Diagnostics of endurance performance on the level of gene expression

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Abstract: Physiological measures of exercise performance provide reference points for physical health and fitness. They allow to evaluate the progression of improvements in work capacity with training in the active sportsman or the injured with rehabilitation. Tests are now established in many Sports Clinics to document task-specific exercise performance. Major developments in the area of the molecular health sciences highlight options to reinforce current performance testing with molecular diagnosis. In the following, a personal view of the perspectives of exercise testing at the molecular level is given with respect to endurance performance. The case is developed that local, biopsy-based measures of the transcript response of exercised muscle to endurance work may be used to estimate specificity, pace, and possibly magnitude of adaptation with repeated endurance stimuli. This expression profiling of muscle's adaptive response to an exercise stimulus complements non-invasive, genomic methodologies that have identified the association of exercise performance with modifications in heritable elements (gene polymorphisms). Research applying these tools highlights the possibility that the molecular analysis of sample collected with minimally invasive methodology from peripheral muscle tissue and blood serum can enhance the diagnostic power of current physiological tests, and lend to a future use in predicting the progression and variability of endurance performance with training.

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Summary

Physiological measures of exercise performance provide reference points for physical health and fitness. They allow to evaluate the progression of improvements in work capacity with training in the active sportsman or the injured with rehabilitation. Tests are now established in many Sports Clinics to document task-specific exercise performance. Major developments in the area of the molecular health sciences highlight options to reinforce current performance testing with molecular diagnosis. In the following, a personal view of the perspectives of exercise testing at the molecular level is given with respect to endurance performance. The case is developed that local, biopsy-based measures of the transcript response of exercised muscle to endurance work may be used to estimate specificity, pace, and possibly magnitude of adaptation with repeated endurance stimuli. This expression profiling of muscle's adaptive response to an exercise stimulus complements non-invasive, genomic methodologies that have identified the association of exercise performance with modifications in heritable elements (gene polymorphisms). Research applying these tools highlights the possibility that the molecular analysis of sample collected with minimally invasive methodology from peripheral muscle tissue and blood serum can enhance the diagnostic power of current physiological tests, and lend to a future use in predicting the progression and variability of endurance performance with training.

Keywords: Skeletal muscle, biopsy, serum, gene, prognosis

Classical exercise physiology has provided valuable tools to quantify maximal work capacity over a large spectrum of power output and forms of physical activity. This covers lab-based tests measuring force production and power output of single, isolated contractions, as well as field tests assessing energy expenditure of repeated contractions in relation to discipline specific performance (table 1). A major benefit of these tests is that the conversion of metabolic energy into mechanical output is assessed in an integrated manner in a whole body context.

Limitations in current performance testing - A major drawback of functional tests into work capacity is that these are often too coarse to resolve improvements or bottlenecks in the processes that conspire to set maximal performance. Maximal performance depends on a number of interrelated biological pathways/organ systems (Fig. 1). However these do only demonstrate minute adjustments with each bout of exercise. In consequence standard performance tests such as ergospirometry do not typically resolve the individual processes [1-3]. In consequence they cannot provide specific answers on functional improvements before an important period of training has passed. This limits their use in the selection and fine-tuning of training and probably explains why experienced coaches prefer to explore psychological options to maximize performance through motivating the recruitment of functional reserves in Athletes. Recent additions to exercise testing involve the assessment of genetic constitution through the measure of gene variants, or polymorphisms, being associated with performance-related phenotypes. According to leading experts, however, current genetic testing has zero predictive power on talent identification [4].

Endurance training – The ultimate purpose of endurance training is to increase the duration during which force production of repeated contractions can be maintained. Classically this is achieved through the completion of a protocol of repeated exercise sessions with a high number of contractions at low load. Thereby the distinction to power sports is typically drawn based on soft parameters respective to the individual maximal values of power output during a workout and the duration during which this can be performed (such as a VO2max test).

Improvements in endurance performance of running type exercise for instance can be assessed via the measure of running economy, peak velocity and time-to-exhaustion in time trials [5, 6]. The adequacy of these measures is amply demonstrated for elite athletes [7-9] but they are rarely performed in routine diagnosis. At best maximal oxygen uptake (VO2max) is

assessed in an incremental test, which allows, when accompanied with measures of mechanical work, to estimate the economy of a given exercise. VO2max, however, is a steady state measure of maximal exercise performed at relatively short duration. This parameter, while reporting maximal aerobic performance, does not necessarily document fatigue resistance, which is probably more relevant to characterize performance of long distance events, which rely on the capacity to maintain substrate metabolism and metabolic stores. This distinction is also important because the improvement in endurance performance with training is more pronounced for factors reflecting the time-to-exhaustion or velocity at a high metabolic strain, than for VO2max, which typically only improves moderately [7, 8]. Towards a representative diagnostics of endurance performance it therefore appears sensitive to target the tests as close as possible to the muscle groups being trained and quantify both the maximal capacity for aerobic work and the time-to-exhaustion.

Myocellular underpinning of exercise performance – As pointed out in a number of research papers, measures comparing structure-function relationships in the effector organ of mechanical work, skeletal muscle, can reveal unprecedented detail on the features that set the endurance training state. For instance, muscles of endurance-trained subjects have been shown to differ to un-trained subjects in terms of muscle size and fat content, slow fiber type composition and myocellular constituents of lipid metabolism [10, 11]. The theme of these adaptations is the promotion of aerobic substrate pathways that increased the economy of muscle contractions.

Performance in most individual sports disciplines has its foundation in two, partially exclusive traits that reflect the maximization of power output or fatigue-resistance of a motor task, i.e. strength vs. endurance (reviewed in [12]). Skeletal muscle contributes in a specific way to the furthering of both traits with training. It has been appreciated that this is mostly explained by the composition in motor units [13]. Three major motor unit types are described based on electrophysiological measures of contractile and metabolic characteristics, i.e. a slow-fatigue-resistant, a fast-fatigue-resistant, and a fast-fatigable type [14]. They can be mapped with a histological or immunological analysis of sections from muscle samples for mitochondrial enzymes (i.e. succinate dehydrogenase, cytochrome c oxidase, nicotinamide adenine dinucleotide-dehydrogenase, alpha glycero-phosphate dehydrogenase) and the major myosin isoforms that confer motor units their typical contractile characteristics (i.e. I, IIA, IID/X)[15]. For the comprehensive characterization, the distribution and cross sectional area of fiber types fiber is then determined by the quantification of area or volume content [16].

This characterization can be complemented with fiber specific measures of capillary density to reveal the local capacity for substrate supply [17, 18].

Owing to the different metabolic and mechanical characteristics of motor unit types, the optimization of power and endurance is reflected at the level of muscle, and muscle fiber, composition [19]. For instance, endurance athletes demonstrate a high content of slow-type fibers which contract more economically than fast-type fibers, and increased capillarity along with elevated volume density of mitochondria in the individual muscle fibers [17]. By contrast increased muscle strength and power, is associated with a high content in fast type muscle fibers and elevated myofibrillar volume density, and a concomitantly reduced density of mitochondria owing to their dilution in a larger volume. A major drawback of these myocellular measures is that they are rarely carried out due to the lack of medical infrastructure being required to collect, and characterize, muscle samples.

Exercise-induced muscle malleability - Skeletal muscle is known to demonstrate a large degree of cellular plasticity to work-related stimuli compared to other tissues being recruited with exercise (Fig. 1). For instance, local aerobic capacity in skeletal muscle of untrained subjects can nearly double after 6-weeks of bicycle-type endurance training. By contrast, the contribution of this adaptation to whole body aerobic capacity is curtailed to a level of a few percent, only [1] and this is probably not always be resolved at the level of VO2max with ergospirometry. It follows that the characterization of muscle's aerobic capacity can provide a valuable complement for classical physiological tests to estimate endurance capacity and its malleability with training.

The suitability of muscle-targeted tests is illustrated by increases in the volume density of capillaries, mitochondria and intramyocellular lipid stores in the knee extensor muscle, *vastus lateralis*, after 6-weeks of a training paradigm on a stationary bicycle composed of thirty, half-an-hour-exercise sessions at the considerable intensity of 65% of peak aerobic power output. These adaptations are corroborated at the level of protein by an increased content of mitochondrial proteins and factors involved in glucose metabolism (Fig. 2). With continuation of the training stimulus the adjustments may further progress [10] but the extent of mitochondrial plasticity is generally reduced in extent in relation to the training state.

The molecular approach - With the introduction of molecular biology, the mechanism underpinning adaptations of muscle organelles to endurance training have been resolved in

unprecedented detail. This has been demonstrated through the quantification of adjustments in biochemical processes and the underlying gene expression in recruited muscle groups.

Gene expression is the process whereby genome-encoded information instructs the synthesis of proteins that constitute the building blocks of cellular organelles. In this process transcripts of single genes (messenger ribonucleic acids, mRNAs) provide templates for the translation of the encoded information into proteins (Fig. 3A). For instance the synthesis of mitochondrial proteins in *vastus lateralis* muscle is nearly three-fold increased within the first 4 hours after bicycle-type endurance exercise in untrained subjects [20, 21]. This response of mitochondrial protein synthesis is preserved in the trained state and is paralleled by transient elevations in transcript levels for mitochondrial factors 3-8 hours after a single bout of bicycle-type endurance exercise [22, 23]. In consequence it is thought that the improvement in local aerobic capacity with endurance training results from the accumulation of micro-adaptations in mitochondrial protein synthesis, following bursts in transcript expression, with repetition of the exercise stimulus [22, 24].

The molecular measures emphasize that transcript expression post exercise specifies the remodeling response to training. For instance it is found that the response of protein synthesis in untrained muscle to single bicycle-type endurance exercise is rather unspecific and concerns both myofibrillar and mitochondrial protein [20]. By contrast in the endurance trained-state, when mRNA templates for the synthesis of mitochondrial proteins are broadly increased, protein synthesis of pooled mitochondrial proteins, only, is specifically elevated. This is reflected by the reactivity of transcript expression after both acute endurance exercise and endurance training. This concerns gene ontologies associated with processes of aerobic metabolism such as fatty acid transport, mitochondrial beta-oxidation and respiration [25]; known to be collectively improved with endurance training (Fig. 3B). As well, in the trained state myofibrillar protein synthesis is blunted in line with moderate alterations in transcript levels for myogenic processes. This response distinguishes to transcript alterations seen after endurance exercise under a high-load complement [26, 27]. The observations support the view that the accumulation of mRNA templates with the repeated impact of endurance exercise specifies the set of proteins being synthesized with endurance training [21]. This view is supported by the significant linear correspondence between transcript expression of lipid metabolism and corresponding organelles, mitochondria and intramyocellular lipids, in an endurance-trained state [22]. Collectively, the findings indicate that measurements of the

transcript response can be used to estimate specificity, pace, and possibly magnitude of adaptation with the repetition of an endurance stimulus.

Resolving the effect of training paradigms - The molecular characterization of muscle's response to exercise appears particularly useful to develop and consolidate endurance-training paradigms. Such an example is endurance training in hypoxia for which proof of efficiency is debated. We have shown that the co-stimulus of hypoxia promotes a specific response of mitochondrial processes to bicycle-type endurance work. The characterization of muscle ultrastructure emphasizes that a volumetric increase in subsarcolemmal mitochondria is a hallmark of endurance-type training under lowered oxygenation [28]. Subsarcolemmal mitochondria localize in the neighborhood of capillaries and are thought to improve substrate supply to working muscle and constitute a possible bottleneck when exercising under the constraint of hypoxia. This is given because the latter environmental factor lowers the capacity for economic energy production via aerobic processes in mitochondria through the reduction of arterial oxygen saturation.

The observed muscle adjustments to bicycle type endurance training in hypoxia are corroborated by findings on the effect of interval-type endurance training based on running. These show that expressional adaptations of metabolic processes are associated with an enhanced efficiency of mitochondrial energy production and improved coupling of energy transfer from mitochondria to the myofibrillar motor via the creatine kinase shuttle [2, 29]. In this case an increase in transcripts for the facilitative glucose transport protein 4, mono-carboxylate transporter 1 and carbonic anhydrase 3 are correlated with changes in time-to-exhaustion after intermittent hypoxia training. The few examples emphasize that molecular measures tackling adaptations in metabolic pathways can expose the tuning of muscle performance by endurance training paradigms.

Lately we have extended our investigation on hypoxia-regulated adaptations in gene transcript expression with endurance exercise by a broad characterization using microarrays. The measures identify that transcript level alterations post-exercise are correlated with changes in arterial oxygenation and serum lactate. Thereby it is found that the larger the lactate concentration in serum, the higher the level alteration in transcript expression [28]. This observation suggests that at the muscle level the disagreement on the effect of added hypoxia may be related to differences in metabolic strain achieved during exercise [30].

A major theme when assessing functional and structural adjustments of skeletal muscle to endurance exercise is that the magnitude of phenotypic malleability is reduced in a trained-state. For instance the increase in mitochondrial volume density in *vastus lateralis* muscle of untrained subjects can amount to a mean accumulation rate of mitochondria per muscle structure of 0.1% per exercise session (as calculated from an increase in mitochondrial volume density from 3 to 6% over 30 exercise sessions). By contrast, mitochondrial density does rarely increase above 10% even after years of endurance training with hundreds of exercise sessions. From this it is calculated that the rate for the accumulation of mitochondrial volume may drop to 0.01% per total muscle volume and session in a trained state. Meanwhile, the increase in synthesis of mitochondrial protein in muscle persists in the trained state [20]. This implies that degradation of mitochondrial protein post exercise is considerably increased in an endurance-trained state and blunts the synthesis in mitochondrial protein [21, 31]. The observations suggest that the plateau in adaptation of local aerobic capacity by mitochondrial biogenesis reflects to a considerable degree increased turnover of muscle [21].

In this regard, caution should apply when assessing regulatory mechanism in elite athletes because limited information exists on muscle adaptation in this population. For instance, we identify that elite runners do not typically increase mitochondrial gene expression with a further endurance-training paradigm as seen in untrained subjects [2]. Possibly this reflects the reduced scope for adaptation in aerobic metabolism after years of endurance training. By contrast the inclusion of an interval-type running paradigm under hypoxia increases transcript expression of metabolic factors after 6 weeks in line with functional improvements in time-to-exhaustion [2]. The observations suggest that gene expression may serve to replenish proteins that are lost with work-related wear-and-tear of muscle tissue. Possibly this reflects changes in set points of regulation as has been pointed out in a cohort of professional cyclists [3].

A personalized approach to endurance training - Variability in the response to exercise has always been a confounder in cohort studies, which was accepted as biological noise inherent to such kind of studies [32]. The early seminal paper of Petit and Klissouras pointed out the contribution of hereditary factors to the experimentally observed variability in VO2max [33]. It was however only relatively recently, with the sophistication of genetic methodology, that research on the role of hereditary factors in regulating exercise performance has gained in importance [34]. Genetic predisposition is now understood to importantly modify the chances to excel in a given Athletic Discipline.

The power of a genomic approach has been pointed out in a yearly series of papers by Bouchard and colleagues on the 'Human Gene Map for Performance and Health-related Fitness Phenotypes'. These articles emphasize the biological relevance of gene x environment interactions for the two major traits of human performance (i.e. strength and endurance; [12, 35]). Two recent reviews are worth mentioning in this regard as they focus on genetic studies in athletic cohorts. Pitsiladis and colleagues comment that over 200 small nuclear polymorphisms (SNPs) were found to be associated with physical- performance traits, and over 20 SNPs were associated with elite athletic status [4]. However, these authors conclude that 'current genetic testing has zero predictive power on talent identification and should not be used by athletes, coaches or parents'. In their outlook on the future of Sports Genetics, they comment that beside the R577X mutation in the actinin 3 gene (ACTN3) and an I-allele insertion polymorphism, in the gene for angiotensin converting enzyme (ACE) 'the vast majority of candidate genes for sporting performance discovered to date are not the key candidates seriously implicated in the phenotypes of interest'. These authors argue that 'new approaches involving large, well-funded consortia and utilizing well-phenotyped large cohorts and genome-wide technologies will be necessary for meaningful progress to be made.' In a modified pronunciation of this theme, the review by Bouchard and colleagues on Advances in Exercise, Fitness, and Performance Genomics comments on the identification of quantitative trait loci on chromosome 13q12 that explain 20% of the variance in training-induced changes in submaximal exercise capacity i.e. 60% of VO2max [36]. Distinct genome regions, holding candidate genes with muscle relevant functions, were mapped, but the responsible gene sequence was not identified. These authors propose that 'new genomic targets should be further investigated to establish their true relevance for targeted exercise recommendations in the context of personalized exercise medicine'.

Owing to the role of muscle plasticity in the enhancement of exercise performance it is perceived that monitoring the molecular processes, such as transcript expression, that are set in motion in recruited skeletal muscle post exercise is a critical step towards the development of personalized, effective training programs (reviewed in [37]). Towards this end we recently pointed out that exercise-induced transcript expression provides a mechanistic connection between heritable factors and inter-individual variability in the phenotypic response to endurance exercise [38]. The investigation shows that the polymorphism for a 287bp gene insertion in intron 16 (the I-allele) of the gene for the producer of the major vasoconstrictor angiotensin 2, angiotensin-converting enzyme (ACE), affects plasticity of aerobic metabolism in skeletal muscle. This gene variant is the prototype for gene-exercise phenotype associations

[36, 39]. It was originally identified after a search of genetic elements conferring risk to coronary hypertension and stroke and is understood to occur in a frequency of nearly 50% ration in Caucasian populations [40]. Specifically, the presence of the I-allele is associated with 2-fold larger local improvements in subsarcolemmal mitochondria and intramyocellular lipids in knee extensor muscle compared to subjects being homozygous for the absence of the I-allele [38]. The latter genotype holds the D-allele, which is characterized by the absence of a control sequence known to reduce ACE gene transcript levels. We identify that the varying trainability in local aerobic capacity in function of the I-allele relates to differences in serum angiotensin 2 levels post exercise (data not shown). Lately we confirmed the functional relevance of ACE-genotype modulated regulation of endurance performance during the field test of a marathon [41] by demonstrating elevated serum glucose levels and finishing times in ACE-DD genotypes. This indicates the contribution of exercise-induced vasodilatation and substrate supply to the effect of the ACE I /D polymorphism on endurance performance.

These observations provide a first mechanistic explanation on the contribution of muscle tissue to the documented association between the homozygous presence, and absence, respectively, of the ACE I-allele with the two extremes of human performance, i.e. endurance and power [34]. The findings allow conclusions on the predisposition for endurance sports and provide clues as how to tailor exercise interventions for subjects with a normally reduced response in aerobic fitness to endurance training. Given that the ACE I-allele occurs in near every other subject in White Caucasians, our observations may be applicable to a considerable proportion of the general public in the Western hemisphere.

Translating basic sciences in diagnostic practice – Research investigations highlight the feasibility of assessing endurance performance based on the characterization of transcript expression in muscle biopsies (Fig. 3B). Transcript expression can be assessed with a few milligrams of muscle material using standard methods of molecular biology. Sampling can be achieved under local anesthesia with fine needle biopsies from defined portions of muscle tissue. The tissue is then processed to isolate, and reverse-transcribe, the labile mRNAs with commercial kits to reveal stable coding deoxyribonucleic acids (cDNA). These can then be subjected to a number of techniques such as polymerase chain reaction or microarray to assess single up to thousands of gene transcripts in one assay. These methods are sensitive and allow assessing gene transcript regulation in tissue in as little material as representing a few dozen, capped muscle fibers in a biopsy, only.

Two main areas are identified which remain to be addressed to apply molecular diagnostics to a larger audience: Firstly this concerns the collection of tissue sample. While it is appreciated that muscle quality can be characterized based on a microbioptic sample, not all sportsmen interested in this knowledge will be comfortable with the idea of being subject to this ambulatory gesture. An alternative may be the sampling of blood serum as it has been demonstrated that transcript expression in blood cells recapitulates changes in gene expression seen in skeletal muscle [42]. A second area of investment is indicated for quantitative aspects of molecular techniques. Hence beyond the basic feasibility of measuring transcript and protein concentration in minute amounts of tissue, routine molecular tests have not established a generic standard or reference that would allow calculating effective concentrations. Another area of interest is the application of proton magnetic resonance spectroscopy of carnosine to draw conclusion on muscle composition [43], yet the robustness of conclusions from this determination needs to be validated.

In consequence minimally invasive means relying on biopsy sampling may be most promising in a setting that aims to develop, or refine, training protocols. We established with our studies that molecular parameters exist which reflect muscle performance and which can foretell the predisposition of exercised skeletal muscle to adapt to endurance training. All of these relate to metabolic fitness. This is shown for genotyping of the ACE I/D polymorphism based on mucosal samples, as well as for the quantification of changes in angiotensin 2, arterial oxygen saturation, serum lactate and glucose in blood serum after a maximal aerobic workout [28, 38, 41]. While the prospective power and robustness of these molecular candidates as biomarkers for muscle performance and its malleability remains to be evaluated; the current data indicate that the step from diagnosis to prognosis may be in close reach.

Ethical considerations of muscle diagnostics– It is now amply appreciated that physical performance is shaped by heritable (i.e. genetic) and environmental (i.e. training, nutrition) factors. Muscle malleability plays an important part in shaping this quality through its influence on motor function. Thereby the expression of a muscle phenotype and its reprogramming by training is reflected at the level of muscle gene expression [22]. These local adaptations can be readily assessed in samples of peripheral muscle tissue as collected with the percutaneous bioptic technique. Biopsy collection does not pose concern from the medical point of view when correctly performed [44] and is in routine use for diagnostic purposes of qualifying pathological samples [45]. From this standpoint, the diagnostic exploitation of bioptic material towards the tailoring of Sport performance is well supported.

As a matter of fact biopsies are now considered by prominent Sports institutions to complement classical physiological measures [46]. However, this benefit has – alike the one of genetic testing - to be weighed against possible negative effects. For instance, biopsies cause a moderate level pain due to muscle soreness which is not always compatible with more than recreational sports for a few days. As well (negative) discriminatory issues may be considered along with the fact that talent selection based on a default biological profile interferes with the concept of fairness which still is idealized in Sports. In this regard, an interesting lesson can be learned from the case of a former Olympic medalist in shot putter. This athlete, who was not affiliated with Germany, demonstrated an unusually high proportion of slow type fibers [47]. Therefore he likely would not have qualified for a talent program for strength type athletes as the default selection strategy for these types of exercise would have been based on a high content of fast fiber types. As shown from the unusual hypertrophy of the fast type fiber population in comparison to fellow competitors, his talent for explosive Sports was, however readily becoming apparent from the assessment of muscle plasticity to resistance type training. Thus a bioptic approach being targeted at the dynamic response to training, but not baseline measures from passive muscle and genetic predisposition alone, appears to offer the resolution to identify the skill for a high level athlete.

Future challenges –Given the spurt of interest in the discovery of biomarkers for successful endurance training it is likely that the modern Sports and Rehabilitation Clinics will see the entry of molecular tests before long [48-50]. These will allow screening with relative ease for constitutional factors affecting the activation of major pathways of adaptation. Yet these methods are not stand-alone and should be accompanied by relevant measures of functional variables to avoid false positive conclusions. This will be particularly attractive when tailoring training interventions and monitoring subject performance through the years.

Conclusion - Highly resolving molecular-biological tests targeted at the mechanism of phenotypic muscle plasticity appear on the horizon to quantify athletic potential and foretell its conditioning by training. A main topic in this regard is the quest to develop personalized protocols, which take different responsiveness of subjects into account. This may be achieved by quantifying malleability of skeletal muscle in a functional, whole body context, with a

combination of molecular tools and classical performance tests. In this regard a complementary approach monitoring the expression response in sampled muscle material in relation to major gene variants, which enhance the effect size of training, is tentatively identified to offer the power to predict the individual trainability of endurance performance. This stands and falls with the technical refinement of available methodologies and applied studies with trained populations to consolidate our knowledge on the underpinning adaptability of muscle's work capacity.

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maximal aerobic capacity following endurance exercise training in humans, J Appl Physiol 108 (6) (2010) 1487-1496.
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Legends:

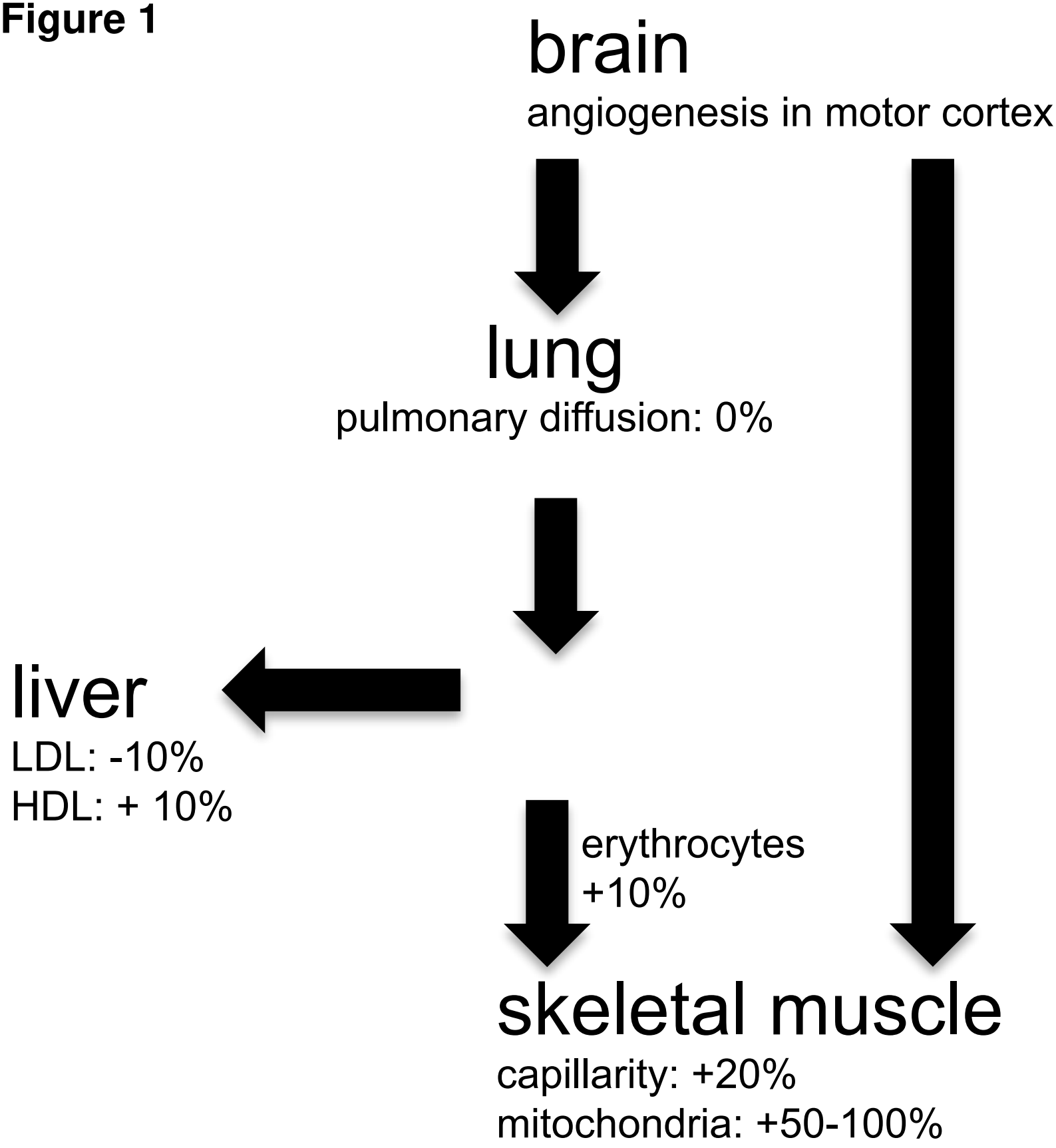
Figure 1: Drawing illustrating the interrelationship between adaptations in organs that set endurance performance.
The typical range of improvement for selected parameters related to endurance performance in untrained subjects after 6-10 weeks of endurance training is indicated.

Figure 2: Adjustments of metabolic proteins after endurance training.
A) Sketch of main metabolic pathways fueling ATP production during muscle work. Arrows indicate the flow of metabolic processes. Protein species being assessed are printed underlined in italics. B) Mean and standard error of fold adjustments in content of selected proteins after 30 endurance exercise sessions on a stationary bicycle. * and + denote $p<0.05$ and $0.05 \leq p < 0.10$ vs. values prior to training (paired T-test, n=8). Abbreviations: AcCoA, Acetyl coenzyme A; ATP5A1, mitochondrial ATP synthase subunit alpha; B-OX, beta oxidation; CO I – CO V, complex I to complex V of the mitochondrial respiration chain; COX4; cytochrome c oxidase subunit 4; CS, citrate synthase; GLUT4, facilitative glucose transporter 4; HADH, 3-hydroxyacyl-CoA dehydrogenase; LDH, lactate dehydrogenase; NDUFA9, NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9; SDH, succinate dehydrogenase; UQCRC1, subunit 1 of cytochrome b-c1 complex.

Figure 3: The molecular response to endurance exercise highlights the responders to a given training scheme.
A) Drawing illustrating the paradigm of gene expression. B) Illustration summarizing the protocol to quantify transcript expression after and endurance exercise test, and an example of possible results. Subjects completed a 0.5-hour bout of bicycle type exercise at 65% of peak aerobic power output. Fine needle biopsies were collected in alternate fashion from different locations of *vastus lateralis* muscle and subjected to the quantification of expression levels of 231 gene transcripts using a custom microarray. For details see Schmutz et al. (2006). Expression data were normalized and centered to the mean transcript signals of pre-exercise measures and subjected to cluster analysis to visualize expression profiles and map groups of transcripts that demonstrate co-regulation post exercise. This analysis illustrates that expression profiles 8-24 hours post exercise differ to the profiles seen prior and 1-hour post exercise. Expression signals are displayed in color-coding in a dendrogram. Examples of the mean values of change of up-regulated (red) and down-regulated (blue) transcripts of main gene ontologies related to aerobic myogenesis are shown to the right. Red and blue color reflects increased and reduced, respectively, transcript content relative to pre exercise levels. Arrowheads point to profiles visualizing an increased (red) and reduced (blue) molecular responsiveness between subjects. Abbreviations: COX4, cytochrome c oxidase subunit 4; ECHS1, Enoyl-CoA hydratase; HSL, hormone sensitive lipase; LPL, lipoprotein lipase; MEF2A, MEF2B, MEF2C; myocyte enhancer factor 2A, 2B and 2C, respectively; VLDLR, Very-low density lipoprotein receptor.

Figure 4: Sketch illustrating a protocol to refine current tests of endurance performance with the inclusion of molecular tests.

Figure 1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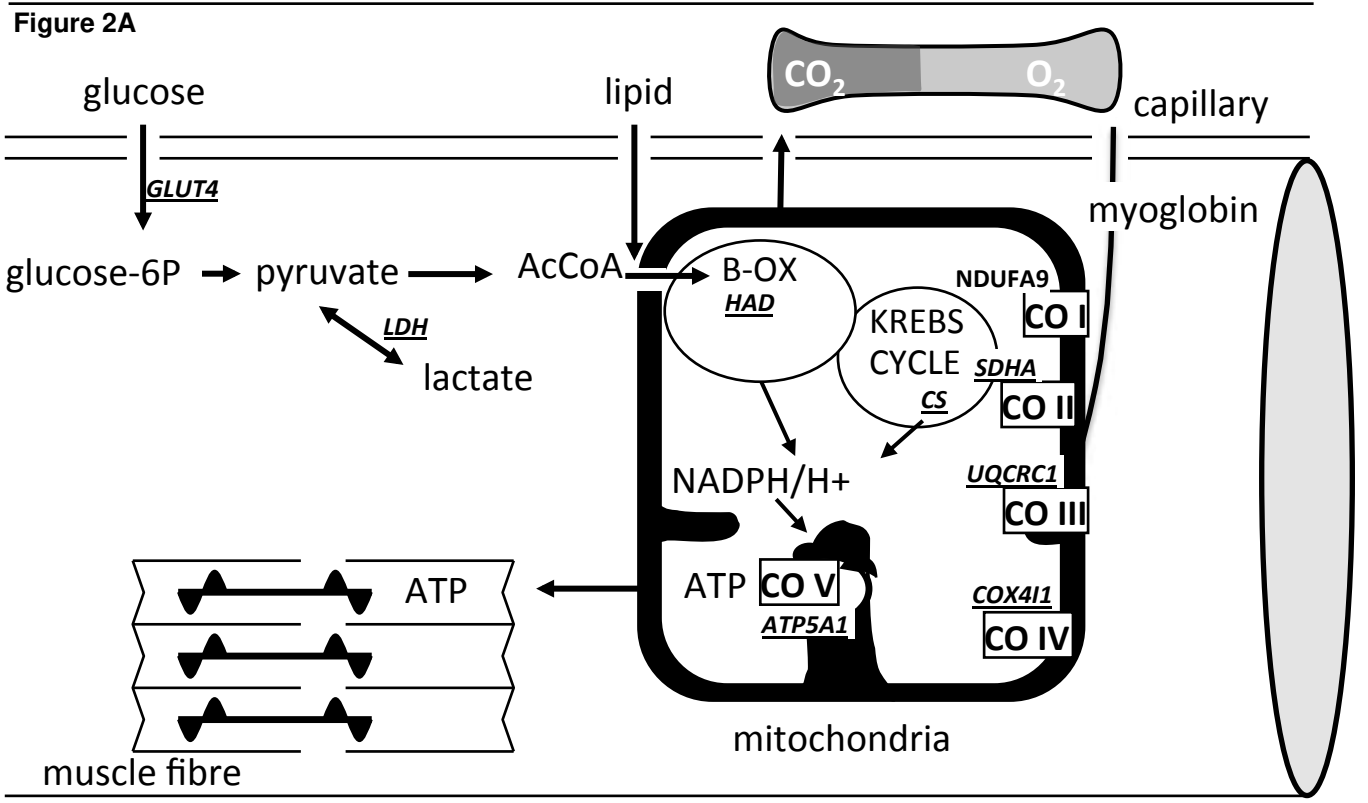
lung
pulmonary diffusion: 0%
liver
LDL: -10%
HDL: + 10%
erythrocytes
+10%
skeletal muscle
capillarity: +20%
mitochondria: +50-100%

Figure 2A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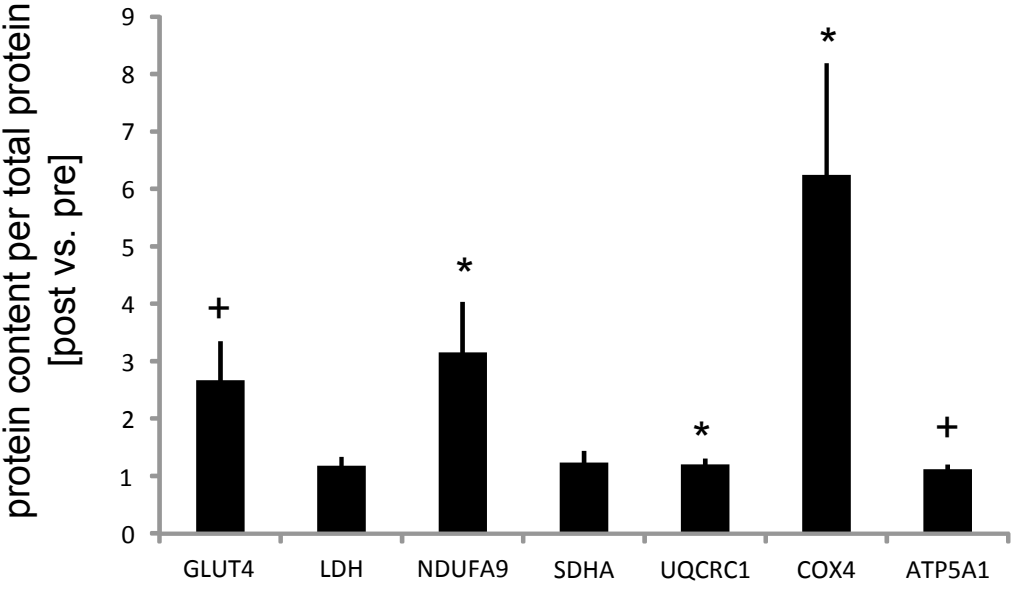
O_2
capillary
GLUT4
myoglobin
glucose-6P
pyruvate
AcCoA
B-OX
HAD
NDUFA9
CO I
KREBS
CYCLE
SDHA
CS
CO II
LDH
lactate
NADPH/H+
UQCRC1
CO III
ATP
CO V
ATP5A1
COX4I1
CO IV
ATP
mitochondria
muscle fibre

Figure 2B


protein content per total protein
[post vs. pre]
9
8
7
6
5
4
3
2
1
0
+
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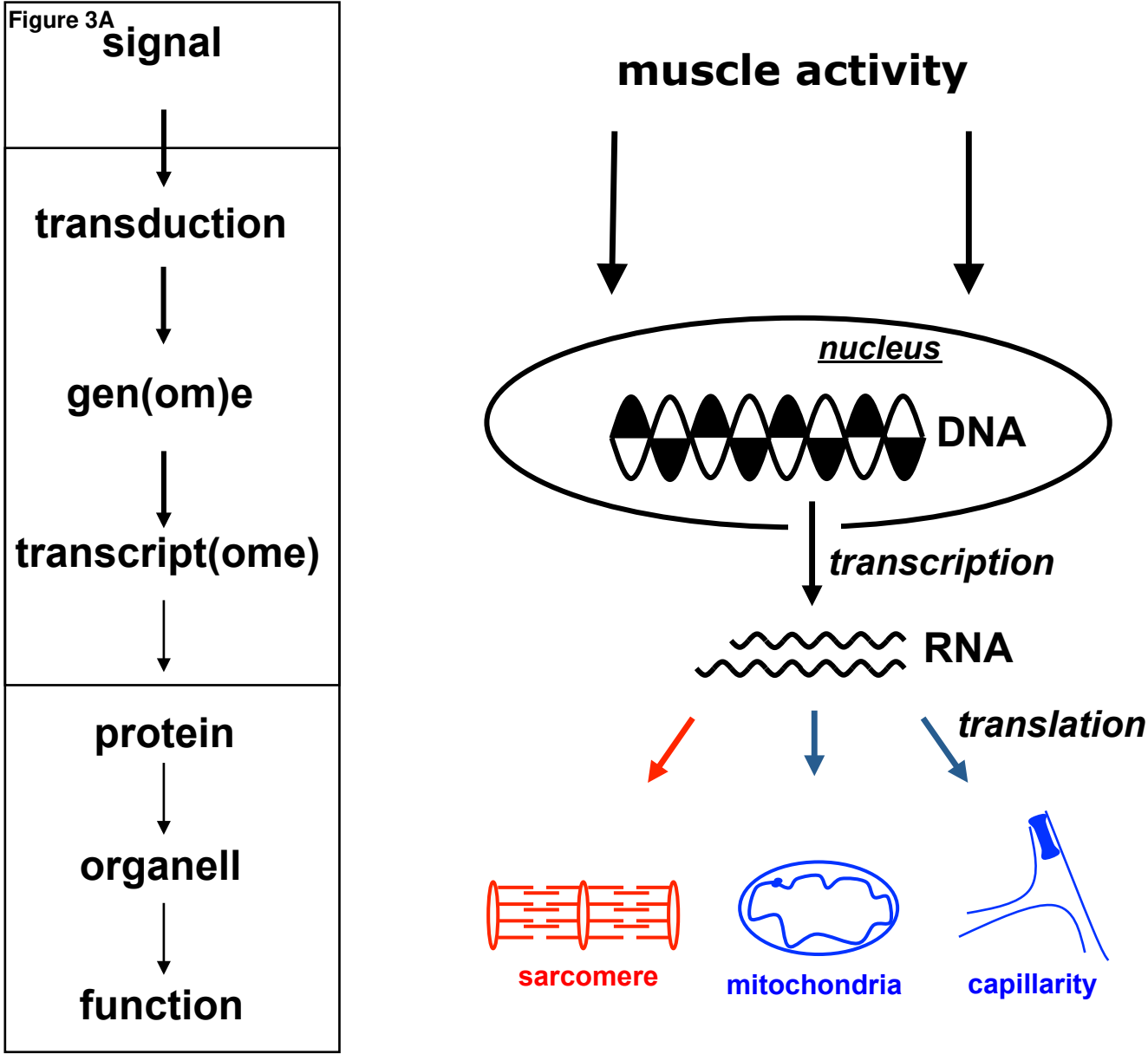
*
+
GLUT4
LDH
NDUFA9
SDHA
UQCRC1
COX4
ATP5A1

Figure 3A


signal
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gen(om)e
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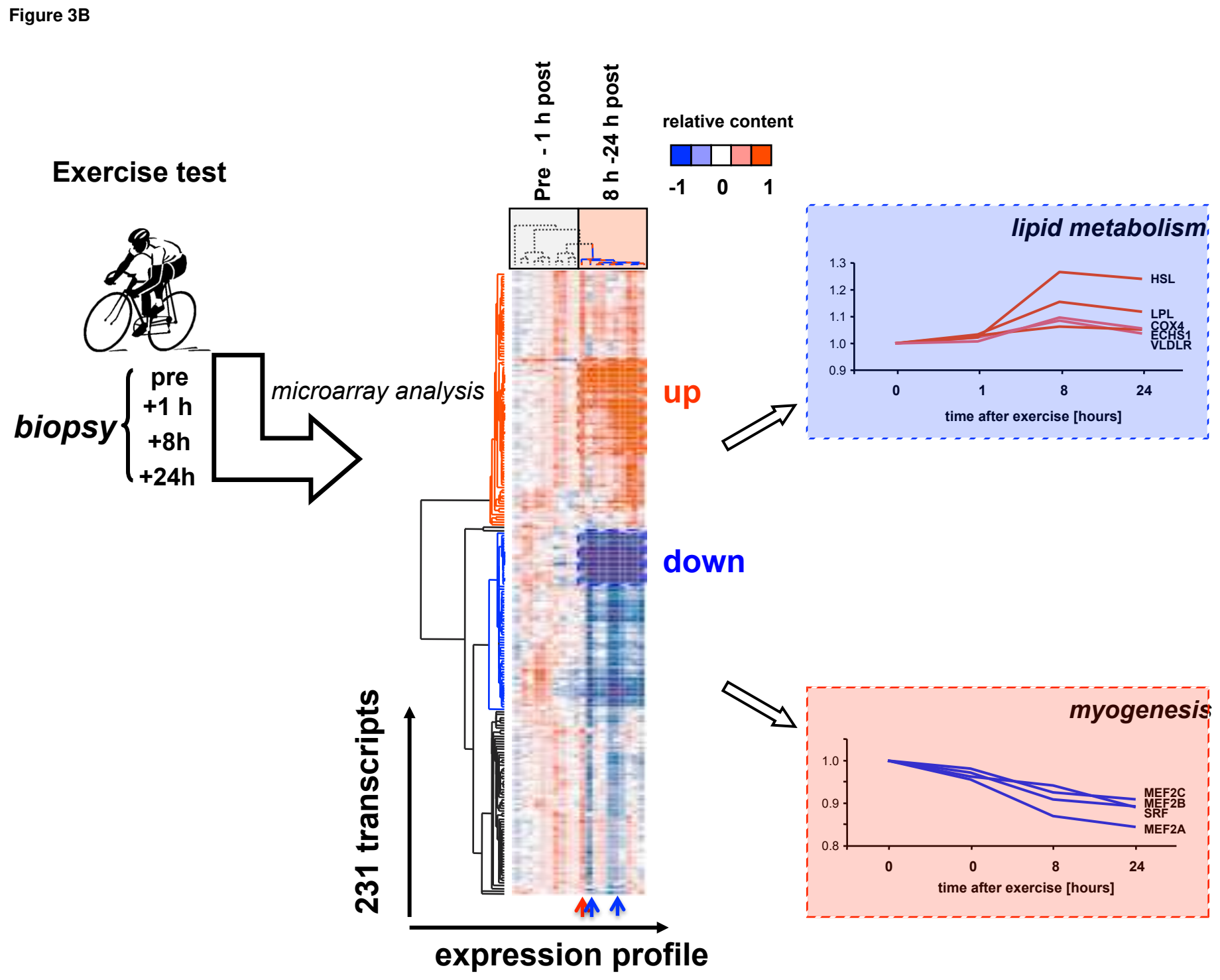
RNA
translation
sarcomere
mitochondria
capillarity

Figure 3B


Exercise test
biopsy
pre
+1 h
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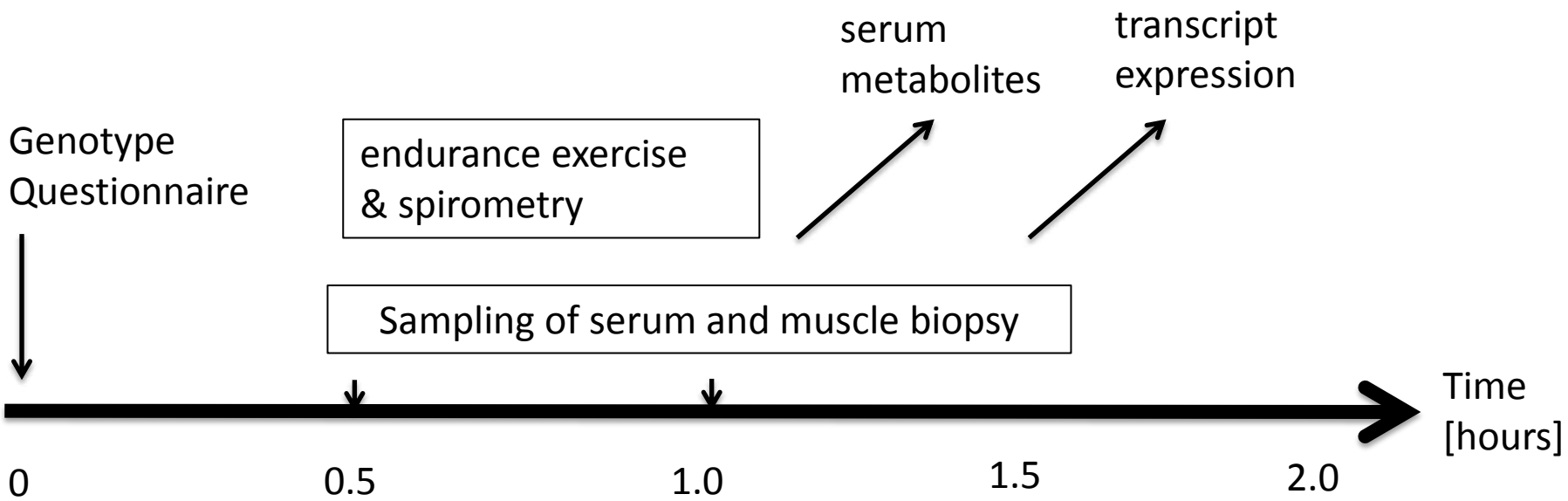
microarray analysis
Pre - 1 h post
8 h -24 h post
relative content
-1
0
1
up
down
231 transcripts
expression profile
lipid metabolism
HSL
LPL
COX4
ECHS1
VLDLR
time after exercise [hours]
myogenesis
MEF2C
MEF2B
SRF
MEF2A
time after exercise [hours]

Figure 4


serum
metabolites
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Questionnaire
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Sampling of serum and muscle biopsy
Time
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2.0

Table 1: ***Features and drawbacks of standardized tests to assess parameters that affect endurance performance***

Name	*parameter being assessed*	*investment*	*advantage*	*limitation*
generic tests:				
anthropometric assessment	biometric data	30-minutes	simple measures	
spiroergometry	VO2max test, respiration exchange ratio ventilation	1-hour	indications on aerobic substrate metabolism	confounded by changes in economy
time-to-exhaustion	fatigue resistance	1-hour	specifically trained	variability
time trial	sustainable maximal velocity	1-hour	simulates competition reduced variability	subject motivation
apprehended tests:				
serum metabolites	concentration of lactate, pyruvate, acetoacetate, 3-hydroxybutyrate	15-minutes	minimally invasive	not always repeated post exercise
heart frequency	maximal heart rate during exercise	30-minutes	precise training indications location independent	objectivity, perturbation through environmental variables
strength and power test	force, power, torque	15-minutess	assessment of functional parameters	standardization of measurement position
Further characterization:				
health screening	blood pressure, nutrition	30-minutes	assessment of body homeostasis	representativeness often not verified
questionnaire (SF-36 or other)	well-being and lifestyle	15-minutes	sampling of environmental and psychological variables	parameters often not numerical