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**Modular regulation analysis of integrative effects of hypoxia
on the energetics of contracting skeletal muscle *in vivo***

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SHORT TITLE : hypoxic regulation of muscle ATP turnover: top-down analysis

Synopsis

In exercising muscle, acute reduction in ambient oxygen impairs muscle contraction due to effects of hypoxia on mitochondrial ATP supply. The less marked impairment reported after long-term exposure to hypoxia points to changes in regulation of the energetic system of contraction in hypoxic conditioned animals. This energetic system was conceptually defined here as two modules, ATP/PCr-producer and ATP/PCr-consumer connected by energetic intermediates. Modular control analysis (MoCA) that combines top-down control analysis with non-invasive ^{31}P -NMR spectroscopy was used to describe *in vivo* the effects of hypoxia and adaptation to hypoxia on each module.

Modulations of steady levels of ATP turnover (indirectly assessed as force output) and muscle PCr were obtained in hypoxic conditioned (HC) rats (6 weeks at 10.5% O_2) vs. normoxic (N) rats. Modular control and regulation analyses quantified the elasticity to PCr of each module in N and HC rats as well as the direct effect of acute hypoxia on the PCr/ATP-producer module.

Similar elasticities in N and HC rats indicate the absence of response to long-term hypoxia in internal regulations of the ATP supply and demand pathways. The less marked impairment of contraction by acute hypoxia in HC rats ($-9\pm 6\%$ vs. $-17\pm 14\%$ in N rats, $P < 0.05$) was therefore fully explained by a lower direct effect (HC $-31\pm 13\%$ vs. N $-44\pm 23\%$, $P < 0.05$) of acute hypoxia on mitochondrial ATP supply. This points up a positive adaptation to chronic hypoxia.

MoCA *in vivo* may provide powerful tools to find out improved function (alternatively dysfunction) at system level in conditioned animals.

Keywords. Metabolic control analysis; ^{31}P -MR Spectroscopy; ATP turnover; hypoxic conditioning; oxidative phosphorylation

Introduction

As an effective oxygen sink, mitochondria house the ultimate step of the fate of oxygen pathway from air through the cardiovascular and pulmonary systems. Oxygen is used for substrate oxidation by mitochondria and is therefore involved in the main mechanism responsible for energy supply in moderately exercising skeletal muscle. An intriguing question for physiologists over the last three decades has been the influence of acute hypoxia over muscle contraction in an integrated context. Accordingly, the effect of breathing gas mixtures with varied fractions of inspired oxygen on muscle respiration has been extensively studied. Acute and severe reduction in oxygen delivery typically results in a wide range of metabolic disturbance, e.g. decrease in the steady level of phosphocreatine (PCr) [1] and alteration in other energetic intermediates (ATP, Pi, and ADP) [2], ultimately culminating in a total loss of contractile function [3, 4]. A less marked perturbation by acute hypoxia of the bioenergetic system of contraction in skeletal muscle is generally reported in humans as well as in rodents after exposure to hypoxia for several weeks as well as in humans indigenous to high altitudes [5, 6]. To date yet, the hypoxia-induced responses of integrative regulatory features of muscle energetics have not been systematically studied.

Recent successful analyses suggest that an improved understanding of the integrative regulation by O₂ could be gained from a modular approach of the energy metabolism of contracting muscle. In such integrative approaches, the energy metabolism of intact contracting muscle (either heart or skeletal muscle) can be conceptually divided into two modules: ATP/PCr-producer and ATP/PCr-consumer [7–10]. Modules communicate only via a group of energetic intermediates (ADP, ATP, Pi, Cr and PCr) whose concentrations are connected to each other and to the phosphorylation potential. Since muscle PCr dynamics can be detected non-invasively by ³¹P-MR spectroscopy with high signal/noise ratio, PCr concentration ([PCr]) is systematically the studied variable in the above experimental works. The reactivity of each module to PCr is quantified by the elasticity coefficient (or elasticity). Since the whole set of elasticities determine internal regulation in a metabolic pathway [11–15] as well as in modular systems [7–10, 14, 16, 17], these parameters may be used to objective the regulation of ATP turnover or so-called ‘energy demand-supply coupling’ [2,

5, 6, 18] in contracting muscle of hypoxic animals. Additionally, once modular elasticities are known, the regulatory role of any effector on the energetic system can be quantified [9, 12]. So, the overall regulation analysis would likely make it possible to find out the origin of the less marked perturbation by O₂ of the energetic system of contraction after hypoxic conditioning.

In the present study, ³¹P-MR spectroscopy was used to quantify *in vivo* the elasticity to PCr of ATP/PCr-producer and ATP/PCr-consumer modules in normoxic rats (N) vs. hypoxic conditioned rats (HC) exposed for 6 weeks to 10.5% O₂. The determination of elasticity values then served for the determination of control exerted by each module on ATP turnover and contraction, as quantified by flux-control coefficients. At last, thanks to the determination of control distribution, the direct effect of O₂ on the producer module was quantified in N and HC rats to uncover regulatory features of acute hypoxia on the energetic system.

Experimental procedures

Animal preparation. Female Wistar rats weighting 280 to 340g were used for these experiments according to the guidelines of the National Research council's *Guide for the Care and Use of Laboratory Animals*. All animals were housed four to cage in an environmentally controlled facility (12h/12h light-dark cycle, 22°C) and received food and water *ad libitum*. They were separated in two groups: normoxic (N) vs. chronically hypoxic (CH), each group housed in specific compartments.

In the hypoxic compartment, long-term reduction in the fraction of inspired oxygen (FiO₂) to 10.5%, as achieved by mixing air with nitrogen, was used to simulate chronic hypoxia during six weeks. Circulating the gas mixture through a water volume into a mixing chamber preserved humidity. FiO₂ was continuously monitored in the hypoxic compartment with an alarm oxygen analyser (GasAlert Extreme, BW technologies, Calgary, Canada).

For experiments, rats were carefully taken out of their cage then anesthetized in a plexiglass box flushed by air containing 3% isoflurane. Throughout the experiment, anesthesia was

maintained by continuous inhalation of a gas mixture containing 1.5% isoflurane delivered in a facemask.

Experimental set up. The experimental set up was described elsewhere [7]. Briefly, the anesthetized rat was placed in a supine position into a supraconducting magnet (4.7 Tesla, 47/50 Biospec Avance MR system Bruker, Ettlingen, Germany) so that the right segment of the lower limb was centred inside a 30 mm diameter ^1H Helmutz imaging coil tuned to the proton frequency (200.3MHz). A circular homebuilt transmit-receive ^{31}P probe (18mm) was placed horizontally under the gastrocnemius and tuned to the phosphorus frequency (81.1MHz). Proton shimming was achieved locally in a $20 \times 20 \times 20 \text{ mm}^3$ voxel by using the water proton resonance, with typical widths at half heights of 30-35Hz.

Before measurements in contracting muscles, a proton MR image was acquired axially by using the ^1H coil to verify muscle group position and to improve field homogeneity.

The plantar flexor muscles group was stimulated electrically (Compex 2 stimulator, Ecublens, Switzerland) directly with transcutaneous electrodes located at the knee and heel levels. For the study of moderate intensity of exercise, the current intensity was set at 6 mA in N rats as well as in CH rats.

A total of thirty ^{31}P spectra (100 μs rectangular pulse, 60° flip angle in the middle of the coil; 64 accumulations 2.8 s recovery time; 3.3kHz bandwidth; 1024 data points) were acquired in 180s blocks throughout the experimental protocol.

Two ^{31}P -MRS spectra were acquired to assess the level of muscle phosphocreatine at rest. Then, during a warm-up period muscle stimulation was increased progressively and hypoxia was gradually initiated by F_iO_2 reduction of inhaled gas (21% to 9% O_2). No measurements were taken into account for the subsequent analysis until contraction and spectra reached a steady state. Typically this transitory period lasted about 40 min (13 spectra).

Once the hypoxic (9% O_2) steady state was reached and maintained for 15 min, a first switch to air for additional 15 min (5 spectra) followed by a second switch to a lower electrical stimulation (5 additional spectra) provided modulations around the studied steady state of PCr

and muscle work rate. The elasticity coefficient of the producer and consumer blocks, flux-control coefficients, and direct effect of O₂ on producer (see below) were determined around this steady state.

System variables. The conceptual system of muscle energetics used here is composed of two modules, ATP/PCr-producer and ATP/PCr-consumer (fig 1). The analysis of the system requires the measurements of two variables: the intermediate and the flux through each module; the later is equal to the overall flux through the system under steady state conditions. The representative 'intermediate' in the system was PCr concentration, as assessed by ³¹P MR spectroscopy; muscle work rate represented the 'flux' as assessed by plantar flexion force exerted on a pedal [7].

Concentrations of metabolic intermediates were assessed from MR-spectroscopy spectra. MRS spectra were routinely deconvolved into Lorentzian lines (Igor Pro Wavemetrics, Lake Oswego, Oregon, USA). The phosphocreatine, Pi, and β-ATP peak areas were calculated and were converted to concentrations assuming [ATP]=8.2 mM.

Contraction resulting from muscle electrical stimulation was measured with a homebuilt ergometer consisting in a foot pedal connected to a hydraulic piston. A hydraulic circuit filled with water connected the piston to a force transducer (ref. N° MLT0699, AD Instruments powerLab Systems) that was placed outside the magnet. Changes in pressure induced by pedal stroke were recorded at 200Hz. The magnitude of strokes was computed as a function of time (Igor Pro, Wavemetrics, Oregon, USA) to provide the work rate (contraction) signal.

Modular Control Analysis. The experimental setup made it possible to quantify concomitant changes in steady levels of PCr and contraction during modulations around the studied steady state called 'reference'. Following the principles of Metabolic Control Analysis, the modulation by acute hypoxia of steady levels of PCr and contraction made it possible to determine two parameters critical for our understanding of integrative regulation

of the modular system by acute hypoxia: (i) the consumer elasticity (ϵ_c) and (ii) the role of oxygen as an external effector of the producer module. The consumer elasticity was determined by relative change in contraction divided by relative change in PCr, induced by acute hypoxia. The direct effect of acute hypoxia on the producer was determined by the relative change in contraction divided by the flux-control coefficient of the producer (see below). The modulation by electrical stimulation allowed the quantification of the producer elasticity (ϵ_p). The producer elasticity was determined by the relative change in contraction divided by the relative change in PCr, induced by lowering electrical stimulation. Summation and connectivity theorems allowed the calculation of flux-control coefficients from the experimental elasticities. The flux-control coefficient of the producer (C_p) was calculated as $\epsilon_c/(\epsilon_c-\epsilon_p)$; the flux control coefficient of the consumer (C_c) equals: $\epsilon_p/(\epsilon_p-\epsilon_c)$.

Enzymatic analysis. Once all the non-invasive measurements were performed, the animal was sacrificed by the injection of a lethal dose of pentobarbital. A portion of the rat soleus muscle (about 80 mg) was rapidly excised with scissors and immediately cooled in ice-cold buffer consisting of 25mM Sucrose, 5mM MOPS and 0.2mM EDTA. Whole tissue homogenates were prepared in the same buffer by hand homogenisation using a Polytron (PT 1200E; Kinematica AG) at 4°C. An aliquot of the homogenate (150 μ l) was used for measuring cytochrome c oxidase (Cox) activity, by following the decrease in absorbance at 550nm in spectrometer (Cary 50 UV-Vis spectrophotometer; Varian Inc.) with chemically reduced horse heart cytochrome c as electron donor. The reaction mixture contained (in mM): 100 KCl, 40 saccharose, 10 KH_2PO_4 , 5 $\text{MgCl}_2/\text{H}_2\text{O}$, 1 EGTA and 0.1% (w/v) BSA (pH= 7.2). The reaction was started by addition of reduced cytochrome c (50 μ M). Cox activity was expressed in $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of protein.

Blood analysis. The hematocrit (Ht) was determined using blood collected from rat heart and centrifuged in micro capillary tube.

Results

Hypoxic conditioned (HC) rats exposed to 10.5% O₂ during 6-weeks had higher hematocrit than control normoxic (N) rats (52±3% vs. 42±3%, P<0.05), thus indicating improved oxygen transport capacity. HC rats had also lower Cox activity (250±12 vs 343±27 μmol·min⁻¹·g⁻¹, P<0.05) possibly indicating a lower maximal oxidative capacity in the *soleus* muscle. Taken together, these results indicate that our rats exposed for 6 weeks to 10.5% O₂ were effectively conditioned.

The present elasticity analysis was based on simultaneous recordings of steady levels of contraction and PCr during a steady state exercise modulated by acute hypoxia (9% O₂) and slight decrease in electrical stimulation as illustrated in figure 2. Figure 3 provides a comparative overview of averaged changes in PCr and contraction due to modulations by 9%O₂ and lowering electrical stimulation in N rats and HC rats. Solid lines represent the effect of each modulation around the studied steady state called 'reference'. As quantified below by the elasticity analysis, figure 3 shows similar slopes (solid lines) in HC and N rats in response to each modulation. It also worth noting that acute hypoxia (9%O₂) induced a lower decrease in both PCr and contraction in HC rats when compared to N rats.

Averaged elasticities of the ATP/PCr-producer and ATP/PCr-consumer module in each population (HC vs. N) are shown in table 1. One main finding of the present study was the absence of difference between elasticities in HC rats and in N rats.

Flux-control coefficients calculated from these experimental elasticities are reported in table 1. A very similar control distribution was quantified in N and HC rats. In the present study where moderate work intensity was stimulated, the control over ATP turnover and contraction was dominantly on the consumer (C_c=65%). Yet, since the producer had substantial control over contraction (C_p=35%), any external effector that can affect directly the producer activity is expected to alter contraction in some extent. Acute hypoxia (9% O₂) was just the effector whose direct effect on producer was quantified here. Since contraction was less inhibited by acute hypoxia in HC rats while the producer, which is the target of acute hypoxia, had similar control over contraction, a lower direct effect of acute hypoxia on

producer must be concluded. Accordingly, the quantified direct effect of acute hypoxia on the ATP/PCr-producer module in HC rats was only 70% of the direct effect in N rats (table 1).

Discussion

The present study demonstrates that, in moderately contracting skeletal muscle, internal regulatory features of ATP turnover as determined here by elasticity coefficients *in vivo* are likely not affected by long-term exposure to 10.5% O₂. In agreement with previous works e.g. [6] we additionally observed a lower impact of acute hypoxia (9%O₂) on contraction in hypoxic conditioned animals, which can be viewed as a positive adaptation to chronic hypoxia. The application of regulation analysis indicates that in absence of change in internal regulation of ATP turnover, this adaptation in HC rats is fully explained by a lower direct effect of ambient hypoxia on the producer.

There is a growing appreciation of the concept that an improved understanding of complex regulatory processes could be gained from integrative determination of modular elasticity coefficients in intact organs. By conceptually defining heart energetics as a two-module system, ATP/PCr-producer and ATP/PCr-consumer, Dolez and colleagues [9] highlighted the routes taken by regulatory effects of calcium in beating heart. Korzeniewski and colleagues [10] incorporated elasticity coefficients in the Proportional Activation Approach and demonstrated so, that both ATP/PCr-producer and ATP/PCr-consumer modules are directly activated to a similar extent during physiological activation of the heart by adrenaline. As regards skeletal muscle, Jeneson and colleagues [17] and recently Arzac and colleagues [7] proposed theoretical and experimental approaches respectively for the assessment *in vivo*, of elasticity coefficients of ATP/PCr-producer and ATP/PCr-consumer modules. Although such methods are usually crude, they have been demonstrated to significantly strengthen the field of physiological regulations in skeletal muscle [16]. Since in the present study, the producer elasticity reached similar values in normoxic rats and hypoxic conditioned rats, we conclude that hypoxic conditioning has no effect on the reactivity of oxidative phosphorylation to energetic intermediates (ADP, ATP, Pi, PCr, Cr and the phosphorylation

potential) during moderate exercise. In other words, the substrate feedback control [19, 20] of tissue respiration is likely unaltered by chronic hypoxia. Although our approach actually did not evaluate variations in all these key regulators but focused on PCr as representative, the degree to which PCr is broken down in our conditions fits well with changes of the energy charge of the cell [2, 18, 21, 22]. Force output, as a surrogate of ATP turnover, was the other critical variable measured in the present approach. For reliable MCA, one should assume that contractile efficiency is not affected by hypoxia, incidentally fatigue due to prolonged work, or stimulation intensity. In humans cycling at submaximal intensities, no change in efficiency was observed at sea level, acute, and chronic altitude of 4300m [23]. As well, in electrically stimulated canine gastrocnemius under control, ischemic and hypoxemic conditions, there was clearly no effect of a lower O₂ delivery on efficiency, as indicated by proportional decrease in muscle force output and O₂ uptake [24]. In steady states prolonged for hours (>2h, personal results) we noted no decline in force output or in PCr concentration, even at the highest (sub-maximal) intensities used in these experiments. We concluded that electrical stimulation intensities generate non-fatiguing conditions and that efficiency therefore is unlikely to change with respect to time in our conditions. At last, an obvious candidate for a change in efficiency due to different stimulation intensities is a shift in the recruitment of oxidative and glycolytic fibers. Our previous T₂-weighted images (based on ¹H MR imaging) indicated that fibers of the mixed gastrocnemius are recruited at whatever electrical intensity [7]. Furthermore, muscle pH during the periods of interest reached 6.97±0.04 in N rats and 6.94±0.07 in HC rats with no value below 6.87, thus indicating that ATP synthesis is fully, or at least to a large extent, oxidative in every experiment.

Although the producer elasticity is a critical parameter that likely reveals kinetic or thermodynamic 'driving functions' for mitochondrial metabolism, the comprehensive understanding of ATP turnover regulation requires paying attention to the reactivity of ATP/PCr consumption as well. In the present study, we obtained similar consumer elasticities in N and HC rats. Therefore, similar ϵ_p as well as similar ϵ_c in both populations suggest that

internal kinetic functions involved in the regulation of muscle ATP turnover *in vivo* are essentially not altered in hypoxic conditioned rats, as compared to normoxic rats.

The absence of changes in elasticity to the intermediates of ATP production and ATP consumption in hypoxic animals is not documented elsewhere in the literature. Our results in normoxic animals can yet be compared to experiments based on electrically stimulated skeletal muscle of animals *in situ*. Hogan and colleagues [2] obtained concomitant recordings of contraction and PCr levels in electrically stimulated canine gastrocnemius *in situ* at two levels of stimulation and under normal and low O₂ conditions. So, the elasticity of conceptual producer and consumer modules in their normoxic animals can be calculated from concomitant changes in oxygen uptake and [PCr] in response to hypoxemia and electrical stimulation. The calculated values of -1.1 for the producer elasticity and 1.2 for the consumer elasticity are consistent with the present quantification in N rats (table 1). These similarities in elasticity coefficients obtained in intact muscle in previous studies [2, 7] as well as in the present study indicate that, although the proposed experimental methods might be crude, elasticity coefficients can be determined in intact muscle with fine consistency. Therefore, we exclude that HC rats might have altered elasticities undetected by our present approach and point definitively to the absence of alteration in internal regulatory features of ATP turnover and contraction in our hypoxic conditioned rats.

The theory of metabolic control analysis has shown that control over the flux through a given pathway can be distributed over many steps in the pathway and that the degree of control of any given step can be quantified by its flux control coefficient [15]. In the present study, the control over ATP turnover and contraction derived from elasticity values was effectively distributed between the two modules in each population (table 1). The quantification of flux-control coefficients highlights similar C_p, amounting to 35% in N and HC rats. The critical consequence for our understanding of regulation by O₂ of muscle energetics is that any direct effect of O₂ on the producer activity will be transmitted to contraction with similar 'strength' (35%) in N and HC rats. Therefore similar control strength over the flux by the producer module in N and HC rats has to be analyzed in concert with the overall effect of 9% O₂ on contraction (so-called global effect in the framework of regulation analysis). This global

effect was only $-9\pm 6\%$ in HC rats but amounted to $-17\pm 14\%$ in N rats (figure 3). Since this difference in global effect cannot be explained by different control by the producer over contraction, it is fully explained by a lower direct effect of $9\% \text{ O}_2$ on the producer module, amounting to -31% in HC rats but -44% in N rats ($P < 0.05$). It is therefore concluded that the target of hypoxic conditioning in the integrated energetic system of contraction *in vivo* is not the sensitivity of ATP/PCr producing processes to energetic intermediates - as reflected by elasticity coefficients - but the sensitivity of these processes to ambient O_2 . This is a critical advantage of regulation analysis to quantify direct effects of external effectors on a metabolic pathway. The 30% lower direct effect of O_2 on mitochondrial ATP/PCr production quantified here in HC rats was obtained after 6 weeks at $10.5\% \text{ O}_2$ in initially healthy rats. Different conditioning patterns may generate different strength of adaptation. Similarly, any initial pathology in the cardio-respiratory system of the animal may result in more or less severe change in the direct effect of acute hypoxia. Modular regulation analysis likely has the potential to quantify these particular integrated responses.

Our study was not designed to specifically identify the very mechanisms responsible for the lower sensitivity to ambient O_2 of ATP/PCr production in hypoxic rats. In our conceptual definition of the two-module system, the producer module includes all the steps from O_2 and substrates uptake to phosphorylation. So, the concept of direct effect of O_2 quantified here embodies integrative properties of cellular steps as well as steps involved in the transfer of O_2 from lung to mitochondria. The increase in hematocrit in HC rats (52 vs. 42%) indicates improved O_2 transport capacity; the lower Cox activity might indicate down-regulations in cell metabolism. Yet, these responses are not sufficient to assess in what extent the lower sensitivity of ATP production to O_2 is due to adaptations in systemic O_2 delivery [23] or due to cellular adaptations to chronic hypoxia [25]. A better understanding might come from *in vivo* assessments of muscle PO_2 as achieved for instance by proton MR spectroscopy of deoxymyoglobin [26]. Unfortunately, due the low sensitivity of magnetic resonance, we are not aware of deoxymyoglobin assessments in small muscles of animals.

An interesting relationship to other ^{31}P MR approaches could be mentioned. As force is supposed to reflect the oxidative ATP rate (called J) in our conditions and assuming

(reasonably, in this largely aerobic exercise) usual linearity of J to PCr, the location of the hypoxia point in N and HC rats in figure 3 correspond to a decreased $\Delta J/\Delta PCr$, which would correspond to a reduced rate constant (increased time constant) of PCr kinetics during onset or offset of exercise (recovery from stimulation). Such a decrease in PCr recovery rate constant has been noted in a variety of experimental and pathological muscle-hypoxic states, and amounts to about -50% in healthy humans breathing 10% O₂ [27]. This value could be compared to -44%, the direct effect of hypoxia on producer assessed here by regulation analysis in N rats. We are not aware of assessment of PCr recovery rate constants in HC animals breathing hypoxic mixtures. At last, it is worth noting that PCr recovery rate (or time) constants say nothing about the elasticity of ATP/PCr production and ATP/PCr consumption. Elasticities determine the regulation of ATP turnover in contracting muscle, are uniquely determined by MCA, and may thus be used to uncover the origin of the observed dysfunctions under hypoxia conditions [8].

In conclusion, the present study provides a quantitative approach of the effects of acute and long-term hypoxia on muscle energetics. *In vivo* assessments of elasticity coefficients and direct effect of O₂ on ATP production illustrate how internal and external regulation of ATP turnover and contraction can be uncovered. We anticipate that the approach is useful to detect dysfunctions or improved functions at system levels [28] and brings new tools for integrative physiology [29].

	Normoxic rats (N)	Hypoxic conditioned rats (HC)
<i>Elasticity Coefficients</i>		
ATP/PCr-producer	-1.6 ± 1.4 ⁽²⁰⁾	-1.7 ± 0.8 ⁽¹⁷⁾
ATP/PCr -consumer	0.8 ± 0.6 ⁽²⁸⁾	1.1 ± 0.7 ⁽²³⁾
<i>Flux Control Coefficients</i>		
ATP/PCr -producer	34 ± 23 % ⁽²⁰⁾	35 ± 17 % ⁽¹⁷⁾
ATP/PCr -consumer	66 ± 23 % ⁽²⁰⁾	65 ± 17 % ⁽¹⁷⁾
<i>Direct effect of acute hypoxia</i>		
on ATP/PCr -Producer	-44 ± 23 % ⁽²⁰⁾	* -31 ± 13 % ⁽¹⁷⁾

Table 1. Coefficients determined by the control and regulation analyses
 Values are mean \pm standard deviation. The number of experiments is indicated in parentheses.
 See text for the definition of the calculated coefficients. * $P < 0.05$

Figure legends

Figure 1. The defined modular system and elasticities quantified by control and

regulation analyses. The energetic system of contraction in skeletal muscle *in vivo* was defined as two modules: ATP/PCr -producer and ATP/PCr -consumer. The consumer module embodies all the cellular ATPases that hydrolysis ATP. The producer module embodies all systemic and cellular steps that allow ATP production in active myocytes.

Figure 2. Typical recordings in a hypoxic conditioned (HC) rat.

Recordings were obtained after the transitory period (not shown). Lowest peaks of PCr (front) indicate the period during which the HC rat inhaled 9%O₂; correspondingly contraction was low as indicated by pedal strokes due to plantar flexion. Highest peaks of PCr (back) were obtained during the low-electrical stimulation period; correspondingly contraction was low. The 'reference' steady state is characterized by PCr-peaks of intermediate height and intermediate level of contraction. Elasticities were calculated from averaged pedal strokes and spectra. Pi, *inorganic phosphate*, NTP, *nucleosides triphosphate*.

Figure 3. Effects of modulations on PCr and contraction.

Lines indicate effects of acute hypoxia and lowering electrical stimulation on steady values of PCr and contraction in N rats (squares) and HC rats (circles). Therefore, slopes provide a rough illustration of elasticities. Slopes look similar in N and HC rats, as confirmed by the actual calculation of elasticity coefficients (table 1) thanks to control analysis. Arrows show the more marked effect of acute hypoxia in N rats, studied here by tools of regulation analysis.

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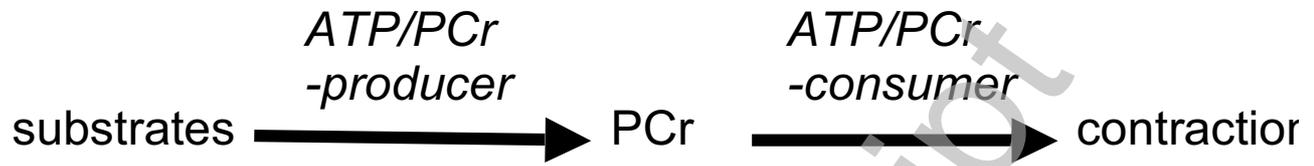


figure 1

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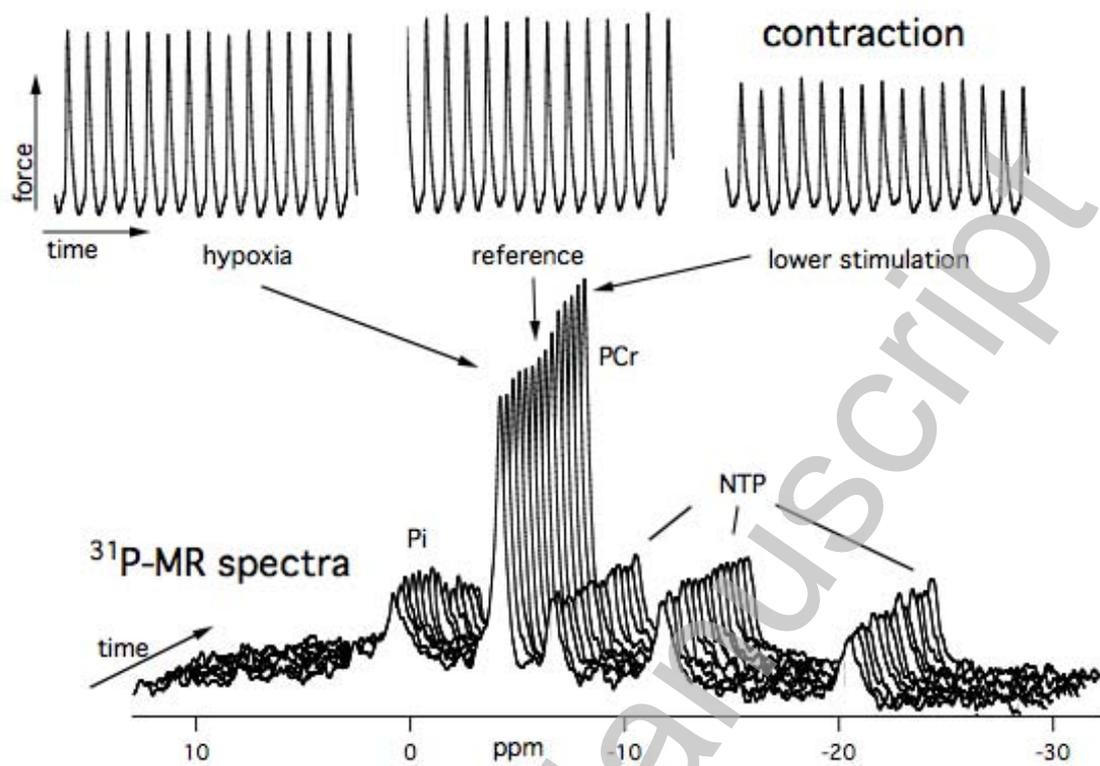


figure 2

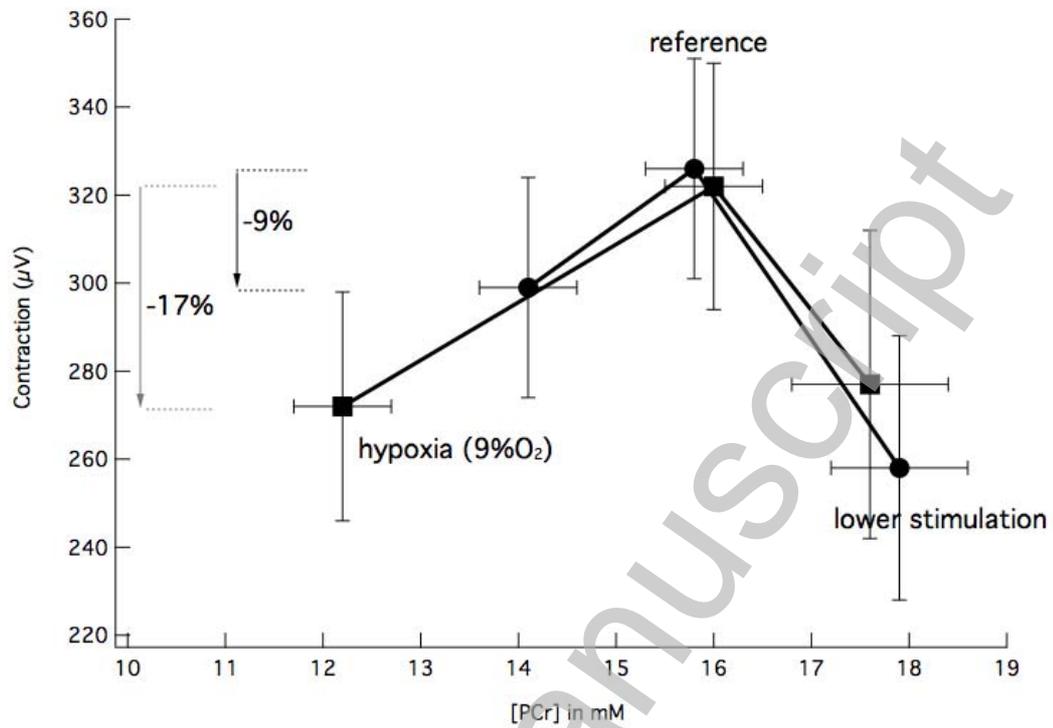


figure 3

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