Journal of Magnetic Resonance **137,** 350–357 (1999)
Article ID jmre.1998.1683, available online at http://www.idealibrary.com on IDEAL®

Effect of Exercise on the Creatine Resonances in ^{1}H MR Spectra of Human Skeletal Muscle

R. Kreis, B. Jung, J. Slotboom, J. Felblinger, and C. Boesch

Department for Clinical Research, Unit for Magnetic Resonance Spectroscopy and Methodology, University Bern, CH-3010 Bern, Switzerland

Received July 27, 1998; revised December 7, 1998

^{1}H MR spectra of human muscles were recorded before, during, and after fatiguing exercise. In contrast to expectations, it was found that the spectral contributions of creatine/phosphocreatine (Cr/PCr) were subject to change as a function of exercise. In particular, the dipolar-coupled methylene protons of Cr/PCr were found to be reduced in intensity in proportion to the co-registered PCr levels. Recovery after exercise and behavior under ischemic conditions provide further evidence to suggest that the contributions of the CH_2 protons of Cr/PCr to ^{1}H MR spectra of human muscle *in vivo* reflect PCr rather than Cr levels. Variation of experimental parameters showed that this effect is not due to a trivial change in relaxation times. At present it can only be speculated about why the Cr resonances have reduced NMR visibility. If temporary binding to macromolecules should be involved, the free Cr concentration—important for equilibrium calculations of the creatine kinase reaction—might be different from what was previously assumed. © 1999 Academic Press

Key Words: **phosphocreatine; visibility; dipolar coupling; quantitation; compartmentation; creatine shuttle; ^{1}H MR spectroscopy; skeletal muscle; exercise; creatine.**

INTRODUCTION

The *in vivo* ^{1}H MR spectrum of human skeletal muscle has recently been shown to feature orientation-dependent dipolar splittings for several compounds (*1, 2*). In a creatine (Cr) loading study (*3*), one of these metabolites was identified as Cr and/or phosphocreatine (PCr). In muscle fibers oriented parallel to the static magnetic field B_0, the methylene group of Cr/PCr (Cr2) appears as a prominent dipolar doublet and the methyl group (Cr3) as a less well-defined triplet. The doublet was shown to disappear from the spectrum of rat muscle *post mortem* on a time scale similar to the disappearance of PCr (*4*). It has been speculated that Cr2 may therefore represent PCr, rather than total Cr (*4*). The aim of the current study was to test this hypothesis by investigating whether variation of muscular PCr content by exercise influences the spectral appearance of Cr/PCr (*5*). From numerous studies of ^{31}P MRS it is well known that the PCr level of muscle decreases with exercise and that it recovers monoexponentially under aerobic conditions, if the workload has not led to strong acidosis. ^{1}H MR spectra of human muscle were thus recorded after workloads of different intensity, during recovery, when work was performed under ischemic conditions, and also together with ^{31}P MR spectra. Earlier studies on muscle metabolism using localized ^{1}H MRS had been performed either in resting muscle while concentrating on peak assignment (*6, 7*) or lipid metabolism (*8*) or during exercise and aiming at the determination of lactate levels and pH shifts (*9–11*). The constancy of the Cr peaks had not been questioned.

RESULTS

Strenuous exercise leading to near exhaustion in a specific muscle leads to the disappearance of the CH_2 resonances of Cr/PCr in ^{1}H MR spectra. This is illustrated in Fig. 1 which contains two spectra of the same ROI located in m. tibialis anterior (TA). Trace B was recorded directly after a strong isometric workload leading to pain and fatigue, while trace A was obtained after 5 min of recovery. With exercise the Cr2 doublet has virtually disappeared and the Cr3 triplet (*3*) and possibly the TMA peak have been broadened and/or reduced, while there has hardly been any effect on X3 and the lipid resonances at 2.4 ppm. The orientation-dependent peak X3, which is currently unassigned but may be due to taurine, has been broadened only marginally. A global frequency shift of ~5 Hz due to exercise can be noted in Fig. 1. Such an unspecific shift in resonance frequency is always observed as a consequence of exercise or application of a pressure cuff. Its extent depends on the particulars of the exercise.

If at the end of exercise the fatigued muscle remains ischemic, the ^{1}H MR spectra do not recover to their pre-exercise appearance; i.e., the Cr2 doublet remains depleted until reperfusion (not shown). This fact was used to test whether the spectral alterations are related to changes in longitudinal or transverse relaxation times by recording spectra with different acquisition parameters (TR, TE) after exercise and under ischemic conditions. Figures 2 and 3 contain results obtained for variations of TR and TE, respectively. At rest, reduction of TR from 4.0 to 1.5 s led to an almost identical decrease in signal amplitude for Cr3 and TMA, while Cr2 is less affected which is in accordance with reported differences in T_1 (*2*). After exercise in ischemia, a very similar pattern was observed.

1090-7807/99 $30.00
Copyright © 1999 by Academic Press
All rights of reproduction in any form reserved.

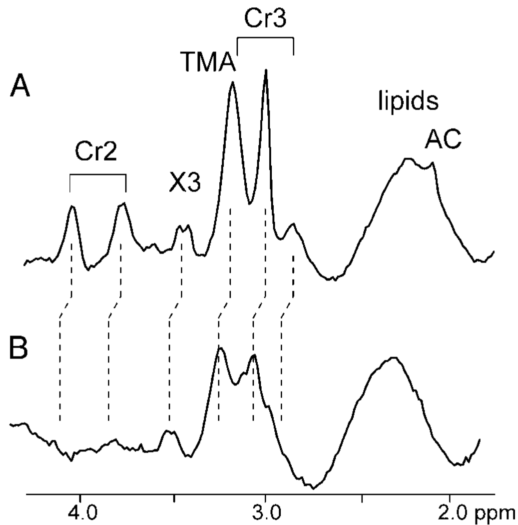

FIG. 1. Effect of fatiguing exercise on the localized ^{1}H MR spectrum of skeletal muscle. Trace A contains a ^{1}H MR spectrum of m. tibialis anterior at rest (after recovery) and B the corresponding spectrum after exhausting exercise in ischemia. Methylene (Cr2) and methyl (Cr3) protons of Cr/PCr appear as a dipolar-coupled doublet and triplet, respectively (*3*). Cr2 disappears as a result of exercise, while Cr3 changes its spectral pattern in a more complicated way. X3 remains unchanged, except for a global frequency shift of 4.7 Hz toward lower field. The trimethylammonium (TMA) peak consists to a large part of carnitine, X3 is unassigned, and AC has been attributed to acetylcarnitine (*30*). (PRESS, TE 20 ms, TR 1.5 s, 16 acquisitions, 5.4-cm^3 ROI, 45-year-old male subject, isometric work against a constant force.)

Thus, variation of TR does not have any strong effect on the extent of exercise-related signal changes in Cr/PCr.

To demonstrate the effects of a TE variation, the workload was adjusted such that it led to partial Cr2 depletion for the shortest TE. (In case of a complete signal reduction at short TE, further signal reduction with increasing TE would not be observable.) At rest, signal behavior for TA parallel to B_0 was as expected (*2*). The Cr2 doublet shows increasing dispersion character with increasing TE and refocuses at ~120 ms (more striking when comparing with intermediate TE spectra which are not shown). After exercise, Cr2 is reduced to about the same extent at short TEs and again refocuses for a TE of ~120 ms. The apparent T_2 may be somewhat smaller after exercise (on the order of 70 vs 110 ms in the case presented, but unchanged within the error of the measurement in other cases). The Cr3 triplet is even more difficult to analyze. Its shoulder at 2.8 ppm appears to behave somewhat differently under the two physiologic conditions, possibly indicating a change in the effective coupling constant. The other dipolar-coupled resonances also feature nonmonoexponential signal decay with TE. This is striking for X3 when comparing its appearance at TE 40 with that at TE 10 and 120 ms. This behavior is not affected by exercise.

To test whether the signal reduction of Cr2 depends on the extent of work performed, spectra were recorded from the same ROI after different periods of isometric exercise. The middle panel of Fig. 4 contains a set of ^{1}H MR spectra obtained at rest and after 11 to 40 s of work. The intensity of the Cr2 doublet diminishes with increasing workload. Similarly, the peak height of the central Cr3 peak decreases in parallel. The outer line of the Cr3 triplet appears to broaden and to move inward. X3 is largely unchanged. To correlate these spectral alterations with the energy state of the muscle, ^{31}P MR spectra of a larger ROI in the same muscle were recorded immediately following ^{1}H MRS under conservation of the PCr/Cr ratio by ischemia. The corresponding ^{31}P MR spectra are displayed in part I of Fig. 4. As expected, the PCr level decreases with increasing workload, while the P_i peak increases and shifts to smaller ppm values (pH of 6.9 ppm with the strongest workload). For the case presented, numerical evaluation yielded an almost perfect correlation between the Cr2 peak areas in ^{1}H MRS and the PCr levels as determined from the ^{31}P MR spectra ($r^2 = 0.98$; $p = 0.001$).

This experiment was performed in six subjects and evaluation of peak areas obtained with exercise relative to signals at rest showed a highly significant correlation between PCr levels and Cr2 ($r = 0.82$, slope $= 0.85$, $p \ll 0.001$; offset 15%, $p = 0.08$). This is illustrated in Fig. 5A.

For the methyl protons of Cr/PCr the correlation depends on

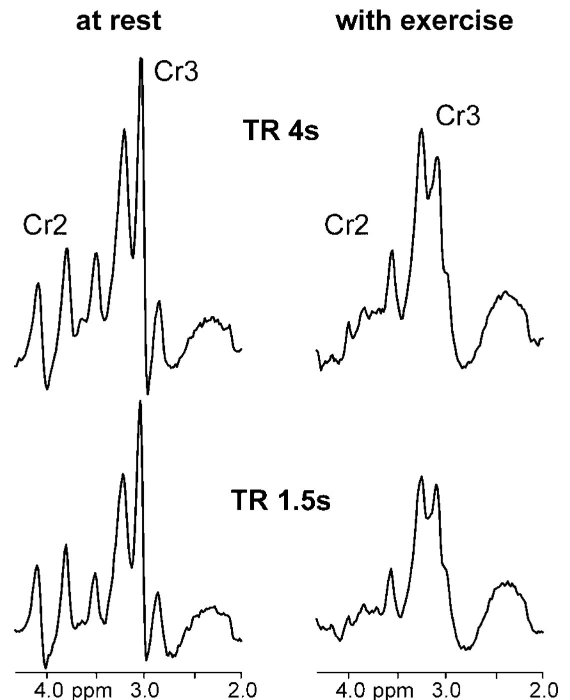

FIG. 2. Influence of TR variation upon the exercise-related signal changes. Localized ^{1}H MR spectra at rest and after exercise (left vs right) recorded with different repetition times (top vs bottom) in the same session while PCr recovery was inhibited by ischemia. The Cr/PCr resonances are altered to the same extent irrespective of acquisition parameters. (PRESS, TE 20 ms, TR 4.0 vs 1.5 s, 16 acquisitions, 7.4-cm^3 ROI in TA, 33-year-old male subject, corrected for a global frequency shift, isometric work against a constant force.)

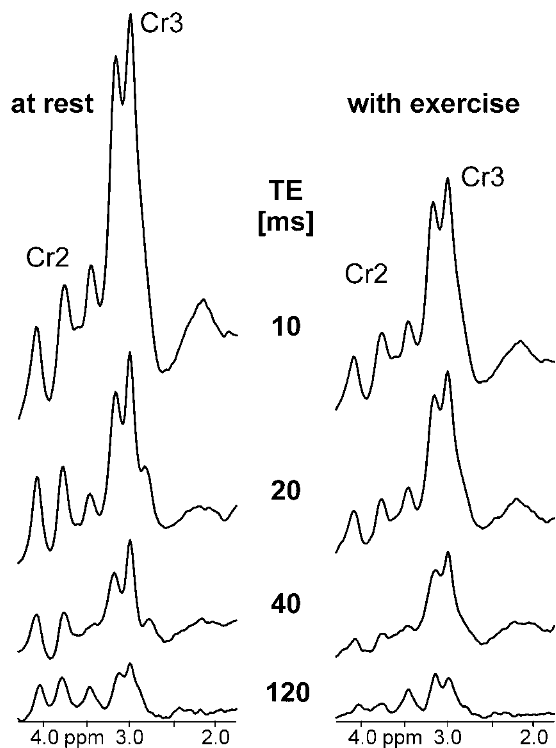

FIG. 3. Influence of echo time upon the exercise-related signal changes. Localized ^{1}H MR spectra at rest and after exercise recorded with different TE (top to bottom) in the same session while PCr recovery was inhibited by ischemia. The Cr2 peaks are reduced to a similar extent irrespective of TE; T_2 may change a little with exercise, but certainly not enough to explain the depletion at short TE. The Cr3 triplet appears to show a slightly different evolution due to an altered dipolar coupling constant. (STEAM, TE 10 to 120 ms, TR 1.26 s, 32 acquisitions, 8.8-cm^3 ROI in TA, 33-year-old male subject, isometric work against a constant force.)

the model lineshape used for fitting. If only the central peak is evaluated one arrives at a significant correlation with PCr levels ($r = 0.55$; slope: 0.20 ± 0.05, $p = 0.001$; offset: 0.80 ± 0.04, $p \ll 0.001$); however, if Cr3 is modeled by a triplet with equal outer lines the total peak area appears to remain constant ($r = 0.15$; slope: -0.05 ± 0.07, $p > 0.1$; offset: 1.03 ± 0.05, $p \ll 0.001$), independent of exercise, as shown in Fig. 5B.

Panel III of Fig. 4 yields evidence for the claim that T_2^* does not change much with increasing exercise intensity. An excerpt of the ^{1}H MR spectra including the Cr2 doublet was scaled individually for each exercise level to its size in the resting spectrum in order to demonstrate that the linewidth of the residual Cr2 peaks stays essentially constant. This was verified for eight subjects (two to five workloads each). Some line broadening (Gaussian, rather than Lorentzian) was observed in about half of the cases.

The recovery of ^{1}H MR spectra after exercise is demonstrated in Fig. 6. Part A contains four spectra obtained at different times during recovery after 3 min of isometric exercise. The first spectrum after exercise (bottom trace) again documents the disappearance of Cr2 and the outer line of the Cr3 triplet due to exercise, while X3 remained constant. The top spectrum recorded after 4 min of recovery is very similar to the pre-exercise spectrum. Difference spectra with respect to the recovered state reveal the spectral alterations (Fig. 6B). Cr2 and Cr3 show doublet and triplet character in the difference spectra.

The dynamics of recovery are displayed in Fig. 7, where the spectral changes in the difference spectrum are plotted against time. The patterns of Cr2 and Cr3 in the difference spectrum were modeled as dispersive doublet and triplet, respectively. The resulting data were fitted to monoexponential decays, yielding recovery constants of 0.016 and 0.027 s^{-1}, respectively. For Cr3, this time constant represents changes in lineshape rather than area, because the total area for Cr3 obtained by modeling of the Cr3 triplet without taking a difference does not change much (Fig. 5B) and does not yield such a monoexponential decay. Determination of the PCr recovery constant with ^{31}P MRS in the same muscle of the same subject, but at a later stage, yielded 0.025 s^{-1}.

DISCUSSION

In contrast to expectation, the localized ^{1}H MR spectrum of human muscle is strongly affected by exercise. The spectral appearances of both the methylene and the methyl protons of Cr/PCr change with exercise. Exercise or application of a pressure cuff also leads to a global shift in resonance frequency of up to 0.1 ppm (Fig. 1) and may be due to deoxymyoglobin and/or deoxyhemoglobin acting as a shift reagent (*12*).

The doublet due to Cr2 (*3*) almost vanishes with strong exercise (Figs. 1, 2, and 6), while the changes of the Cr3 triplet are less straightforward. The central line appears to broaden and possibly shrink, and the outer lines (X4 in earlier notations (*1*)) seem to move inward. The resulting difference spectra between spectra in recovered and exercised states consist of a doublet for Cr2 and a triplet with strong negative antiphase character for Cr3 (Fig. 6). The doublet corresponds to the *in vivo* spectral pattern attributed to the methylene protons in Cr/PCr as obtained from oral Cr supplementation (*3*). The difference spectra for Cr3 between rest and exercise feature stronger negative lobes than observed for Cr loading. This may arise because a broadened line is subtracted from a narrower line.

The severity of these changes scales with the workload performed as seen from Fig. 4 II. In particular, the Cr2 doublet vanishes gradually with an increase in workload. Relating the area under the Cr2 doublet to the level of PCr as determined with ^{31}P MRS in the same muscle yields a highly significant linear correlation (Fig. 5A). However, it should be cautioned that such quantitative comparisons of the Cr2 and PCr areas are difficult, because the small Cr2 peaks are superposed on an ill-defined baseline and can only be quantitated using prior knowledge constraints. In the current evaluation, where the

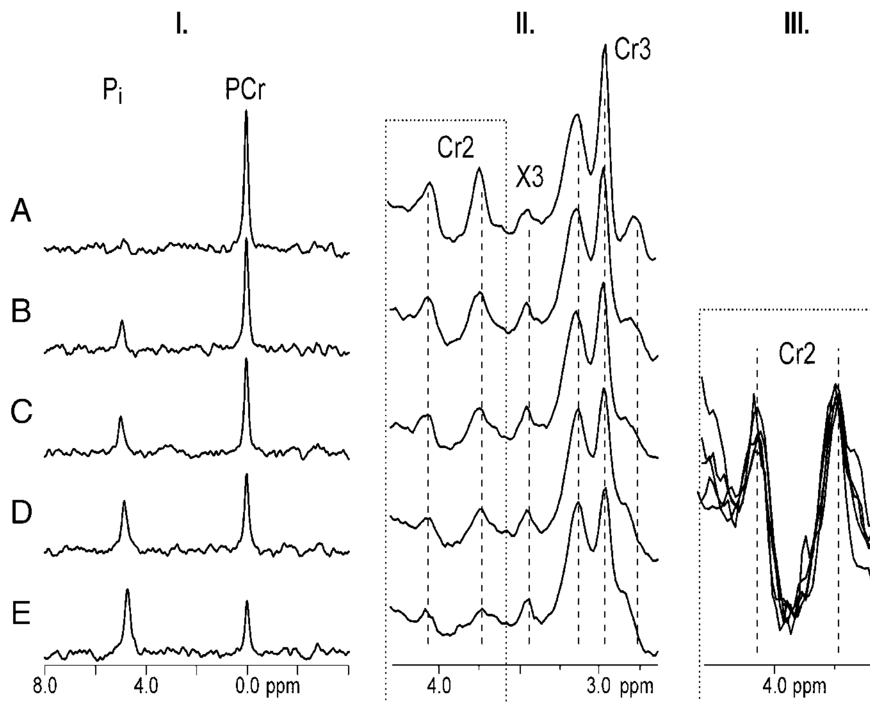

FIG. 4. ^{1}H and ^{31}P MR spectra of human skeletal muscle as a function of increasing workloads (A to E: 0, 11, 21, 30, and 40 s of isometric exercise in ischemia, 30-year-old male subject). (I) ^{31}P spectra of TA acquired with a PRESS sequence (TE 20 ms, TR 6 s, 20 cm^3) display the expected behavior of decreasing PCr and increasing P_i levels. (II) The ^{1}H MR spectra from the same muscle (10.8 cm^3 centered at the same position, PRESS, TE 20 ms, TR 1.5 s) show a decrease in Cr2 proportional to the decrease in PCr. While X3 remains unchanged, the outer line of the Cr3 triplet moves inward with increasing load. (III) An excerpt of the ^{1}H MR spectra (dotted rectangle in II) is scaled line by line to enable a comparison of lineshape for the Cr2 doublet. The residual Cr2 peaks appear not to be subject to large changes in T_2^*.

peaks were modeled as Voigt lines (*13*), Cr2 areas were fitted assuming that the Lorentzian linewidths of most fitted metabolite peaks (Cr2, X3, TMA, center peak of Cr3, but not the outer lines of the Cr3 triplet) stay constant with exercise and that the Gaussian widths vary in parallel. Judging from the T_2 and lineshape analyses (Figs. 3 and 4) these assumptions are

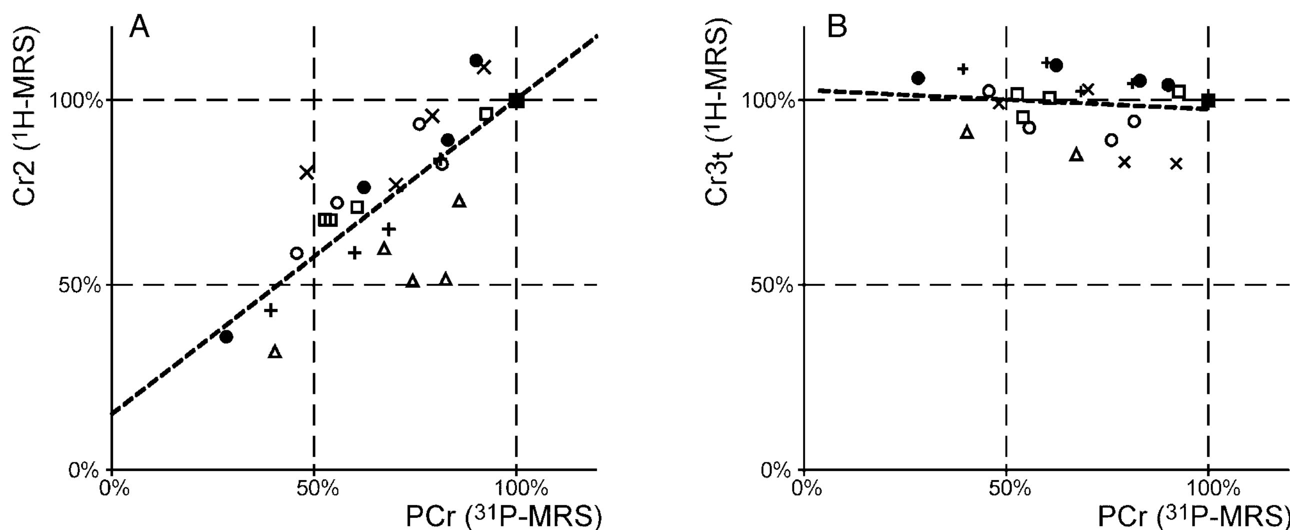

FIG. 5. Correlation between Cr/PCr signals from ^{1}H and ^{31}P MRS fitted from spectra obtained after different workloads in ischemia. (A) Cr2 (area of doublet) vs PCr; (B) $Cr3_t$ (total area of Cr3 modeled as symmetric triplet) vs PCr. Data from rest spectra were set to 100% for each subject. Different symbols identify different subjects; dashed lines represent linear regression lines ($p \ll 0.05$ for Cr2, $p > 0.5$ for $Cr3_t$). (Three data points of one subject were omitted from B because of obvious misfit of the Cr3 triplet.)

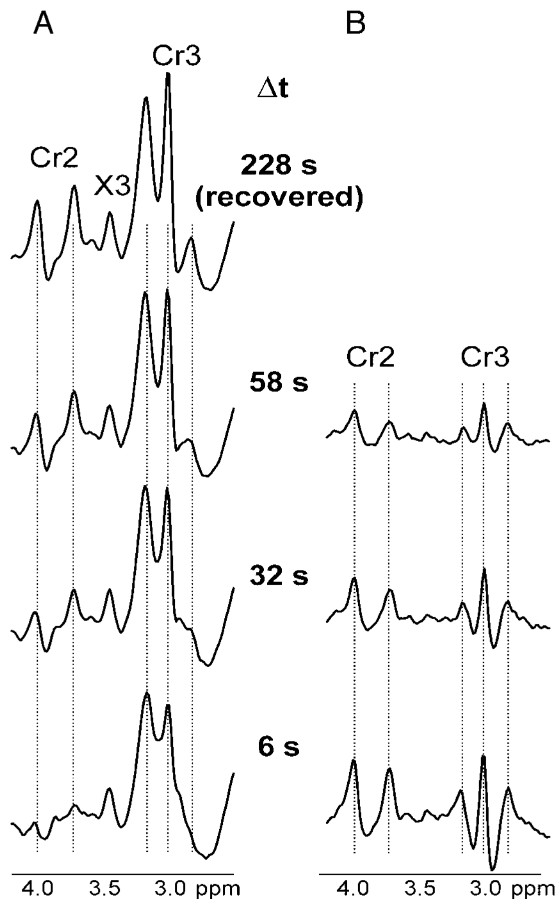

FIG. 6. Recovery of a localized ^{1}H MR spectrum of TA as a function of time after end of exercise: (A) original (frequency-adjusted) spectrum; (B) difference spectra with respect to the recovered spectrum in the top row. The difference spectra consist of a doublet for Cr2 and a triplet-like signal for Cr3. (PRESS, TE 20 ms, TR 1.6 s, eight acquisitions/spectrum, 8.0-cm^3 ROI, 34-year-old male subject, isometric work against a constant force, indicated times represent the middle of the eight acquisitions.)

well justified in practice for short TE spectra and the given signal-to-noise, even if they are not truly fulfilled.

The ^{1}H MR spectrum recovers after exercise to the pre-exercise appearance gradually within minutes under aerobic conditions. During ischemia the ^{1}H MR spectrum remains unchanged. Initial results of quantitative evaluations for the recovery of the Cr2 areas and the Cr3 lineshapes suggest that the time course is similar to that of aerobic PCr resynthesis (k around 0.03 s^{-1}) as measured by ^{31}P MRS. Further careful experiments with parallel or subsequent recordings of ^{31}P and ^{1}H spectra are necessary to prove this relationship unequivocally.

These effects of exercise on the Cr/PCr resonances cannot be the consequence of simple artifacts, although the comparison of spectra recorded during and after exercise to those acquired at rest is susceptible to experimental influences. Motion and displacement effects upon local shim and water suppression quality have been excluded by optimization of the experimental setup (fixation of leg; improvements of ergometer and exercise regime; choice of muscle, localization sequence, and echo time) and evaluation of reproducibility in numerous studies. In this context, it is best to compare exercise spectra with spectra recorded after recovery and not to an initial pre-exercise spectrum at rest, because degradation of field homogeneity usually occurs during exercise, but not during recovery.

It was also excluded that the observed phenomena are a mere consequence of changes in relaxation times (Figs. 2, 3, 4 III). It was found that effects of exercise did not alter when repetition times were varied between 1.5 and 8.0 s, while already a T_1-related 25% signal reduction at TR 4 s would imply a highly unlikely increase in T_1 from 1 to 3 s. It could also be shown that changes in overall T_2 cannot be responsible for the disappearance of the Cr2 signal in short TE spectra, although they may contribute to signal reductions at longer TE. As an example, a drop in signal amplitude by 50% at TE 10 ms would require doubling of the observed width (say from 7 to 14 Hz for Cr2). This would imply that T_2 drops from ~100 ms at rest to 30 ms with exercise. Such a drastic T_2 change would in turn cause a 95% drop in signal at TE 140 ms, which was clearly not observed.

Of course T_2 (Fig. 3) and lineshape (i.e., T_2^*, Fig. 4 III) can be determined only on the remaining signal and not on the apparently missing signal, such that it cannot be excluded that the missing signal is in fact lost due to severe signal broadening, if there is a compartmentation of metabolites without fast chemical exchange. For Cr3 the current data suggest that the changes seen at TE 20 ms are at least partly caused by changes in T_2^* or the effective diploar coupling constant. Unlike the Cr2 doublet, the total area of the Cr3 resonance pattern does not vanish for strongly exercised muscle, but rather remains more or less constant.

The interpretation of these puzzling effects is by no means evident. The Cr signals in ^{1}H MRS have hitherto always been associated with total creatine which does not alter with exercise. The fact that Cr2 scales with exercise level and recovers

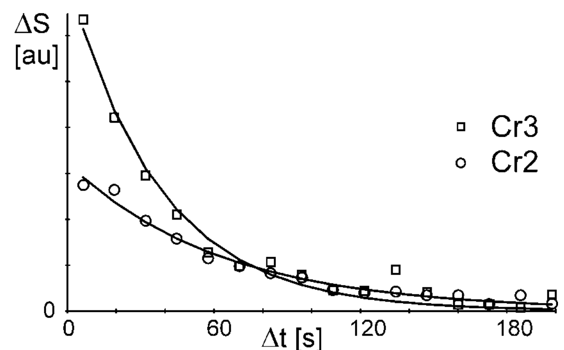

FIG. 7. Decay of the difference patterns in Fig. 6B fitted as doublet and dispersive triplet. (Cr2, sum of both peaks; Cr3, sum of all three areas.) The decays were fitted to monoexponential curves yielding time constants of 0.016 and 0.027 s^{-1}, whereas the recovery constant for PCr resynthesis determined in the same subject and muscle at a later date was 0.025 s^{-1}.

in a fashion similar to PCr can be explained in two principally different ways that will be elaborated on below. (1) Exercise alters the physicochemical conditions of the cell, inducing the observed NMR changes in both Cr and PCr; or (2) exercise is only relevant as much as it alters the relative concentrations of Cr and PCr such that intrinsic differences in the NMR properties of Cr and PCr become evident.

The first hypothesis would mean that parameters like temperature, pH, water content, deoxy- vs oxymyoglobin and hemoglobin contents, muscle fiber orientations, or cellular compartmentation would influence the NMR visibility of Cr2 and Cr3 in such a way as to yield an incidental and approximate correlation with PCr concentration. Such an explanation would also have to account for the fact that there is no smooth transition in peak shape from visible to invisibly broad or other peak shapes. This necessitates a model of physical or chemical compartmentation without chemical exchange on the NMR time scale.

Most of the effects that could lead to an incidental correlation are fairly unlikely. *A. Temperature:* Temperature changes do not respond so quickly to the start and end of exercise, nor would one expect the observed correlation with ischemia. *B. pH:* Although pH may change the resonance position of Cr2 strongly (*14*), it is unlikely that overall pH is responsible for the observed effects. pH does not recover immediately after the end of exercise, but usually continues to drop for some time. Additionally, severe effects on Cr2 are already seen with minor pH changes. One would therefore need to invoke compartmentation effects due to strongly deviating local pH values (e.g., for mitochondria). *C. Compartmentation:* The Cr/PCr pool acts as an energy buffering system to stabilize short-term energy demands. It is also involved in energy shuttling between energy producing and consuming sites (*15*). It is not very likely that the transport aspects associated with increased flux during exercise would render Cr partially invisible, because general diffusion would still dominate overall flux. However, increased flux through mitochondria—though disputed by ^{31}P MRS measurements—could provide an explanation, but it would not cover the observations *post mortem* and in ischemia. Restricted mobility in some compartments is a possibility for accounting for the residual dipolar coupling (*4*). One could speculate that this might also be involved in producing the reduced visibility of free Cr irrespective of exercise. *D. Edema:* Muscular exercise is followed by local edema as shown by MRI (*16, 17*). The time scale of this excludes it from being involved in causing the spectral changes. *E. Muscle fiber orientation:* The pennation angle of some muscles changes with contraction and could therefore induce changes in dipolar coupling constants and possibly lineshape. For TA such effects are expected to be minimal and would not occur during recovery in the relaxed muscle. *F. Oxygen saturation of hemo- and myoglobin:* It is believed that the ratio of deoxy- to oxymyo- and hemoglobin can induce a bulk shift in resonance frequency (*12*), and that oxygen tension may play a major role in the regulation of oxidative phosphorylation (*18*). In principle, shift reagents also act as relaxation agents, such that T_2 and T_2^* could be affected. However, it is difficult to see how a distinct and specific effect on the Cr/PCr resonances could ensue. A global resonance shift (including the lipid signals) was observed and could be due to changes in oxygen saturation.

The second and—in the eyes of the authors—more plausible hypothesis is that the Cr2 signals in ^{1}H MR muscle spectra represent PCr content rather than total creatine. This would also explain the disappearance of Cr2 as observed in rat muscle *post mortem* (*4*). This implies that methylene protons in unphosphorylated Cr are NMR invisible under the given experimental parameters, while the methyl protons remain at least in part visible, which could be fulfilled if Cr binds to larger molecular structures. The effective T_2 of Cr2 could then become extremely short (due to efficient motional modes or spin diffusion), while Cr3 could still be visible because of internal rotational uncoupling. Alternatively, temporary immobilization in a partially ordered state could increase the effective dipolar coupling constant by orders of magnitude which would also render the doublet invisible. The reverse reasoning, i.e., that free Cr does not experience any dipolar coupling and that only the larger and doubly charged PCr ion is subject to motional constrictions inducing the residual dipolar coupling, seems more convincing at first, but is ruled out by the fact that with this explanation there should be a central singlet to replace the doublet during exercise. This, however, is observed neither after exercise nor *post mortem.* A small peak visible at ~3.9 ppm in Figs. 2 and 6 is probably present already in spectra at rest, but is inconspicuous because of the much larger Cr2 peaks. The hypothesis of temporary binding of the Cr molecule might also explain why magnetization transfer (MT) effects are strongest for Cr (*19, 20*), and mostly unobservable for other metabolites (*21*). With this background, it would even be possible that MT effects due to water presaturation could in principle produce the apparent disappearance of Cr2 with exercise.

Considering the fact that Cr and PCr are in fast equilibrium via creatine kinase, it is not strictly correct to speak of completely separate NMR entities. However, although the interconversion is termed fast, with k_{forward} (PCr $\rightarrow$ Cr) and k_{rev} between 0.07 and 0.5 s^{-1} (*22–24*), it is just slow enough to lead to separate spin entities on the time scale of a single acquisition ($T_2 \ll k^{-1}$). On the time scale of T_1 of the methyl protons, the two entities start to melt into a single one.

If Cr2 scales with the concentration of PCr and not total Cr also at rest, absolute quantitation of both Cr2 and Cr3 patterns should be able to answer whether the total or only the phosphorylated pool of Cr as determined chemically from biopsies contributes to the *in vivo* Cr resonances. Absolute quantitation by localized MRS is feasible for spectra of the brain (*25*). MR method-related variation has been determined to be about 5% with the sequence used in the present study (*26*) which would be sufficient to answer this question. However, quantitation

studies from different laboratories vary with a CV of as much as 40% (*25*), indicating that the systematic error in single studies is fairly large. In addition, absolute quantitation of muscle spectra is even more prone to systematic error than that of cerebral spectra, because the model lineshapes used for muscle spectra are complicated by orientation-dependent dipolar couplings, such that resolution of the current puzzle by absolute quantitation of Cr2 is challenging, since it would require an accuracy of around 10%. Accordingly, quantitation of the Cr3 peak has led to a wide range of resulting concentrations (*27–29*).

CONCLUSIONS

^{1}H MRS of exercising muscle yields a number of unexpected results. Besides a frequency shift and the appearance of an additional metabolite peak (*30*), it is of particular interest that the Cr/PCr signals in ^{1}H MR spectra of muscle change with exercise. The Cr2 peaks in ^{1}H MR spectra correlate with the PCr levels as determined from ^{31}P MRS. It is unclear whether the ^{1}H MR changes are caused by the changes in PCr levels or only incidentally and approximately coincide with them. At present, it can only be speculated how the observed changes are brought about. Care was taken to exclude artifacts and trivial changes in relaxation times as causes for the spectral changes. In any case the total Cr/PCr pool must be divided into physical compartments or chemical entities without fast exchange to explain the observed gradual disappearance of the Cr2 signals without major effect on lineshape. One possible explanation for the observed changes in the Cr/PCr resonances is that free creatine is NMR-invisible. If it should turn out that ^{1}H MRS can indeed measure PCr levels as opposed to total creatine, ^{1}H MRS may provide a tool for examining muscle energetics that is more sensitive and better suited to localized studies than ^{31}P MRS. If the observed spectral changes are caused by invisibility of free Cr due to binding of free Cr, there might be repercussions for the interpretation of some of the ^{31}P MRS literature on muscle energetics, which to a large extent relied on an assumed free Cr concentration to calculate ADP levels (*18, 31*).

EXPERIMENTAL

Data acquisition. Localized ^{1}H MR spectra were acquired on a 1.5-T whole-body MR scanner (SIGNA, General Electric, Milwaukee, WI) using a pair of 5-cm phased array receive coils with the body coil for RF transmit or a ^{1}H/^{31}P double-tuned flex coil (Medical Advances, Model 710GE-64). Most spectra were recorded from a region of interest (ROI) in m. tibialis anterior (TA) in the lower extremity. ROI size varied from ~5 to 16 cm^3. Further standard acquisition parameters for ^{1}H MRS included (*26*) PRESS sequence with outer volume suppression, water presaturation and phase rotation, 20-ms TE, 1.5–3 s TR, 8–512 averages, 1953.1 Hz spectral width, and 1024 data points. To test for T_1 effects, TR was varied from 1.5 to 8 s. For T_2 evaluation, STEAM localization (*32*) was used with TE 10–280 ms, TR 1.5 s, and 16–128 acquisitions. In some experiments the water signal was used as internal standard and for water referencing.

PCr levels were determined with localized ^{31}P MRS using the same PRESS sequence without outer volume suppression (TE 20 ms, TR 6.0 s, 1953 Hz spectral width, 1024 points, 32–64 averages, double-tuned flex coil (Medical Advances, Model 710GE-64)). PCr recovery in TA was observed with a pulse and acquire sequence (adiabatic half-passage RF pulse, 2500 Hz spectral width, 120 μs dead time, 1024 data points, 2 s TR, 240 scans, add/subtract cycle of 2) using coplanar transmit/receive coils, with a diameter of 7 cm for the receive and 22 cm for the transmit coil.

Data processing. Spectra were analyzed either visually using difference spectroscopy or numerically with a fitting routine allowing for incorporation of prior knowledge and Voigt or Lorentz lineshape models (*13*).

Subjects and exercise. Twelve subjects (males, unsportive or moderately trained, aged 24–46) were examined in 28 sessions (specifically: T_1 effects, 1 session; T_2 effects, 8 sessions; ^{1}H/^{31}P correlation, 6 sessions). With the lower extremity well fastened to the bed, TA was exercised within the magnet in two different schemes: First, the work load was applied isometrically using rubber strings attached to a nonmagnetic foothold or later using a pneumatic ergometer for the lower extremity. The force was adjusted individually such that 1–5 min of exercise would lead to fatigue, i.e., to a substantial decrease in PCr. In the second setup, the subject was asked to lift his foot every 2 s against strong resistance (rubber strings). MR spectra were acquired before exercise, during isometric work or in between the movements (TR 2 s), and during recovery.

For T_1 and T_2 determination in ^{1}H MRS and for recording of both ^{1}H and ^{31}P MR spectra at the same PCr level, the time at a specific lowered PCr level was prolonged by inducing ischemia with a pressure cuff above the knee to inhibit PCr recovery ((*33*) and references therein).

ACKNOWLEDGMENT

Support by the Swiss National Foundation (3100-042162.94) is gratefully acknowledged.

REFERENCES

1. R. Kreis and C. Boesch, Liquid-crystal-like structures of human muscle demonstrated by *in vivo* observation of direct dipolar coupling in localized proton magnetic resonance spectroscopy, *J. Magn. Reson. B* **104**, 189–192 (1994).
2. R. Kreis and C. Boesch, Spatially localized, one- and two-dimensional NMR spectroscopy and *in vivo* application to human muscle, *J. Magn. Reson. B* **113**, 103–118 (1996).
3. R. Kreis, M. Koster, M. Kamber, H. Hoppeler, and C. Boesch, Peak

assignment in localized ^{1}H MR spectra based on oral creatine supplementation, *Magn. Reson. Med.* **37,** 159–163 (1997).

4. V. Ntziachristos, R. Kreis, C. Boesch, and B. Quistorff, Dipolar resonance frequency shifts in ^{1}H MR spectra of skeletal muscle: Confirmation in rats at 4.7T *in vivo* and observation of changes *post mortem, Magn. Reson. Med.* **38,** 33–39 (1997).
5. R. Kreis, J. Slotboom, J. Felblinger, B. Jung, and C. Boesch, Can proton MRS detect changes in phosphocreatine? *in* "Proceedings, 5th ISMRM, Vancouver," p. 1338 (1997).
6. P. A. Narayana, J. D. Hazle, E. F. Jackson, L. K. Fotedar, and M. V. Kulkarni, In vivo ^{1}H spectroscopic studies of human gastrocnemius muscle at 1.5 T, *Magn. Reson. Imaging* **6,** 481–485 (1988).
7. H. Bruhn, J. Frahm, M. L. Gyngell, K. D. Merboldt, W. Haenicke, and R. Sauter, Localized proton NMR spectroscopy using stimulated echoes: Applications to human skeletal muscle in vivo, *Magn. Reson. Med.* **17,** 82–94 (1991).
8. C. Boesch, J. Slotboom, H. Hoppeler, and R. Kreis, In vivo determination of intra-myocellular lipids in human muscle by means of localized ^{1}H-MR spectroscopy, *Magn. Reson. Med.* **37,** 484–493 (1997).
9. J. W. Pan, J. R. Hamm, H. P. Hetherington, D. L. Rothman, and R. G. Shulman, Correlation of lactate and pH in human skeletal muscle after exercise by ^{1}H NMR, *Magn. Reson. Med.* **20,** 57–65 (1991).
10. H. P. Hetherington, J. R. Hamm, J. W. Pan, D. L. Rothman, and R. G. Shulman, A fully localized ^{1}H homonuclear editing sequence to observe lactate in human skeletal muscle after exercise, *J. Magn. Reson.* **82,** 86–96 (1989).
11. L. Jouvensal, P. G. Carlier, and G. Bloch, Practical implementation of single-voxel double-quantum editing on a whole-body NMR spectrometer: Localized monitoring of lactate in the human leg during and after exercise, *Magn. Reson. Med.* **36,** 487–490 (1996).
12. D. Shen, C. D. Gregory, and M. J. Dawson, Observation and quantitation of lactate in oxidative and glycolytic fibers of skeletal muscles, *Magn. Reson. Med.* **36,** 30–38 (1996).
13. J. Slotboom, C. Boesch, and R. Kreis, Versatile frequency domain fitting using time domain models and prior knowledge, *Magn. Reson. Med.* **39,** 899–911 (1998).
14. Y. L. Chung, W. S. Wassif, G. Jenkinson, D. L. Scott, and J. D. Bell, Correlation between in vivo and in vitro ^{1}H-MRS of human muscle, *in* "Proceedings, 5th ISMRM, Vancouver," p. 1335 (1997).
15. T. Wallimann, M. Wyss, D. Brdiczka, K. Nicolay, and H. M. Eppenberger, Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: The 'phosphocreatine circuit' for cellular energy homeostasis, *Biochem. J.* **281,** 21–40 (1992).
16. J. L. Fleckenstein, P. T. Weatherall, L. A. Bertocci, M. Ezaki, R. G. Haller, R. Greenlee, W. W. Bryan, and R. M. Peshock, Locomotor system assessment by muscle magnetic resonance imaging, *Magn. Reson. Q.* **7**(2), 79–103 (1991).
17. J. B. Rodenburg, R. W. de Boer, P. Schiereck, C. J. van Echteld, and P. R. Bär, Changes in phosphorus compounds and water content in skeletal muscle due to eccentric exercise, *Eur. J. Appl. Physiol.* **68,** 205–213 (1994).
18. R. S. Balaban, Regulation of oxidative phosphorylation in the mammalian cell, *Am. J. Physiol.* **258,** C377–C389 (1990).
19. W. Dreher, D. G. Norris, and D. Leibfritz, Magnetization transfer affects the proton creatine/phosphocreatine signal intensity: In vivo demonstration in the rat brain, *Magn. Reson. Med.* **31,** 81–84 (1994).
20. M. J. Kruiskamp, R. A. De Graaf, G. van Vliet, and K. Nicolay, The ^{1}H-NMR signal of creatine/phosphocreatine from rat skeletal muscle shows a strong off-resonance magnetization transfer effect, *MAGMA* **5**(Suppl.), 27–28 (1997). [Abstract]
21. R. A. De Graaf, A. van Kranenburg, and K. Nicolay, Off resonance magnetization transfer measurements on rat brain *in situ, in* "Proceedings, 6th ISMRM, Sydney," p. 329 (1998).
22. D. Rees, M. B. Smith, J. Harley, and G. K. Radda, In vivo functioning of creatine phosphokinase in human forearm muscle, studied by ^{31}P NMR saturation transfer, *Magn. Reson. Med.* **9,** 39–52 (1989).
23. M. Rudin, Measurement of exchange reactions in vivo using NMR magnetization transfer techniques, *Magn. Reson. Med. Biol.* **2,** 177–195 (1989).
24. A. Horska and R. G. S. Spencer, Correctly accounting for radiofrequency spillover in saturation transfer experiments: Application to measurement of the creatine kinase reaction rate in human forearm muscle, *MAGMA* **5,** 159–163 (1997).
25. R. Kreis, Quantitative localized ^{1}H-MR spectroscopy for clinical use, *Prog. NMR Spectrosc.* **31,** 155–195 (1997).
26. R. Kreis, C. Fusch, P. Maloca, J. Felblinger, and C. Boesch, Supposed pathology may be individuality: Interindividual and regional differences of brain metabolite concentrations determined by ^{1}H MRS, *in* "Proceedings, 2nd SMR, San Francisco," p. 45 (1994).
27. R. Kreis, T. Pfaeffli, E. Suter, H. Hoppeler, and C. Boesch, Quantitative ^{1}H MRS of human muscle: In vivo metabolite concentrations, muscle specific spectral patterns . . . and a puzzle with shifting peaks, *in* "Proceedings, 12th SMRM, New York," p. 402 (1993).
28. P. Styles, R. M. Dixon, and G. K. Radda, Unexpected heterogeneity in the ^{1}H creatine signal from human skeletal muscle, *in* "Proceedings, 3rd SMR, Nice," p. 1890 (1995).
29. P. A. Bottomley, Y. Lee, and R. G. Weiss, Total creatine in muscle: Imaging and quantification with proton MR spectroscopy, *Radiology* **204,** 403–410 (1997).
30. R. Kreis, B. Jung, S. Rotman, J. Slotboom, J. Felblinger, and C. Boesch, Acetyl-group buffering in exercising human muscle observable by ^{1}H-MRS? *in* "Proceedings, 5th ISMRM, Vancouver," p. 162 (1997).
31. G. J. Kemp and G. K. Radda, Quantitative interpretation of bioenergetic data from ^{31}P and ^{1}H magnetic resonance spectroscopic studies of skeletal muscle: An analytical review, *Magn. Reson. Q.* **10,** 43–63 (1994).
32. P. G. Webb, N. Sailasuta, S. J. Kohler, T. Raidy, R. A. Moats, and R. E. Hurd, Automated single-voxel proton MRS: Technical development and multisite verification, *Magn. Reson. Med.* **31,** 365–373 (1994).
33. B. Quistorff, L. Johansen, and K. Sahlin, Absence of phosphocreatine resynthesis in human calf muscle during ischaemic recovery, *Biochem. J.* **291,** 681–686 (1993).