Time Averaging and Fitting of Nonlinear Metabolic Changes: The Issue of the Time Index Choice Applied to ^{31}P MRS Investigation of Muscle Energetics

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We present an exact analytical method dedicated to fitting time-dependent exponential-like changes in MR spectra. As an illustration, this method has been applied to fitting metabolic changes recorded by ^{31}P MRS in human skeletal muscle occurring during a rest–exercise–recovery protocol. When recording metabolic changes with the accumulative method, the time averaging of the MR signals implies the choice of a time index for fitting any changes in the features of the associated MR spectra. A critical examination of the different ways (constant, linear, and exponential) of choosing the time index is reported. By numerical analysis, we have calculated the errors generated by the three methods and we have compared their sensitivity to noise. In the case of skeletal muscle, both constant and linear methods introduce large and uncontrolled errors for the whole set of metabolic parameters derived from [PCr] changes. In contrast, the exponential method affords a reliable estimation of critical parameters in muscle bioenergetics in both normal and pathological situations. This method is very easy to implement and provides an exact analytical solution to fitting changes in MR spectra recorded by the accumulative method. © 2001 Academic Press

Key Words: **time averaging; fitting method; ^{31}P MRS; human skeletal muscle.**

INTRODUCTION

^{31}P magnetic resonance spectroscopy (MRS) has been largely used to investigate *in vivo* the energetic status of organs including heart, liver, brain, and kidney. More particularly, analysis of muscle energetics in humans has provided significant results regarding (i) metabolic control of energy supply during muscle contraction and recovery in healthy subjects and (ii) altered muscular energetics in patients suffering from various muscular diseases (*1–17*).

Unlike muscle biopsy, which samples metabolite concentrations at a single moment in time, MRS gives an average value of concentrations sampled at consecutive moments. MRS widely uses the accumulative method in the time domain to improve the sensitivity, i.e., to obtain a reliable signal-to-noise ratio in the frequency domain. At the end of the total acquisition time

P, spectrometers produce a MR signal that results from the time averaging of NS (number of scans) successive and elementary signals, each of them being sampled during a time T within the TR cycle (time of repetition).

Because the Fourier transform is a linear operator, the accumulative aspect is also true in the frequency domain. Consequently, any metabolite concentration calculated from a MR spectrum is the average of NS successive and elementary concentrations calculated from each elementary MR spectrum obtained during each successive TR. This averaged concentration is subsequently "associated" with the total acquisition time $P = \text{NS} \times \text{TR}$.

For muscle investigations, NS and P depend on the magnetic field strength, the observed muscle mass, and the specific experimental protocol used. Analysis of literature indicates that NS ranges from 4 to 128 accumulations while P varies from several seconds to several minutes.

Concentration values, obtained for every P, are generally used in a modeling and fitting process to calculate several critical metabolic parameters. Due to the averaging of the NS successive and elementary values, a time index must be provided for each metabolic concentration value in the fitting process. In other words, one must implicitly or explicitly characterize the time-dependent evolution of metabolite levels during each acquisition time P.

During a rest–exercise–recovery muscle protocol, analysis of phosphocreatine (PCr) concentration time-dependent changes provides important information on muscle bioenergetics, such as the initial rate of [PCr] variations at the start of the exercise period or at the start of the recovery period (*13, 18–20*). Over the past years, different ways of choosing the time index have been reported and no critical analysis of all methods is yet available. We aimed in this work at providing such a critical analysis and we propose a method well suited to exponential-like[1] phenomena

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[1] Exponential-like is used to describe a linear combination of exponential functions with a constant term: $x \mapsto a_0 + \sum_{n=1}^{N}\{a_n \cdot \exp(b_n \cdot x)\}$, with a_n and b_n being complex numbers.

such as metabolic changes occurring in exercising/recovering muscle.

METHODS

Definitions of Parameters Used in Modeling [PCr] Changes

During muscle contraction, PCr is consumed, thereby buffering [ATP] changes and leading to inorganic phosphate (Pi) accumulation. The initial [PCr] decrease is rapid and then becomes curvilinear as oxidative phosphorylation and glycogenolysis resynthetize ATP. Throughout the exercise, the time-dependent evolution of [PCr] is commonly described as an exponential-like model (*13, 18–21*):

$$[PCr](t) = BEG_{exe} - MAX_{exe} \times [1 - \exp(-K_{exe} \times t)]. \quad [1]$$

This model is valid between time $t = 0$ (beginning of the exercise) and time $t = t_{endexe}$ (end of the exercise). BEG_{exe} refers to [PCr] value at the beginning of the exercise; it must be equal to $[PCr]_{rest}$, the average value of [PCr] during rest before exercise. K_{exe} (min^{-1}) is the kinetic constant of PCr consumption during exercise. MAX_{exe} represents the amount of PCr consumed when steady-state conditions of the exercise are reached, i.e., when the duration of the exercise is very long compared to $1/K_{exe}$.

The initial rate of [PCr] decrease (IR_{exe}), the [PCr] value at end of the exercise (END_{exe}), and the amount of PCr consumed during exercise (CONSUM) can be calculated from Eq. [1] as

$$IR_{exe} = -K_{exe} \times MAX_{exe};$$
$$END_{exe} = BEG_{exe} - MAX_{exe} \times [1 - \exp(-K_{exe} \times t_{endexe})];$$
$$CONSUM = BEG_{exe} - END_{exe}.$$

Only three of the above parameters are necessary and sufficient to completely define the model (3 degrees of freedom model).

It is noteworthy that [PCr] increase during the recovery period is governed by a similar exponential-like model:

$$[PCr](t) = BEG_{rec} + MAX_{rec} \times [1 - \exp(-K_{rec} \times t)]. \quad [2]$$

This model is valid between time $t = 0$ (beginning of the recovery) and time $t = t_{endrec}$ (end of the recovery). BEG_{rec} refers to [PCr] value at the beginning of the recovery; it must be equal to END_{exe}. K_{rec} (min^{-1}) is the kinetic constant of PCr resynthesis during recovery. MAX_{rec} represents the amount of PCr resynthetized when steady-state conditions of full [PCr] recovery are reached, i.e., when the duration of the recovery is very long compared to $1/K_{rec}$. The value of full recovery $[PCr]_{full} = BEG_{rec} + MAX_{rec}$ is expected to be equal to $[PCr]_{rest}$.

The initial rate of [PCr] increase (IR_{rec}), the [PCr] value at end of the recovery (END_{rec}), and the amount of PCr resynthetized

during recovery (RECOV) can be calculated from Eq. [2] as

$$IR_{rec} = +K_{rec} \times MAX_{rec};$$
$$END_{rec} = BEG_{rec} + MAX_{rec} \times [1 - \exp(-K_{rec} \times t_{endrec})];$$
$$RECOV = END_{rec} - BEG_{rec}.$$

The Choice of the Time Index for the Fitting Process

We have used the widely accepted global models describing [PCr] changes during the whole duration of the exercise (Eq. [1]) and the whole duration of the recovery (Eq. [2]). To compute the global model parameters in the fitting process, one must define a submodel for the time-dependent evolution of [PCr] during the acquisition time P; i.e., one needs to associate a time index to each average [PCr] value produced by the spectrometer during each P. Two main methods have already been reported and we propose a third one.

The constant method. As in the usual approach of time-averaging methods, one could consider that the time-dependent evolution of [PCr] is constant during P and choose the time index given by the spectrometer at the endpoint of P. This method implicitly assumes that the global model is piecewise constant during the whole duration of the exercise or recovery. This assumption is fully valid for the time-dependent evolution of [PCr] during rest before exercise; it is an approximation for the steady-state periods of the exercise or recovery.

The linear method. Some authors have introduced another method consisting in choosing the midpoint of P as the time index (*13–15, 22*). This method is implicitly based on the assumption that the time-dependent evolution of [PCr] is linear during P. This method assumes that the global model is piecewise linear during the whole period of the exercise or recovery. This assumption may be considered as approximately valid for a period with nearly constant rate of [PCr] variations, but is obviously inaccurate when the rate varies rapidly, as at the start of the exercise or at the start of the recovery.

The exponential method. In coherence with Eq. [1] and Eq. [2], we propose that the time-dependent evolution of [PCr] during P follows exactly Eq. [1] for exercise and Eq. [2] for recovery. For the exercise period and for each successive P of rank $m = 1, 2, \ldots$, the theoretical averaged value of [PCr] can be analytically calculated as the arithmetic mean of the NS elementary concentration values of PCr. If DL is the time delay between the start of TR and the middle of the sampling period T, then during the current P of rank m, the current sampling time is

$$t_n = n \times TR + DL, \quad \text{with } n = (m-1) \times NS,$$
$$(m-1) \times NS + 1, \ldots, m \times NS - 1.$$

Under conditions of stationary concentration during T, the current elementary concentration of PCr is

$$[PCr](t_n) = BEG_{exe} - MAX_{exe} \times [1 - \exp(-K_{exe} \times t_n)], \quad [3]$$

and can be rewritten as

$$[PCr](t_n) = BEG_{exe} - MAX_{exe} \times [1 - KDL \times q^n],$$

with $q = \exp(-K_{exe} \times TR)$ being the decreasing factor between successive elementary concentrations and $KDL = \exp(-K_{exe} \times DL)$ being the decreasing factor due to the delay.

During the current P of rank m, the average $[PCr]_m$ of the NS values is

$$[PCr]_m = \frac{1}{NS} \sum_{n=(m-1)NS}^{n=mNS-1} \{[PCr](t_n)\}$$

$$= BEG_{exe} - MAX_{exe} \times \left[1 - \frac{KDL}{NS} \sum_{n=(m-1)NS}^{n=mNS-1} \{q^n\} \right].$$

The last term is the sum of a finite geometric progression and the resulting average value can be expressed as

$$[PCr]_m = BEG_{exe} - MAX_{exe} \times \left[1 - \frac{KDL}{NS} \times \frac{Q^{(m-1)}(1-Q)}{(1-Q^{(1/NS)})} \right],$$
[4]

with $Q = q^{NS} = \exp(-K_{exe} \times P)$ being the decreasing factor between successive spectra. Similar calculations can be performed throughout the recovery period, using the appropriate corresponding parameters.

In the exponential method, values given by Eq. [4] (average of theoretical values), indexed by m, must be fitted with the corresponding experimental values (averaged measures produced by the spectrometer). Interestingly, this method does not require any choice of time index at all; it only requires the rank m of each spectrum, independent of the collection time. The fitting is conducted with time-averaged values and not with simple time-point values (a time-averaged value is "associated" with the total time of averaging).

We can observe that Eq. [4]:

(i) is the exact analytical form of the average value of the NS elementary concentrations during P, whatever the number of scans;

(ii) may be easily extended to the very general case of a linear combination of complex exponential functions with different complex coefficients, since averaging is a linear operator; and

(iii) can be used for all phenomena measured by the accumulative method, in all domains.

Evaluation of the Methods

The overall evaluation was based on the three following studies.

Analysis of errors[2]. First, we have analyzed the errors introduced by each method (constant, linear, and exponential) using a priori given values (reference values) of parameters for each model (Eq. [1], Eq. [2]). Parameter values were obtained from our data bank (*23*). For the exercise period, for the independent parameters selected, and for each combination of their reference values shown in Table 1, we have followed these three successive steps.

Step 1. For each $P(m = 1, 2, \ldots)$: (i) we have numerically calculated the NS elementary values $[PCr](t_n)$ given by Eq. [3], and (ii) we have numerically obtained, by arithmetic mean, the corresponding theoretical average values A_m ($m = 1, 2, \ldots$).

Step 2. We have looked for the values of parameters that have led to the best fit (χ^2 minimization) between the theoretical values $\{[PCr]_{rest}, A_1, A_2, \ldots\}$ and the following values:

(i) $[PCr](t)$ given by Eq. [1] at time $\{0, P, 2P, \ldots\}$ for the constant method;

(ii) $[PCr](t)$ given by Eq. [1] at time $\{0, P/2, 3P/2, \ldots\}$ for the linear method; and

(iii) $\{BEG_{exe}, [PCr]_1, [PCr]_2, \ldots\}$ given by Eq. [4] for the exponential method.

Step 3. For each individual parameter (independent or dependent), we have calculated the agreement between the fitted value and the a priori given value (reference value in Table 1) as

$$agreement(\%) = 100 \times \frac{fitted\ value}{reference\ value} = 100 + error(\%).$$

For the recovery period, we have applied the same process using the corresponding appropriate parameters and subscripts. Moreover, we have always used the reference value of each parameter as the starting values of the fitting iterative process.

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[2] Error: the difference between a computed, estimated, or measured value and the true, specified, or theoretically correct value (FED-STD-1037C).

Estimation of sensitivity to noise[3].   Since real data are always noisy, we have estimated, in the second study, the sensitivity to noise for each method. The noise effects were simulated by the Monte Carlo method. Noisy $[PCr](t_n)$ values were obtained by adding a distinct random value to each value given by Eq. [3]. Characterization of noise agreed with what we have experimentally obtained in the exploration of human muscle. A Gaussian noise was used with a zero mean and a standard deviation value equal to 30% of $[PCr]_{rest}$.

For each method, for each period (exercise and recovery), and for each combination of the parameter reference values shown in Table 1, the three steps of the first study were run 1000 times with different samples of noise. For each individual parameter, the 1000 corresponding agreements (or errors) were obtained. These agreements were obviously scattered. The distribution of the 1000 agreements was fitted by χ^2 minimization with a normal curve. The mean value of the normal curve gives an experimental estimation of the most probable agreement among the 1000 agreements. The standard deviation of the normal curve gives an experimental estimation of the dispersion produced by the noise. In this study, we have also used the reference values of the parameters as the starting values of the fitting iterative process.

Analysis of data from healthy subjects.   Finally, in the third study, data from 42 healthy subjects (mean age $\pm$ SD, 37 $\pm$8) were examined using the three different methods.

For the three studies, the fixed protocol parameters were NS = 32; DL = 0; P = 1 min; exercise duration = 3 min (m = 1, 2, 3) and recovery duration = 20 min (m = 1, 2, ..., 20). Numerical calculations were written and run under IDL (version 5, IRIX mipseb, Research Systems Inc., Boulder, CO). The built-in routine curvefit.pro (version 1.13 1997/01/15) was used for fitting. The internal normal random number generator randomn.pro was applied for noise simulation.

RESULTS AND DISCUSSION

Analysis of Errors

For the constant and linear methods, all results show that the agreement (or error) is a nonlinear function of theoretical values, as can be observed for K_{exe} in Fig. 1. This error is not a bias[4] since it is neither constant nor proportional nor unidirectional. There is no exact analytical method that can correct this error because of the use of a nonlinear iterative process (χ^2 minimization).

The main results concerning selected critical parameters are displayed in Table 2. Clearly, both constant and linear methods often underestimate most of the theoretical values. For instance, the best value of K_{exe} obtained with the constant method is only 36% of the theoretical value. In contrast, results obtained with
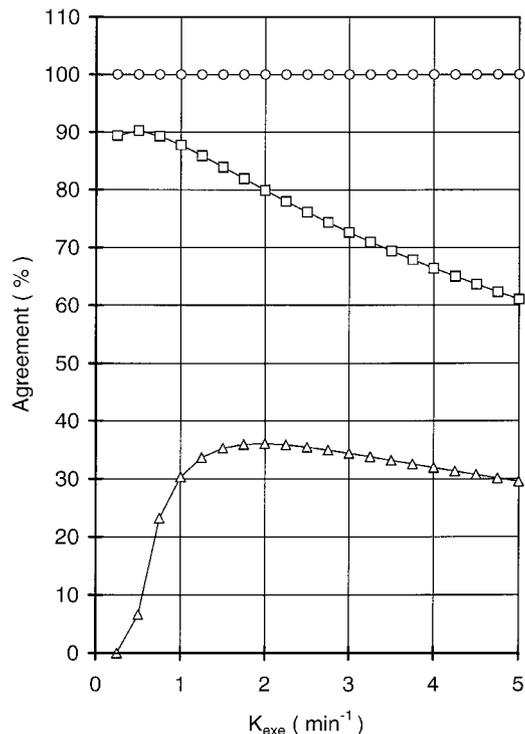
[3] Noise: a random signal of known statistical properties of amplitude, distribution, and spectral density (FED-STD-1037C).

[4] Bias: a systematic deviation of a value from the reference value (FED-STD-1037C).

FIG. 1.   Agreement (percentage ratio of fitted value to reference value) of the kinetic constant K_{exe} calculated according to the constant (triangles), the linear (square), and the exponential (circles) methods. Results are displayed as a function of the reference values of K_{exe} expressed in min^{-1}. These results were obtained with DEB_{exe} = 100% of [PCr] at rest ($[PCr]_{rest}$) and were independent of $ENDexe$ varying from 80 to 20% by increments of 10% of $[PCr]_{rest}$.

the linear method are closer to the theoretical values, for all parameters, the worst value obtained being 61% of the theoretical value. The errors introduced by both methods are smaller for the recovery period, as compared to exercise, but are still

TABLE 2
Agreement (Percentage Ratio of Fitted Value to Reference Value) Calculated for Selected Parameters

Parameters	Agreement (%; min–max)		
	Constant method	Linear method	Exponential method
Exercise			
K_{exe}	0.005–36	61–90	100
IR_{exe}	30–63	61–97	100
CONSUM	90–103	100–102	100
Recovery			
K_{rec}	50–92	84–100	100
IR_{rec}	51–97	84–100	100
RECOV	102–106	100–101	100

Note. IR and K represent respectively the initial rate and kinetic constant of [PCr] changes. CONSUM refers to the amount of PCr consumed at end of the exercise. RECOV refers to the amount of PCr recovered at end of recovery. Results are presented as ranges including minimal and maximal values of the agreement. Independent parameters used for the calculations are shown in Table 1.

TABLE 3

Agreement (Percentage Ratio of Fitted Value to Reference Value) of the Kinetic Constant K_{exe} Calculated by Each Method in 1000 Experiments with Different Samples of Noise

Reference values $K_{exe}(min^{-1})$	Agreement distribution: MPA(%) ± SD(%)		
	Constant method	Linear method	Exponential method
0.25	0.004 ± 0.002	90 ± 49	101 ± 54
1.75	36 ± 5	82 ± 9	101 ± 14
5.0	30 ± 4	61 ± 7	99 ± 23

Note. Results are presented as estimated most probable agreement (MPA) and estimated standard deviation (SD) of the agreement distribution. Independent parameters were BEG_{exe} equal to 100% and END_{exe} equal to 50% of [PCr] at rest ($[PCr]_{rest}$).

significant. It is noteworthy that the smallest errors are calculated for both the amount of PCr consumed at end of the exercise and PCr resynthetized throughout recovery.

The exponential method always gives the correct results, whatever the parameter considered, providing the expected agreement value of 100%. This is not surprising because A_m and $[PCr]_m$ are two different ways to numerically calculate the same average value.

Estimation of Sensitivity to Noise

For each parameter, the estimations of the most probable agreements obtained by both constant and linear methods are very similar to the agreements obtained in the first study, with noiseless data. Agreements reported in Table 3 can be compared with agreements in Fig. 1 for the same theoretical K_{exe}. As shown in Table 3, the best estimation of the most probable agreement of K_{exe} by the constant method is only 36% of the theoretical value, whereas the linear method provides better results (90% of the theoretical value).

Regarding values of K_{exe}, the estimation of the most probable agreement of the agreement distribution associated with the exponential method remains by far the best, i.e., less than 1% different from the theoretical value.

For a given method, the sensitivity to noise for a given parameter can be measured by the ratio between the estimated standard deviation of the agreement distribution and the estimated most probable agreement of the agreement distribution. For each individual value of K_{exe}, the sensitivity has approximately the same value for all methods (Table 3). This sensitivity to noise is the highest (roughly 50%) for the lowest theoretical value of K_{exe}.

Analysis of Data from Healthy Subjects

Based on the results obtained above, the parameter value calculated by the exponential method was chosen as the reference value and compared with values provided by the other two methods. Figure 2 illustrates results of the initial rate of [PCr] post-exercise recovery (IR_{rec}) for each of the 42 subjects. One can note
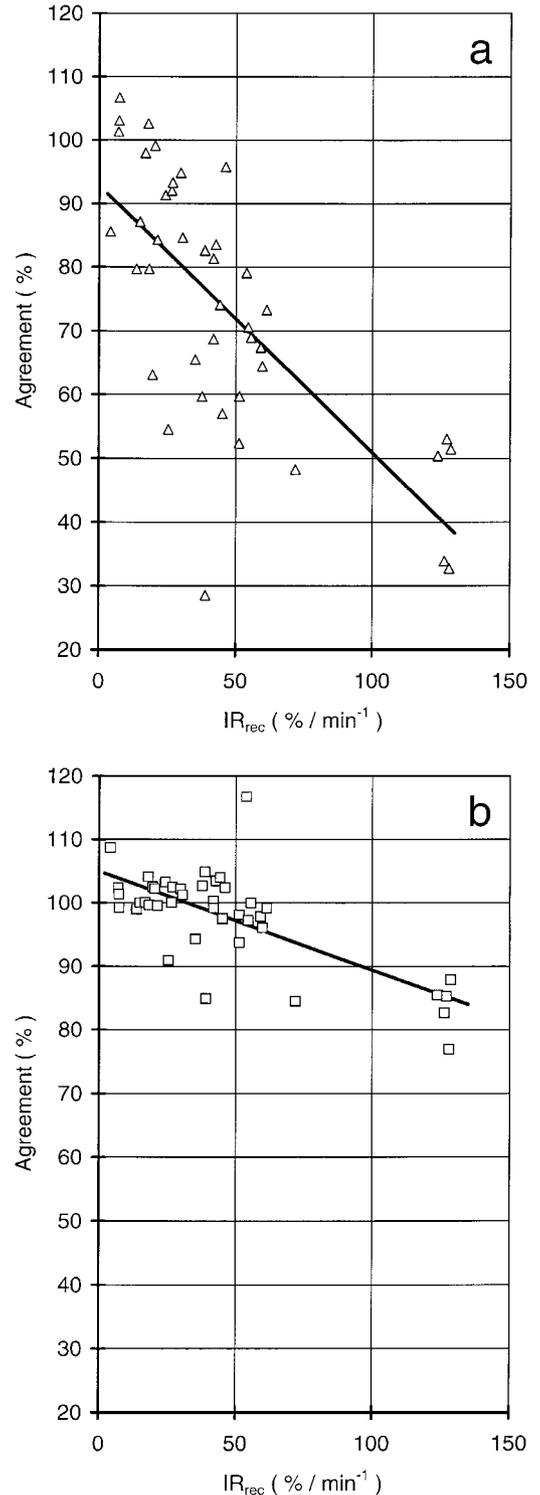

FIG. 2. Agreement (percentage ratio of the value obtained with the constant or the linear method, to the value obtained by the exponential method) of the initial rate of [PCr] post-exercise recovery (IR_{rec}) for healthy subjects ($n = 42$), calculated according to the constant (triangles, a) and to the linear (squares, b) methods. Results are displayed as a function of the values of IR_{rec} obtained by the exponential method and expressed in percentage of [PCr] at rest ($[PCr]_{rest}$) per minute.

that constant and linear methods generate errors that are roughly proportional to the value of IR_{rec}. For the constant method, the agreement ranged from 40 to 90%, while for the linear method it varied from 85 to 105%.

CONCLUSIONS

We have demonstrated that both constant and linear methods introduced uncontrolled errors for the whole set of metabolic parameters. In this regard, one should keep in mind that errors are different from bias. Bias is often characterized by a constant offset and is independent of theoretical values; it can be corrected. In the present case, errors are nonlinear functions of theoretical values and systematic corrections are impossible. When having to select the most reliable fitting model, one should consider that the accuracy of MRS is estimated to be around 10%. As a consequence, any difference up to 10% in the metabolic parameters among healthy subjects or between controls and patients with muscular disorders cannot be considered as significant. Since errors introduced by the constant and the linear methods are often larger than 10%, they are likely to provide unreliable results.

We have proposed an exponential method, which allows a reliable estimation of critical metabolic parameters such as those usually recorded during and after muscular exercise. Those parameters are calculated to analyze muscle bioenergetics in both normal and pathological situations. Among them, IR_{exe} is used to estimate the energy cost of contraction, thereby illustrating metabolic efficiency (*13, 19, 20, 24–27*). Additionally, IR_{rec} is utilized to illustrate aerobic ATP production, thereby giving information on mitochondrial metabolism (*1, 4, 8, 9, 12, 28, 29*). The exponential method can be reliably used for a subtle exploration of metabolism in normal situations and for conducting comparative analyses between control subjects and patients with muscular disorders.

This study demonstrates that, with the use of the exponential method, the accumulative method may be extended to observe and to measure MR signals with exponential-like changes in MR spectra. The only assumed condition is that the MR signal be stationary only during the sampling period T. The exponential method is easy to implement and provides an exact analytical solution to fitting changes in MR spectra.

ACKNOWLEDGMENTS

REFERENCES

1. D. Bendahan, S. Confort-Gouny, G. Kozak-Reiss, and P. J. Cozzone, Heterogeneity of metabolic response to muscular exercise in humans. New criteria of invariance defined by in vivo phosphorus-31 NMR spectroscopy, *FEBS Lett.* **272,** 155–158 (1990).

2. M. J. Kushmerick, Integrated human muscle bioenergetic studies by magnetic resonance methods, *Int. J. Sports Med.* **18,** S304–306 (1997).

3. K. E. Conley, M. L. Blei, T. L. Richards, M. J. Kushmerick, and S. A. Jubrias, Activation of glycolysis in human muscle in vivo, *Am. J. Physiol.* **273,** C306–315 (1997).

4. D. J. Taylor, P. J. Bore, P. Styles, D. G. Gadian, and G. K. Radda, Bioenergetics of intact human muscle. A 31P nuclear magnetic resonance study, *Mol. Biol. Med.* **1,** 77–94 (1983).

5. D. J. Taylor, M. J. Brosnan, D. L. Arnold, P. J. Bore, P. Styles, J. Walton, and G. K. Radda, Ca2+-ATPase deficiency in a patient with an exertional muscle pain syndrome, *J. Neurol. Neurosurg. Psychiatry* **51,** 1425–1433 (1988).

6. Z. Argov, W. J. Bank, J. Maris, P. Peterson, and B. Chance, Bioenergetic heterogeneity of human mitochondrial myopathies: phosphorus magnetic resonance spectroscopy study, *Neurology* **37,** 257–262 (1987).

7. D. L. Arnold, D. J. Taylor, and G. K. Radda, Investigation of human mitochondrial myopathies by phosphorus magnetic resonance spectroscopy, *Ann. Neurol.* **18,** 189–196 (1985).

8. D. L. Arnold, P. M. Matthews, and G. K. Radda, Metabolic recovery after exercise and the assessment of mitochondrial function in vivo in human skeletal muscle by means of 31P NMR, *Magn. Reson. Med.* **1,** 307–315 (1984).

9. D. Bendahan, C. Desnuelle, D. Vanuxem, S. Confort-Gouny, D. Figarella-Branger, J. F. Pellissier, G. Kozak-Ribbens, J. Pouget, G. Serratrice, and P. J. Cozzone, 31P NMR spectroscopy and ergometer exercise test as evidence for muscle oxidative performance improvement with coenzyme Q in mitochondrial myopathies, *Neurology* **42,** 1203–1208 (1992).

10. D. Bendahan, G. Kozak-Ribbens, L. Rodet, S. Confort-Gouny, and P. J. Cozzone, 31Phosphorus magnetic resonance spectroscopy characterization of muscular metabolic anomalies in patients with malignant hyperthermia: Application to diagnosis, *Anesthesiology* **88,** 96–107 (1998).

11. D. Bendahan, M. Badier, Y. Jammes, S. Confort-Gouny, A. M. Salvan, C. Guillot, and P. J. Cozzone, Metabolic and myoelectrical effects of acute hypoxaemia during isometric contraction of forearm muscles in humans: A combined 31P- magnetic resonance spectroscopy-surface electromyogram (MRS-SEMG) study, *Clin. Sci.* **94,** 279–286 (1998).

12. G. J. Kemp, D. J. Taylor, and G. K. Radda, Control of phosphocreatine resynthesis during recovery from exercise in human skeletal muscle, *NMR Biomed.* **6,** 66–72 (1993).

13. G. J. Kemp, D. J. Taylor, C. H. Thompson, L. J. Hands, B. Rajagopalan, P. Styles, and G. K. Radda, Quantitative analysis by 31P magnetic resonance spectroscopy of abnormal mitochondrial oxidation in skeletal muscle during recovery from exercise, *NMR Biomed.* **6,** 302–310 (1993).

14. G. J. Kemp and G. K. Radda, Quantitative interpretation of bioenergetic data from 31P and 1H magnetic resonance spectroscopic studies of skeletal muscle: An analytical review, *Magn. Reson. Q.* **10,** 43–63 (1994).

15. G. J. Kemp, C. H. Thompson, D. J. Taylor, and G. K. Radda, ATP production and mechanical work in exercising skeletal muscle: A theoretical analysis applied to 31P magnetic resonance spectroscopic studies of dialyzed uremic patients, *Magn. Reson. Med.* **33,** 601–609 (1995).

16. G. J. Kemp, C. H. Thompson, J. R. Stratton, F. Brunotte, M. Conway, S. Adamopoulos, L. Arnolda, G. K. Radda, and B. Rajagopalan, Abnormalities in exercising skeletal muscle in congestive heart failure can be explained in terms of decreased mitochondrial ATP synthesis, reduced metabolic efficiency, and increased glycogenolysis, *Heart* **76,** 35–41 (1996).

17. J. A. Kent-Braun, K. R. Sharma, R. G. Miller, and M. W. Weiner, Postexercise phosphocreatine resynthesis is slowed in multiple sclerosis, *Muscle Nerve* **17,** 835–841 (1994).

18. K. K. McCully, K. Vandenborne, K. DeMeirleir, J. D. Posner, and J. S. Leigh, Jr., Muscle metabolism in track athletes, using 31P magnetic resonance spectroscopy, *Can. J. Physiol. Pharmacol.* **70,** 1353–1359 (1992).

19. T. W. Ryschon, M. D. Fowler, R. E. Wysong, A. Anthony, and R. S. Balaban, Efficiency of human skeletal muscle in vivo: Comparison of isometric, concentric, and eccentric muscle action, *J. Appl. Physiol.* **83,** 867–874 (1997).

20. M. Erkintalo, D. Bendahan, J. P. Mattei, C. Fabreguettes, P. Vague, and P. J. Cozzone, Reduced metabolic efficiency of skeletal muscle energetics in hyperthyroid patients evidenced quantitatively by in vivo phosphorus-31 magnetic resonance spectroscopy, *Metabolism* **47,** 769–776 (1998).

21. R. A. Meyer, A linear model of muscle respiration explains monoexponential phosphocreatine changes, *Am. J. Physiol.* **254,** C548–553 (1988).

22. D. J. Taylor, A. Amato, L. J. Hands, G. J. Kemp, G. Ramaswami, A. Nicolaides, and G. K. Radda, Changes in energy metabolism of calf muscle in patients with intermittent claudication assessed by 31P magnetic resonance spectroscopy: A phase II open study, *Vasc. Med.* **1,** 241–245 (1996).

23. P. J. Cozzone and D. Bendahan, P-31 NMR spectroscopy of metabolic changes associated with muscle exercise: Physiopathological applications, *in* "NMR in Physiology and Medicine" (R. G. Gillies, Ed.), Chap. 23 Academic Press, San Diego, 1994.

24. A. Ratkevicius, M. Mizuno, E. Povilonis, and B. Quistorff, Energy metabolism of the gastrocnemius and soleus muscles during isometric voluntary and electrically induced contractions in man, *J. Physiol. (London)* **507,** 593–602 (1998).

25. M. C. Hogan, E. Ingham, and S. S. Kurdak, Contraction duration affects metabolic energy cost and fatigue in skeletal muscle, *Am. J. Physiol.* **274,** E397–402 (1998).

26. M. Boska, Estimating the ATP cost of force production in the human gastrocnemius/soleus muscle group using 31P MRS and 1H MRI, *NMR Biomed.* **4,** 173–181 (1991).

27. J. M. Foley and R. A. Meyer, Energy cost of twitch and tetanic contractions of rat muscle estimated in situ by gated 31P NMR, *NMR Biomed.* **6,** 32–38 (1993).

28. D. J. Taylor and G. K. Radda, Mitochondrial diseases—Noninvasive approaches, *Curr. Topics Bioenerg.* **17,** 99–126 (1994).

29. B. Chance, J. S. Leigh, D. S. Smith, S. Nioka, and B. J. Clark, Phosphorus magnetic resonance spectroscopy studies of the role of mitochondria in the disease process, *Ann. N.Y. Acad. Sci.* **488,** 140–153 (1986).