Most Asked Questions

Q. How easy is it to use the device?

A. The EchoMRI[™] system is exceedingly easy to operate and maintain by customers without prior experience. In fact, a training period of one hour is sufficient for individuals without prior knowledge. Daily system tests are automatic. Measurement results are stored in a separate database for each user. The device is a stand-alone unit, uses standard power sources, and is completely shielded and safe to run.

Q. How does the EchoMRI[™] system work to measure fat, lean, and free water?

A. In general, NMR instruments create contrast between soft tissues by taking advantage of the differences in relaxation times of the hydrogen spins and/or hydrogen density in these tissues. Radio frequency signals are generated by hydrogen nuclei due to precession of the spin axes. The amplitude, duration, and spatial distribution of these radio frequency signals are related to properties of the material investigated by the NMR technique. Tissue contrast is high between fat, body free fluid, and muscle based on NMR signal amplitude and relaxation time, and can be further enhanced by application of certain radio frequency sequences.

Q. Does Echo Medical Systems offer warranty on its products?

A. Our installation and training package includes a complete 1 year warranty. This warranty includes parts, labor and travel expenses. Of course, this service includes all software upgrades and all new application that we are developing. Additionally, we try to visit a customer once or twice during the warranty period for a checkup. Extended warranties are also available. For more information on these, please call our office at (281) 492-0082.

Q. What size of animals can be scanned?

A. Body masses between 20g and 130g are recommended for EchoMRI-100[™] and EchoMRI-130[™] systems for mice. For body mass above 130g, EchoMRI-500[™], EchoMRI-700[™], or EchoMRI-900[™] systems for rats are recommended. At this time, we have three versions of the machine for rats: for masses up to 500g, for up to 700g, and for up to 900g. We also have EchoMRI-3in1[™] for body masses between 15g and 100g, tissue sample masses between 0.2g and 10g, and biopsy masses between 10mg and 500mg. Finally, for body masses between 60Kg and 250Kg there is EchoMRI-AH[™] for adult humans.

Requests for custom-built sizes and specifications are welcome. Please call Echo Medical Systems Sales Department at 281-492-0082, extension 5#.

Q. What are the features and benefits of EchoMRITM systems?

A. The key benefit of the EchoMRI[™] systems to those involved in research is the precision and accuracy they offer when compared to those of DEXA. When Nuclear Magnetic Resonance is applied toward the analysis of body composition during clinical trials, it has an effect on the

accuracy, precision, sensitivity, and specificity of results. It is far superior in its ability to offer precise and accurate results for researchers and clinicians. To learn more, please click <u>here</u>.

EchoMRI System

Q. What are the physical dimensions of EchoMRI[™] equipment?

A. The major components of the EchoMRITM system are one analog electronics enclosure, including power supply; one digital electronics enclosure; a flat LCD display; sensor assembly, including antenna and matching circuitry; two service drawers; and a double-shielded permanent magnet. All system components are enclosed in a rack of the following physical dimensions:

Rat/ Mouse System	3-in-1 System
Height: 58"/ 147cm	Height: 52"/ 132cm
Width: 24"/ 61cm	Width: 24"/ 61cm
Depth: 28"/ 71cm	Depth: 28"/ 71cm
Weight: 6411bs/ 291kg, including shipping	Weight: 641lbs/ 291kg, including shipping
boxes	boxes

4-in-1 System

Height: 52"/ 132cm Width: 48"/ 122cm Depth: 28"/ 71cm Weight: 1100lbs/ 500kg, including shipping boxes

Adult Human System

Rack	Magnet
Height: 58"/ 147cm	Height: 60"/ 152.5cm
Width: 24"/ 61cm	Width: 57"/ 145cm
Depth (including bed): 28"/ 71cm	Depth: 144"/ 366cm
Weight (including bed): 300lbs/ 136kg	4500lbs/ 2000kg, not including shipping
	crates

Q. What power supply is needed (volts, ampere)?

A. One outlet is required (110/220VAC, 50/60Hz). The maximum power consumption of the EchoMRI[™] system is 450 Watts, the equivalent of 1536 BTUs. The device is compatible with international standards required in Europe and Asia.

Q. Does the machine generate heat? If so, how much?

A. The device has five core components: a magnet, sensor assembly, analog electronics, digital electronics, and an LCD monitor. The Rat/Mouse magnets are permanent, not electromagnets, and, therefore, generate no heat. The Adult Human magnet is an electromagnet but generates a relatively small amount of heat, equal to a computer workstation. The operation of the antenna assembly generates a negligible amount of heat. The analog electronics box generates a small amount of heat so that the box does not even need a fan. The most heat generated by the instrument comes from the digital electronics, which has a standard computer-type fan that exhausts about as much heat as a typical personal computer or a typical light bulb. In a normal laboratory environment, no special air conditioning or environmental settings are required.

Q. What are the safety issues with EchoMRI[™]? What is the magnetic field in the vicinity of the EchoMRI[™] system? Is any special shielding required?

A. No special shielding is required. For the Rat/ Mouse/ 3-in-1 systems, the magnetic field induction is less than 5 X 10-4T, excluding volume that is less than 9" (0.2m) from the gantry. The Adult Human system has a 5-gauss field that extends approximately 65"/ 26cm from the magnet covers.

Q. Can we move the EchoMRITM equipment to another room or floor?

A. Yes, you can. However, we recommend that you perform our standard quality assurance postinstallation tests and send us your data so that we may review the results and confirm that your system is operating well. Please contact Echo Medical Systems for more information on postinstallation testing procedures.

Q. May I lease or rent the EchoMRITM system for animals?

A. Please contact Echo Medical Systems for specific information.

Q. What are the features and benefits of EchoMRI[™] systems?

A. The key benefit of the EchoMRI[™] systems to those involved in research is the precision and accuracy they offer when compared to those of DEXA. When Nuclear Magnetic Resonance is applied toward the analysis of body composition during clinical trials, it has an effect on the accuracy, precision, sensitivity, and specificity of results. It is far superior in its ability to offer precise and accurate results for researchers and clinicians. To learn more, please click <u>here</u>.

Q. When will you have a system for humans?

A. Several EchoMRI[™] systems for adult humans are operational. Click <u>here</u> to find more information about EchoMRI-AH[™] system for adult humans and to learn about the latest studies involving EchoMRI-AH[™]. Please contact us if you have an interest in the human system at 281-492-0082 extension 5#.

Measurement Procedure

Q. How does the EchoMRITM system work to measure fat, lean, and free water?

A. In general, NMR instruments create contrast between soft tissues by taking advantage of the differences in relaxation times of the hydrogen spins and/or hydrogen density in these tissues. Radio frequency signals are generated by hydrogen nuclei due to precession of the spin axes. The amplitude, duration, and spatial distribution of these radio frequency signals are related to properties of the material investigated by the NMR technique. Tissue contrast is high between fat, body free fluid, and muscle based on NMR signal amplitude and relaxation times, and can be further enhanced by application of certain radio frequency sequences.

Q. How is an animal scanned?

A. To be measured, an animal is placed in a specially sized, clear plastic holder without sedation or anesthesia. The holder is then inserted into a tubular space in the side of the EchoMRI[™] system. The researcher then presses a button on the LCD screen to scan the animal. The fat, lean mass, body fluids, and total body water are measured in grams.

Q. What is the lean mass as measured by the EchoMRI[™] system?

A. Lean is presented as equivalent of muscle mass. However, this mass includes muscle and organs.

Q. What organs are considered to be lean mass?

A. All organs are eventually presented as lean mass.

Q. What are Free Water and Total Water?

A. Free Water can be found in the stomach and bladder. In experiments, for a limited time, water can be detected after it is injected into abdominal cavity of an animal, until it gets absorbed. Total Water is found from the difference between the total hydrogen in a body and the estimated hydrogen in fat. For normal animals, the hydrogen of lean, estimated as (Total Water - Free Water) / lean is typically within the range 80%±10%.

Q. Why is canola oil used to conduct a system test? Can other fat be used as a test sample?

A. From our experience with testing many animals (including chemical analysis), we found that, on average, the amount of fat in mice and rats can be well represented by the amount of canola oil. EchoMRI measures the relaxation time of hydrogen in molecules of fat within the subject, and on average, canola oil has the same density of hydrogen as animal fat.

Q. Can the animals move in the holders?

A. Animals are able to move in the holders since they are without anesthesia or sedation. It is best, however, to use the smallest volume holders that can hold the animal to restrict its movement, and so reduce the measurement errors caused by motion.

Q. Why does the total body weight differ from the sum of the weight of its components?

A. Our systems do not currently measure and quantify things such as bone, solid intestinal content, and hair. Fat, lean, and free water are directly measured and quantified; everything else is not.

Q. What is the difference between measuring body composition by EchoMRI[™] in a mouse and in a human (or in substantially different sizes of animals)?

A. Due to the vast size differential between the two test subjects, the two systems will obviously operate using different hardware parameters for the system. However, the methodology is exactly the same for our systems, regardless of subject size.

Measurement Objects

Q. What kind of fat is measured?

A. The EchoMRI[™] system measures total body fat, including every fat molecule in the body, such as fat in the brain, muscles, organs, bone marrow, intestines, blood lipids, and fatty acids.

Q. Can EchoMRI[™] measure visceral and subcutaneous fat separately?

A. Our standard EchoMRI[™] System does not distinguish between different types of fat and fat depositions at different sites in a body; instead, it measures total body fat. Some of our customers have successfully excised fat pads and immediately measured them using our device. Please call Echo Medical Systems for more details at 281-492-0082 extension 5# for more information.

Q. Can I measure fat in the liver? Can I measure fat in body tissues?

A. Yes, you can. In 2005, we have launched the EchoMRI-3-in-1[™] system that measures fat in live animals, ex-vivo tissue samples including liver, and biopsies. Please call for availability at 281-492-0082, extension 5#.

With the current EchoMRI-100TM system for mice weighing less than 100 grams, it is possible to measure fat in the liver or body tissues. In practice, please have in mind the following two facts pertinent to measurements done on animal body parts:

- 1. Measurement errors do not decrease with the mass of the sample proportionally to the mass, so relative errors are larger for smaller samples.
- NMR relaxation properties of substances vary with temperature, particularly for fat. The EchoMRI[™] system has technological features that reduce temperature dependence in the 30°-40°C range, and the errors in fact remain small down to about 25°C. However, at temperatures well below 18°C these errors become quite noticeable.

In the whole body EchoMRI[™] systems, all fat will be measured in the body, including the liver and other organs and muscles. Some customers measure fat in the liver and other organs immediately

after excision. In excised organs the system is sensitive to temperature and other changes in dead animals. Therefore, measuring closer to the time of excision is better.

Q. What size of animals can be scanned?

A. Body masses between 20g and 130g are recommended for EchoMRI-100[™] and EchoMRI-130[™] systems for mice. For body mass above 130g, EchoMRI-500[™], EchoMRI-700[™], or EchoMRI-900[™] systems for rats are recommended. At this time, we have three versions of the machine for rats: for masses up to 500g, for up to 700g, and for up to 900g. We also have EchoMRI-3in1[™] for body masses between 15g and 100g, tissue sample masses between 0.2g and 10g, and biopsy masses between 10mg and 500mg. Finally, for body masses between 60Kg and 250Kg there is EchoMRI-AH[™] for adult humans.

Requests for custom-built sizes and specifications are welcome. Please call Echo Medical Systems Sales Department at 281-492-0082, extension 5#.

Q. Can I scan monkeys, piglets, cats, or dogs?

A. Yes, you can. Call Echo Medical Systems Sales Department at 281-492-0082, extension 5# for pricing and information regarding custom-built systems for different animals.

Q. Can I scan a dead animal?

A. Yes, if the animal is scanned soon after death (within several minutes of excision) and is still warm enough, or alternatively is warmed up to temperature above 30°C. It is important under all conditions that the tissues have not yet decayed significantly nor lost too much of the cellular water.

Q. Can I use a single EchoMRITM system to scan both mice and rats?

A. We custom build a device that measures both mice and rats. Please call Echo Medical Systems Sales Department at 281-492-0082, extension 5# for more information regarding custom-built systems.

Q. What size of samples can be scanned in EchoMRI 3-in-1[™] systems?

A. EchoMRI 3-in-1[™] systems allow measuring live mice weighing 15 to 100 grams and enable researchers to perform tissue characterization in samples having weight from 0.2 to 10 grams as well as biopsies with weight between 10mg and 500mg.

Q. Can the EchoMRITM systems measure bone mineral mass?

A. At this time, we do not recommend using our systems for bone analysis. However, we are willing to consult and/or recommend certain CT or DEXA methods for this purpose.

Q. Can the EchoMRI[™] systems measure cartilage mass or composition of joints?

A. At this time, we do not recommend using our system for cartilage mass or joint composition analysis. However, we are willing to consult and/or recommend certain CT methods for this purpose.

Q. Can the EchoMRITM system measure composition of avian eggs?

A. Yes. In fact, we currently do measurements to identify muscle (lean) mass in embryos in order to monitor development.

Q. Can the EchoMRI[™] system measure hydration of animals and tissues?

A. Yes, in many cases we can do this, but please contact us for further details.

Q. Can the EchoMRI[™] systems measure temperature of animals?

A. Yes, in many cases we can do this, but please contact us for further details.

Q. Can I use tags or microchips on animals with EchoMRITM?

A. Yes, this is not a problem. In fact, some customers are already using our equipment with animals containing metal tags and microchips (note that plastic tags will have absolutely no affect on the data produced). It is important to remember that there are two types of metal: one type is magnetic (i.e. steel or iron), and the other type is not magnetic (i.e. aluminum or copper). If the tagging device is metallic, but not magnetic, then there is no dramatic effect on the data or the subject whatsoever. However, if the device is indeed magnetic, there are two potential problems: 1) the strong magnetic attraction could potentially damage the subject, and 2) the magnetic field will be substantially disturbed, causing the data results to be potentially erroneous. In such cases, it is really a judgment call and the effect of the tagging device on the overall data produced will be dependent on the ize of the metallic object.

Precision and Accuracy of Measurements

Q. How do we know that our system is measuring accurately?

A. In order to validate the system performance, a simple experiment can be performed. Take muscle from a rat or a lean chicken breast, measure it, and then combine it with a known amount of canola oil warmed to temperature between 30°C and 40°C, and measure again. Repeat both measurements about 10 times to gather appropriate statistics. The difference should reproduce the weight of the lean and canola oil.

Q. How do we know that our system is measuring precisely?

A. Our determination of precision is based on repeatability. For example, one can measure 1 animal many times, expressing the occurrence of error in terms of Standard Deviation; alternatively, one can measure many animals once and express the occurrence of error in terms of the Standard Error of the Mean. For our instruments, we take one animal and do repeated measurements under the assumption that animal's properties will not change over a short period of time. We believe that this is the best means for defining precision.

Q. Why is the precision different for different EchoMRI[™] systems?

A. The pricing and precision for EchoMRI[™] technology is related to the magnet and antenna sizes that are based on the weight ranges of your animal subjects. Before you order any equipment, we will consult you regarding your required weight ranges, and recommend appropriate solutions.

The key advantage of optimizing your system for different weight ranges is to maintain a higher relative precision. The narrower the weight range is - the higher the relative precision. For example, the precision of measured fat for a 1200 grams rat will be about 3 grams with the system for rats between 500 to 1200 grams. However, if you were to use the same system for a 100 grams rat, the relative measured precision of 3 grams for fat determination would not be acceptable.

In order to serve you better, Echo Medical Systems provides several standard configurations of equipment for mice and rats based on weight ranges 20-100, 100-700, 250-900, and 500-1200 grams. We also offer custom weight ranges as an option to our customers, and we encourage you to discuss your requirements with us.

Q. What is the difference between standard precision, high precision, and extra high precision modes?

A. The major source of imprecision in NMR methodology is due to subject movement. However, in cases where random error plays a significant role in the analysis, it can be reduced by increasing the number of scans conducted on the subject and then averaging all of the data points, thus reducing the effect of the random error. The main source of random error in NMR systems comes from antenna noise.

Q. What are the possible sources of error for the data measured by the EchoMRI[™] system?

A. These include subject movement, random noise (from the antenna which can be mitigated by multiple subject scans), and environmental noise (which we shield for).

Q. How many measurements will it take to achieve the desired precision for my experiment?

A. It depends. The more you measure a single sample (repeatability), the more you will reduce random noise. However, there is no reason to measure to a precision of more than 0.1 grams for mice weighing under 100g. Based on the size of the animals, the systems are pre-calibrated to give the researcher the most precise measurement within a reasonable sample size. The systems can be programmed and adjusted based on a researcher's precision needs, and the process can be optimized (size of holder, time of management, etc.).

Comparisons of EchoMRITM with Other Technologies

Q. What is the difference between measurements made by EchoMRI[™] and DEXA? What is the difference between fat and lean measured by EchoMRI[™] and fat and non-fat measured by DEXA?

A. A major difference between DEXA (Dual Energy X-rays Absorptiometry) and NMR (Nuclear Magnetic Resonance) methods is that NMR receives un-obscured signals from all elements of a three-dimensional body, whereas DEXA calculates the amounts of fat and non-fat from a two-dimensional projection of a body, in which the bones obscure about 40% of the projected body area. Therefore, DEXA actually makes an educated guess on composition of the obscured parts of a body. This is the reason why results of DEXA measurements can vary significantly with changes in body posture.

Another important difference between DEXA and NMR is that, in addition to fat, NMR can also directly evaluate the amounts of lean tissue and free liquids, whereas DEXA distinguishes between just fat and non-fat.

The third important difference for measurements on live animals is that DEXA requires the animals to be sedated and rendered immobile, whereas NMR works well for fully alert animals whose motion needs only be restricted by mechanical means limiting their freedom of motion to some volume.

To see how EchoMRITM compares to DEXA in more detail, please take a look at the following link: <u>5 Advantages of EchoMRITM vs. DEXA</u>

Q. What are the differences between the Bruker small mice body composition analyzer that you sell and the EchoMRITM system?

A. There are several differences:

- The hardware of Bruker small mice body composition analyzer (also known as Minispec LF-50) is produced by Bruker. The software is from Echo Medical Systems. Bruker and Echo Medical Systems are marketing and selling these systems together.
- 2. Bruker Minispec hardware is only for live animals up to 35 g. The EchoMRI[™] systems for mice are suitable for sizes up to 100-130 g (New Zealand Obese Mice weight is 130 grams) and rats up to 1,200 grams.
- 3. The Bruker device requires holder temperature of 37° Centigrade. The EchoMRI[™] system holder temperature is permitted to vary from room temperature to 40°C.
- 4. The Bruker Minispec system is calibrated to measure fat at 40° Centigrade vs. wide temperature range for the EchoMRITM systems.

Q. How does EchoMRI[™] technology compare with MicroCT?

A. MicroCT analyzes three dimensional distributions and has the potential to measure fat content in every pixel. MicroCT is identifying volumes of pixels that are adipose tissue; then contours are drawn around the tissue. Thus, fat in muscle and blood are not being measured. The measurement is not made at the molecular level.