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COMMENTARY

Deciphering $\dot{V}_{O_2,max}$: limits of the genetic approach

Hans Hoppeler

ABSTRACT

Maximal oxygen consumption ($\dot{V}_{O_2,max}$) denotes the upper limit of aerobic energy flux through the cascade of oxygen transfer from the environment to tissue mitochondria, essentially to skeletal muscle mitochondria during intense exercise. A high $\dot{V}_{O_2,max}$ is a key component for athletic success in human and animal endurance sports. From a public health perspective, a high $\dot{V}_{O_2,max}$ is a validated negative predictor for cardiovascular disease and all-cause mortality. $\dot{V}_{O_2,max}$ varies by more than twofold between sedentary subjects and shows a heritability value greater than 50%. Likewise, the capacity for an individual's $\dot{V}_{O_2,max}$ to be increased with exercise training (i.e. its trainability) varies massively between subjects, independent of each subject's $\dot{V}_{O_2,max}$ in the absence of training (i.e. their sedentary $\dot{V}_{O_2,max}$), and with a similarly high heritability. Athletic as well as public health interests have prompted a search for the genetic profile of sedentary $\dot{V}_{O_2,max}$ and of trainability. Candidate-gene studies, gene-expression studies and genome-wide-association studies (GWAS) have not been able to identify a genetic signature that distinguishes subjects or athletes with a favorable $\dot{V}_{O_2,max}$ phenotype or a high trainability from controls. Here, I propose that multigenetic phenotypes such as $\dot{V}_{O_2,max}$ are emergent properties of multiple underlying transcriptomic networks modified by epistasis, the epigenome and the epitranscriptome. The genetic approach is thus considered to be necessary but insufficient for furthering our understanding of multigenetic higher-level functions.

KEY WORDS: Exercise, Heritability, GWAS, Epistasis, Epigenetic, Epitranscriptome

What is $\dot{V}_{O_2,max}$?

Maximal oxygen uptake ($\dot{V}_{O_2,max}$) denotes the upper limit for energy flux through the cardiorespiratory system whereby exercising muscles consume more than 90% of the oxygen taken up by the lungs. $\dot{V}_{O_2,max}$ is an identifiable and reproducible plateau of oxygen consumption above which the energetic requirements of additional power production are met by anaerobic glycolysis. $\dot{V}_{O_2,max}$ reflects the limitation of oxidative metabolism of active muscle cells (peripheral limitation) as well as the capacity for oxygen delivery by the heart and the vascular system (central limitation) (Hoppeler and Weibel, 2000). Each step of the pathway for oxygen delivery affects maximal oxygen transport individually in a non-linear fashion (Wagner et al., 1997). It is generally accepted that in humans under normoxic conditions and at sea level, cardiac output is responsible for more than 50% of the 'limitation' of $\dot{V}_{O_2,max}$ during heavy exercise with a large muscle mass.

$\dot{V}_{O_2,max}$ is not genetically fixed in any individual but is subject to phenotypic plasticity and can be increased with exercise training of sufficient duration and intensity. In humans, the malleability of exercise phenotypes is often called 'trainability' (see Glossary). Sedentary $\dot{V}_{O_2,max}$ (i.e. the $\dot{V}_{O_2,max}$ of individuals who do not engage in exercise training) varies substantially between individuals of a species but is also systematically different in mammals with different ecological backgrounds (Weibel et al., 1991). With regard to the variability of the $\dot{V}_{O_2,max}$ response, it is convenient to study skeletal muscle tissue, as endurance exercise training leads to easily detected and large changes of skeletal muscle morphology and function. The molecular mechanisms of this plasticity can be studied in humans, as muscle biopsies can readily be obtained. The focus of this Commentary is thus strongly on the molecular aspects of the control of muscle plasticity.

In humans, $\dot{V}_{O_2,max}$ can directly and reliably be measured by established exercise procedures (cardiopulmonary exercise testing, CPX; Poole and Richardson, 1997). This is routinely done in athletes who perform in classical endurance sports such as bicycling, cross-country skiing and long-distance running, including the marathon. In these sports, a high $\dot{V}_{O_2,max}$ is a key characteristic for outstanding performance and success in competition. $\dot{V}_{O_2,max}$ in Olympic endurance athletes typically reaches 80 ml O_2 min^{-1} kg^{-1} and above. Values for female top endurance athletes are about 15% lower (Tønnessen et al., 2015). In the general population, $\dot{V}_{O_2,max}$ values are approximately half those of top athletes.

$\dot{V}_{O_2,max}$ as estimated by CPX is not only a prime determinant of endurance athletic prowess but also considered to be the gold standard for assessing cardiorespiratory fitness (CRF) in the general population (Kodama et al., 2009). In a meta-analysis, Kodama et al. (2009) showed that subjects with a $\dot{V}_{O_2,max}$ in excess of 7.9 MET (metabolic equivalents, see Glossary) had a substantially lowered risk both for all-cause mortality as well as for coronary heart disease (CHD) and cardiovascular disease (CVD). The data indicate that a 1 MET higher exercise capacity decreases all-cause and CHD–CVD mortality by as much as 15%, possibly more for subjects with very low METs (Harber et al., 2017). There seems to be broad consensus that $\dot{V}_{O_2,max}$ represents the best validated predictor of endurance prowess and all-cause, as well as disease-specific, mortality.

From the interest in $\dot{V}_{O_2,max}$ in athletic and general populations, it is evident that much thought and research has gone into understanding the variability of $\dot{V}_{O_2,max}$ in a sedentary population as well as the extent to which $\dot{V}_{O_2,max}$ can be changed with exercise training. In athletics, understanding the genetic signature of sedentary $\dot{V}_{O_2,max}$ and trainability would be a key asset in the selection of promising talent and in using the most effective training regimes. Furthermore, in a public health setting, a mechanistic understanding of the variability of sedentary $\dot{V}_{O_2,max}$ and trainability could allow for evidence-based, targeted interventions, with the aim of improving CRF and overall health in the population. This Commentary explores current knowledge of the genetic underpinning of the variability of sedentary $\dot{V}_{O_2,max}$ and its phenotypic malleability, with a focus on skeletal muscle. The analysis indicates that genetics alone are not sufficient to explain the $\dot{V}_{O_2,max}$ phenotype – additional levels of the control of gene

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Glossary

Allele
An allele is a variant of a gene. Diploid organisms have two variants of each gene, one inherited from the mother, the other from the father. These two variants can be identical (homozygous) or different (heterozygous).

Epigenome
Biochemical changes to the DNA (DNA methylation, histone modification) that alter gene expression without altering the gene sequence. The epigenome responds to internal and external (environmental) cues and can act transgenerationally.

Epistasis
Dependence of gene action on the presence or absence of other genes or mutations in other genes or within the same gene.

Epitranscriptomics
Biochemical changes to the RNA that change gene expression in the absence of changes in the ribonucleotide sequence.

Haplotype
Alleles inherited from the same parent. Also, the set of linked SNPs that occur together on an allele.

Ingenuity pathway analysis (IPA)
A software tool to identify pathways and regulatory networks from 'omic data.

KEGG (Kyoto encyclopedia of genes and genomes) pathways
A collection of pathway maps representing the knowledge available on the molecular interaction networks of cellular processes.

Kroghian approach or Krogh's principle
'For such a large number of problems there will be some animal of choice or a few such animals on which it can be most conveniently studied' Krogh (1929).

Metabolic equivalent (MET)
Quantifies the level of energy expenditure of an individual as compared with rest. 1 MET=metabolism during sitting; 5 MET=5×resting metabolism.

Quantitative trait locus (QTL)
The portion of DNA that is responsible for a particular phenotype. A complex phenotype may depend on many QTLs that can be located on different genes. Typically, QTLs are identified by SNPs that correlate with the trait of interest.

Single nucleotide polymorphism (SNP)
Variation of a single nucleotide at a particular location in a gene. A tagSNP is a single nucleotide polymorphism representative of a group of SNPs, often called a haplotype.

Trainability
Responsiveness of an individual to a particular training regime.

expression, such as the epigenome, epistasis and the epitranscriptome (see Glossary), must be involved.

The variability of sedentary $\dot{V}_{O_2,max}$

There is substantial evidence that $\dot{V}_{O_2,max}$ varies considerably among subjects who claim to abstain from systematic exercise training. Bouchard et al. (1998) found $\dot{V}_{O_2,max}$ to vary by more than twofold among 429 sedentary individuals from 86 families, as reported in his pioneering HERITAGE (HEalth, RIsk factors, exercise Training And GEnetics) family study. Bouchard et al. (1998) defined sedentary subjects as subjects that did not engage in more than one weekly exercise session of maximally 30 min duration at an energy expenditure of 7 METs for subjects ≥50 years and 8 METs for subjects <50 years.

This enormous interindividual difference in sedentary $\dot{V}_{O_2,max}$ begs the question as to the hereditary contribution to this phenotypic trait. Klissouras (1971) was the first to study the heritability of $\dot{V}_{O_2,max}$ in a systematic way, comparing pairs of monozygotic twins (MZ, n=15) with pairs of dizygotic twins (DZ, n=10). Klissouras (1971) found $\dot{V}_{O_2,max}$ to be virtually identical between MZ and DZ; however, when $\dot{V}_{O_2,max}$ values of twins were pairwise regressed, the correlation coefficient r for MZ was 0.91 while that for DZ was only 0.44. From this, Klissouras (1971) calculated the heritable component of $\dot{V}_{O_2,max}$ to be 0.93.

Since then there have been a number of twin-sibling studies, as reviewed by Schutte et al. (2016). These authors performed a sample size weighted meta-analysis on all heritability studies of maximal oxygen consumption in children, adolescents and young adults. They found that 59% (n=1088) and 72% (n=1004) of the variability of measured $\dot{V}_{O_2,max}$ or of $\dot{V}_{O_2,max}$ relative to body mass ($\dot{V}_{O_2,max}/M_b$), respectively, could be explained by genetic influences. Overall, they concluded that innate factors determine more than 50% of the inter-individual differences in $\dot{V}_{O_2,max}$ in the sampled populations.

The heredity estimates for $\dot{V}_{O_2,max}$ reported by Schutte et al. (2016) are higher than those reported by Bouchard et al. (1998) for the HERITAGE family study. Bouchard et al. (1998) analyzed sedentary $\dot{V}_{O_2,max}$ data obtained from the members of 86 nuclear families using stepwise multiple regression procedures. An analysis of variance (ANOVA) was performed to verify the family aggregation of $\dot{V}_{O_2,max}$ by comparing between-family and within-family variance. The analysis showed this variance to be 2.6 to 2.9 times larger between than within families. Depending on adjustments (for sex, body mass, fat mass and fat-free mass), heritability estimates between 51% and 59% were calculated. Using appropriate regression models, Bouchard et al. (1998) were also able to calculate the maternal (mitochondrial) contribution to general heritability – they reported maternal contribution to be 29–36% (more than half of total heritability). Bouchard et al. (1998) denote these heritability estimates as 'maximal' as their approach did not allow for isolating and subtracting the contribution of familial environment to overall heritability.

In summary, the currently available data indicate a large inter-individual variability of sedentary $\dot{V}_{O_2,max}$, spanning at least a twofold range between the lowest and the highest estimates. Twin-sibling studies and familial-resemblance studies both indicate that at least 50% of this variability is of genetic origin, with maternal, mitochondrial inheritance being more than half of the total inheritance.

The trainability of $\dot{V}_{O_2,max}$

It is commonly observed that identical training regimes lead to different training outcomes in individuals (Vesterinen et al., 2016). A meta-analysis of Scribbans et al. (2016) indicates that high-intensity interval training (HIIT) and low-intensity long-duration training (classical endurance training) lead to similar (average) improvements in $\dot{V}_{O_2,max}$. Other meta-analyses produce different results and find slightly larger increases in $\dot{V}_{O_2,max}$ with HIIT protocols (Milanović et al., 2015), in particular in subjects with cardiometabolic diseases (Weston et al., 2014) and participants of cardiac rehabilitation programs (Hannan et al., 2018).

The HERITAGE family study, for which sedentary $\dot{V}_{O_2,max}$ baseline data are discussed above, was set up to test the hypothesis of familial aggregation of the $\dot{V}_{O_2,max}$ response to a standardized training program (Bouchard et al., 1999). A total of 481 sedentary Caucasian adults from 98 two-generation families were subjected to the same training protocol. Subjects trained 3 times per week for 20 weeks. Training duration and intensity were increased over the training period, such that subjects trained at 75% of their heart rate at $\dot{V}_{O_2,max}$ for 50 min per training session over the last 6 weeks of the intervention period. Bouchard et al. (1999) used the familial correlation model presented above to calculate familial resemblance. They found the average age- and sex-adjusted increase

of $\dot{V}_{O_2,max}$ to be 393 ml min^{-1} O_2. However, they also found a massive inter-individual difference in training-induced $\dot{V}_{O_2,max}$, ranging from actual decreases in some subjects to increases larger than 1000 ml O_2 min^{-1} in other subjects. The training-induced increase in $\dot{V}_{O_2,max}$ was between 200 and 600 ml O_2 min^{-1} for over 60% of the subjects. The maximum general genetic contribution to the increase of $\dot{V}_{O_2,max}$ was calculated to be 47%, with a maximum maternal contribution reaching 28%. From this, Bouchard et al. (1999) concluded that the familial contribution to an increase in $\dot{V}_{O_2,max}$ with exercise training was similar to the underlying genetic factors responsible for sedentary $\dot{V}_{O_2,max}$. Similar to the variability of sedentary $\dot{V}_{O_2,max}$, these authors found a 2.5 times larger variance for the $\dot{V}_{O_2,max}$ response between than within families.

Skinner et al. (2001) expanded the Bouchard et al. (1999) study by adding the training response of 198 Afro-Americans to the training protocol described above. This brought the total number of men to 287 and women to 346 (aged 17–65 years). Skinner and colleagues (2001) estimated the effect of age, sex, race and initial aerobic fitness on the training response of $\dot{V}_{O_2,max}$. They found trainability to be independent of age, sex and race. Most surprisingly, they also found the training-induced gain in $\dot{V}_{O_2,max}$ following the standardized 20 week training protocol to be independent of sedentary $\dot{V}_{O_2,max}$ (Skinner et al., 2001).

In terms of selecting athletes for endurance events, this indicates that one would have an interest in selecting individuals with a high sedentary $\dot{V}_{O_2,max}$ that at the same time also have a high trainability. With regard to the general population, we currently do not know how differences in sedentary $\dot{V}_{O_2,max}$ and trainability would contribute to the positive health outcomes observed with a high aerobic fitness.

The search for the genetic underpinning of the variability of $\dot{V}_{O_2,max}$ and the training response

In view of the large genetic contribution to both sedentary $\dot{V}_{O_2,max}$ and trainability discussed above, the question arises as to how genetics can explain these differences. Most pertinently, which genes are involved in setting sedentary $\dot{V}_{O_2,max}$ and which genes are responsible for the degree to which $\dot{V}_{O_2,max}$ can be increased with exercise training? Studies which address these issues are discussed in some detail below.

The candidate gene approach

The candidate gene approach questions the effect of gene variants (alleles; see Glossary) of a potentially contributing gene on a phenotype by an association study. One study using this approach focused on the genetics of exercise-induced left ventricular (LV) hypertrophy. Such hypertrophy can be taken as a reasonable proxy for an increase in $\dot{V}_{O_2,max}$, as cardiac hypertrophy (permitting a larger stroke volume) is a commonly observed feature of an exercise-induced increase in cardiac output. Montgomery et al. (1997) suspected cardiac hypertrophy to be induced by BNP (brain natriuretic protein), a peptide secreted when the heart dilates. This secretion was suspected to depend on an insertion/deletion (I/D) polymorphism of the ACE (angiotensine converting enzyme) gene. This highly influential study indicated that, over a training period of 10 weeks, the increase in LV mass estimated by echocardiography in 140 army recruits was dependent on the I/D polymorphism of the ACE genotype. Recruits with an II configuration increased LV mass negligibly, whereas recruits with an I/D or DD genotype increased LV mass by 22% and 26%, respectively. The observed differences in LV mass were significant and independent of subject height, pre-training LV mass or systolic blood pressure.

The Montgomery et al. (1997) study spurred massive interest in studies exploring the gene associations of fitness phenotypes with exercise training. Between 2000 and 2007, a panel of experts annually catalogued the published literature on fitness-associated phenotypes. The last of these updates appeared in 2009 (Bray et al., 2009). To that date, 214 autosomal gene variants and quantitative trait loci (QTL; see Glossary) had been reported, plus seven on the X-chromosome and 18 on mitochondrial genes (Bray et al., 2009). However, the interest in gene association studies then waned as the candidate-gene approach failed expectations. Bouchard (2012), one of the experts of the panel noted: ‘Unfortunately, the vast majority of the studies substantiating these associations were based on observational data, were targeting poorly justified candidates and were grossly statistically underpowered’. Bouchard (2012) also pointed out that virtually all positive findings were later contradicted by further studies.

Gene expression profiling

Gene expression profiling characterizes phenotypes by establishing patterns of transcriptomic response to a particular stress. Timmons et al. (2010) performed two independent exercise training studies to identify transcripts associated with a gain in $\dot{V}_{O_2,max}$ in a global set of muscle genes. This was based on the assumption that baseline RNA expression profiling could be used to identify genes containing the genetic variants responsible for the variability of the $\dot{V}_{O_2,max}$ response. In a first training study (n=24), a set of 29 genes was found to be strongly associated with the gain in $\dot{V}_{O_2,max}$. In a second study (n=17), the predictive value of these genes was confirmed. Timmons et al. (2010) then proceeded to identify the genetic variants of the genes responsive to exercise training. This was achieved by genotyping the tagSNPs (single-nucleotide polymorphisms; see Glossary) for the predictor transcripts in a re-analysis of the HERITAGE family study (WHITE cohort; n=473; Bouchard et al., 1999). A set of transcriptome-derived SNPs (n=7) and a set of SNPs (n=4) identified by positional cloning of the HERITAGE family study together explained a total of 23% of the $\dot{V}_{O_2,max}$ response, after multivariate regression. This was almost half of the heritability gain in $\dot{V}_{O_2,max}$ of 47% observed in this population previously (Bouchard et al., 2011). Timmons et al. (2010) also noted that the RNA abundance of predictor genes was not changed by exercise training, indicating that genetic variation was responsible for the difference in gene expression. As 11 SNPs identified by Timmons et al. (2010) were responsible for approximately half the genetic variance in the $\dot{V}_{O_2,max}$ response, this suggested that the $\dot{V}_{O_2,max}$ response could be predicted from a pre-training RNA expression signature of some 30 muscle genes. The approach taken by Timmons et al. (2010) was not pursued, as later studies did not corroborate the findings of these authors (see below).

Genome-wide association study (GWAS)

A genome-wide association study is a hypothesis-free query of genetic variants (SNPs) for association with a trait. Bouchard et al. (2011) tested 324,611 SNPs for association with the $\dot{V}_{O_2,max}$ response of the 473 subjects of the HERITAGE WHITE cohort. The average increase in $\dot{V}_{O_2,max}$ was ∼400 ml O_2, with 7% of the subjects showing an increase of 100 ml O_2 or less, and 8% improving by 700 ml O_2 or more. Bouchard et al. (2011) identified 39 SNPs that were significantly associated with gains in $\dot{V}_{O_2,max}$ (at $P<1.4\times10^{-4}$). After stepwise multiple regressions, a panel of 21 SNPs was found explaining 47% of the variability of the $\dot{V}_{O_2,max}$ response. From the panel of 21 SNPs, a predictor score was calculated. Subjects that had

nine or fewer of the favorable alleles improved $\dot{V}_{O_2,max}$ on average by 221 ml O_2 min^{-1}, whereas those that had 19 or more positive alleles improved $\dot{V}_{O_2,max}$ by 604 ml O_2 min^{-1}. However, Bouchard et al. (2011) noted that none of the predictor SNPs reached genome-wide significance and only five out of the strongest 15 predictor SNPs could partially be replicated in three cohorts that used similar or somewhat less-stringent exercise protocols. Likewise, none of the 11 SNPs previously identified by Timmons et al. (2010) was part of the 21 predictor SNPs of the HERITAGE WHITE cohort. In an accompanying Invited Editorial, Pitsiladis and Wang (2011) cautioned against too much optimism, pointing out that genome-wide linkage analyses had been successful in identifying disease genes related to monogenic disorders but had been less useful in detecting traits related to multiple genes.

More recently, an international consortium published data obtained in a GWAS performed on a total of 1520 elite endurance athletes and 2760 controls to compare world-class endurance athletes with ethnicity-matched controls (Rankinen et al., 2016). A panel of 45 promising genetic SNP markers was identified in two cohorts of endurance athletes and controls for replication in seven additional cohorts of endurance athletes and controls. The meta-analysis of all studies performed revealed a single significant marker (rs558129 at the GALNTL6 locus, chromosome 4q34.1, P=0.0002) that was expressed at a lower level in all athlete populations. This marker identifies a gene that encodes *N*-acetylgalactosaminyltransferase, the functional role of which is currently not clear. Rankinen et al. (2016) identified various limitations; most prominently, the inherent difficulty in obtaining samples of a large enough number of outstanding athletes. They concluded that, with the currently available technology, it is not possible to find a genomic signature that distinguishes endurance athletes from controls. They pointed to the possibility of using whole-genome sequencing in the future to explore not only common polymorphisms but also rare variants and copy-number variants. They also point out the need for considering the epigenome. It is evident that all heritability estimates for the variability of sedentary $\dot{V}_{O_2,max}$, as well as of the phenotypic plasticity of $\dot{V}_{O_2,max}$ reported above, include potential effects of the epigenome and the epitranscriptome, as discussed below.

Limitations of the genomic approach

Recently, Bouchard (2015) called for a paradigm shift in exercise genomics. He pointed to the enormously complex multisite regulation of gene expression, in addition to the host of post-transcriptional and post-translational modulators, and proposed the abandonment of observational studies with their poorly selected candidate genes and small sample sizes. He also pointed to the necessity for replication experiments even in well-designed experimental studies with adequate statistical power of replication. He argued that, instead, unbiased large-scale experimental studies that combine the power of genomics, epigenomics and transcriptomics should be performed. The need for a shift in focus away from a purely genomics approach is highlighted in a recent study by Williams et al. (2017). The authors reviewed 35 articles describing 15 cohorts collectively identifying 97 genes with an impact on trainability of $\dot{V}_{O_2,max}$. Of the 97 genes, only 13 gene variants were described in more than two studies. Williams et al. (2017) suggest that differences in training programs and diet together with environmental gene expression modulators affect the traits for $\dot{V}_{O_2,max}$ trainability.

In a recent review, the Bouchard's group (Sarzynski et al., 2017) revisited the genomic approaches that had been used in the study of the variability of the $\dot{V}_{O_2,max}$ response. As none of the SNPs associated with the gain in $\dot{V}_{O_2,max}$ had reached genome-wide significance in the HERITAGE family study (Bouchard et al., 2011), no single gene could be identified that explained a significant proportion of the genetic variation in the training response. Consequently, the training response had to be modelled from modest contributions from several genes – that is, on the gene-set level. For this purpose, gene-level trait association scores were formulated to query KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways (see Glossary). This allowed for the identification of several candidate pathways related to immune function, cardiomyopathy, extracellular matrix regulation and metabolism to be significantly associated with the $\dot{V}_{O_2,max}$ response. Through a web-based software application known as Ingenuity Pathway Analysis (IPA; see Glossary), further networks of genes associated with the training-induced changes in $\dot{V}_{O_2,max}$ could be identified. This analysis pointed to pathways related to calcium signaling, nitric oxide signaling and protein kinase A (PKA) signaling influencing the training response through accumulation of polymorphisms in their constitutive genes. Sarzynski et al. (2017) propose that genomic analyses and wider bioinformatics explorations do suggest pathways related to calcium signaling, energy sensing and partitioning, mitochondrial biogenesis, angiogenesis, immune function and the regulation of apoptosis and autophagy to be the main players mediating the physiological response to exercise training. For future research, Sarzynski et al. (2017) also regard next-generation sequencing, epigenetic mapping and the analysis of non-coding RNA as crucial for furthering our understanding of trainability. Sarzynski et al. (2017) propose a hierarchical model of concentric circles that govern training-induced structural and functional modifications. In the outermost circle, environmental stressors and physiological signals drive signaling cascades that, in turn, regulate the molecules directly involved in the cellular response to the training stress. This model is conceptually similar to the more detailed model of the transcriptomic plasticity of skeletal muscle tissue with endurance exercise training proposed by Hoppeler (2016) (Fig. 1).

Variability and trainability of $\dot{V}_{O_2,max}$ as a system response

As discussed above, the variability of the $\dot{V}_{O_2,max}$ response to endurance exercise training is a highly malleable phenotypic trait with a strong hereditary component. In skeletal muscle tissue, an increase in mitochondrial content of up to 40% is associated with an increase in $\dot{V}_{O_2,max}$ in typical training protocols (Hoppeler, 1986). It is the overall functioning of the complex transcriptomic network that drives mitochondrial biogenesis in skeletal muscle (Hoppeler, 2016). This transcriptomic network is determined by several internal and external modulators, each with their own signaling cascades impinging on a transcriptional coactivator, PGC-1α (Fig. 1). This transcriptional coactivator orchestrates mitochondrial biogenesis by integrating the signals of the different signaling cascades. This muscle transcriptomic network sets the individual baseline mitochondrial content of muscle as well as the gain in mitochondria observed with exercise training (Hoppeler, 1986). The total number of partners or nodes in this network is currently unknown, but must be in excess of 20, and is more likely close to 100 (Hoppeler, 2016). Each one of these nodes is a protein encoded by a gene and potentially existing in several isoforms (see Fig. 2). These proteins are subject to epistasis. Epistasis refers to functional interactions between or within genes in the genome (Wei et al., 2014). In a narrow functional sense, epistasis means that even if a gene is involved in a particular function, this functionality can be modified

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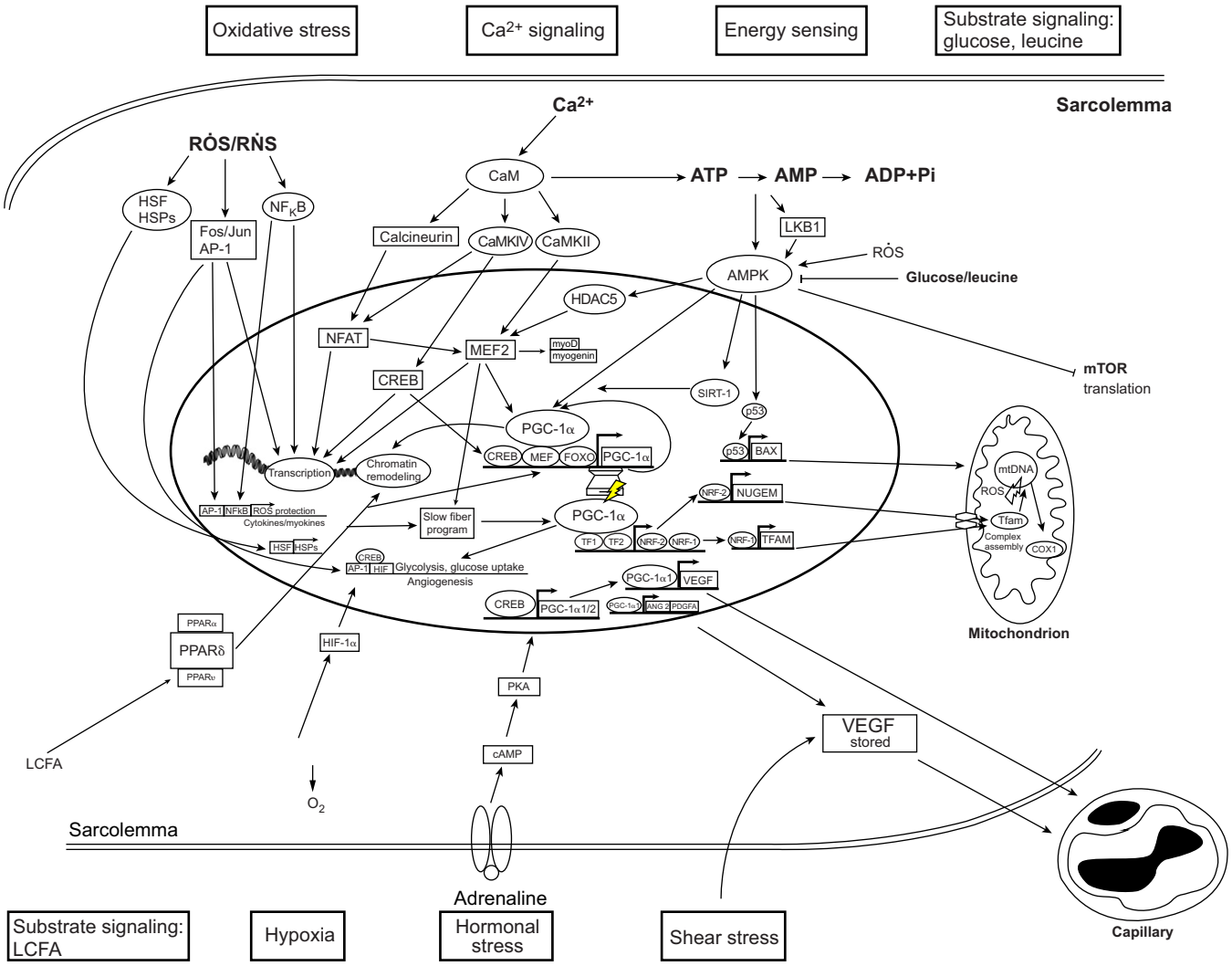

Fig. 1. Model of the transcriptomic network activated by endurance exercise training in skeletal muscle. Modified from Hoppeler (2016). A number of external stressors activate interlinked signaling cascades that in turn invoke activators of gene expression. This model represents an incomplete subset of proteins that are involved in the response of mitochondria and capillaries to various training cues, all of which are subject to epigenomic and epitranscriptomic regulation.

by actions of other gene products or buffered in the transcriptomic network. However, the extent to which genomic variants associated with the $\dot{V}_{O_2,max}$ phenotype are subject to epistasis has not been explored.

The transcriptomic machinery is central to setting the mitochondrial content of skeletal muscle, but its output is modulated upstream by the epigenome (Willyard, 2017). Epigenetic modifications are responsible for changes in gene function that do not involve changes to the gene sequence and may be inheritable. The epigenome is significant for its role in mediating the influence of the environment on the transcriptomic machinery by mechanisms such as DNA methylation and histone modification. It is recognized that acute and chronic physical exercise is an important epigenetic modulator of the transcriptome of skeletal muscle (Voisin et al., 2015; McGee and Walder, 2017).

In addition to epigenetics modulating gene transcription, it has recently become clear that epitranscriptomic mechanisms, mechanisms downstream of the transcription process, can also influence gene expression (Licht and Jantsch, 2016). The epitranscriptome denotes biochemical modifications of any type of cellular RNA that result in functionally relevant changes of the transcriptome without involving changes in the sequence or abundance of ribonucleotides. The epitranscriptome has a relationship to RNA that is akin to that of the epigenome and its relationship to DNA. Several readable and erasable tags have been identified that decorate various types of RNA and impinge on gene expression. However, it is currently not known how epitranscriptomic changes are relevant to exercise-related gene regulation.

As mentioned above, this Commentary considers the variability of the $\dot{V}_{O_2,max}$ response from a perspective that focuses on the molecular aspects of skeletal muscle plasticity. However, it must be pointed out that any change in $\dot{V}_{O_2,max}$ is a system change depending on more than the changes in the muscle mitochondrial complement. Changes in $\dot{V}_{O_2,max}$ also strongly depend on an increase in oxygen delivery to muscle tissue brought about mainly by an increase in cardiac stroke volume (Bassett and Howley, 2000). Moreover, endurance exercise training involves major modifications of many organs such as adipose tissue, liver, pancreas, bone, brain, and the cardiovascular and endocrine system. These changes are mediated

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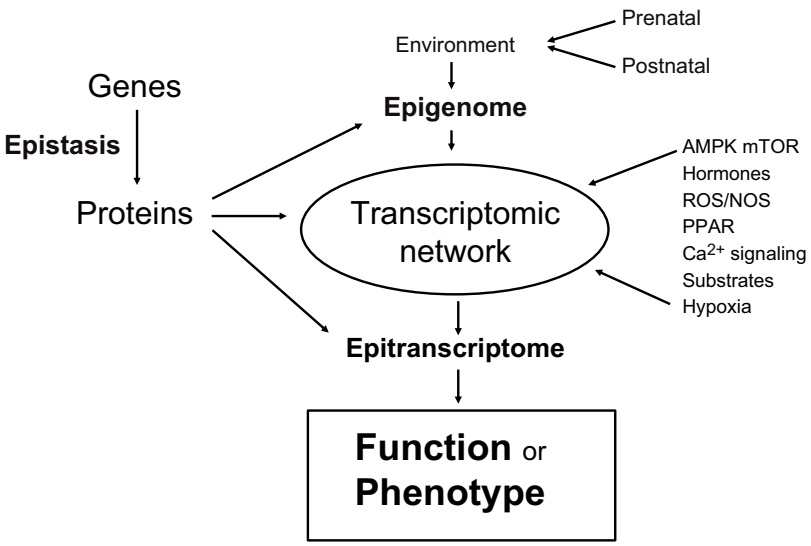

Fig. 2. The malleable phenotype of $\dot{V}_{O_2,max}$ is determined by the interconnectivity of the transcriptomic network that integrates the relevant internal and external cues. Gene expression is modified by epistasis, the environment influences the transcriptome via the epigenome and possibly by epitranscriptomic mechanisms that modify RNA expression. A large number of proteins and their isoforms intervene at every level of the phenotype cascade.

by peptides released by working skeletal muscle, collectively termed myokines. Myokines, such as interleukin-6, irisin, myostatin, interleukin-15, brain-derived neurotrophic factor, β-aminoisobutyric acid and several others are responsible for tissue crosstalk, and regulate muscle metabolism as well as systemic metabolism through autocrine, paracrine and endocrine mechanisms (Pedersen, 2013). The myokines released by working skeletal muscle during endurance exercise training activate a host of signaling cascades in all organs concerned and lead to functional modifications permitting an increase in $\dot{V}_{O_2,max}$ by all contributing tissues. It is safe to assume that, in most cases and in all organs, several signaling cascades are activated as a consequence of endurance exercise training. This means in essence that the same proteins, or isoforms thereof, perform different functions in different cells of the body at the same time. Together, this shifts the exercise phenotype of the organism towards a higher capacity to transport and utilize energy. This global enhancement of the organism's ability to take-up, transport and utilize energy manifests itself by a readily determined increase in $\dot{V}_{O_2,max}$ – the malleable $\dot{V}_{O_2,max}$ phenotype.

Variability and trainability of $\dot{V}_{O_2,max}$ as an emergent property of molecular networks

As noted above, the regulation of baseline $\dot{V}_{O_2,max}$ and its increase with exercise training is incredibly complex. At the level of a single muscle cell, a transcriptomic network regulates mitochondrial content and thus sets a limit to how much power a muscle cell can produce aerobically (Fig. 1). At the level of the cardiovascular system, the energy demand for muscular activity must be met by a commensurate energy delivery. An increase in $\dot{V}_{O_2,max}$ necessitates a larger oxygen supply, ultimately achieved by structural modifications of the heart. As discussed above, the process of exercise training in itself leads to a host of changes in multiple organs mediated by the release of myokines by working skeletal muscle.

The enormous complexity of the regulatory processes that set $\dot{V}_{O_2,max}$ and support its increase with exercise training begs the question as to whether any hypothesis-free genomic approach could be expected to lead to significant insight. It must seriously be considered whether larger numbers of subjects and better technologies would solve the problem of finding the genetic signature of the trainability of $\dot{V}_{O_2,max}$, as proposed by Rankinen et al. (2016). Might it be that relevant information on the $\dot{V}_{O_2,max}$ phenotype cannot be obtained by a bottom-up approach (Noble, 2016)?

Let us consider the $\dot{V}_{O_2,max}$ phenotype of skeletal muscle tissue to be a higher-level physiological function of muscle tissue. We observe that this higher-level function emerges through the collective connectivities of the proteins of the transcriptomic network, their interactions and their regulation by feedback and feedforward. Noble (2016) points out that: '…the question … of function depends on the level we are considering … It has no meaning below the level at which the relevant function is integrated'. This could indicate that it is fundamentally impossible to infer higher-level functions such as the $\dot{V}_{O_2,max}$ phenotype from the constituents of the underlying interactive networks. Trying to understand the phenomenon of $\dot{V}_{O_2,max}$ at the level of the proteins or their constituent genes would thus be doomed to failure.

Considering higher-level functions such as $\dot{V}_{O_2,max}$ as emergent properties of (multiple) transcriptomic networks subject to epistasis and modulated by epigenomics and epitranscriptomics does not invalidate the genetic approach. It merely means that knowledge of the genetic underpinning of the functionally relevant transcriptomes is a necessary – but not sufficient – condition for understanding such higher-level functions. It also indicates that the bottom-up approach may not be able to provide insight into the function in question and that alternative approaches might have to be considered. It is difficult to see at present how the relative importance of the genotype as modified by epistasis, epigenetics and epitranscriptomics could experimentally be accessed.

In the case of the $\dot{V}_{O_2,max}$ phenotype, we might have to study species that set or modulate their $\dot{V}_{O_2,max}$ phenotype differently from humans. In essence, we could use a classical 'Kroghian' approach (see Glossary) to decipher the $\dot{V}_{O_2,max}$ phenotype. Mass-specific $\dot{V}_{O_2,max}$ varies by as much as fivefold when comparing the performance of mice with that of cows. Within each size class, athletic animals such as dogs and horses systematically have a more than twofold larger $\dot{V}_{O_2,max}$ than the more sedentary species such as goats and cattle, with the pronghorn antelope setting the record at 272 ml O_2 min^{-1} kg^{-1} for the most 'aerobic' land-borne animal (Lindstedt et al., 1991; Weibel and Hoppeler, 2005). These mammals all use essentially the same transcriptomic networks as humans to modulate mitochondrial content in skeletal muscle and to set and vary $\dot{V}_{O_2,max}$. It is quite possible that different species have different ways to achieve this.

With regard to trainability, it has been shown that feeding sedentary quails (a migratory game bird) a diet rich in unsaturated dietary lipids increases muscle oxidative capacity (estimated by the level of activity of citrate synthase) by up to 90% (Nagahuedi et al., 2009). By contrast, in humans, it has been shown that a fat-rich diet can change muscle substrate selection but has no impact on muscle oxidative capacity (Vogt et al., 2003). The red knot, a migratory wading bird, changes its muscle mass seasonally. In periods without exercise, muscle mass may increase by up to 40% (Dietz et al., 2013). This is obviously not observed in humans. Muscle mass gains in humans are only seen as a consequence of strenuous strength training. These examples indicate that the way the relevant transcriptomic networks in muscle tissue operate may differ significantly among species. Another potential avenue to apply genetic techniques in an informed way is to study mice selectively bred over many generations for a high running performance (Xu and Garland, 2017). This could provide unique opportunities for the study of multigenetic traits such as $\dot{V}_{O_2,max}$, their phenotypic malleability and their genetic underpinning.

Concluding remarks

The $\dot{V}_{O_2,max}$ phenotype and its malleability vary massively between humans, with heritability being responsible for more than 50% of this variability. So far, the candidate-gene approach, gene-expression studies and GWAS have been unable to identify causal variants of the genome with predictive value for the $\dot{V}_{O_2,max}$ phenotype. It appears that multigenetic phenotypes such as $\dot{V}_{O_2,max}$ are emergent properties of the underlying transcriptomic networks, subject to epistasis and modified by the epigenome and the epitranscriptome. The genetic approach is thus a necessary but insufficient condition for understanding multigenetic higher-level functions. The currently available genetic tools do not seem to be capable of making assertions with predictive power for multigenetic phenotypes. I see it as a challenge for the future to enhance the available genetic knowledge by adding relevant information on the additional levels of genetic control. This would deepen our understanding not only of the factors determining individual $\dot{V}_{O_2,max}$ but also of other multigenetic phenotypes.

Competing interests

The author declares no competing or financial interests.

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