Published in final edited form as:

J Magn Reson Imaging. 2014 April ; 39(4): 1039–1046. doi:10.1002/jmri.24231.

***In vivo* absolute quantification for mouse muscle metabolites using an inductively coupled synthetic signal injection method and newly developed $^1\text{H}/^{31}\text{P}$ dual tuned probe**

Donghoon Lee, PhD, Kenneth Marro, PhD, Mark Mathis, BS, Eric Shankland, PhD, and Cecil Hayes, PhD

Department of Radiology, University of Washington, Seattle, WA

Abstract

Purpose—To obtain robust estimates of ^{31}P metabolite content in mouse skeletal muscles using our recently developed MR absolute quantification method and a custom-built $^1\text{H}/^{31}\text{P}$ dual tuned radiofrequency (RF) coil optimized for mouse leg.

Materials and Methods—We designed and fabricated a probe consisting of two dual tuned $^1\text{H}/^{31}\text{P}$ solenoid coils: one leg was inserted to each solenoid. The mouse leg volume coil was incorporated with injector coils for MR absolute quantification. The absolute quantification method uses a synthetic reference signal injection approach and solves several challenges in MR absolute quantification including changes of coil loading and receiver gains.

Results—The $^1\text{H}/^{31}\text{P}$ dual tuned probe was composed of two separate solenoid coils, one for each leg, to increase coil filling factors and signal-to-noise ratio. Each solenoid was equipped with a second coil to allow injection of reference signals. ^{31}P metabolite concentrations determined for normal mice were well within the expected range reported in the literature.

Conclusion—We developed an RF probe and an absolute quantification approach adapted for mouse skeletal muscle.

Keywords

Absolute quantification; dual tuned RF coil; mouse muscle; ^{31}P metabolites; inductive coupling; signal injection method

Genetically engineered mouse models are becoming useful tools for studying various muscle disorders such as the mdx mouse model of Duchenne muscular dystrophy (1) and the dystrophin and utrophin double knockout mdx:utrophin^{-/-} model (2,3). Studies that utilize genetically engineered mice usually rely on biopsy followed by histology as a means for obtaining metabolite concentrations. Muscle biopsy has been a gold standard to investigate both disease progression and treatment responses but is invasive so multi-time point procedures are not feasible. More important, muscle biopsy is a local procedure performed for a specific muscle type and has a substantial limit in examining the rest of muscle regions (4-9). Noninvasive MR measurements could enhance these studies by allowing longitudinal measurements during disease progression and therapy in individual mice. A surface coil and volume coils have been used to acquire ^{31}P MR spectra for small animals (10,11). However, MR measurements in individual muscles are difficult to conduct in mice because the tissue volume is inherently small, so the signal-to-noise ratio (SNR) is typically very low for non-

proton (or x-nucleus) MRS. Further, the results are usually reported as ratios, making interpretation ambiguous (12,13). In particular, absolute quantification is important for ^{31}P MR, as none of the phosphorous containing metabolites is necessarily constant in pathology (14).

A synthetic reference signal injection method has been developed to ease the burden of converting MR signals into quantitative units of concentration (15-19). This approach is similar to the ERETIC (Electronic Reference To In vivo Concentrations) method (20). The distinguishing feature of the method is that the synthetic reference signal is generated by inductive coupling rather than RF radiation. The method uses inductive coupling to inject artificial reference signals, which are called pseudo-signals that serve as calibration parameters for absolute quantification. All parameters downstream of the RF coil that affect quantification have equal effect on the pseudo-signal and the real signals. Any changes in these parameters, in particular coil loading and receiver gain, which might occur between subjects or in a single subject due to physiological perturbations, are transparent and have no effect on the quantification process.

In this study, we applied the pseudo-signal injection method to determine ^{31}P metabolite contents in mice. To improve the SNR, we developed a dual tuned $^1\text{H}/^{31}\text{P}$ probe optimized for MRI and MRS measurements in mouse hind limb muscles. The probe was equipped with two dual tuned $^1\text{H}/^{31}\text{P}$ solenoid coils, one for each leg, each of which was individually interfaced with an injector coil for pseudo-signal injections. ^1H MRI was conducted to measure muscle volumes and ^{31}P MRS was performed to quantify phosphorous metabolites. We provide a detailed description of the RF probe, demonstrating how it improves SNR, and provide quantitative measurements of average inorganic phosphate (Pi), phosphocreatine (PCr), and adenosine triphosphate (ATP) concentrations that agree with published values for normal mice. The approach we developed in this study would be applicable to longitudinal quantification of mouse muscle for any $^1\text{H}/\text{x-nucleus}$ metabolites where SNR is expected low.

MATERIALS AND METHODS

MRI/MRS data were acquired from five normal mice on a Bruker 4.7 T horizontal bore magnet (Bruker Medical Systems, Karlsruhe, Germany) equipped with a Varian INOVA spectrometer (Varian, Inc., Palo Alto, CA, USA). Mouse husbandry and experimental procedures were performed in accord with the NIH Guide for the Care and Use of Experimental Animals and with approvals from our Institutional Animal Care and Use Committee.

RF coil design and construction

To allow ^1H imaging and ^{31}P spectroscopy without repositioning the mice, a dual tuned $^1\text{H}/^{31}\text{P}$ volume coil (see Fig. 1) was designed and fabricated. The probe was composed of two dual tuned $^1\text{H}/^{31}\text{P}$ solenoid coils: one leg was inserted into each solenoid. The circuit for each solenoid (see Fig. 2) was based on the design that Najim *et al.* proposed for a single coil and double tuned for proton and heteronucleus (or x-nucleus) frequencies (21). A second coil, which we call the injector coil, was incorporated into each solenoid to allow injection of the pseudo-signals. For efficiency, the probe design allowed both legs to be imaged simultaneously at the ^1H resonance frequency. The solenoid coils were used independently for non-localized ^{31}P spectroscopy in individual legs. Hence, the ^{31}P signals acquired individually by the two solenoid coils were detected by the same ^{31}P receiver rather than the signal combined in one receiver channel. With this method of operation it was critical to minimize interactions between the active and inactive solenoids. We used a detune stub length of 0.88 m to make a short at junction “A” of the ^{31}P match and ^{31}P tune

capacitors to ground to disable the second unused coil as shown in Figure 2. One end of the detune stub was connected to the ^{31}P feed point and the other end shorted. The ^{31}P feed cable (velocity factor $\text{VF} = 0.73$) was one wavelength (1.0 m) at the ^1H resonance frequency and 145.7° of phase shift for the ^{31}P frequency at 81.2 MHz. The detune stub ($\text{VF} = 0.73$) represents 117.4° for the ^{31}P frequency. Hence, the total phase shift for the detune stub and the ^{31}P feed cable is 263.1° of phase shift for the ^{31}P frequency. The short at the end of the stub, shifted by 263.1° , creates an impedance of $j413$ ohms at the other end of the two coaxial cables that connects to the ^{31}P match capacitor. The variable capacitor used for the ^{31}P match had a range from 1 to 30 pF. If the match capacitor is set to a nominal capacitance of 4.75 pF, the two coaxial cables (the ^{31}P feed cable and detune stub) will resonate and short to ground at junction “A”, disabling the inactive solenoid. We set the detune stub to tune it with a nominal capacitance for the ^{31}P match capacitor through the ^{31}P feed coaxial cable until the tune display shows minimal interaction.

The two solenoid coils were separated by 20 mm (from one coil center to the other): each coil (inner diameter of 7 mm, length of 7 mm) accommodated one lower leg, covering only the tibialis anterior and gastrocnemius muscles to increase the filling factor (see Fig. 3). Baluns were included to minimize cross talk (22,23). The proton channels were separately used to acquire ^1H images for each leg and also combined with a Wilkinson RF power combiner (24) to image both legs at once. A manual switch was used to change frequencies between the ^1H and ^{31}P channels.

SNR comparison between one solenoid and $^1\text{H}/^{31}\text{P}$ 25 mm commercial coil

A 5 mm NMR tube filled with a 200 mM inorganic phosphate solution was prepared to examine signal-to-noise ratio (SNR) for both ^1H and ^{31}P nuclei. SNR values determined for a $^1\text{H}/^{31}\text{P}$ solenoid coil were compared with those for a commercial double tuned $^1\text{H}/^{31}\text{P}$ 25 mm diameter volume coil (Doty Scientific Inc., Columbia, SC; inner diameter = 25 mm, length = 22 mm) in a linear Litz RF coil type (25,26). The commercial RF coil was composed of two Litz coils (tuned at the ^1H and ^{31}P resonance frequencies) mounted on two coaxial cylinders and located axially with the magnet bore. Two dimensional multi-slice spin echo ^1H images were acquired to compare SNR values for the central slices from both the $^1\text{H}/^{31}\text{P}$ dual tuned solenoid and the commercial $^1\text{H}/^{31}\text{P}$ 25 mm RF coil. The parameters for the ^1H images were the following: $\text{TR}/\text{TE} = 4 \text{ s} / 9 \text{ ms}$, number of averages of 2, field of view of 30 mm and matrix size of 256×256 . A single slice of 1 mm thickness at the coil center was selected from 9 slices for ^1H SNR determination. Then ^{31}P MR spectra were acquired with both RF coils to compare their corresponding SNR values with the following parameters: recycle delay of 60 s, number of averages of 16 and sweep width of 5 kHz. T_1 of the solution was determined to be 10.22 seconds. The SNR in the ^1H images was determined by dividing the average signal intensity per pixel within a region in the phantom by the standard deviation of the pixel intensities in a region outside the phantom. Similarly, the SNR for the ^{31}P MR spectra was calculated by a ratio of the maximum ^{31}P signal intensity acquired for the phantom and a standard deviation as a noise in a non-signal region of the spectrum.

Coil coupling

The probe design was intended to minimize coupling between the solenoids to allow efficient individual operation for ^{31}P MRS. To examine coupling, both solenoid coils were tuned and matched at the ^{31}P resonance frequency (81.2 MHz). Using the decoupling scheme described in the RF coil design and construction section (above), one solenoid was configured to be active and the other to be inactive. An on-resonance driving signal was introduced to the active solenoid and pick up loops (1 mm diameter) were used to measure

the power in each solenoid to provide the coupling constants, C_a and C_i , in the active and inactive coils, respectively:

$$C_a = 10 \log_{10} \left(\frac{p_a}{p_d} \right) \quad C_i = 10 \log_{10} \left(\frac{p_i}{p_d} \right) \quad [1]$$

where p_d is the power used to drive the active coil, p_a is the power measured with the pickup loop placed at the center of the active coil, and p_i is the power measured with the pickup loop at the center of the inactive coil. These results were rearranged to get an equation defining the relative sensitivity p_a/p_i :

$$\frac{p_a}{p_i} = \kappa = 10^{(C_a - C_i)/10} = 10^{\Delta C/10} \quad [2]$$

The value of κ can be used to estimate the flip angle, θ , that would occur in the inactive solenoid due to cross coupling when a nominal 90 degree pulse is generated in the active coil:

$$\theta = \frac{90^\circ}{\kappa} \quad [3]$$

The relative signal, η , (given in percent) *produced* in the inactive coil and *received* in the active coil due to cross coupling is then given by:

$$\eta = \frac{\sin \theta}{\kappa} \times 100 \quad [4]$$

Animal preparation and setup for in vivo MR experiments

Five normal C57BL/6 mice were purchased from Harlan laboratories (Kent, WA). For metabolite measurements, the mice were positioned on a plastic holder with one leg in each of the solenoid coils (see Fig. 3B). The probe and mouse holder were placed inside a 9 cm inner diameter radiofrequency (RF) shield. A water jacket, circulating warm water, was used to maintain the animal body temperature at 37 °C throughout the course of the procedures. The animal's body temperature was monitored by a rectal temperature probe in place during the animal setup for MR acquisitions.

After the adjustment of magnetic field shimming, three dimensional (3D) ^1H images of both hind legs were acquired simultaneously. A gradient echo sequence was used with the following parameters: TR/TE = 100/2.2 ms, flip angle = 10 degrees, number of averages (NA) of 2, matrix = $128 \times 64 \times 64$, field of view of $30 \times 30 \times 40$ mm. The images were used to determine muscle volume, excluding bone and subcutaneous lipids, so that the non-localized ^{31}P MRS measurements could be converted to units of average concentration. Muscle volumes were determined from segmented regions of interest using the editor module of the 3D Slicer Version 3.6.2 (<http://www.slicer.org>). Each volume was calculated by delineating leg muscles covering from ankle joint to knee joint with bone regions eliminated.

Subsequent to imaging, each solenoid coil was used separately for quantification of ^{31}P metabolites using a non-localized, pulse-acquire sequence with the following parameters: TR = 15 s, NA = 128, sweep width = 10 kHz, number of complex points = 16,384. Intracellular pH (pHi) values were determined using the chemical shift between PCr and Pi (27).

Phantoms and setup for calibration experiments

A single *in vitro* session was required to calibrate the amplitude of the pseudo-signal against a known concentration. This session was conducted on two 5 ml NMR tubes, one for each solenoid, containing 50 mM inorganic phosphate in distilled water with the filling height of the phantom of 40 mm. 3D ^1H images were acquired to determine the excited volume of the NMR tubes and then ^{31}P spectra were acquired from each tube independently using a non-localized, pulse acquire sequence (TR = 30 s, NA = 16, sweep width = 10 kHz, number of complex points = 16,384). We have assumed that the excitation volumes for both ^1H and ^{31}P resonance frequencies are equal. Since the same coil is used for both frequencies and it is small compared to the wavelengths (1.5 m and 3.7 m for ^1H and ^{31}P resonances at 4.7T, respectively), the same RF field strength should occur spatially. Using the second RF channel, a modulated free induction decay waveform was transmitted to the injector coils during the data acquisition window so that an artificial peak, the pseudo-signal, appeared in the processed spectra. The transmitter power was adjusted so that the integrated area of the pseudo-signal peak was approximately the same as the real signal arising from the inorganic phosphate in the NMR tubes. The same transmitter power settings were used to inject pseudo-signals into the real data during the *in vivo* sessions.

^{31}P metabolite determination using the synthetic signal injection method

The spectroscopy data were analyzed using the Advanced Method for Accurate, Robust and Efficient Spectral fitting (AMARES) (28) time domain fitting algorithm as included in the Java-based Magnetic Resonance User Interface (jMRUI) software package (29,30). ^{31}P metabolite concentrations were determined by the following formula.

$$C_{m,abs} = C_{m,ref} \times \frac{A_m}{A_p} \times \frac{A_{p,ref}}{A_{m,ref}} \times \frac{V_{m,ref}}{V_m}, \quad [5]$$

where $C_{m,abs}$ is the muscle metabolite concentration for metabolite m, $C_{m,ref}$ is the concentration of the reference molecule used for the calibration session (i.e. 50 mM inorganic phosphate), A_m is the area of the real *in vivo* peak corresponding to metabolite m, A_p is the area of the pseudo-signal acquired during the *in vivo* session, $A_{p,ref}$ is the area of the pseudo-signal acquired during the calibration session, $A_{m,ref}$ is the area of the pseudo-signal acquired during the calibration session, $V_{m,ref}$ is the excitation volume for the calibration session and V_m is the muscle volume for the *in vivo* sessions. Equation 5 was used to determine the average *in vivo* muscle concentrations of ATP, Pi, and PCr within the measurement volumes. The ATP concentration was determined from the peak corresponding to the α -ATP nucleus.

RESULTS

The pair of dual tuned solenoids allowed simultaneous imaging of both mouse legs as well as ^{31}P MRS for individual legs without the need to reposition the animals. The SNR was sufficient to allow accurate quantification of ^{31}P metabolites in each mouse leg.

The goal of the decoupling scheme was to allow the active solenoid to operate efficiently and to minimize the undesirable signal that cross coupling could generate within the inactive solenoid and that could then be received by the active solenoid during the ^{31}P MRS measurements. Using equations 1 through 4, we estimated the relative sensitivity, κ , within the inactive coil, the expected flip angle, θ , that could be generated within the inactive coil when a nominal 90 degree pulse is generated within the active coil, and the relative amplitude of the undesirable signal, κ , compared to the desirable signal that is generated within, and received by, the active solenoid. These results are summarized in Table 1. The

calculations show that, when they were in active mode, the amplitude of the undesirable signals potentially generated within the solenoids enclosing the right and left legs were 0.01% and 0.06%, respectively, of the desirable signal generated within the corresponding active solenoid. Our comparisons against a state of the art, high quality Litz coil (Doty Scientific Inc., Columbia, SC) demonstrate that the increased filling factor provided by our dual tuned solenoid probe improved the SNR by a factor of 7 for ^1H imaging. Side-by-side ^1H images (1 mm slice thickness) are shown in Fig. 4, demonstrating the SNR enhancement. SNR comparisons between ^{31}P MR spectra showed a factor of 2 improvement for our solenoid probe. Reduced SNR enhancement for the ^{31}P MRS comparing to that for the ^1H MRI was obtained due to the shorter RF coverage of our solenoid coil for the ^{31}P MRS comparison to that of the commercial coil. The 3D *in vivo* images allowed calculation of muscle volume within the homogenous regions of the solenoid coils (see Fig 5). Muscle volumes measured for the left legs of all 5 mice are provided in Table 2.

A typical *in vivo* ^{31}P spectrum is shown in Fig. 6. The PCr, ATP and Pi peaks all have sufficient SNR to allow robust fitting with AMARES. The pseudo-signal appears at about 12 ppm. Similar spectra, acquired from all 5 mice, were used to determine concentrations of the individual metabolites ($\text{Pi} = (10.9 \pm 5.8) \text{ mM}$, $\text{PCr} = (30.5 \pm 4.1) \text{ mM}$, and $\text{ATP} = (11.8 \pm 3.5) \text{ mM}$) using Eq. [5]. For these metabolite concentrations, we assumed a cellular water fraction of 0.82 in skeletal muscles (31). Intracellular pH level of leg muscles was determined 7.03 ± 0.03 using the chemical shift between PCr and Pi.

DISCUSSION

In this study, we successfully quantified ^{31}P metabolites in mouse skeletal muscles using a custom designed $^1\text{H}/^{31}\text{P}$ dual tuned probe that allowed injection of pre-calibrated artificial reference signals. The custom built probe provided several advantages: 1) It allowed simultaneous imaging of both legs, reducing the total acquisition time and eliminating the need to reposition the animal. 2) It guaranteed identical regions of interest for the imaging and spectroscopy measurements. 3) It allowed ^{31}P MRS measurements to be acquired from individual legs. This will be useful in future studies where we expect to acquire serial measurements in treated legs with the untreated, contralateral leg serving as a control. 4) The use of two solenoids maximized the filling factor and provided excellent spatial resolution in the images and high SNR in the spectra. There are, however, design challenges that must be considered when building dual solenoid probes. Steps must be taken to eliminate cross talk between the two solenoids, which could lead to artifacts in the images and/or contamination of the spectra due to unwanted signal generated in the unused solenoid. The method of switching from the ^1H to the ^{31}P resonance must also be considered. For the current design, we used a manual switch to change frequencies. However, a TTL type switch, controlled by commands within the pulse sequence, could also be used and would allow fast-interleaved acquisitions between the two RF channels. The solenoid coils fabricated for this study can be replaced with different types of RF coils with which coil axes can be aligned with the main magnetic field direction, which is often favorable for the study of extremities.

The Pi values determined in this study and their standard deviation are large for normal resting muscles. One explanation for this is that the *in vivo* setup for some animals may have reduced blood flow to the skeletal muscles under examination. As shown in Fig. 3B, both legs were snugly positioned into two solenoid coils with the feet securely taped to the holder. The upper regions of both hind-limb muscles may have experienced slight pressure at the upper edges of the two solenoid coils, possibly reducing blood flow to the entire limb.

The increased SNR using our custom-built coil compared to that for the Litz coil was due to the significant improvement of the filling factor for our solenoid coils. While the relationship between filling factor and SNR is well known (32), and this result is not unexpected, we felt it was important to compare performance against a commercially available volume coil that might be considered for MRI and MRS in mouse legs. In most protocols where data is to be acquired from both legs of a mouse, or any other animal, users must decide whether to use a large, inefficient coil to image both legs without moving the animal or to seek higher SNR by using a smaller coil, which requires repositioning the animal. Our results demonstrate the value of building or purchasing a dedicated, optimized probe that provides improved SNR without having to reposition the animal. For some studies, similar probe designs may be worth the added cost, particularly for studies where the SNR is expected to be critical and the increased filling factor could allow the study to be successfully completed.

We used a simple pulse-acquire sequence with nonlocalized RF excitation to maximize the ^{31}P SNR. For the nonlocalized RF excitation, the ^{31}P signal from non-homogeneous region will contribute. It is difficult to estimate how much the signal from the non-homogeneous region contributed in the total ^{31}P signal. We assumed that the contribution would be significantly smaller than that from the homogeneous region. However, our concentration results may be slightly high due to the exclusion of the non-homogeneous region in the muscle volume calculations. Further improvement for quantification accuracy could be achieved by focusing on localized regions of interest rather than nonlocalized excitations, which will minimize potential inhomogeneity of RF fields and eliminate the need to calculate muscle volumes.

The metabolite quantification algorithm we employed was reliable enough to provide reasonable levels of ^{31}P metabolites for the normal mouse muscles. PCr levels of about 30.5 mM determined from this study are within the range of PCr levels of about 30 mM acquired by others (33,34). The concentrations for the observed ^{31}P metabolites, shown in Table 3, were compared to those determined in the literature. The conventional method of ^{31}P metabolite quantification uses high performance liquid chromatography (HPLC) determined ATP concentration to determine other phosphorous metabolite contents. The synthetic reference signal injection method described in this study does not require an additional HPLC measurement for the determination of ATP concentration yet generates reliable quantification outcomes. A benefit of using our approach would be the possibility to take ^1H and ^{31}P MR spectra from the same volume by the same double tuned coil. This might be an important feature of studies in which the tCr (total creatine)/PCr ratio might be an important parameter or by taking in the H_2O signal as an internal reference for absolute quantification. In addition both the coil design and quantification method shown in this study would be applicable to human leg muscle studies for which both ^1H and non-proton MR quantitative measurements are required.

In conclusion, we developed an optimized dual tuned $^1\text{H}/^{31}\text{P}$ RF coil equipped with injectors for the MR absolute quantification. This approach allows reliable ^{31}P metabolite determination in small animal skeletal muscles.

Acknowledgments

We thank Daniel Chen for image segmentation to determine muscle volumes. We acknowledge the use of resources and equipment at the Diagnostic Imaging Science Center and Animal Bioimaging Center at the University of Washington.

Grant Support: R21EB008166, R21EB009438 and R01AR041928

REFERENCES

1. Bulfield G, Siller WG, Wight PA, Moore KJ. X chromosome-linked muscular dystrophy (mdx) in the mouse. *Proceedings of the National Academy of Sciences of the United States of America*. 1984; 81(4):1189–1192. [PubMed: 6583703]
2. Deconinck AE, Rafael JA, Skinner JA, et al. Utrophin-dystrophin-deficient mice as a model for Duchenne muscular dystrophy. *Cell*. 1997; 90(4):717–727. [PubMed: 9288751]
3. Grady RM, Teng H, Nichol MC, Cunningham JC, Wilkinson RS, Sanes JR. Skeletal and cardiac myopathies in mice lacking utrophin and dystrophin: a model for Duchenne muscular dystrophy. *Cell*. 1997; 90(4):729–738. [PubMed: 9288752]
4. Coggan AR. Muscle biopsy as a tool in the study of aging. *J Gerontol A Biol Sci Med Sci*. 1995; 50:30–34. Spec No. [PubMed: 7493214]
5. Prayson RA. Diagnostic yield associated with multiple simultaneous skeletal muscle biopsies. *Am J Clin Pathol*. 2006; 126(6):843–848. [PubMed: 17074688]
6. Roth SM, Martel GF, Rogers MA. Muscle biopsy and muscle fiber hypercontraction: a brief review. *Eur J Appl Physiol*. 2000; 83(4-5):239–245. [PubMed: 11138560]
7. Salinero-Paniagua E, Esteban-Garcia A, Traba A, Palencia-Herrejon E, Garzo-Fernandez C, Prieto-Montalvo J. [Modified aspiration needle muscular biopsy in neuromuscular diseases. Use and advantages over open surgical biopsy]. *Rev Neurol*. 2004; 39(9):821–825. [PubMed: 15543496]
8. Filosto M, Tonin P, Vattemi G, et al. The role of muscle biopsy in investigating isolated muscle pain. *Neurology*. 2007; 68(3):181–186. [PubMed: 17224570]
9. Chahin N, Engel AG. Correlation of muscle biopsy, clinical course, and outcome in PM and sporadic IBM. *Neurology*. 2008; 70(6):418–424. [PubMed: 17881720]
10. Masad IS, Grant SC. A retunable surface coil for high field 31P and 1H magnetic resonance evaluations of the living mouse leg. *Physiological measurement*. 2011; 32(8):1061–1081. [PubMed: 21677364]
11. Heerschap A, Sommers MG, in 't Zandt HJ, Renema WK, Veltien AA, Klomp DW. Nuclear magnetic resonance in laboratory animals. *Methods in enzymology*. 2004; 385:41–63. [PubMed: 15130732]
12. Cady E. Determination of absolute concentrations of metabolites from NMR spectra. *NMR Basic Princ Prog*. 1992; 26:249–281.
13. Jansen JF, Backes WH, Nicolay K, Kooi ME. 1H MR spectroscopy of the brain: absolute quantification of metabolites. *Radiology*. 2006; 240(2):318–332. [PubMed: 16864664]
14. Kreis R, Slotboom J, Pietz J, Jung B, Boesch C. Quantitation of localized (31)P magnetic resonance spectra based on the reciprocity principle. *J Magn Reson*. 2001; 149(2):245–250. [PubMed: 11318624]
15. Marro KI, Lee D, Shankland EG, et al. Synthetic signal injection using inductive coupling. *J Magn Reson*. 2008; 194(1):67–75. [PubMed: 18595750]
16. Lee D, Marro K, Shankland E, Mathis M. Quantitative 19F imaging using inductively coupled reference signal injection. *Magn Reson Med*. 2010; 63(3):570–573. [PubMed: 20187163]
17. Marro KI, Lee D, Shankland EG, et al. Quantitative in vivo magnetic resonance spectroscopy using synthetic signal injection. *PLoS One*. 2010; 5(12):e15166. [PubMed: 21203385]
18. Marro KI, Lee D, Shankland EG, Mathis CM, Hayes CE. Synthetic signal injection using a single radiofrequency channel. *J Magn Reson Imaging*. 2011; 34(6):1414–1421. [PubMed: 22113992]
19. Chen X, Pavan M, Heinzer-Schweizer S, Boesiger P, Henning A. Optically transmitted and inductively coupled electric reference to access in vivo concentrations for quantitative proton-decoupled (1)(3)C magnetic resonance spectroscopy. *Magn Reson Med*. 2012; 67(1):1–7. [PubMed: 22084025]
20. Barantin L, Le Pape A, Akoka S. A new method for absolute quantitation of MRS metabolites. *Magn Reson Med*. 1997; 38(2):179–182. [PubMed: 9256094]
21. Najim E, Grivet JP. A double-tuned probe for metabolic NMR studies. *Magn Reson Med*. 1992; 23(2):367–371. [PubMed: 1549050]
22. Davidson MD. Design of a low cost, half wave dipole antenna. *Applied Microwave and Wireless*. 2002; 14:62–66.

23. Kubichek K. WA6TEY 144/220 MHz RDF quad antenna. 73 Amateur Radio. 1989; 343:26, 51.
24. Hagen, JB. Radio-frequency electronics : circuits and applications. Vol. xiii. Cambridge University Press; Cambridge ; New York: 1996. p. 358
25. Doty FD, Entzminger G Jr. Hauck CD. Error-tolerant RF litz coils for NMR/MRI. J Magn Reson. 1999; 140(1):17–31. [PubMed: 10479546]
26. Doty FD, Entzminger G, Kulkarni J, Pamarthy K, Staab JP. Radio frequency coil technology for small-animal MRI. NMR Biomed. 2007; 20(3):304–325. [PubMed: 17451180]
27. Prichard JW, Alger JR, Behar KL, Petroff OA, Shulman RG. Cerebral metabolic studies in vivo by ³¹P NMR. Proc Natl Acad Sci U S A. 1983; 80(9):2748–2751. [PubMed: 6573678]
28. Vanhamme L, van den Boogaart A, Van Huffel S. Improved method for accurate and efficient quantification of MRS data with use of prior knowledge. J Magn Reson. 1997; 129(1):35–43. [PubMed: 9405214]
29. Naressi A, Couturier C, Devos JM, et al. Java-based graphical user interface for the MRUI quantitation package. MAGMA. 2001; 12(2-3):141–152. [PubMed: 11390270]
30. Naressi A, Couturier C, Castang I, de Beer R, Graveron-Demilly D. Java-based graphical user interface for MRUI, a software package for quantitation of in vivo/medical magnetic resonance spectroscopy signals. Comput Biol Med. 2001; 31(4):269–286. [PubMed: 11334636]
31. Hazlewood CF, Chang DC, Nichols BL, Woessner DE. Nuclear magnetic resonance transverse relaxation times of water protons in skeletal muscle. Biophys J. 1974; 14(8):583–606. [PubMed: 4853385]
32. Abragam, A. The principles of nuclear magnetism. Clarendon Press; Oxford: 1961. p. 599
33. Marcinek DJ, Ciesielski WA, Conley KE, Schenkman KA. Oxygen regulation and limitation to cellular respiration in mouse skeletal muscle in vivo. Am J Physiol Heart Circ Physiol. 2003; 285(5):H1900–1908. [PubMed: 12775561]
34. Siegel MP, Kruse SE, Knowels G, et al. Reduced coupling of oxidative phosphorylation in vivo precedes electron transport chain defects due to mild oxidative stress in mice. PLoS One. 2011; 6(11):e26963. [PubMed: 22132085]

Figure 1.

Dual tuned $^1\text{H}/^{31}\text{P}$ probe for two mouse legs *in vivo*. A. Two solenoid RF coils are shown with their tune/match networks and $^1\text{H}/^{31}\text{P}$ baluns for all cables. B. RF homogeneity for ^{31}P signal was examined to generate up-to 270 degree flip angles by varying pulse width (ranging from 10 to 250 μsec) with a constant RF power of 30 dB. C. An end view of the dual tuned $^1\text{H}/^{31}\text{P}$ probe showing 8 tuning rods for all variable capacitors.

J Magn Reson Imaging. Author manuscript; available in PMC 2015 April 01.

Figure 3.

A. Solenoid coil for mouse leg probe. Variable capacitors PT, HT, PM and HM are tuning capacitors for both ^{31}P and ^1H and matching capacitors for both ^{31}P and ^1H , respectively. A frequency trap, shown under a phantom holder, is to isolate two resonance frequencies for ^{31}P and ^1H . The phantom, containing 30 mM of phosphoric acid solution, is placed in the coil to test the coil performance. B. *In vivo* setup for a live mouse with both legs placed in separate solenoid coils. An injector coil is located close to the lowest end of a solenoid coil and separated by a radius (1 mm) of the injector coil (see the white arrow for an injector coil for the right solenoid coil).

Figure 4.

Signal-to-noise ratio (SNR) comparison for the ^1H resonance between the 25 mm diameter commercial coil and the custom-built mouse leg solenoid for this study. Images were cropped from 256×256 ^1H images acquired by the commercial coil (A) and the solenoid (B) and their corresponding profiles in (C) and (D) along the yellow horizontal lines to generate SNR values of 87 and 602, respectively. Spin echo sequence was used with $\text{TR/TE} = 4 \text{ s}/9 \text{ ms}$ and 2 NA.

Figure 5.

Three orthogonal images selected from a 3D volume imaging for a mouse hind-leg: (A) a coronal view showing a cross-sectional view of leg muscles, (B) sagittal view and (C) axial view. The segmented regions, colored in blue, for muscle volume measurements were overlaid on the three images with bones and skin layers excluded.

Figure 6. One example ^{31}P MR spectrum acquired for a mouse hind-leg muscle at rest. A synthetic reference signal (or pseudo-signal) was injected during the acquisition of *in vivo* signals.

Table 1

Coil coupling measured for the two solenoid dual tuned coils.

RF input	Location of pick-up loop	Coupling (dB)	Coupling difference, ΔC (dB)	Relative sensitivity, κ	Flip angle in inactive coil, θ (°)	Relative signal received by active coil, η (%)
Left coil	Left coil	-4.9	20.6	115	0.78	0.01
	Right coil	-25.5				
Right coil	Right coil	-6.1	17.0	50.1	1.80	0.06
	Left coil	-23.1				

Table 2

Muscle volumes of left legs for five mice measured by 3D MRI.

Mouse number	Volume (μL)
1	241.6
2	223.4
3	223.4
4	238.8
5	173.8

Table 3Concentrations of ^{31}P metabolites compared to other works

	Pi (mM)	PCr (mM)	ATP (mM)
Marcinek <i>et al.</i> (33)	3.83 ± 0.40	$\sim 30^*$	8.23 ± 0.30
Siegel <i>et al.</i> (34)	3.11 ± 0.17	28.90 ± 2.56	8.00 ± 0.92
This study	10.9 ± 5.8	30.5 ± 4.1	11.8 ± 3.5

* Value estimated from the graph presented in the literature.