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Basic considerations on the energetic metabolism are presented elsewhere in this proceeding (See: Energy metabolism of the performance horse by I. Vervuert). In this paper the training-induced modification of all systems involved in the oxygen chain will be briefly reviewed, especially those occurring in the muscles. The scientific basis of training regimens and the tests available to evaluate fitness in horses will also be presented.

**Introduction**

_The horse, a genuine athlete with physiological specificities_

Exercise represents the most important stress factor that can be encountered by the organism (Figure 1). When a horse starts to run, it is able to increase dramatically its oxygen consumption and pulmonary ventilation that may be multiplied by more than 50 and 30 respectively (Art et al., 1990; Art and Lekeux, 1995; Eaton et al., 1995). This increase in oxygen consumption is provided by both the increase of oxygen extraction as well as of the cardiac output (multiplied by 10) (Evans and Rose, 1987; Eaton et al, 1995), the latter being related to the increase in heart rate (that changes from 25-30 bpm at rest to 220-240 bpm during maximal exercise). Another important physiological adjustment is its ability to empty its spleen by an autonomic contraction reflex at the beginning of exercise (Evans and Rose, 1988a) which doubles the number of erythrocytes and consequently the blood oxygen transport capacity in the same proportion.

Among multiple physiological specificities to exercise, horses are known to support arterial hypoxemia and sometime hypercapnia during strenuous effort, a strategy which seems to avoid metabolic energetic wastage to maintain extremely high level of ventilation (Bayly et al., 1989; Lekeux and Art, 1994).
Figure 1: A brief summary of the exercise-induced adjustments of the oxygen chain in the horse

What is conditioning?

The aim of conditioning is to minimize the exercise-induced stress and to postpone the occurrence of fatigue. Conditioning is obtained by the chronic repetition of exercise which results in physiological and morphological adaptations at the level of all systems involved in the realization of exercise. Some tissues or functions are highly malleable and are significantly improved by conditioning, i.e. muscles (Snow and Valberg, 1994) and cardiac function (Evans and Rose 1988b, Foreman et al., 1990) while others remain unchanged i.e. lung and respiratory function (Art and Lekeux, 1993). In general, among the adaptable biological systems, the soft tissues (skeletal and cardiac muscles for example) adapt rather rapidly while hard tissues (tendons and bones) take longer to improve.

The major objective of training is, consequently, to prepare a horse for a specific athletic competition by increasing its physiological capacity to adequately respond to the increased metabolic demands and by decreasing the risk for metabolic disorders and injury.

*The progression of science in the field of genomics and transcriptomics opens new perspectives in the study of physiology of exercise and training.*

The equine genome has been recently sequenced and annotated by an international consortium (Wade et al., 2009). The equine genome includes 20,322 predicted protein-coding genes. Microsatellites markers and more
recently "single nucleotides polymorphisms" (SNP) chips allow nowadays genome-wide scan studies and consequently offer the opportunity to identify some genes of interest related to health or performance (Gu et al., 2009).

For example, polysaccharide storage myopathy (PSSM) has been now associated with mutation within the gene encoding glycogen synthase 1 (GYS1) and in severe cases in the gene of the ryanodine receptor RYR1: genotyping of these genes could be used to detect horses susceptible to transmit the problem to offspring (McCue et al., 2008, 2009). On the other hand, Hill et al. (2010) have identified a Myostatin (MSTN) sequence polymorphism that is associated with the best race time among elite thoroughbred. They showed that the SNP C/C horses were particularly adapted for short distance runs, C/T for middle-distances races and T/T were suited for longer distances.

Gene expression and regulation (up or downregulation) determine most of the phenotype and allows cellular differentiation, morphogenesis and adaptation to the environment. It is possible to assess the variation of gene expression by the analysis of the cell transcriptome. The so called "transcriptomic studies" are conducted either by indentifying some specific genes expression by RT-qPCR, or by a global but complex analysis using expression DNA micro-array. These emergent and new methods are simple and non invasive, realized on nucleated cells in biological samples (blood, muscles biopsies, pulmonary liquids …) and help to identify factors involved in the occurrence of exercise training-adjustments, but also involved as causes of poor performance, e.g. existence of inflammatory processes. In the near future, this new approach could consequently bring new tools for objective assessment of fitness, or inflammation, or diseases (Barrey, 2010).

Barrey et al. (2006) examined the leucocytes gene expression of endurance horses using a heterologue DNA micro-array. They assessed physiological and pathological modifications associated to the realization of an endurance course: many of the over-expressed genes were involved in inflammatory processes. As well, McGivney et al. (2009) examined gene expression in muscles of Thoroughbred horses after exercise. After the exercise, there was an overexpression of the genes involved in stress response, metabolism and intracellular signaling.

Studies performed on mRNA extracted from muscular biopsies in thoroughbred horses highlight the roles of genes in the regulation of the metabolism of oxygen, glucose and free fatty acid during exercise and training (Eivers et al., 2010).

More recently, our group examined mRNA extracted from muscular micro-biopsies using a specific equine micro-array (Horse Gene Expression Microarray; Agilent® Technologies, Palo Alto, CA). Endurance horses were sampled before and after 10 weeks training as well as before and after a 120 km endurance race (Fraipont et al., 2011). Training induced a variation of 25 genes expression within genes showing an increase in mitochondrial respiration (BDH), morphological changes (VCAN, CHSY1) and some inflammatory response (CTL1). The race induced also important changes in genes expression (more than 300 genes), showing an increase in mitochondrial respiration and biogenesis (PGC1α, ANT2, MCT2), an increased utilization of fatty acids (LPL, CD36), structural
modification and activation of immune and inflammatory responses (PTX3, CD55, PAR3) (Fraipont et al., 2011).

The repetitions of exercise lead to the training-induced adaptation by inducing a modulation in gene expression.

During exercise, numerous physiological factors (composition of the biological fluids, i.e. pH, K⁺, O₂, CO₂, and lactate; mechanical stress; oxidative stress; changes in temperature; …) will induce modification in gene expression, either as an immediate (effect of effort) or delayed response (effect of training) (Figure 2). The increased transcription, associated to the increase in translation of mRNA to proteins -and appropriate post-translational events-, results in a production of proteins, including (1) enzymes that control various metabolisms and namely those which control the rate-limiting functions associated to these waste products; and (2) structural proteins. At an organic level, these changes result in modifications in functional and in morphological characteristics, with, generally, an increase in the organ size and cellular content (McGivney et al., 2009). For example, hypoxia seems to be an inducer of gene activity, favoring erythropoiesis, capillary growth and synthesis of glycolytic enzymes.

![Figure 2: Mechanisms of genes expression regulation through different stimuli related to exercise, leading to training-induced adjustments](image)

The choice of appropriate training schedules should be done by keeping this rule in mind. If exercise intensity is too weak, if training sessions are too rare or too short, they will not result in condition improvement. If the subject does not work anymore, the benefit of the training will be rapidly lost, especially in the "soft tissues". The basal level will be recovered after a several weeks of detraining (Snow and Valberg, 1994; Rivero, 2007).
Physiological adaptations to training

Muscular adaptations to training

Depending on the nature (type, frequency, intensity, duration) of the stimulus (exercise), the adaptative response in muscles can take the form of (1) hypertrophy (myofibers increase in size but retain their basal structural, physiological and biochemical properties) or (2) remodeling (without hypertrophy but changes in enzymatic and structural characteristics as well as changes in microvasculature) or (3) a mixed response. Generally, adaptation to endurance training takes the form of remodeling without muscle hypertrophy, but possibly hyperplasia. High resistance muscle activity probably stimulates muscle hypertrophy (Rivero 2007).

Muscle fiber type differentiation is nowadays based on an immuno-histochemical analysis that allows discriminating the specific myosin heavy chain (MyHC) isoforms expressed by each fiber. Briefly, 3 types MyHC are identified in the horse's muscle and designated as types I, IIA, and IIX. Muscles fibers may contain only a single isoform (pure type I, IIA or IIX) or have 2 coexpressing isoforms (Hybrids type I+IIA or IIA+IIX). On a physiological point of view, oxidative capacity of the fibers is decreasing from type I → type IIA → type IIX. It was previously thought that the relative distribution of type I and type II fibers was genetically determined and unchangeable by any kind of training. In the light of the most recent works, it is reasonable to assume that muscle fiber type is not immutable, and that training may progressively change the fast glycolytic fibres (IIX) into oxidative fibers (I), in a sequential and graded process, as shown in figure 3 (Rivero et al., 2006).
With training, the enzymatic machinery contained in the sarcoplasm and in the mitochondria change: the muscle increases its capacity for utilization of free fatty acid (FFA) resulting in a delay of the onset of fatigue, according to the fact that there is a direct relationship between depletion of the glycogen stores and the occurrence of fatigue especially in endurance exercise. As well, during heavy exercise, training delays the production of lactate and hydrogen ions, reducing the potential adverse effects of these by-products on the contractile processes, a factor known to induce fatigue during intense exercise (McCutcheon et al., 1987). From previous studies performed on human athletes, it has been demonstrated that the major component of VO$_2$\textsubscript{max} of an individual results from mitochondrial oxygen consumption. High-resolution respirometry (HRR) is a novel technique for measuring mitochondrial oxygen consumption, recently validated in horses (Votion et al., 2010). HRR evaluates the coupling between ATP production and oxygen consumption in mitochondria harvested from a muscular microbiopsy. Preliminary data shows that HRR is correlated to performance and could be used an important tool to assess both aerobic and anaerobic capacity of the equine muscle and its evolution over time with training (Votion et al., 2010).

The capillaries surrounding the muscular fibers increase in number with, as a consequence, an enlargement of the surface for exchanges and a prolongation of the transit time of the blood in the capillary bed of the muscle. The exchanges of substrates (glucose, FFA), oxygen, by-product (CO$_2$, lactate, H$^+$) and heat will hence be improved. The glycogen concentration in horse's muscle is very high (50% higher than what is reported for humans) and training results in a slight further increase of this concentration. There are apparently few effects of the
nutrition on the rate of glycogen depletion. The feeding strategies leading to "supercompensation" in muscle glycogen stores used for human athlete seem to have no effect in the horse (Snow and Valberg, 1994).

Horses possess a large intramuscular buffering capacity to offset the effects of H⁺ accumulation. This capacity is namely related to the presence of proteins, inorganic phosphate and carnosine. Training seems to improve the buffering capacity allowing the horse to tolerate a higher H⁺ load in the working muscle (McGowan et al., 2002). Enzymatic transporters of lactate (LA) out of the muscle fibers, called MCTs, have also been shown to be upregulated by exercise and training (Revold et al., 2010).

In summary, muscle is a remarkable ‘plastic’ tissue that is rapidly modified when exposed to the strain of training: increase in mitochondrial density, increase in the enzymes of the tricarboxylic acid cycle (Krebs) and of the lipid metabolic pathways, finally contributing to a dramatic increase in muscle aerobic capacity. This will delay the formation of lactate as well as the time when LA starts to accumulate in muscle and in circulation (onset of blood lactate accumulation). The derived variable which is VLA4 (velocity of the horse when LA becomes higher than 4 mmol/L) will consequently be increased.

**Cardiovascular and hematologic adaptations to training**

The pool of erythrocytes, which are vital for the oxygen transport, is reported to increase with training, especially within the first few weeks. As well, the plasma volume is significantly increased by training. In endurance horses, the need for sweating is so high that the increase in plasma volume results in a reduction of the packed cell volume and other red cells indices (McKeever et al., 1987)

As in the skeletal muscles, the myocardium evidences a great plasticity. Training induces a reduction of the heart rate (HR) during submaximal exercise, while it does not affect maximal HR. Recovery HR within the 5 minutes after exercise has been suggested to be correlated with fitness. The cardiac output (CO) and the stroke volume (SV) are increased by training. While training induces morphological changes in the cardiac muscle (increase of the mass, volume, internal diameter and thickness of walls), the increase in SV is also related to the increase in blood volume and therefore to the end-diastolic volume, which increases ventricular preload and, consequently, improves force and velocity of contraction of the myocardial fibers (Bayly et al., 1983a, Evans and Rose, 1988b)

The use of cardiac ultrasound techniques has demonstrated a significant relationship between heart size and VO₂ max, as well as significant changes occurring on a morphological point of view with training (Bulh et al., 2005; Young et al., 2005)

The maximal oxygen consumption, which is the reflection of both the muscular oxidative capacity and the capacity for oxygen transport by the cardio-vascular system, is significantly increased, with the most substantial increases occurring within the first few weeks (Evans and Rose, 1987).
Respiratory system adaptation to training

Surprisingly, while all other systems involved in exercise show improvement at different degrees, there is no morphological or physiological adjustments to training of the respiratory system. Since this system is not anatomically enlarged nor functionally improved by training, it is likely to become a limiting factor to performance in well trained animals (Figure 4) (Art et al., 1990; Art et al., 2002).

This limiting role of the respiratory system is probably more important in horses racing at high speed on short and middle distances than in horses racing on very short (quarter horses) or very long distances (endurance horses). Because, on the one hand, O_{2} extraction at the muscular level is increased with training for several reasons mentioned before (increase in muscles oxidative capacity, capillarity ….), and on the other hand, (1) pulmonary ventilation is not increased because of the cost of breathing at high ventilator level and (2) pulmonary gas exchange is not improved (on the contrary the higher cardiac output reduces the transit-time in the exchanging alveolo-capillary zone), the arterial oxygen tension (PaO_{2}) for a given exercise intensity will be lower in well trained horses.
Thermoregulatory adjustments to training

Training modifies the thermoregulatory response to exercise and enhances the ability to dissipate heat. It has been shown that, after 8 weeks of training, there is an expansion of plasma volume, an increase in cutaneous blood flow, an earlier onset of superficial vasodilatation and sweating, and a decrease in recovery sweating rate, resulting in a overall reduction in fluid losses, with a better thermoregulatory capacity.

Moreover, because the use of fat as provider of energy is favored in trained horses and because FFA are more efficient than carbohydrates, total heat production is lower in trained horses.

Assessment of fitness

Up to now, no measurement performed at rest provides reliable information regarding the horse's fitness, except perhaps some cardiac measurements by echocardiography. Consequently, physical condition has to be assessed during exercise (Evans, 2007).

Standardized tests, on a race track or on a treadmill (Sloet van Oldruitenborgh-Oosterbaan and Clayton, 1999), may be performed either by demanding a maximal or a submaximal effort. During the maximal test, we may determine the time taken to complete a given distance: the lower the time and the faster the recovery of HR, the more likely the horse is fit for competition (Evans, 2007).

During a submaximal test, measurements are generally based on HR and LA at different increasing speeds (Figure 5). The purpose of these measurements is to established curves of both values in function of speed, to interpolate VLA4 (i.e. the speed at which blood lactate is equal to 4 mmol/l) and V200 (i.e. the speed at which the heart rate equals 200 beats/min) (Persson, 1983). Both values are likely to be indicators of aerobic capacity (VLA4 and V200) and cardiovascular capacity (V200) (Couroucé, 1999; Couroucé et al., 2002; Kingston et al., 2006).
Figure 5: The curves of heart rate (HR) and plasma lactate (LA) as a function of speed allow interpolating V200 and VLA4.
Although several exercise protocols have been published to assess fitness in thoroughbred and standardbred horses, there is a lack of scientific horses. From our own experience, it is very difficult for several reasons (behavior of the horse, type of discipline, owner's psychological factors) to ask these horses to perform an exercise at intensities sufficiently high to reach a HR around 200/min and/or LA above 4 mmol/L. Therefore, studies should be realized to design exercise test specific to saddle horses.

**Principles of Training**

Because of the multiple differences existing between the exercise physiology of horses and human, it is hazardous to directly adapt to horses knowledge and techniques of training coming from experiences in the human athlete. Researches on the different schedules of training in horses are expensive and time-consuming; they take place on long periods and the risk of losing experimental subjects for several reasons (injury ...) is high. It is consequently difficult to assess quantitatively -and to compare the efficiency of- different methods of training and therefore, the gains in knowledge in this area are not rapid, especially -once again! - for saddle horses (endurance, dressage, 3-day event) (Rivero, 2007). However, some general principles of training will be presented here after.

Conditioning is based on the chronic repetition of exercise to induce physiological and morphological adaptations. The three principles of training, expressed for human exercise physiology are: (1) repetition: to induce an effect, the strain must be regularly repeated; (2) summation, which refers to the total amount of work; (3) duration: the stimulus must be of a sufficient duration to induce a measurable effect.

"*Endurance training involves prolonged exercise at low intensity (i.e. HR lower than 160b/min and no LA increase)*"

It is generally employed in the first weeks or months of all training programs. It is designed to improve the aerobic capacity and osteo-articular strength, and educate the horse (Hinchcliff et al., 2008). The duration of this period varies greatly among disciplines or trainers. For example, some thoroughbreds undergo only 4 or 5 weeks of slow training before moving on to fast exercise. However, prolonged endurance training has been shown to stimulate continued adaptation of skeletal muscles even beyond 9 months. The principal advantage for the use of longer periods of low-intensity training is the development of stronger limbs, and consequently the reduction of limb injury frequency.

Yet, we must keep in mind that, to be efficient in conditioning, there must be a certain degree of "over-reaching", i.e. a physical activity at sufficient intensity and duration to induce some strain into the organism.
“Preparation of racehorses for racing necessitates gradual increases in speed of exercise”

It is only at exercise intensities near the maximum that improvement of anaerobic capacity and power may be expected. Such training necessitates careful monitoring of exercise intensity: too low level will limit the rate of adjustments; too high level may lead to a state of overtraining or to injuries. **Interval training** is defined as repetition of multiple exercise bouts separated by recovery periods. It allows increasing the total amount of work, and consequently training stimuli, during a training session. It is a method frequently used in human athletes that has been also proposed for the training of racing horses. In this species, up to now, no study has concluded that it is superior to conventional training (Hinchliff et al, 2008). On the other hand, it is not always easy to apply it in thoroughbred horses, namely for behavioral reasons.

Other **alternative methods of training** have been proposed such as weight lifting, swimming, working on a treadmill with a slope, work at altitude… Once again, no definitive conclusion is available about the efficiency of these alternative strategies in horses. **Overtraining** is an imbalance between training and recovery periods. It leads to under-performance, not related to clinical problems, and persisting despite 2 weeks of rest. In humans, the condition is characterized by high HR, LA, loss of body weight and changes in mood. Some studies have shown the same trends for horses. Despite numerous studies, the underlying mechanisms of this condition are poorly understood and there are no objective biomarkers for this syndrome (Hamlin et al., 2002; Rivero et al., 2008).

![Figure 6](image.png)

**Figure 6:** The condition of the subject improves rapidly with training to reach a plateau after 8 to 12 weeks. Training should continue then sufficiently to maintain the condition (and avoid detraining) but without further increase of intensity to avoid overtraining.
Detraining refers to the cession of training. Many horses may have their preparation interrupted by illness or injury. There have been few studies on the effect of detraining and results are contradictory. Generally, there is a loss of training-induced adjustments, with a time-course seeming to be related to the rapidity of occurrence of the training-induced adjustments (Foreman et al., 1990) (Figure 6).

Conclusions

Exercise is the most potent stress experienced by the organism. The chronic repetition of this stress leads to physiological adaptations, called conditioning effects that will decrease the level of stress for a given exercise. Adaptations occur through changes in gene expression induced by various stimuli related to the stress of exercise. To obtain an appropriate effect, exercise has to be sufficiently intense and long and to be repeated frequently. However, the limit between training and overtraining is tight and all horses should be medically followed during the training period. Research in equine sports medicine has improved the scientific knowledge about exercise- and training-induced adjustments, about the tests that can be used to assess fitness and performance ability, about the techniques that may be used to identify the causes of exercise intolerance. However, most of these works have been performed on thoroughbred and standardbred race horses. Moreover, objective information on the success of different training regimens is rare. Therefore, further research should focus on the effect of different training protocols as well as on the management of saddlebred horses, namely to propose exercise tests and training protocols for disciplines such as endurance, 3-day event, show jumping,...
References


