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MUSCLE RESEARCH WORK WITH BRITTON CHANCE FROM *IN VIVO* MAGNETIC RESONANCE SPECTROSCOPY TO NEAR-INFRARED SPECTROSCOPY

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Britton Chance has pioneered magnetic resonance spectroscopy (MRS) and near-infrared (NIR) spectroscopy (NIRS) as noninvasive methods for measuring muscle metabolism *in vivo* from the late 1970s. This review honoring Britton Chance will highlight the progress that has been made in developing and utilizing MRS and NIRS technologies for evaluating skeletal muscle O_2 dynamics and energetics. Adaptation of MRS and NIRS technology has focused on the validity and reliability of the measurements and extending the methods in physiological and clinical research. Britton Chance has conducted MRS and NIRS research on elite athletes and a number of chronic health conditions, including patients with chronic heart failure, peripheral vascular disease, and neuromuscular myopathies. As MRS and NIRS technologies are practical and useful for measuring human muscle metabolism, we will strive to continue Chance's legacy by advancing muscle MRS and NIRS studies.

Keywords: Muscle; near-infrared spectroscopy; magnetic resonance spectroscopy; muscle oxygenation; muscle energy metabolism; phosphorus metabolites; intramuscular pH; exercise.

1. Introduction

The purpose of this article is to highlight research conducted by Britton Chance using noninvasive magnetic resonance spectroscopy (MRS) and near-infrared spectroscopy (NIRS) for evaluating skeletal muscle O_2 dynamics and energy metabolism. Because of the large amount of research that Dr. Chance has published, we will limit our review, for the most part, to papers we have published with Dr. Chance. This is a total of 44 papers with 1898 citations (science citation index, 8 March 2011), impressive considering that Britton Chance has published over 1000 papers with other authors.[1] A brief background on MRS and NIRS methodologies is presented, along with

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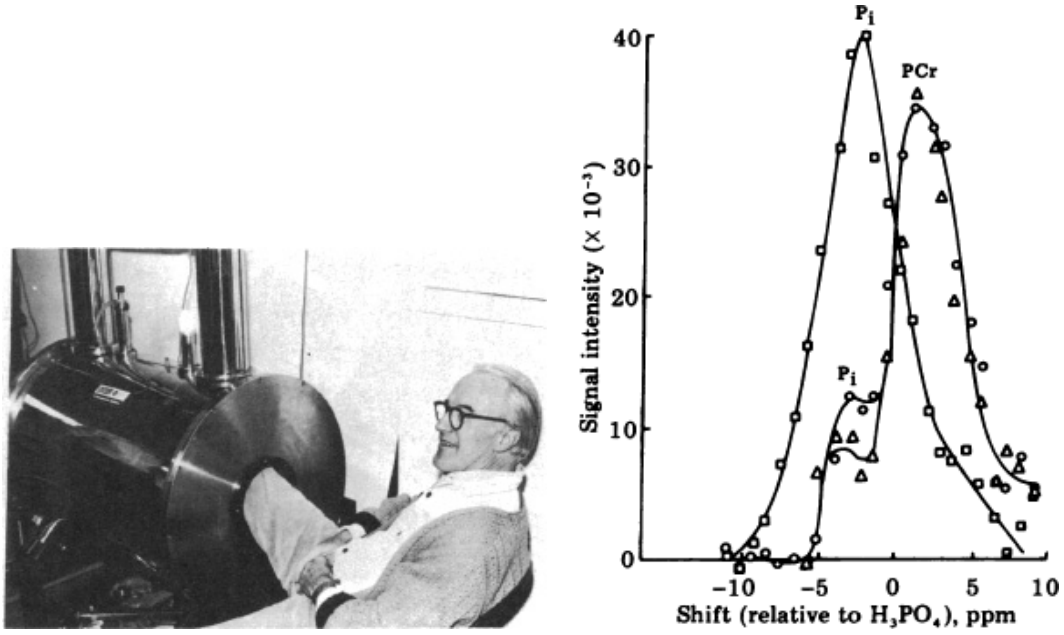
collaborative studies with Britton Chance on how these methodologies have contributed to understanding muscle biochemistry, physiology, and pathology.

The primary reason MRS and NIRS technologies are extremely useful for the study of muscle is the visibility of phosphorus molecules and heme/copper molecules, respectively. Traditional methods using analytical biochemistry are based on obtaining biopsy samples.[2] Analytical biochemical techniques have provided information on muscle phosphorus compounds,[3] NAD^+/NADH (nicotinamide adenine dinucleotide/reduced NAD),[4] and other unique biochemical metabolites. Myoglobin (Mb) O_2 saturation and NADH redox state can be detected using freeze clamped tissue.[5] The strength of the biopsy approach is that a wide array of metabolites can be measured to study specific metabolic pathways. The disadvantage of biopsy procedure is the invasive nature of sampling method and the difficulty of repeated measurement, resulting in the obtained data with poor time resolution.[6] In addition, values for metabolites include both bound and free forms and thus do not provide biologically active concentrations. Thus, there has been a strong need for noninvasive approaches to measuring phosphorus metabolites using MRS. MRS was first used to measure free (active) forms of phosphate compounds in skeletal muscle in the late 1970s by Chance, Radda, and other collaborators.[7] Especially, Leigh at University of Pennsylvania played an important role in collaboration with Chance for developing ^{31}phosphorus-MRS and related human muscle studies.[8,9] Shortly after, Britton Chance published one of the first examples of the use of MRS to monitor exercising skeletal muscle in humans.[8] At this time, because only relatively small MR machines were available, it took a bit of imagination to follow changes in the muscle spectra (Fig. 1).

The light in the visible region has been used for monitoring of changes in tissue oxygenation since the 1930s.[10] Chance[11] and Chance and Connelly[12] discovered that the mitochondrial NADH signal showed a rapid change by the electrical muscle stimulation in a fraction of a second even at less than 10°C, indicating the coupling of muscle contraction and mitochondrial activity. Jobsis[13] together with Ramirez, Weber, and others followed up with *in vitro* optical studies of various organs. Later, Jobsis[14] discovered that the NIR light easily goes through the skull and set the stage for the recent application of NIRS to scientific research area as well as varying clinical settings. Chance developed a series of NIRS systems, which used both continuous wavelength and time-resolved



(a) (b)

Fig. 1. (a) Insertion of limb into the 18-cm superconducting magnet for detection of PCr and Pi. The calf muscle lies over the probe.[8] (b) Effect of strenuous exercise on PCr and Pi levels in the human forearm. ^{31}P NMR scans were for 1.5 min. The forearm was tested before, immediately after 1 min of violent exercise, and 20 min into the course of recovery.[8]

spectroscopy (TRS) to evaluate human skeletal muscle.[15] This was followed by commercial development of the continuous wavelength "RunMan" device[16,17] that served as one of the first models to provide us with opportunities for further clinical muscle research in a noninvasive and portable way.

2. Control of Oxidative Energy Metabolism

To better understand how NIRS and MRS function in the muscle studies, the control of oxidative energy metabolism in working muscles is presented here. Skeletal muscle has some unique metabolic characteristics, which include rapid changes in oxygen delivery that can reach some 10-fold changes and metabolic rates that can reach over 100-fold changes. The metabolic control of skeletal muscle has been of great interest to historic biochemists such as Meyerhof, Krebs, and others for many years. Chance has performed research to understand the metabolic control of mitochondrial function in earnest since the 1950s.[18,19] Because of the strong dependence of muscle metabolism on the oxidative pathway, alteration in either oxygen consumption (VO_2) or oxygen delivery (DO_2) will influence functional capacity. The net oxidative energy pathway in muscle tissues can be described by the following equation:

$$3\mathrm{ADP} + 3\mathrm{Pi} + \mathrm{NADH} + \mathrm{H}^+ + 1/2\mathrm{O}_2 = 3\mathrm{ATP} + \mathrm{NAD}^+ + \mathrm{H}_2\mathrm{O},$$

where ADP is adenosine diphosphate, Pi is inorganic phosphate, and ATP is adenosine triphosphate.

The kinetic control model, which describes metabolic rate as a function of regulatory substrate concentrations using the Michaelis−Menten equation, is listed below[9]:

$$\mathrm{V/Vm} = 1/(1 + \mathrm{k1/ADP} + \mathrm{k2/Pi} + \mathrm{k3/O}_2 + \mathrm{k4/NADH}),$$

where V is the observed velocity, Vm is the maximal velocity, K1−K4 represent affinity constants for the various substrates, NADH is reduced nicotinamide adenine dinucleotide, and NAD is nicotinamide adenine dinucleotide.

As the *in vivo* mitochondrial concentrations of ADP, Pi, O_2, and NADH are 20 μM, 1000 μM, 1 μM, and 100 μM, respectively; and the Km (half maximum velocity) *in vitro* values for ADP, Pi, O_2, and NADH are 20 μM, 300 μM, 0.1 μM, and ~10 μM, respectively; the primary candidate for metabolic control is ADP. It has been proposed that the rate of mitochondrial respiration can be determined by the rate of adenine nucleotide translocation and, therefore, the [ATP]/[ADP] ratio regulates the respiratory rate under physiological conditions.[20] Chance *et al.* illustrated the reversibility of electron transport in isolated mitochondria under anaerobic conditions. Meyer *et al.*[21] proposed the thermodynamic control model to describe that the relationship between the PCr level and the mitochondrial respiration rate is linear.

3. A Brief Description of Muscle ^{31}P-MRS Methodology

Magnetic resonance (MR) has become a popular tool in the fields of both biochemistry and physiology since 1980s, its first use on human subjects.[22] While MR imaging (MRI) has shown its extraordinary capabilities in terms of imaging anatomical structures, MR spectroscopy (MRS) provides varying biochemical information. ^{31}Phosphorus (^{31}P) MRS spectra contain five major peaks corresponding to concentrations of Pi, PCr, and the three phosphates of ATP (Fig. 2).[9] Free ADP concentrations are too low to be directly visible but can be calculated via the creatine kinase equilibrium

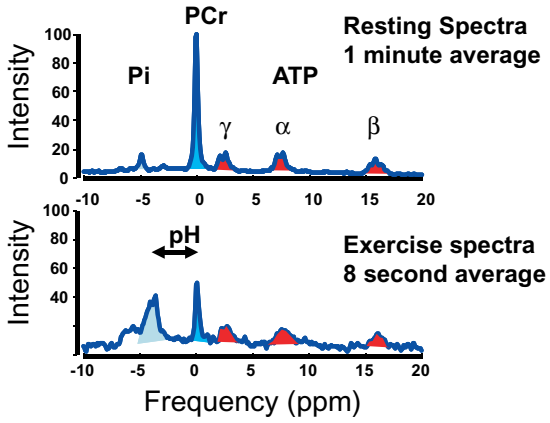


Fig. 2. Typical phosphorus spectra from the calf muscle. The top spectra is a 1-min average taken at rest. The major peaks are inorganic phosphate (Pi), phosphocreatine (PCr) and the three peaks of ATP. The bottom spectra is a typical spectra and was collected in 8 s. The PCr peak is reduced and Pi peak is increased. Muscle pH is measured by the distance between the Pi and PCr peaks. Decreased distance indicates lower pH values.

reaction. In addition, ^{31}P-MRS allows the measurement of intracellular pH, based on a shift of the frequency of the Pi peak due to different concentrations of the mono- and di-protonated forms of Pi (pK of 6.75 in muscle).[8] In human studies, the muscles most easily studied were those in the calf, forearm, and later the thigh, as the bore size increased from 30-cm bore magnets to 100-cm. Increasing bore size and magnet strength (from 1.5 T to as high as 7.0 T in human studies) has reduced the time needed to obtain adequate signals from 1.5−5 min in the early studies to as short as 1−3 s.

4. A Description of Muscle NIRS Methodology

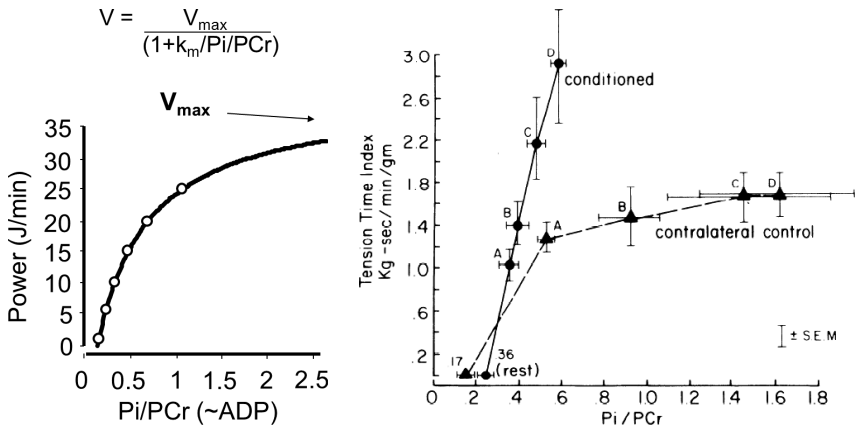
Wavelengths ranging from 700−3000 nm show less scattering and thus better penetration into biological tissue than visible light. However, light absorption by water limits the tissue penetration above 900 nm wavelength, leaving the 650−900 nm range. The major absorbing compounds of this wavelength region are intravascular hemoglobin (Hb), intramuscular Mb, skin melanin, and mitochondrial cytochrome c oxidase.[14] The most common, commercially available NIRS devices use single-distance continuous wavelight (NIR_{SDCWS}). To calculate the changes in oxy-Hb/Mb, deoxy-Hb/Mb, or total-Hb/Mb, the equation of a two-, or multiple-wavelength method can be applied according to the modified Beer−Lambert law.

Chance demonstrated that the pattern of the light path from the light source to the detector is that it follows a banana-shaped curve in which the penetration depth into the tissue is approximately equal to half the distance between the light source and the detector.[17] Chance also found that penetration depth would be 1−2 cm and the measured volume would be approximately 4 cm^3 with a 3-cm light-detector separation.[23] The pathlength of light will vary due to variations in tissue composition (adipose tissue versus muscle), blood volume (can increase or decrease heme concentrations over time), and muscle shape (altered during muscle contractions). Subcutaneous adipose tissue thickness, in particular, influences the light path resulting in the reduced NIRS signal intensity.[24] Chance also conducted a pioneer research on the methodology such as time-resolved spectroscopy (NIR_{TRS}) and phase modulation spectroscopy (NIR_{PMS}) that is able to measure pathlength of NIR light.[25,26] NIR_{TRS} uses expensive single-photon detectors to measure the time the light spends in the tissue, while NIR_{PMS} uses the change in phase of coherent light to determine the time the light spends in the tissue. These approaches provide absolute values of oxygenated and deoxygenated Hb/Mb and Hb/Mb O_2 saturation (SO_2) in the skeletal muscle. Spatially-resolved NIR_{SRCWS} (NIR_{SRCWS})[27] provides relative changes in Hb/Mb and absolute values of SO_2. NIR_{SRCWS}, using multiple light sources coupled to one detector, solves multiple equations for pathlength. We have still limited information available whether pathlength shows any significant change during exercise, recovery, and other intervention periods.[25,26] Measurement of changes in pathlength is needed in a wide range of exercise mode/intensity and amongst varying subjects. The other technological limitation is the similar absorption spectra for Hb and Mb. This makes it difficult to distinguish between the two by the optical properties alone although ^{1}H-MRS is able to distinguish the two by the deoxygenated proximal histidyl $N_\delta H$ signals of myoglobin. From large stationary devices using one source detector pair, recent devices can have either variable source detector separation distances or up to 16 individual source detector pairs, allowing for either depth resolution or spacial resolution of the NIRS signal. Signals are also routinely collected at 1−3 Hz.

5. Muscle MRS and NIRS Studies in Collaboration with and Supervised by Britton Chance

5.1. *Physiological studies*

Early MRS studies measured Pi, PCr, ATP, and pH values during steady-state exercise. In steady state, levels of ATP are normally quite constant in skeletal muscle and PCr decreases with an increase in exercise intensity. Chance *et al.*[9] pioneered the use of the ratio of Pi to PCr as an indicator of ADP levels with little change in muscle pH. These studies showed that the primary control of oxidative metabolism during steady-state exercise was ADP. Fitting the Pi to PCr ratios and work levels to a Michaelis−Menten type of equation yields a maximal velocity (Vmax) of the reaction, which was considered a measurement of oxidative capacity (Fig. 3, left panel).[9,28] This relationship has been

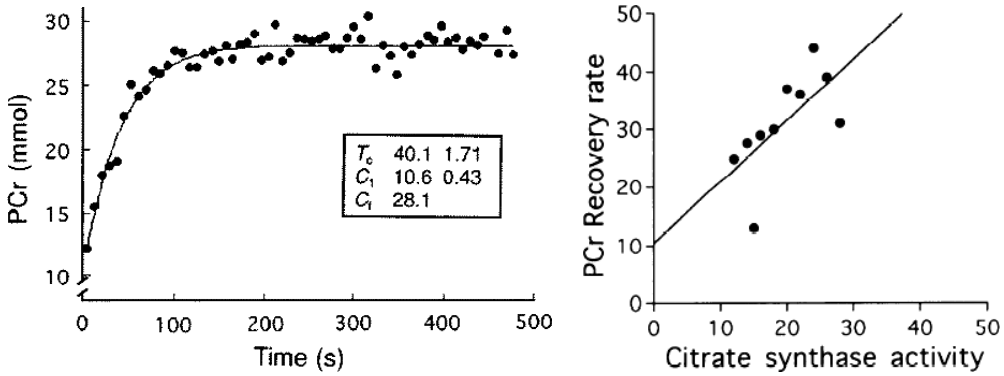


(a) (b)

Fig. 3. (a) Theoretical relationship between the work performed by a muscle (power) and the metabolic response (Pi/PCr).[9] (b) Changes in the transfer function in canine skeletal muscle with chronic electrical stimulation.[28]

described as the "transfer function", indicating the relationship between the transduction of chemical energy and physical work. A number of studies have used steady-state measurements of Pi to PCr ratios to demonstrate differences between athletes, sedentary normal subjects, and patients with various diseases (Fig. 3, right panel).[28,29] Improvements in MRS signal quality allowed for a new kinetic approach to assessing muscle mitochondrial function.[21] PCr recovery closely reflects oxygen consumption and the rate constant of PCr resynthesis is equivalent to the "Vmax" calculated from the steady-state measurements (Fig. 4, left panel).[30,31] Submaximal exercise is used because decreases in muscle pH with higher levels of exercise slow the rate of PCr recovery due to mitochondrial inhibition. This approach has been used to "validate" MRS measurements with those from muscle biopsies[31] and to evaluate a variety of human populations.[32–34] A major advantage of the recovery technique is that it allows for comparison between population groups without having to normalize for differing amounts of muscle mass or use of synergistic muscles, always a concern in studies of voluntary human exercise.[35]

In the early studies, Chance[17] had hypothesized that reoxygenation kinetics during induced-hyperemic response after exercise has similar information as PCr recovery kinetics does, which was confirmed in a later study that MRS measurements of PCr recovery and NIRS measurements of recovery of HbO_2 saturation provide similar information as long as muscle pH remains near 7.0 (Fig. 5).[32] Thereafter, several studies reported that the recovery time of muscle reoxygenation after submaximal to maximal exercise[32,36] is one of the indicators for evaluating muscle oxidative capacity. These studies have



(a) (b)

Fig. 4. (a) Levels of PCr after exercise in a young sprint athlete. Values of PCr were fit to an exponential curve. Tc, rate constant; Ci, initial level; Cf, final level.[30] (b) Comparison between biopsy-measured citrate synthase activity and post exercise PCr recovery from the gastrocnemius muscles of healthy men.[31]

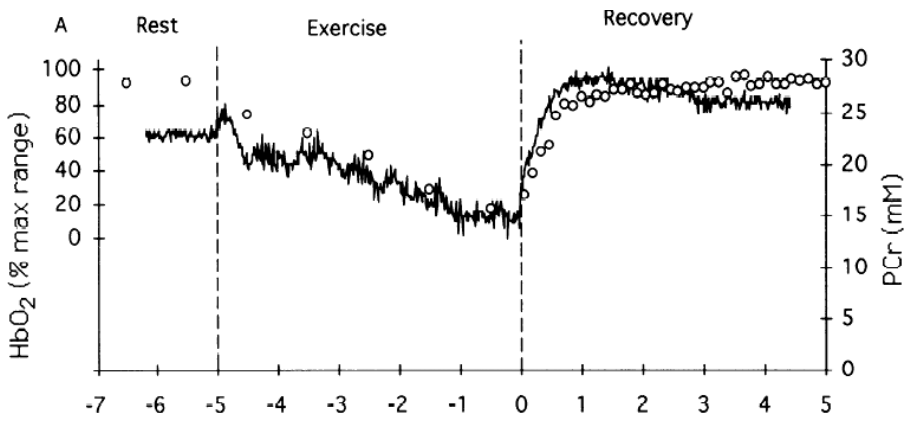


Fig. 5. Simultaneous NIRS and MRS measurements during progressive plantar flexion exercise. This study shows the generally good agreement between the changes in oxygen saturation and PCr concentrations.[32]

reported a good agreement between faster PCr recovery kinetics and faster oxygenation kinetics measured with NIRS. A calibration is needed to compare the values obtained using NIR_{SDCWS} among subjects and different measurement sites.[17] The arterial occlusion is one of the popular methods and based on the assumptions that 5−6 min of ischemia will result in the complete disappearance of O_2Hb and that the reactive hyperemia after occlusion will almost completely eliminate deoxygenated Hb.

Evaluation of muscle energy metabolism using NIRS is difficult because the measured oxygenation levels do not specifically reflect muscle oxygen consumption (mVO_2), rather they reflect the balance between muscle DO_2 in relation to mVO_2. In order to dissociate mVO_2 from DO_2 using NIRS, the transient arterial occlusion method is one of the popular approaches used in Chance's group. The transient arterial occlusion uses 10−30 s of arterial occlusion provided by a pneumatic tourniquet to interrupt DO_2 to the monitored muscle.[37−39] Quantitative measurement of resting metabolic rate is possible in a combination with MRS measurement by applying a 15-min ischemia to the muscles.[38] The rate of decline of muscle O_2Hb during ischemia can be compared with that of muscle PCr in mM per second or a conversion to mVO_2 in mM per second. NIR_{TRS} has also been used to measure resting mVO_2, providing results in absolute units.[26] The transient arterial occlusion method has also been applied to measure forearm muscle metabolism during exercise.[38]

NIRS has also been used for evaluating acute and chronic (training) effects of exercise on muscle oxygenation for athletes. Chance was very interested in exercise performance of varying athletes because of his experience as a gold medalist in Helsinki Olympics in 1952. He examined varying athletes' performance ranging such as triathletes,[40] cyclist,[41] rowers,[17,42] cross-country skier,[43] resistance-trained athletes,[44] and skaters[45] using NIRS in a cross-sectional study design. For a longitudinal study, changes in skeletal muscle oxidative function were measured by NIRS in immobilized forearm muscles evaluating the preventive effect of the endurance training protocol on deterioration of skeletal muscle.[39] Muscle oxidative function was determined by the time constant for the recovery of mVO_2 applying repeated transient arterial occlusions after exercise (Fig. 6). This study suggested that NIRS is useful clinically for noninvasive monitoring of deconditioning and reconditioning of skeletal muscle oxidative function.

5.2. *Pathological measurements*

Chance was the first to detect functional deterioration of muscles in patients with muscle diseases using MRS and NIRS. Previous studies using MRS have shown the utility of measuring muscle energetics in patients with cytochrome b deficiency.[46,47] Altered resting phosphorous metabolites have also been used to identify various muscle-wasting disorders.[48−50] Using NIRS, an increase in muscle oxygenation at the onset of treadmill exercise has been detected in patients with cytochrome c oxidase deficiency,[51] in patients with mitochondrial myopathy caused by the mitochondrial DNA mutations,[52] and in patients with Friedreich's ataxia.[53] Muscle hyperoxygenation at the exercise onset measured with NIRS

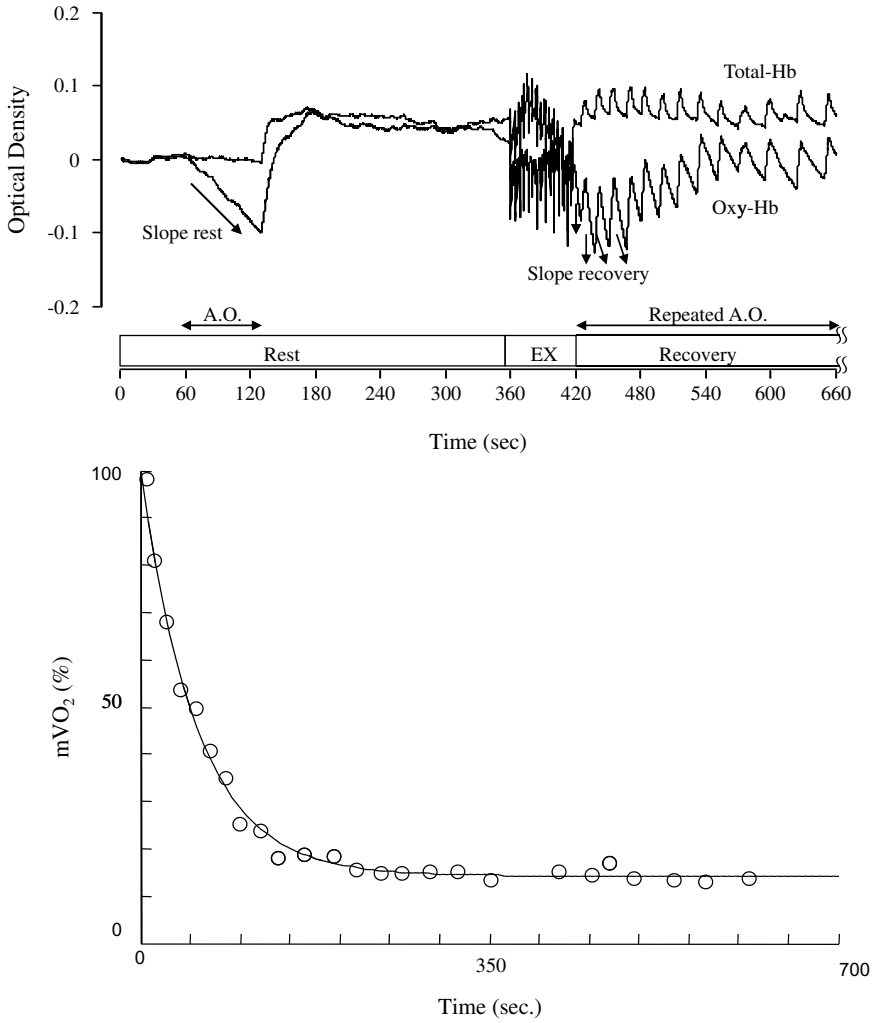


Fig. 6. (a) Schematic representation of mVO_2 and typical changes in muscle oxygenated Hb/Mb. Schematic representation of mVO_2 and typical changes in muscle oxygenated Hb/Mb at rest, during exercise, and recovery. mVO_2 was calculated from the rate of the decline of the oxygenated Hb/Mb during arterial occlusion at rest (slope rest) and recovery period (slope recovery); (b) typical kinetics of mVO_2 recovery after exercise. Typical kinetics of mVO_2 recovery after exercise. Time constant for this subject was 55.8 s (pre) $\rightarrow$ 54.7 s (post). Adapted from Ref. 39.

has been used as a diagnostic in many cases of suspected mitochondrial disease. Recently, patients with mitochondrial myopathies (MM) or myophosphorylase deficiency (McArdle's disease, McA) were tested for changes in the capacity for O_2 extraction, maximal aerobic power, and exercise tolerance during cycle exercise using NIRS.[54] NIRS is a promising noninvasive tool for monitoring metabolic deterioration in the settings of follow-up and in the assessment of therapies and interventions.

A number of studies have used MRS and NIRS to evaluate patients with peripheral vessel diseases. Peripheral arterial disease (PAD) involves partial occlusion of arterial flow, usually to the legs, that

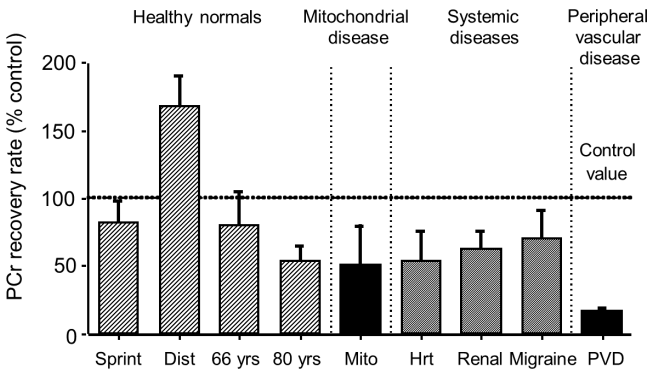


Fig. 7. MRS-measured PCr recovery rates for various populations of human subjects. Each group is expressed as a percentage of the age and or activity matched control group.[36]

impairs function. A consistent finding with NIRS measurements in PAD patients is slower rates of muscle PCr recovery and reoxygenation after exercise (Fig. 7).[55,56] The magnitude of the impairment could be very large, with recovery rates being up to five times slower than healthy control subjects.[57] Taking into account that not all studies have shown positive results, NIRS appears to be able to identify and quantify the severity of patients with PAD.

Chance collaborated with cardiologists to evaluate skeletal muscle oxygenation in patients with heart disease.[15,58] Patients with chronic heart failure exhibited greater deoxygenation compared with the controls due partly to the pump failure of the heart and the consequent skeletal muscle hypoperfusion. Recently, it is also reported in the heart transplant recipients (HTR) that NIRS allows the detection of an impairment of both DO_2 and O_2 extraction in the skeletal muscle.[59] Muscle oxygenation and metabolism were examined by using NIRS in children with end-stage renal disease (ESRD) one week before and four weeks after renal transplantation during submaximal hand-grip exercise.[60] Recovery time (Rec-time) for reoxygenation after exercise was significantly lower in patients before renal transplantation compared with the control group. Rec-time after exercise improved significantly after renal transplantation and it was not significantly different from that of controls, indicating that

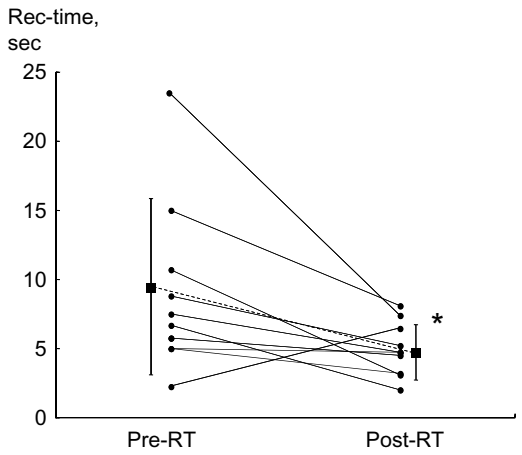


Fig. 8. Changes in recovery time (Rec-time) for reoxygenation after renal transplantation (RT) in 10 renal transplant recipients. Individual data shown in black circles and solid lines, mean and SD shown in black squares and dotted line. Significant difference ($p < 0.05$) is marked by asteriks (*). After RT, Rec-time was shortened in nine patients and delayed in one patient who showed the shortest TR before RT. Adapted from Ref. 60.

Fig. 9. *In vivo* NIRS pioneers got together in Japan. From the left to the right: Tamura, Nakase, Unknown, Hamaoka, Chance, Jobsis, Delpy, Ferrari, Okada, Koizumi, Benaron, Kagaya.

oxidative metabolism in skeletal muscle during exercise is impaired in children with ESRD and recovers after renal transplantation (Fig. 8).[60]

NIRS measurement has also been applied to the study of the other chronic diseases, including patients with chronic obstructive pulmonary disease,[61,62] spinal cord injury,[63,64] diabetes mellitus,[65] and chronic fatigue syndrome.[66]

6. Conclusion

There is an increasing need for monitoring skeletal muscle oxygenation and metabolism in humans. MRS has been developed by Chance's group as the "gold standard" for noninvasive evaluation of skeletal muscle bioenergetics. Chance has also developed portable NIRS system called "RunMan" that served as one of the first models to provide us with opportunities for further clinical muscle research in a noninvasive and portable way. MRS and NIRS indicators have been shown to be useful for monitoring changes in muscle metabolism and oxygenation in healthy subjects as well as in patients with various organ diseases as well as muscle specific disorders. As MRS and NIRS technologies are practical and useful for measuring human muscle metabolism, we will strive to continue Chance's legacy by advancing muscle MRS and NIRS studies (Fig. 9).

Acknowledgments

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