in risk identification and mitigation across therapeutic areas. By choosing BioTel Research/AMRA, you ensure a seamless continuum from image acquisition to analysis in all liver imaging modalities. Work with us to discover the right imaging modality, the right imaging acquisition protocol, and the right analysis method for your next NAFLD/NASH study. ■

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