In vivo imaging of phosphocreatine with artificial neural networks

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Phosphocreatine (PCr) plays a vital role in neuron and myocyte energy homeostasis. Currently, there are no routine diagnostic tests to noninvasively map PCr distribution with clinically relevant spatial resolution and scan time. Here, we demonstrate that artificial neural network-based chemical exchange saturation transfer (ANNCEST) can be used to rapidly quantify PCr concentration with robust immunity to commonly seen MRI interferences. High-quality PCr mapping of human skeletal muscle, as well as the information of exchange rate, magnetic field and radio-frequency transmission inhomogeneities, can be obtained within 1.5 min on a 3 T standard MRI scanner using ANNCEST. For further validation, we apply ANNCEST to measure the PCr concentrations in exercised skeletal muscle. The ANNCEST outcomes strongly correlate with those from 31P magnetic resonance spectroscopy (R = 0.813, p < 0.001, t test). These results suggest that ANNCEST has potential as a cost-effective and widely available method for measuring PCr and diagnosing related diseases.

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Phosphocreatine (PCr) is a high-energy phosphate compound that is abundant in muscle and brain and used by creatine kinase isoenzymes to generate adenosine triphosphate from adenosine diphosphate. PCr plays a vital role in cellular energy buffering and energy transport, particularly in tissues with high and fluctuating energy demands, such as skeletal muscle, cardiac muscle, and brain. The measurement of PCr provides a unique way to achieve insight into cellular energetics, and has shown great potential in many areas, such as for evaluating mitochondrial function in vivo, and for identifying peripheral arterial disease and heart failure. PCr concentrations are reduced in several neurodegenerative and muscle diseases. To date, phosphorus-31 magnetic resonance spectroscopy ($^{31}$P MRS) has been the established method for noninvasively detecting and quantifying PCr in vivo. In addition to PCr measurement, $^{31}$P MRS also provides information about pH, inorganic phosphate, and adenosine phosphates (ATP, ADP, and AMP) in tissue. In practice, $^{31}$P MRS is most commonly applied to monitor the time and adenosine phosphates (ATP, ADP, and AMP) in tissue. In peripheral arterial disease and heart failure, PCr concentrations are not used in clinical $^{1}$H MRI scanners, the signifi cation method needs to be robust against the field strengths used in the clinic (1.5 T and 3 T), at which the frequency shifts between the exchangeable protons and the water resonance, as well as the contrast-to-noise ratio of the CEST signal, are reduced. Currently, no prior studies have yet been performed to experimentally evaluate human PCr mapping on clinical MRI systems. To explore and develop high-quality PCr mapping using CEST for clinical practice at low field strengths, optimization of the PCr CEST acquisition is required, and the quantification method needs to be robust against the inevitable static magnetic field ($B_{0}$) and radio-frequency transmit field ($B_{1}$) inhomogeneities, as well as interference from other saturation transfer components in tissues.

Artificial neural networks (ANNs) are increasingly used in many diverse areas to successfully extract relevant features from extremely large, annotated data sets, and utilize them to create predictive tools based on patterns hidden inside. Once trained, ANNs can apply the learned knowledge to analyses of other data and/or solve task-specific problems. In this study, we demonstrate that ANNs can be used for CEST quantification, dubbed hereafter as ANNCHEST. More specifically, we show that this trained neural network can accurately and simultaneously predict multiple important properties, including metabolite concentration, the exchange rate of the exchangeable protons, and $B_{1}/B_{0}$ homogeneity information, with just the simple input of a Z spectrum (water saturation transfer spectrum) for each image volume element (voxel). After first training and validating ANNCHEST using numerical simulations and PCr phantom data at 3 T, we optimize the PCr CEST acquisition to obtain maximum PCr contrast on human skeletal muscle, and again train and apply ANNCHEST. We then show the feasibility of applying ANNCHEST to simultaneously quantify the PCr concentration of human skeletal muscle, the exchange rate of the guanidinium protons from PCr, and the $B_{0}$ and $B_{1}$ maps on a clinical 3 T MRI scanner. As additional validation, the PCr depletion and recovery in exercised human skeletal muscle were detected and quantified by ANNCHEST, and the results were compared with those from $^{31}$P 2D MRS. We also discuss the potential applications of ANNCHEST as well as its advantages and limitations. The results suggest that the exchangeable guanidinium protons of millimolar concentration PCr can be exploited to detect it via the water signal in MRI with greatly enhanced sensitivity (molar signal) using CEST MRI, and its concentration can be quantified using ANNs.

Results

Validation of ANNCHEST. We implemented ANNCHEST with a feed-forward neural network as shown in Fig. 1a. The neural network was trained using Z spectra generated by the Bloch–McConnell equations for various concentrations and exchange rates of exchangeable protons at multiple offset frequencies, and for spatially varying $B_{1}$ and $B_{0}$. Gaussian white noise was added to the training Z spectra to mimic the real situation (Fig. 1b). Numerical simulations were performed to validate that ANNCHEST can accurately and simultaneously predict metabolite concentration, exchange rate of exchangeable protons, and $B_{0}$ with the simple input of a Z spectrum per voxel. The simulated Z spectra were generated based on a PCr phantom model at 3T containing two CEST peaks at 1.95 ppm and 2.5 ppm as shown in Supplementary Fig. 1d–f. The exchange rate ratio between 1.95 ppm and 2.5 ppm was set to 1:2.19 according to measures obtained in a PCr phantom at 37 °C using an inversion recovery technique on a 17.6 T NMR spectrometer (see Supplementary Section 2 and Supplementary Fig. 2). The number of hidden layers was optimized to avoid the risk of overfitting (Fig. 1c), and the initial findings suggested that seven hidden layers can provide adequate predicted capacity, because the improvement of performance with more hidden layers was less than $5 \times 10^{-4}$. Therefore, seven hidden layers were used in this study unless otherwise specified. The statistical analyses of the training results are given in the Supplementary Section 3. To test the performance of the trained neural network for quantifying new data, we generate new Z spectra pixel by pixel based on the ground-truth maps shown in Fig. 1d–f. To give a comparison to ANNCHEST, Bloch equation fitting was also performed. The quantification results and statistical analysis in Fig. 1g–n show that ANNCHEST can yield better fidelity to ground truth compared with Bloch equation fitting, especially for the quantification of exchange rate and $B_{0}$ in regions with low concentration (detailed values are listed in Supplementary Table 1). It should be noted that ANNCHEST is completed within 2 s on a personal computer (Intel i5-6300U CPU with 8 G memory), while the Bloch fitting requires around 18 h on a cluster computer with 8 parallel computing (AMD Opteron 6100 8-core CUP with 16 G memory).

The same neural networks were applied to quantify the Z-spectra obtained from PCr phantoms, and the results are shown in Fig. 2. An excellent correlation ($R = 0.9989$) was observed between the ground truth and predicted phantom PCr concentration. The related Bland–Altman analysis of concentration is shown in Fig. 2f. The exchange rates obtained by ANNCHEST were consistent with those obtained using an inversion recovery approach ($260 \pm 40$ Hz, mean ± s.d.) (detailed values are listed in Supplementary Table 2). The predicted $B_{0}$ map (Fig. 2e) showed a strong correlation ($R = 0.9969$) with that obtained by water
saturation shift referencing (WASSR) MRI\textsuperscript{16}, as illustrated in Fig. 2h.

**Optimize PCr CEST contrast in vivo on human skeletal muscle at 3 T.** Our previous study on mouse skeletal muscle at 11.7 T showed that PCr has two distinct CEST peaks around 1.95 ppm and 2.5 ppm, and that the 1.95-ppm peak also contains contributions from Cr and protein guanidinium protons\textsuperscript{17}. However, the CEST line shapes can be significantly different at lower fields, especially in vivo, where strong magnetization transfer contrast (MTC) exists. Here, CEST experiments with different saturation powers and saturation lengths were performed on human skeletal muscle to experimentally evaluate the PCr contrast on a 3 T clinical system. Due to the presence of MTC, the Z spectra of human skeletal muscle are quite different from those obtained on PCr phantoms (see Supplementary Fig. 1). From the optimization results for saturation powers ranging from 0.2 µT to 0.8 µT (Fig. 3a–d), the Z spectrum obtained with a relatively low
Fig. 1 Validation of ANNCEST with numerical simulation. a Diagrammatic representation of the feed-forward artificial neural network used in this study. This network comprises three layers: an input layer, fully connected hidden layers, and an output layer. The input of the neural network is the intensity of the Z spectrum at different frequency offsets, and the outputs are the predicted values of metabolite concentrations, exchange rates of exchangeable protons, and $B_0/B_0^*$. b Representative Z spectra of PCr phantom generated by three-pool Bloch–McConnell equations. Z spectra from 0.5 to 4.0 ppm were sampled with a 50-saturation offset over equal intervals. Gaussian white noise with a standard deviation of 0.35% and $B_0$ inhomogeneity offsets were added to the Z spectra. The saturation power and length were set to 0.6 $\mu$T and 10 s, respectively. c The performance of neural networks as a function of the number of hidden layers. The error bar was obtained by repeating the neural network training five times. d–f The ground truth maps of concentration, exchange rate at 2.5 ppm, and $B_0$ for generating validated Z spectra. The matrix size of maps is 256 × 256. The maps of concentration, exchange rate, and $B_0$ obtained by Bloch equation fitting (g, h) and ANNCEST (i–l), respectively. m, n Quantified concentrations and exchange rates, respectively. The bar and error bar indicate the mean value and standard deviation across each phantom, respectively ($n = 4523, 3655, 2770, 2610,$ and 2790 pixels for phantom numbers 1–5, respectively).

Fig. 2 Validation of ANNCEST at 3 T (preclinical MRI) on a phantom consisting of test tubes with different concentrations of PCr. All experiments were performed at 37 °C. a The arrangement of the PCr phantoms with different concentrations. b Representative Z-spectra extracted from one pixel of each of the PCr phantoms. The ANNCEST-predicted concentration (c), exchange rate at 2.5 ppm (d), and $B_0$ maps (e) for the CEST experiments collected using a 10-s saturation pulse of 0.6 $\mu$T. The neural network is identical to the one used in numerical simulation. f Bland–Altman plot for the predicted concentration and ground truth. g The exchange rate quantification results. The bar and error bar indicate the mean value and standard deviation across each phantom, respectively ($n = 579, 473, 508, 587,$ and 566 voxels for tubes with concentration from 10 mM to 50 mM, respectively). The ground truth of exchange rate (260 ± 40 Hz) was obtained using inversion recovery technique as specified in Supplementary Materials. h Bland–Altman plot for the predicted $B_0$ map and referenced $B_0$ map obtained via WASSR method.

saturation power of 0.2 $\mu$T exhibits two discernible CEST peaks around 2.5 ppm and 1.95 ppm. With the increase in saturation power, the observed CEST signal ($\Delta Z$) at 2.5 ppm increases at first and then decays after reaching a maximum at 0.6 $\mu$T. The CEST peak at 2 ppm is indiscernible from the Z spectra with the saturation power larger than 0.4 $\mu$T due to the MTC scale-down effect. The saturation length was optimized with a fixed saturation power of 0.6 $\mu$T. From the quantitative analysis shown in Fig. 3i, a CEST protocol with a saturation length of 800 ms yields the maximum $\Delta Z$. Therefore, a saturation power of 0.6 $\mu$T and a saturation length of 800 ms were applied in the remaining studies unless otherwise specified.
the same slice obtained by the established dual-echo and DREAM methods, respectively.

medial (GM), soleus (SOL), tibialis anterior (TA), and peroneus (P).

exchange rate (\( \Delta Z \)) indicating the difference between CEST peak and fitted background at 2.5 ppm is used as the evaluation parameter. a-d Acquired data and fitted results of CEST experiments with fixed saturation length of 800 ms and four saturation powers. e, f Acquired data and fitted results of CEST experiments with fixed saturation power of 0.6 \( \mu \)T and different saturation lengths. h, i Quantitative analysis of \( \Delta Z \) with different saturation powers and saturation lengths, respectively (n = 3 ROIs). Error bars show standard deviations in ROIs.

Fig. 3 Optimization of PCr CEST contrast in human skeletal muscle on a 3 T clinical scanner. The Z spectra were fitted using a polynomial and Lorentzian line-shape fitting (PLOF) CEST method. The observed CEST signal (\( \Delta Z \)) was used as the evaluation parameter. a-d Acquired data and fitted results of CEST experiments with fixed saturation length of 800 ms and four saturation powers. e, f Acquired data and fitted results of CEST experiments with fixed saturation power of 0.6 \( \mu \)T and different saturation lengths. h, i Quantitative analysis of \( \Delta Z \) with different saturation powers and saturation lengths, respectively (n = 3 ROIs). Error bars show standard deviations in ROIs.

Fig. 4 PCr mapping on human skeletal muscle using ANNCEST. a A high-resolution T2 weighted anatomy image with segmentation of gastrocnemius medial (GM), soleus (SOL), tibialis anterior (TA), and peroneus (P). b Concentration map obtained using the PLOF method. The PCr concentration (c) and exchange rate (d) maps together with the \( B_0 \) (e) and \( B_1 \) (f) maps obtained by the ANNCEST method using the CEST images acquired with 0.6 \( \mu \)T saturation power and 800 ms saturation length. The \( B_0 \) map was normalized by the reference power 0.6 \( \mu \)T = 100%. Reference \( B_0 \) (g) and \( B_1 \) (h) maps of the same slice obtained by the established dual-echo and DREAM methods, respectively.

High-quality PCr mapping on human skeletal muscle using ANNCEST. To train the in vivo network, Z spectra of human skeletal muscle at 3 T were generated assuming the experimentally verified situation of a single PCr CEST peak at 2.5 ppm and an additional broad background signal to account for the contributions from MTC and other metabolites that do not show distinct CEST peaks on the Z spectrum. Maps of concentration, exchange rate, \( B_0 \) and \( B_1 \) obtained by applying the newly trained neural network to the in vivo data are shown in Fig. 4c-f. The previously published polynomial and Lorentzian line-shape fitting (PLOF) method was performed for comparison. The results showed that the concentration map obtained by PLOF was degraded due to inherent \( B_0 \) and \( B_1 \) inhomogeneities as indicated by the white arrow (Fig. 4b), while the concentration map
obtained by ANNCEST shows much better robustness against $B_0$ and $B_1$ inhomogeneities. The quantified PCR concentrations of gastrocnemius medial (GM), soleus (SOL), tibialis anterior (TA) and peroneus (P) over five volunteers were $31.9 \pm 2.0$ mM, $31.7 \pm 3.3$ mM, $30.8 \pm 4.1$ mM, and $30.9 \pm 3.9$ mM, consistent with previous total muscle reports ($29 - 36$ mM$^{19,20}$). The quantified exchange rate ($164 \pm 36.8$ Hz across muscle) is consistent with a previously reported value ($140 \pm 50$ Hz$^{21}$). The quantitation of the metabolites and proteins that form the Z-spectrum is challenging. Here, ANNCEST provides a new dimension for CEST quantification since the PCR contrast in vivo is around 1% (Fig. 3h, i). The quantitation of the metabolites and proteins that form the Z-spectrum is challenging in CEST studies due to the concurrence of the CEST signals from solid-like macromolecules and mobile proteins, as well as the water direct saturation. Many techniques have been proposed for quantification in CEST studies. The most common one is the asymmetry analysis method, i.e., subtracting images acquired at two symmetric offsets with respect to the water resonance, which has been used for quantifying various metabolites$^{29-31}$. Some other methods such as the Lorentzian fitting method$^{32}$ and the rotating frame relaxation theory$^{33,34}$ have also been proposed for quantification. In most of the CEST quantification methods, the contribution of the CEST signal in the Z-spectrum was usually simplified to a linear function and all contributions were assumed to be superimposed linearly; however, these simplified models usually only work in certain situations, such as with weak saturation powers. CEST quantification is further complicated by the inevitable $B_0$ and/or $B_1$ inhomogeneities, which can shift or distort the in vivo CEST Z-spectra. Usually, $B_1$ and $B_0$ maps need to be collected and included in the CEST quantitation modeling, which not only lengthens the CEST experimental time but also makes the quantification of CEST more challenging. Here, we present the first evidence that ANNs can be used for quantifying in vivo CEST signal. The initial idea of this study was inspired by the work of Bo Zhu et al.$^{14}$, who demonstrated that conventional image reconstruction methods can be replaced by ANN-based methods with much-improved performance. Similar to image reconstruction, the CEST quantification is a kind of inverse problem, where the useful information (e.g., concentration and exchange rate) needs to be decoded from the acquired data (i.e., the intensities in a Z-spectrum). The encoding process of CEST MRI can be well described by the Bloch–McConnell equations and a training Z-spectrum can be easily generated with known parameters. However, due to the complexity of the Bloch–McConnell equations, an accurate solution is hard to derive especially with the presence of possible $B_0$ and $B_1$ effects, which means decoding quantitative concentrations and exchange rates from Z-spectra is challenging. Here, ANNCEST provides a new dimension for CEST quantification. A fully connected feed-
forward neural network with enough neurons in its hidden layer is known to be good at finding patterns behind one-dimensional data\textsuperscript{35}, which is well suited for CEST quantification. Instead of needing to derive the solution of the Bloch–McConnell equations, we train the ANN with a large annotated dataset to extract the relationship between the Z-spectrum and quantifiable parameters. As shown in Supplementary Section 4, the depth, width, and offset of in vivo PCr peak are related to concentration, exchange rate, and $B_0$ introduced frequency shift, respectively, while $B_1$ variation affects overall Z-spectral background intensity (Supplementary Fig. 5d). These effects can be fully exploited by ANN and applied to simultaneously quantify these parameters (Supplementary Figs. 3 and 4). The ANNC\textsuperscript{E}ST approach proposed here provides an additional dimension for CEST quantification. Similar to other artificial intelligence methods, ANNC\textsuperscript{E}ST is a data-driven quantification method and its accuracy highly depends on the training data. In this study, the training data were generated using the Bloch–McConnell equations with consideration of $B_0$ and $B_1$ inhomogeneities and noise. The relatively homogeneous $T_1$ and MTC across the human skeletal muscle benefit the generation of training data\textsuperscript{24,36,37}. With optimal saturation parameters, training Z-spectra within a limited spectral range (1.3 ppm–3.5 ppm) can be generated using a three-pool Bloch McConnell simulation, namely water protons, PCr guanidinium protons, and background. The background including the contributions from MTC and all other metabolites can be well represented by a single pool (Supplementary Fig. 5e). The discernible PCr guanidinium CEST peak in vivo provides a unique opportunity for ANNC\textsuperscript{E}ST to learn the relationships between the Z-spectrum and PCr concentration, exchange rate, and $B_0$ and $B_1$ (Supplementary Sections 3 and 4), and to apply the learned knowledge to simultaneously quantify these multiple parameters, as demonstrated in Figs. 4, 5. Previous studies\textsuperscript{2,37,38} have shown that the pH, $T_1$, and $T_2$ can change after exercise. We included the effects of pH and $T_2$ on training data by adopting varying exchange rates and $T_2$ values. In addition, we tested the performance of ANNC\textsuperscript{E}ST using simulated data over a range of water and PCr proton $T_1$ and $T_2$ values (Supplementary Figs. 10 and 11). The results showed that the ANNC\textsuperscript{E}ST-determined exchange rates and concentrations are robust over a water $T_1$ range from 1.0 s to 2.0 s and a water $T_2$ range from 15 ms to 50 ms, which are the relevant ones of in vivo ranges\textsuperscript{24}. Concentrations were also very insensitive to the PCr proton $T_1$ and $T_2$. ANNC\textsuperscript{E}ST can be applied to quantify other metabolites under different situations (see Supplementary Figs. 7, 8 and 9). In the case where the Z-spectrum may be difficult to simulate using Bloch–McConnell equations, the training data can be generated by combining acquired Z-spectra with corresponding quantification results obtained through other gold standard methods. An advantage of CEST MRI for collecting adequate training data is that the size of the training data set is proportional to the number of pixels of the CEST image since each pixel has its own Z spectrum, thus providing sufficient samples and enhancing the power of the resulting data (see Supplementary Section 7).

Though ANNC\textsuperscript{E}ST can efficiently exploit the relationship between the Z spectrum and different parameters, the range and frequency offset interval of the Z spectrum and the quantifiable parameters still need to be carefully designed for the successful application of ANNC\textsuperscript{E}ST. For the numerical simulations and phantom experiments, the frequency offsets of the Z-spectra ranged from 0.5 to 4 ppm with a total offset number of 50. Within this limited Z-spectral range, $B_1$ and concentration both affect the depth of CEST peak. Therefore, when varying $B_1$ variation (0.5 $\mu$T–0.7 $\mu$T) in the training, the accuracy of concentration quantification was significantly degraded (the linear regression $R$-value of neural network training dropped from 0.99983 to 0.85054). In this study, due to the relatively small FOV of phantom experiments (30 $\times$ 30 mm$^2$), we, therefore, assumed the $B_1$ to be homogeneous across phantoms and a fixed $B_1$ was adopted in the training Z-spectra for numerical simulation and phantom
experiments. This assumption worked well, and high fidelity of PCr concentrations was obtained, as shown in Fig. 2c, f. In phantom experiments where $B_1$ inhomogeneity cannot be neglected, an additional range of Z-spectrum (e.g., the direct spectrum) was used to evaluate the neural network, where $m_{pw}$ refers to the mean squared normalized error in training data and $m_{pw}$ is a mean of the squares of the network weights and biases, and $\gamma$ is the regularization parameter (set to 0.01 in the current study). The application of $m_{pw}$ causes the network to have smaller weights and biases, and forces the network response to be smoother and less likely to overfit. As demonstrated in the early stopping constraint, the neural network training was stopped once any of the following conditions were met: (1) the calculated gradient was smaller than $10^{-7}$; (2) the mean squared normalized error on validation data was smaller than $10^{-4}$; (3) or the maximum epochs number reached $10^6$. All of the above processing was accomplished on the MATALAB platform (www.mathworks.com, version R4.0;813654).

Z spectra for neural network training. In this study, training data were generated using the Bloch–McConnell equations with consideration of various imperfect situations, such as $B_0$, $B_1$ inhomogeneity and noise. Details of the parameters used in the simulation can be found in Supplementary Table 3 for the phantom and Supplementary Table 4 for the human leg. To mimic the real situation, measurements of water $T_1$ and $T_2$ values were performed for use in this training data. However, it is important to realize that such measurements are not necessary for the ANNCEST application. The segregation of ANN in the neural network is very powerful, and the network training is robust against imperfect situations. For instance, the numerical simulation and phantom experiments, the frequency offsets of the Z-spectra ranged from 0.5 to 4 ppm with a total offset number of 50. The offsets of PCr ANNCEST peaks were set to 1.95 ppm and 2.55 ppm. The exchange rate ratio between 1.95 ppm and 2.55 ppm was set to 1:2.19 according to the measurement in a PCr phantom at 3T using a magnetization recovery measurement (see Supplementary Section 2). The $T_1$ and $T_2$ of water protons were set to 2.6 s and 1.8 s, respectively, according to the measurements on the phantom. The $T_1$ and $T_2$ values for PCr protons were set to 0.05 s and 0.02 s, respectively. The saturation power and duration were 0.6 $\mu$T and 10 s. The concentration, exchange rate at 1.95 ppm, and $B_1$ inhomogeneity were randomly chosen from the range of $8 \pm 4$ to $200$ Hz, $\pm 0.4$ to 0.01 ppm, respectively. Gaussian white noise with zero mean value and 0.0015 standard deviations was imposed on the simulated Z spectra. The number of Z-spectra used for neural network training was 10^5.

For the PCr mapping of human muscle, the frequency range of off-resonance Z-spectra was from 1.3 to 3.5 ppm, with a total offset number of 50. The CEST peak offset was set to 2.5 ppm. The $T_1$ was set to 1.2 s according to the measurement obtained on human skeletal muscle. The $T_2$ was varied from 15 to 35 ms. The $T_1$ and $T_2$ values for PCr protons were set to 0.05 s and 0.02 s, respectively. The robustness of ANNCEST against $T_1$ and $T_2$ variations of PCr protons is shown in Supplementary Fig. 11. The saturation power and duration were, respectively, 0.6 $\mu$T and 800 ms. The concentration, exchange rate, $B_0$ inhomogeneity, and $B_1$ inhomogeneity were randomly chosen from the range of $0$ to $100$ mM, $80$ to $230$ ppm, $230$ to $81$ ppm, and $0.25$ to $0.25$ ppm, respectively. Gaussian white noise with a zero mean value and 0.0035 standard deviations was imposed on the simulated Z spectra. The CEST signal was acquired in a phantom with a concentration of 8 M for the exchanging protons and an exchange rate of 30 Hz. The $T_1$ and $T_2$ values for the background signal were set to 1 s and 9.1 $\times$ 10^{-6} s, respectively. The lineshape for the background signal was a Super Lorentzian function. The goodness of background fitting using the above parameters is shown in Supplementary Fig. 5e. The number of Z-spectra used for neural network training was 10^5.

Numerical simulation. The ground truth concentration, exchange rate, and $B_0$ maps with a matrix size of 256 × 256 are shown in Fig. 1d–f. The Bloch equations were applied to simulate the Z-spectrum for each pixel. In comparison to ANNCEST, Bloch fitting was performed to quantify the concentration, exchange rates, and $B_0$. The initial values for the ranges of concentration, exchange rate at 1.95 ppm, and $B_1$ inhomogeneity were set to 15 to 35 ppm, 50 to 200 Hz, and 0 ppm to 0.4 ppm, respectively. The other parameters were the same as those used for the Z-spectra simulation. The Bloch fitting was performed at a Vicon computer cluster with eight parallel workers (AMD Opteron 6100 8-core CPU and 16 G memory).

Magnetic resonance imaging of in vitro phantom at 3 T. The phantom experiments were performed on a 3 T Bruker Biospec system (Bruker, Ettlingen, Germany). PCr phantoms with different concentrations (i.e., 10 ± 1 mM, 20 ± 1 mM, 40 ± 1 mM, 60 ± 1 mM, and 80 ± 1 mM) were prepared. Phantoms were prepared in phosphate-buffered saline (PBS) titrated to pH 7.3 ± 0.1. All samples were studied in 10 mm glass tubes. A continuous wave saturation module with a saturation power of 0.6 $\mu$T and a saturation time of 10 s was applied. Data were acquired using a turbo spin echo (TSE) sequence with TR/TE = 13 ± 4.5 ms, TSE factor = 12, slice thickness of 2 mm, acquisition matrix size of 32 × 32. Zero filling was applied for the Fourier transform and lead to a reconstructed image with a matrix size of 64 × 64, and a resolution of 0.47 ± 0.04 mm^2. The CEST experiments...
Correlation analysis of PCr maps obtained by ANNCHEST and 31P 2D MRS. A correlation analysis was performed to compare PCr maps obtained by ANNCHEST and gold-standard 31P 2D MRS on resting and exercised human skeletal muscle. To remove the location mismatch in the CEST images caused by motion, image registration was applied to ANNCHEST results using the Medical Imaging Registration Toolbox (MIRT). Because 31P 2D MRS is less sensitive to motion with worse spatial resolution, the ANNCHEST results were downsampled from 72 × 72 to 16 × 16 to match the matrix size of 31P 2D MRS. The downsampling map was calculated by averaging the intensity within the corresponding patch on high-quality CEST mapping. The PCr maps of baseline, during holding, and 0.75 min of recovery were chosen for comparison. Because the 31P was placed beneath the skeletal muscle and may lead to poor sensitivity to the upper-half FOV, only the regions close to the coil were chosen for correlation analysis. The pixel-by-pixel correlation analysis was accomplished using Matlab built-in function "corcoor".

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The source data underlying Figs. 1d–n, 2c–g, 3a–i, 4b–h, 5b–g are provided as a Source Data file. The other data that support the findings of this study are available from the corresponding author upon reasonable request.

Code availability
The code used in this study is provided in Supplementary Data 1. We also deposit the code in https://github.com/LinChenMRI/ANNCEST.git.

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Author contributions
L.C. and J.X. designed and performed experiments, processed data, and wrote the paper; P.V.Z. and R.W. helped with designing the study, interpreting the data, and paper editing; R.W. and M.S. helped with the 31P 2D MRS experiments and processing. K.C. and J.H. prepared the phantoms and performed the phantom MRI experiments; M.S. and Q.Q. provided pulse sequence design and technical guidance on the human subject scanning. H.L. and Z.W. helped with human experiments.

Competing interests
The authors declare no competing interests.

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