A better understanding of the permittivity property of skeletal muscle is essential for the development of new diagnostic tools and approaches for neuromuscular evaluation. However, there remain important knowledge gaps in our understanding of this property in healthy and diseased skeletal muscle, which hinder its translation into clinical application. Here, we report the permittivity of gastrocnemius muscle in healthy wild type mice and murine models of spinal muscular atrophy, muscular dystrophy, diabetes, amyotrophic lateral sclerosis and in a model of myofiber hypertrophy. Data were measured ex vivo from 10 kHz to 1 MHz using the four-electrode impedance technique. Additional quantitative histology information were obtained. Ultimately, the normative data reported will offer the scientific community the opportunity to develop more accurate models for the validation and prediction of experimental observations in both pre-clinical and clinical neuromuscular disease research.

Background & Summary
Electromagnetism constitutes a basic physical principle widely used in the field of biomedical engineering, designed to monitor and treat a broad spectrum of conditions including Parkinson's disease and brain tumors. Understanding how different biological tissues and fluids interact with electromagnetic fields is essential for improving the accuracy of existing analytical techniques as well as developing new diagnostic tools and therapies.

In electromagnetism, permittivity is one fundamental material parameter affecting the propagation of electromagnetic fields. When exposed to an electromagnetic field, the dipole moment of the material's molecules opposes the external electric field and so the net electric field is reduced within the material. In other words, the permittivity is a measure of the ability to store an electric charge in the polarization of the material.

Basic and applied scientific endeavors have reported the permittivity property for well over 100 years in a collective effort to understand the propagation of electromagnetic fields in the human body. The birth of biomedical engineering and the field of electrophysiology arose from these pioneers' early multidisciplinary studies applying new theory, tools and methods. For example, voltage clamp and telegrapher's equations made it possible to understand how ionic currents give rise to the action potential. Another prominent contribution that emerged from studying tissues' permittivity includes the development of the bidomain model to study cardiac muscle, which laid the foundation for the development of implantable cardiac pacemakers.

Previous studies have reported permittivity values of specific tissue as a function of the applied frequency. However, a recent meta-analysis revealed that major gaps in the knowledge of the frequency-dependence of the conductivity and relative permittivity in many tissues still exist, especially for those tissues such as skeletal muscle, in which these properties are directionally dependent. For example, prior authors acknowledged the technical limitations of their reported paravertebral muscle permittivity values nominally measured along and across the muscle fiber directions.

In addition, some previous studies of the permittivity property of biological specimens did not specify the state of the tissue examined, even though it is known that the permittivity values change postmortem and with temperature, nor did they specify the extent of disease, if any, present, and some did not include healthy control samples.
tissue for comparison. Other noteworthy factors that have not been exhaustively evaluated include the variation of tissues’ permittivity with age, gender, and disease progression. This missing information highlights critical gaps in our understanding of the factors that affect the permittivity property of biological tissues required to aid in identification of clinically abnormal results in pathological tissue.

The present study is motivated by the need to obtain permittivity values from both healthy and diseased skeletal muscle to aid in the diagnosis and monitoring of patients with neuromuscular disorders (NMDs). Analysis of

Fig. 1 Permittivity in a mice model of spinal muscular atrophy. (a) Longitudinal and transverse conductivity and relative permittivity of healthy wild type (WT) and spinal muscular atrophy (SMA) mice. Mean ± standard error of the mean (SEM). (b) Representative histology images from the gastrocnemius muscle. Scale bar: 50 μm. Quantification of the myofiber cross sectional area (CSA) in WT and SMA mice. (c) Mean CSA. *p < 0.05.
muscle histology performed over the course of disease progression has shown that the composition and structure of skeletal muscle tissue changes as a function of the specific NMD. For example, in amyotrophic lateral sclerosis (ALS) muscle fibers tend to atrophy over time\textsuperscript{24}, whereas in Duchenne muscular dystrophy, the lack of dystrophin protein\textsuperscript{25}, causes a loss of muscle fibers and their progressive substitution by fat and fibrous tissue\textsuperscript{26}.

Changes in the permittivity property in diseased muscle establishes the underlying scientific premise of electrical impedance myography (EIM)\textsuperscript{27,28}, a relatively new electrodiagnostic technique that is gradually

![Figure 2](attachment:image.png)

**Fig. 2** Permittivity in a mice model of muscular dystrophy. (a) Longitudinal and transverse conductivity and relative permittivity of healthy wild type (WT) and muscular dystrophy (MDX) mice. Mean ± standard error of the mean (SEM). (B) Representative histology images from the gastrocnemius muscle. Scale bar: 50 μm. Quantification of the myofiber cross sectional area (CSA) in WT and MDX mice. (c) Mean CSA. **p < 0.01.
finding its niche for the assessment of NMD progression and the success of therapeutic intervention. EIM is a non-destructive technique that is based on the measurement of the electrical impedance of individual muscles or groups of muscles. The permittivity property of the muscle can be extracted from the EIM values by accounting for the experimental setup. To date, however, EIM studies that have reported permittivity values of diseased muscle are scarce and often the data are incomplete, evaluating permittivity values at only a single frequency. For

Fig. 3 Permittivity in a mice model of diabetes. (a) Longitudinal and transverse conductivity and relative permittivity of healthy wild type (WT) and diabetes (DB/DB) mice. Mean ± standard error of the mean (SEM). (b) Representative histology images from the gastrocnemius muscle. Scale bar: 50 μm. Quantification of the myofiber cross sectional area (CSA) in WT and DB/DB mice. (c) Mean CSA. **p < 0.01; ***p < 0.001.
EIM to achieve its full potential, it is paramount to first establish normative permittivity values in the frequency range where the histological alterations in disease would be expected to have an effect.

Here, using EIM technique, we report the conductivity and relative permittivity values of mouse muscle in healthy and four disease models (i.e., spinal muscular atrophy, muscular dystrophy, diabetes, amyotrophic lateral sclerosis) and in a drug-induced model of myofiber hypertrophy, which represents an all-encompassing effort by

![Fig. 4](https://example.com/figure4)

**Fig. 4** Permittivity in a mice model of amyotrophic lateral sclerosis. (a) Longitudinal and transverse conductivity and relative permittivity of male and female amyotrophic lateral sclerosis (ALS) mice. Mean ± standard error of the mean (SEM). (b) Representative histology images from the gastrocnemius muscle. Scale bar: 50 μm. Quantification of the myofiber cross sectional area (CSA) in male and female ALS mice. (c) Mean CSA.
our group over the course of the last three years. The objective of this report is to equip the reader with a comprehensive database of the permittivity properties of healthy and diseased skeletal muscle from 10 kHz to 1 MHz, in both longitudinal and transverse directions. We also include additional quantitative histology data and periodic measurements obtained during disease progression. Providing the permittivity of healthy and diseased muscle.
using these various mouse models will open new venues for the development and improvement of the clinical diagnosis and monitoring of patients with NMD.

**Methods**

**Terminology and definitions.** The permittivity $\varepsilon$ determines the dielectric behavior of materials when exposed to an applied electric field and it is defined as (dimensionless)
where \( \varepsilon \) is the relative permittivity (dimensionless), \( \sigma \) is the total conductivity of the material (S m\(^{-1}\)), \( \omega \) is the (angular) frequency of the field measured (rad s\(^{-1}\)), \( \varepsilon_0 = 8.85 \times 10^{-12} \) is the permittivity of the vacuum (F m\(^{-1}\)), and \( i \) is the imaginary unit (dimensionless).

In skeletal muscle, due to its highly organized cellular and fascicular structure, the permittivity is different along and perpendicular to the direction determined by the myofibers orientation\(^{31}\). Here, we calculated the longitudinal and transverse conductivity and relative permittivity from longitudinal and transverse resistance \( R \) and reactance \( X \) muscle data measured using a dielectric cell with the four-electrode technique,

\[
\sigma_{[L,T]} = \frac{R_{[L,T]}}{R_{[L,T]}^2 + X_{[L,T]}^2} \quad \text{and} \quad \varepsilon_{r,[L,T]} = \frac{X_{[L,T]}}{\omega \sigma_{[L,T]}^r},
\]

where \( K \) (m\(^{-1}\)) is a geometrical factor determined from measurements in saline solution.

**Ethical approval and informed consent.** All animal procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH and approved by the Institutional Animal Care and Use Committee at Beth Israel Deaconess Medical Center (Protocol #087–2016).

**Animal experimentation.** Animal experimentation was the same for all animals. All animal procedures were carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the NIH and approved by the Institutional Animal Care and Use Committee at Beth Israel Deaconess Medical Center (Protocol #087–2016). Mice were given ad libitum access to food (FormulaLab Diet 5008, LabDiet, MO, USA) and water. Animals were allowed to acclimate at least 72 h prior to testing. Prior to measurements, animals were humanely euthanized with CO2 and the gastrocnemius muscle harvested from both left and right legs. Muscle impedance measurements were made immediately after with a heating pad under the dielectric cell to maintain a constant temperature 37 °C. Unless otherwise stated, the animals were obtained from Jackson Laboratories (JAX, Bar Harbor, ME, USA).

**Spinal muscular atrophy (SMA) mice.** Five spinal muscular atrophy (SMA) Model (“Smn 2B/2B-Neo”) (B6.129-Smn1tm1.1Cdid/tm1Cdid) mice were generated by intercrossing SMN 2B mice with SMN 2B-Neo mice. The official name of the Smn 2B allele is B6.129-Smn1tm1.1Cdid. 2B mice were generated from the progenitor line Smn 2B-Neo (B6.129-Smn1tm1Cdid) through removal of the flox-neo cassette\(^{32}\). Germline mice were subsequently crossed to C57BL/6J mice (JAX stock #000664) for at least 3 generations prior to use in these studies. The median survival of 2B/2B-Neo mice is ~13 months for males and 24 months for females. Details of this model will be reported elsewhere. Mice were studied at 40 weeks of age. Five wild type littermates served as controls for EIM analysis.

**Muscular dystrophy (MDX) mice.** The D2.B10 (DBA/2-congenic) Dmd\(^{mdx}\) mouse (also referred in the literature as DBA/2-mdx) was chosen as a model of Duchenne muscular dystrophy model as it recapitulates several of the human characteristics of DMD myopathy including lower hind limb muscle weight, fewer myofibers, increased fibrosis and fat accumulation, and muscle weakness relative to strains with this mutant allele on other genetic backgrounds\(^{33–35}\). These genetically altered mice develop the disease without additional intervention and live at least one year. Fifteen male D2.B10-Dmd\(^{mdx}\)/J mice hemizygous for Dmd\(^{mdx}\) (6–9 weeks of age, JAX strain #013141) and studied at various ages from 6 to 43 weeks. Fifteen male wild type mice (DBA/2J, JAX strain #000671) served as controls (5 mice per time point).

**Obese/Diabetic mice.** The DBA/D2 mouse (DBA/D2J, JAX strain #013141) was chosen as a model of diabetes type II and obesity\(^{36–38}\). Mice homozygous for the diabetes spontaneous mutation (Leprdb) become obese at approximately three to four weeks of age. Elevations of plasma insulin begin at 10 to 14 days and elevations of blood sugar at four to eight weeks. Homozygous mutant mice are polyphagic, polydipsic, and polyuric. These mice are well known for their obesity and for developing substantial intramuscular fat deposition by approximately 8 weeks of age. Ten male mice (5 weeks, JAX strain #000642) were studied at 6 and 20 weeks (5 mice per time point). Ten WT type C57BLKS/J (JAX strain #000662) served as controls (5 mice per time point).

**Amyotrophic lateral sclerosis (ALS) mice.** Breeding pairs of ALS B6SJL-Tg(SOD1\(^{G93A}\))1Gur/J mice (JAX strain #002726) were obtained and bred to obtain 37 animals (approximately half female and half male). To study varying fiber size\(^{40}\), animals were studied at various ages ranging from 8–18 weeks (approximately 6–7 animals per fortnight, at 8, 10, 12, 14, 16, and 18 weeks).

**Mice with myofiber hypertrophy.** Twenty male wild type mice (C57BL/6J, 8 weeks of age, JAX strain #000664) were obtained. Starting at 9 weeks of age, mice were divided randomly into two groups of 10 mice per group. Mice were treated twice weekly with subcutaneous injections of either phosphate-buffered saline (PBS) or the myostatin ligand trap ActRIIB-mFc (Acceleron Pharma, Cambridge, MA, USA) at a dose of 3.3 mg/kg. ActRIIB-mFc (also termed RAP-031)\(^{41}\) is a protein comprised of a form of the extracellular domain of ActRIIB fused to a mouse Fc that acts as a ligand trap to inhibit myostatin signaling. Animals were weighed weekly with an analytical balance (AS64, Adventurer SL, Ohaus Corporation, Pine Brook, NJ, USA) to ensure correct dosing.
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Author Contributions
Conceived and designed the experiments: J.A.N., C.J.D., S.B.R., B.S. Performed the experiments: J.A.N. Analyzed the data: J.A.N., B.S. Wrote the paper: B.S. All authors revised and approved the manuscript.

Additional Information
Competing Interests: Dr. Rutkove serves as scientific advisor and consultant to Myolex, Inc., a company that commercializes impedance technology. Dr. Rutkove is also a member of the company’s Board of Directors. Dr. Sanchez serves as a consultant to Myolex, Inc., and Impedimed, Inc., a company that develop impedance technology for research and clinical use. Dr. Sanchez and Dr. Rutkove are Co-Founders of Haystack Diagnostics, Inc., a company that commercializes needle impedance technology. Haystack Diagnostics, Inc., has the option to license patented needle impedance technology of which Dr. Sanchez and Dr. Rutkove are named inventors.

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