The genetics of human performance

Daniel Seung Kim[1], *Matthew T. Wheeler*[1,2] *and Euan A. Ashley*[1,2,3,4] ✉

Abstract | Human physiology is likely to have been selected for endurance physical activity. However, modern humans have become largely sedentary, with physical activity becoming a leisure-time pursuit for most. Whereas inactivity is a strong risk factor for disease, regular physical activity reduces the risk of chronic disease and mortality. Although substantial epidemiological evidence supports the beneficial effects of exercise, comparatively little is known about the molecular mechanisms through which these effects operate. Genetic and genomic analyses have identified genetic variation associated with human performance and, together with recent proteomic, metabolomic and multi-omic analyses, are beginning to elucidate the molecular genetic mechanisms underlying the beneficial effects of physical activity on human health.

Endurance physical activity (EPA). Human performance that optimizes sustained activity over an extended period of time, such as running, cycling and cross-country skiing. Maximal oxygen uptake (VO_2 max) is commonly used as a proxy for maximal capacity for EPA.

Resistance physical activity (RPA). Human performance that requires muscles to work against an external force, such as weightlifting; individual muscle groups (such as biceps or triceps) can be measured accurately in isolation using dynamometry.

[1]*Division of Cardiovascular Medicine, Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA.*

[2]*Stanford Cardiovascular Institute, Stanford University, Stanford, CA, USA.*

[3]*Department of Genetics, Stanford University School of Medicine, Stanford, CA, USA.*

[4]*Department of Biomedical Data Science, Stanford University School of Medicine, Stanford, CA, USA.*

✉*e-mail: euan@stanford.edu*

https://doi.org/10.1038/s41576-021-00400-5

Compared with our recent hominid ancestors, humans seem to have uniquely evolved for endurance physical activity (EPA)[1,2] (BOX 1). Evidence for this theory derives from observations in modern hunter-gatherer tribes, who walk an average of 9–15 km daily to forage for food, in addition to the sustained endurance activity used in persistence hunting[3]. However, the rapid increase in industrialization during the twentieth century has seen modern humans become largely sedentary, with an accompanying increase in the prevalence and incidence of chronic diseases, such as cancer, cardiometabolic disease and dementia. All forms of exercise have been shown to be protective against the onset of chronic diseases[4,5], suggesting a central role for physical activity in our evolutionary history[6].

Human performance synergizes numerous organ systems (see FIG. 1) to optimize EPA and resistance physical activity (RPA). Moreover, as exercise extends healthspan in the population, the study of the molecular pathways through which exercise exerts its beneficial effects can inform our understanding of biology and even identify potential therapeutic targets. It is largely accepted that an individual's maximal performance is strongly heritable[7], with few able to realize Olympic level performance; for example, an inherited performance-enhancing variant in the erythropoietin receptor, which contributes to increased red blood cell mass, was found to be at least partly responsible for the Olympic success of cross-country skiing champion, Eero Mäntyranta[8]. However, genome-wide association studies (GWAS) of maximal oxygen uptake (VO_2 max) and elite endurance athletes have yet to yield substantial insight into the genes and mechanisms involved in optimizing performance[9] because of sample size limitations and inconsistent protocols for studying EPA. As a result, our current knowledge of the genetics of EPA have emerged from candidate gene studies and mouse models.

More recent GWAS have focused on narrower 'intermediate' phenotypes (for example, factors that affect VO_2 max, such as maximal exercise heart rate) to achieve greater sensitivity in detecting association signals[10,11]. These studies have identified loci that may contribute via single organ system mechanisms to optimizing human performance. Separately, advances in 'omic' technologies (such as RNA-seq, ATAC-seq and high-throughput proteomics, metabolomics and lipidomics), have enabled the multi-omic study of changes that occur with acute exercise and chronic exercise training; integration of these data can identify interacting molecular networks that affect EPA.

Here, we review our current understanding of the genetics of human performance, including several recent large studies that successfully identified and replicated variants associated with VO_2 max[12,13]. We then explore recent studies of defined intermediate phenotypes involved in human performance. We review advances in the multi-omics of acute and chronic endurance training, and highlight molecular pathways identified through longitudinal multi-omic approaches. Of note, this Review does not discuss the role of microRNAs in acute exercise-mediated gene expression changes, which are reviewed in detail elsewhere for skeletal muscle[14] and cardiac function[15]. We conclude by introducing ongoing consortia work that aims to illuminate the molecular mechanisms through which exercise, and in particular EPA, exert their health-promoting effects.

Genetics of endurance physical activity

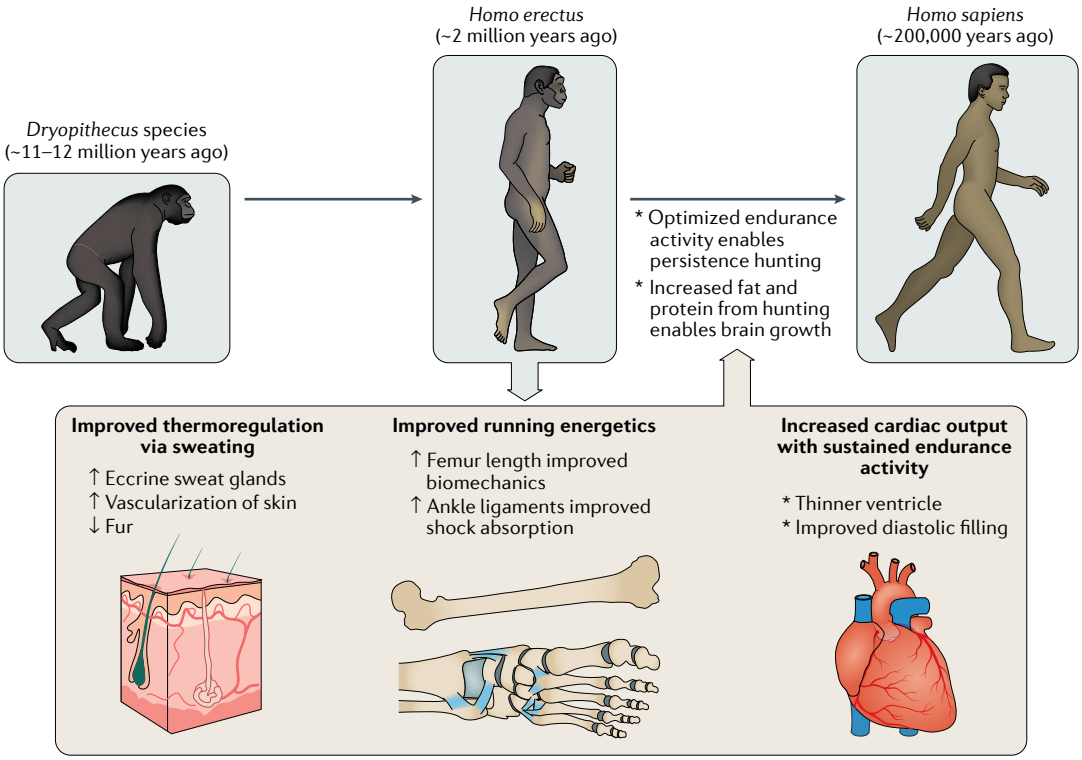
Heritability of endurance physical activity. A substantial proportion of knowledge regarding the genetics of EPA has come from the HERITAGE study[16]. In HERITAGE, 90 European and 30 African ancestry families (each with ≥3 adult children) were recruited from multiple centres across North America. From this familial data,

Box 1 | **Evolutionary pressure favours EPA in *Homo* species**

The emergence of *Homo erectus* ~2 million years ago, with a body like that of modern humans, marked the onset of the rapid geographical expansion of *Homo* species[141]. There remains vigorous debate about which *Homo*-specific adaptations enabled them to spread throughout the world; however, the evolution of larger brains is thought to be critically important.

One prominent theory (proposed by Carrier et al.[1] and popularized by Bramble and Lieberman[2]) integrates the fossil/biomechanical and thermoregulation hypotheses and proposes that humankind evolved to optimize endurance physical activity (EPA) to obtain the fat and/or protein necessary for expanded brain development (see figure). First, *Homo* species evolved a unique musculoskeletal system associated with optimized EPA: compared with the more ape-like *Australopithecus afarensis* species that likely preceded it, *H. erectus* had a ~50% longer femur, more compact feet and shorter toes[142]. Combined with the evolution of shock-absorbing foot and ankle ligaments, these skeletal changes likely allowed for improved running energetics[143]. Second, early hominids shed the fur prominent amongst our chimpanzee relatives, allowing for improved thermoregulation. Unlike most other mammals, humans can sweat[144], which is facilitated by the greater density of eccrine sweat glands throughout their skin[145]. Human skin is also more densely vascularized, which enables loss of body heat via blood flow to the skin and by peripheral sweat production[146]. Finally, the loss of fur in *Homo* species provides a greater surface area for heat exchange[147]. Additional support comes from recent work comparing human hearts with chimpanzee and gorilla hearts (which together represent the inferred form of the last common ancestor of modern humans and apes), which indicates that the human heart is optimized for cardiac output (thinner walls, larger ventricular cavity, improved diastolic filling) and therefore better suited for EPA, such as running[148]. By contrast, the thicker, more spherical hearts of chimpanzees and gorillas are well adapted for resistance activity, such as climbing or fighting[149]. Taken together, these observations suggest that the numerous inferred adaptations of ancient *Homo* species are consistent with evolutionary pressure towards efficiency of EPA.

Notably, although humans cannot sprint or gallop as fast as most quadrupeds[150], they are able to thermoregulate[144] and use the bioenergetics of foot strike to continue running longer than their prey[151]. By contrast, quadrupeds must pant to dispel excess heat[152], placing them at a significant disadvantage as they cannot simultaneously pant and run[153]. This observation has led to the hypothesis that the adaptations first seen in *H. erectus* ~2 million years ago enabled ancient hominids to fill the evolutionary niche of hunter-gatherers via persistence hunting in an era without weapons[2]; hunting could explain how our ancient ancestors obtained the large quantities of fat and protein necessary for expanded brain development[1,2]. Persistence hunting is still practised by the Kalahari Bushmen, and involves alternating cycles of chasing and tracking. By ensuring their prey cannot fully thermoregulate between chases, the hunters force their prey to eventually collapse from hyperthermia. Hunters can then dispatch the prey without fear of injury[154].



Healthspan
The length of time that a person is healthy and independent in their activities; by contrast, lifespan simply measures the length of time a person is alive but does not factor in quality of life.

Maximal oxygen uptake
(VO_2 max). Represents the maximum capacity for endurance physical activity (EPA). It is the maximal rate of oxygen uptake (O_2 in $ml\,kg^{-1}\,min^{-1}$) measured during incremental increases in EPA activity intensity, commonly on a treadmill or stationary bike.

investigators were able to estimate the heritability of baseline VO_2 max[17] (heritability approximately 60%) and post-training improvements to VO_2 max[7] (heritability approximately 50%). Interestingly, both studies showed a significant contribution of maternal heritability (approximately 30% for both baseline and post-training VO_2 max)[7,17], suggesting that mitochondrial inheritance has a role in EPA genetics.

Although small, the HERITAGE study provided evidence for both genetically determined EPA capacity and for differential training response dependent on underlying familial genetics. Improvements in genotyping

Haplogroups
Combinations of alleles at different regions of the genome (organellar or nuclear) that share polymorphisms inherited from a common ancestor.

technology subsequently enabled mitochondrial variant association studies and GWAS of VO_2 max to be performed to identify candidate genes responsible for the heritability of EPA.

Mitochondrial genetics and endurance physical activity. One explanation for the strong maternal contribution to the heritability of VO_2 max (both at baseline and after training)[7,17] is that variation in mitochondrial DNA (mtDNA) results in altered endurance capacity. Associations between mtDNA haplogroups and EPA have been investigated, but with inconsistent results[18,19]. Specifically, given the ancestral inheritance of haplogroups (for example, populations of African ancestry tend to have different mtDNA haplogroups from individuals of European ancestry), trans-ethnic comparisons of haplogroup association with EPA have not been reliably replicated[18]. Moreover, a recent review of mitochondrial genome variants did not find consistent signals between mtDNA haplogroups and EPA, even when separating studies by genetic ancestry[19], and no association was observed between mouse haplogroups and EPA in mice specifically bred for voluntary wheel running[20]. This failure to detect associations can partially be explained by small sample sizes, but it should also be considered that most mitochondrial proteins are encoded by nuclear DNA rather than mtDNA[18,19,21]. Indeed, recent work found that only nuclear DNA (but not mtDNA) variants encoding mitochondrial proteins were associated with response to exercise training[21]. There is a need for a gold standard study of mtDNA and EPA and standardization of mtDNA genotyping and/or sequencing; for instance, numerous studies only consider the hypervariable regions of mtDNA and thus omit non-coding mtDNA variation[21].

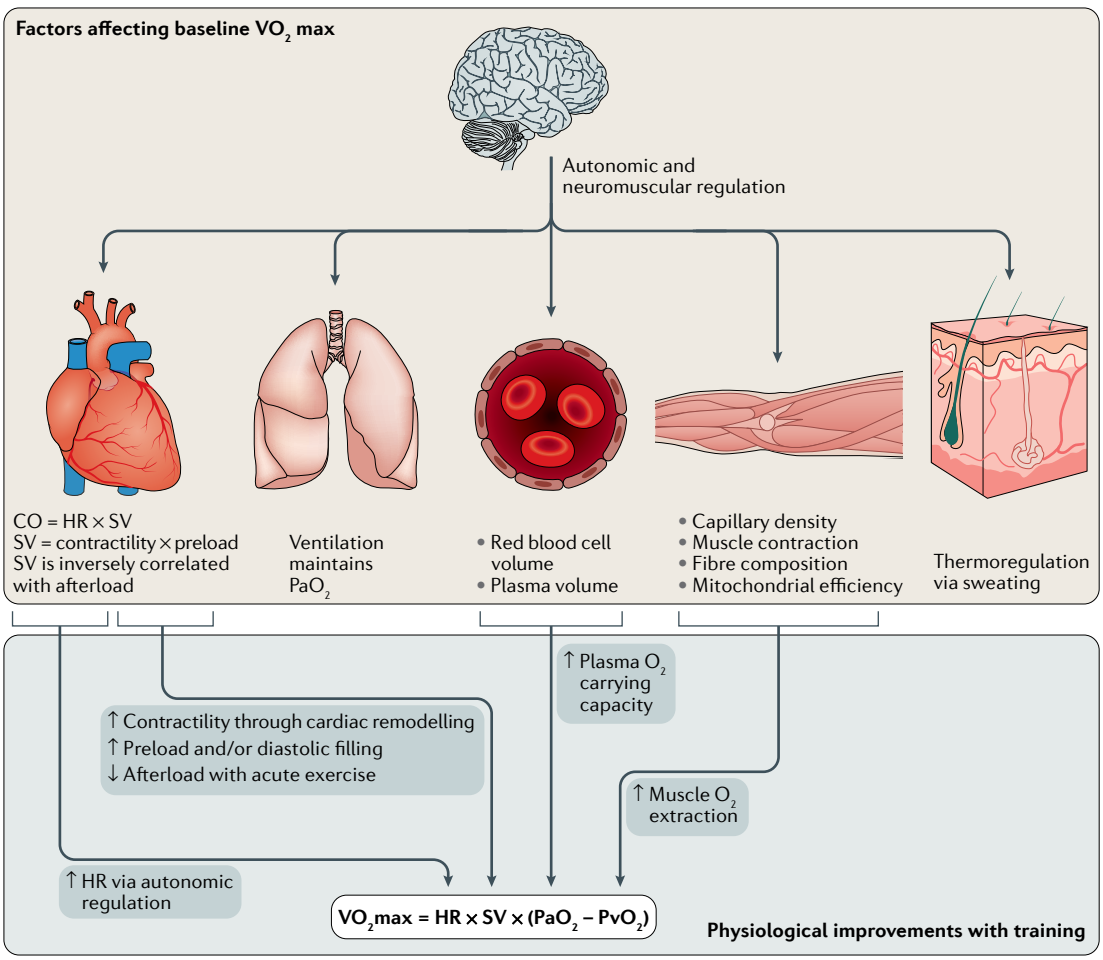


Fig. 1 | **VO_2 max is affected by the function of numerous organs.** At baseline, VO_2 max (a surrogate for endurance physical activity) is determined by the product of cardiac output (CO, measured as heart rate (HR) multiplied by stroke volume (SV)) and oxygen extraction (measured as mixed arterial oxygen tension (PaO_2) minus mixed venous oxygen tension (PvO_2)). Organs such as the heart, lungs, blood system, skeletal muscle and skin all contribute to baseline VO_2 max. However, only the heart, blood system and skeletal muscle significantly contribute to increased VO_2 max with exercise; ventilatory capacity (via the lungs) and sweating (via skin) are generally not changed with exercise training and hence do not greatly influence changes to VO_2 max. With exercise training, cardiac remodelling occurs to allow for greater SV (via passive flow, or preload). The autonomic nervous system and cardiac-specific remodelling increase the HR response to exercise. Plasma oxygen-carrying capacity also increases via erythrocytosis, mediated by hypoxia-inducible factor (HIF) family signalling. Finally, there are numerous changes to skeletal muscle that allow for greater extraction of muscle oxygen and its utilization for metabolism during future exercise.

Polygenic score
A measure constructed from additive genotypes of variants across multiple genes associated with a trait that can be used to predict from an individual's genetic sequence their likelihood of having that trait.

Type II/fast-twitch muscle fibre
This muscle fibre type is optimized for resistance physical activity, and is characterized by high force generation but low endurance capability.

Type I/oxidative muscle fibre
This 'slow-twitch oxidative' muscle fibre type is optimized for endurance physical activity; it has greater mitochondrial density, which leads to lower force generation but increased resistance to fatigue.

GWAS of endurance physical activity. Peak EPA is typically assessed from VO_2 max measurements obtained during laboratory exercise testing, which makes it difficult to obtain the large samples necessary for sufficiently powered GWAS[12,13,22–25]. Indeed, most GWAS have focused largely on the phenotype of baseline VO_2 max[12,13,25], but those that have studied changes in VO_2 max with training have been extremely small and have used partially overlapping data from the HERITAGE study[22–24]. Similarly, performing a case–control GWAS (with cases represented by persons with elite endurance capacity) requires careful ascertainment and recruitment, which also limits sample size[26,27]. Hence, only a handful of GWAS for EPA have been published[12,13,22–27]; the remainder of the genetic studies have typically focused on identifying EPA-associated genetic variation in candidate genes, and these associations could not be reliably replicated[28], likely because of a lack of study participants with both VO_2 max and genotype data.

The most recent of these GWAS have been performed in non-HERITAGE cohorts and have identified several single nucleotide variants (SNVs) and genes associated with EPA (TABLE 1). A small discovery study of Russian endurance athletes ($n = 80$) identified six significant SNVs associated with VO_2 max[25], of which only one SNV that mapped close to the *NFIA-AS2* locus (which is involved in erythropoiesis — see 'GWAS of EPA-related phenotypes') was later replicated[29]. More recently, a GWAS using pooled data from endurance athletes of European ancestry ($n = 173$) identified (via false discovery rate filtering) an *IL6* intronic variant associated with VO_2 max[13]. Finally, in the most comprehensive GWAS to date, data from the HUNT3 ($n = 3{,}470$) and Generation100 ($n = 718$) population-based studies of non-athletes of European ancestry were leveraged to identify and replicate SNV associations with VO_2 max in the loci *PROX1*, *EDN1*, *MYLIP*, *VIPR2*, *ADRB3*, *ACTN3*, *KCNQ1*, *BAHD1* and *MYOCD*[12]. Using these novel findings, a nine-SNV polygenic score was constructed and demonstrated an allelic dose-dependent increase in VO_2 max and the presence of fewer cardiovascular risk factors (such as cholesterol, blood pressure and body mass index) in individuals with a more favourable polygenic score[12].

Two GWAS that used elite endurance athletes as cases and sedentary participants as controls have identified replicating SNVs associated with VO_2 max (TABLE 1). The first study was a meta-analysis of 1,520 endurance athletes and 2,760 sedentary controls; a coding variant in *GALNTL6* was the only SNV identified in trans-ethnic analyses as significantly associated with elite endurance athlete status[26]. More recently, a GWAS of European endurance athletes ($n = 662$) identified and replicated (in separate Russian and Japanese cohorts) a synonymous SNV in *MYBPC3* that was significantly associated with increased odds of being an elite endurance athlete[27]. Notably, *MYBPC3* is one of the most commonly associated genes in hypertrophic cardiomyopathy[30].

Human GWAS of EPA have been limited by statistical power because of the highly specialized nature of maximal exercise testing. As a result, GWAS of EPA have identified and replicated only a limited number of possible candidate genes (TABLE 1), although we suspect that sufficiently powered studies of the trainability of VO_2 max may identify novel genetic variants and genes that influence EPA. Moreover, unlike common diseases such as type 2 diabetes[31], a functional and mechanistic understanding of how these candidate genes affect EPA is largely lacking (apart from *ACTN3*, which is discussed below). Consequently, investigators have instead identified plausible biological targets and examined their effects in mice.

Genes identified in model organisms with a role in endurance physical activity. Transgenic mice can be used to test the effects on EPA of specific genes, with endurance capacity typically measured as time or distance to exhaustion via either swimming or running. A recent systematic literature review identified 31 mouse genes the knockout or overexpression of which led to increased endurance capacity[32], including nine with human orthologues in which variants have been implicated in endurance traits: *ACTN3*, *ADCY5*, *ADRB2*, *BDKRB2*, *HIF1A*, *PPARD*, *PPARGC1A*, *PPARGC1B* and *PPP3CA*[32]. Of these, strong evidence for a role in human performance exists for *ACTN3*, *HIF1A*, *PPARGC1A* and *PPARD* (TABLE 1).

ACTN3 has previously been reported[22] and replicated[12] in human GWAS for VO_2 max. It encodes α-actinin-3, an actin-binding protein expressed in muscles, and is required for the formation of type II/fast-twitch muscle fibres (glycolytic fibres used for RPA)[33]. *ACTN3* has been extensively studied since it was first reported that a common nonsense mutation ($ACTN3^{R577X}$) resulted in α-actinin-3 deficiency in the human population[34]. Given the role of *ACTN3* in type II/fast-twitch muscle fibre formation, interest in the gene intensified when a follow-up case–control study demonstrated that $ACTN3^{R577X}$ was associated with decreased sprint and/or power performance, but possibly conferred increased endurance capability[35]. Subsequent candidate gene studies in humans have consistently found $ACTN3^{R577X}$ to be associated with decreased RPA, but results for EPA were more inconsistent[36]. By contrast, *Actn3*-knockout mice show a consistent, modest increase in endurance capacity[37]. Moreover, *Actn3*-knockout mice show a greater differentiation from wild-type mice with endurance training; that is, whereas baseline endurance capacity does not significantly differ between *Actn3*-knockout mice and wild-type mice, the improvement in endurance capacity with endurance training is significantly greater in *Actn3*-knockout mice[36]. This exercise training-dependent increase in endurance capacity in *Actn3*-knockout mice is mediated through muscle fibre switching to type I/oxidative muscle fibres, in line with the crucial role of *ACTN3* in type II/fast-twitch muscle fibre formation[33].

Hypoxia-inducible factor 1 (HIF1) is a transcription factor that is activated by hypoxic conditions and subsequently increases oxygen-carrying capacity by upregulating genes related to angiogenesis and/or erythropoiesis[38]. While an acute session of endurance exercise will increase expression of HIF1-regulated genes, fit and trained endurance athletes tend to have

Table 1 | **Summary of genes confidently associated with human performance**

Trait	Gene function	Gene	Other trait associations	Refs
GWAS of endurance performance activity				
VO_2 max	*NFIA* gene regulation, erythropoiesis	*NFIA-AS2*	–	25
VO_2 max	Inflammation-regulating cytokine	*IL6*	Autoimmune disease[117], T2D[118]	13
VO_2 max	Skeletal muscle contraction	*ACTN3*[a]	–	12,13,34,35,37
VO_2 max	Adrenergic receptor; thermogenesis/ lipolysis	*ADRB3*	Metabolic syndrome[119]	12,13
VO_2 max	Endothelin-1, smooth muscle vasoconstriction	*EDN1*	HDL-C[120]	12,13
VO_2 max	Cardiac potassium channel	*KCNQ1*	Long QT syndrome[121], AFib[122]	12
VO_2 max	Myocardin; smooth muscle differentiation	*MYOCD*	–	12
VO_2 max	Lymphatic system development	*PROX1*	–	12
VO_2 max	Actin linking of membrane proteins	*MYLIP*	–	12
VO_2 max	Neurointestinal signalling of food intake	*VIPR2*	–	12
VO_2 max	Heterochromatin formation	*BAHD1*	–	12
Elite endurance athlete (cases)	Glycoprotein transfer	*GALNTL6*	–	26
Elite endurance athlete (cases)	Cardiac myosin binding protein	*MYBPC3*	Hypertrophic cardiomyopathy[123]	27
Transgenic mouse studies with strong in vivo and/or in vitro evidence[b]				
Time or distance to exhaustion	Skeletal muscle contraction	*ACTN3*[a]	–	12,13,34,35,37
Time or distance to exhaustion	Increased erythrocytosis to hypoxia	*HIF1A*[a]	Inflammation[124], CNS development[125]	32,41
Time or distance to exhaustion	Lipid metabolism, mitochondrial biogenesis	*PPARD*[a]	Exercise mimetics and/or doping[48,127]	32,126
Time or distance to exhaustion	Mitochondrial biogenesis	*PPARGC1A*[a]	Dilated cardiomyopathy[128], thermogenesis[129]	13,32,52
GWAS of performance-related traits[b]				
Hand-grip strength[c]	Axonal outgrowth, neurogenesis	*BDNF*	Neuropsychiatric disorders[130–132]	59
Hand-grip strength[c]	Microtubule motor, axonal outgrowth	*KIF1B*	Charcot–Marie–Tooth disease 2A1 (REF.[133])	59
Cardiac function	Cardiac muscle assembly; force generation	*TTN*	Hypertrophic[30] and dilated[134] cardiomyopathy	69,71
Cardiac function	Cytoprotective; binds to Hsp70 chaperones	*BAG3*	Hypertrophic[30] and dilated[135] cardiomyopathy	69,71,72
HR recovery after exercise	Cardiac muscarinic ACh receptor	*CHRM2*	Neuropsychiatric disorders[136,137]	10,11
Resting HR and HR recovery after exercise	E3 ubiquitin ligase	*RNF220*	Cardiac development[138]	10,11,79

ACh, acetylcholine; AFib, atrial fibrillation; CNS, central nervous system; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; QT, Q-to-T interval on electrocardiogram measured in milliseconds; T2D, type 2 diabetes. [a]Denotes a human gene with a mouse orthologue that has been studied (via overexpression or knockout experiments) for endurance-related phenotypes. [b]Although many candidates have been identified, we include only the subset of loci that we consider most likely to affect human performance. [c]Hand-grip strength is adjusted for body size, to normalize strength across differing heights and weights.

an attenuated HIF1 response owing to increased levels of HIF1 inhibitors[39]. A variant in *EGLN1* (which encodes PHD2, one of these HIF1 inhibitors) inhibits HIF pathway activation in Tibetan populations living at high altitudes and is thought to contribute to their improved high-altitude performance compared with low-altitude populations[40] (FIG. 2). Similarly, mice with skeletal muscle-specific deletions of *Hif1a* have significantly greater mitochondrial density and improved endurance capacity (albeit with greater muscle damage) than wild-type mice — suggesting that HIF1 inactivation leads to improved oxidative capacity and performance[41]. Hence, activity of the HIF family pathway seems to be required for changes that occur during the acute phase of endurance training but are then inhibited in the trained state, allowing for greater oxidative metabolism and EPA[38].

The *PPARD*, *PPARGC1A* and *PPARGC1B* genes converge upon the exercise metabolism pathway. *PPARGC1A* encodes peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), which is widely considered to be the master regulator of mitochondrial biogenesis[42]. *PPARGC1B* encodes PGC1β, a homologue of PGC1α with similar function[43], but the expression of which alone cannot compensate for the loss of *PPARGC1A* in knockout experiments[44]. PGC1α is activated by AMP kinase (AMPK) signalling[45]. AMPK senses intracellular energy balance and is activated by the high concentrations of cytoplasmic calcium and AMP[46] (FIG. 3) that result from repeated muscle contraction and ATP use, respectively. Once PGC1α has been activated by AMPK, it translocates to the nucleus and mitochondria to regulate expression of genes necessary for mitochondrial biogenesis[42]. As part of this process, PGC1α

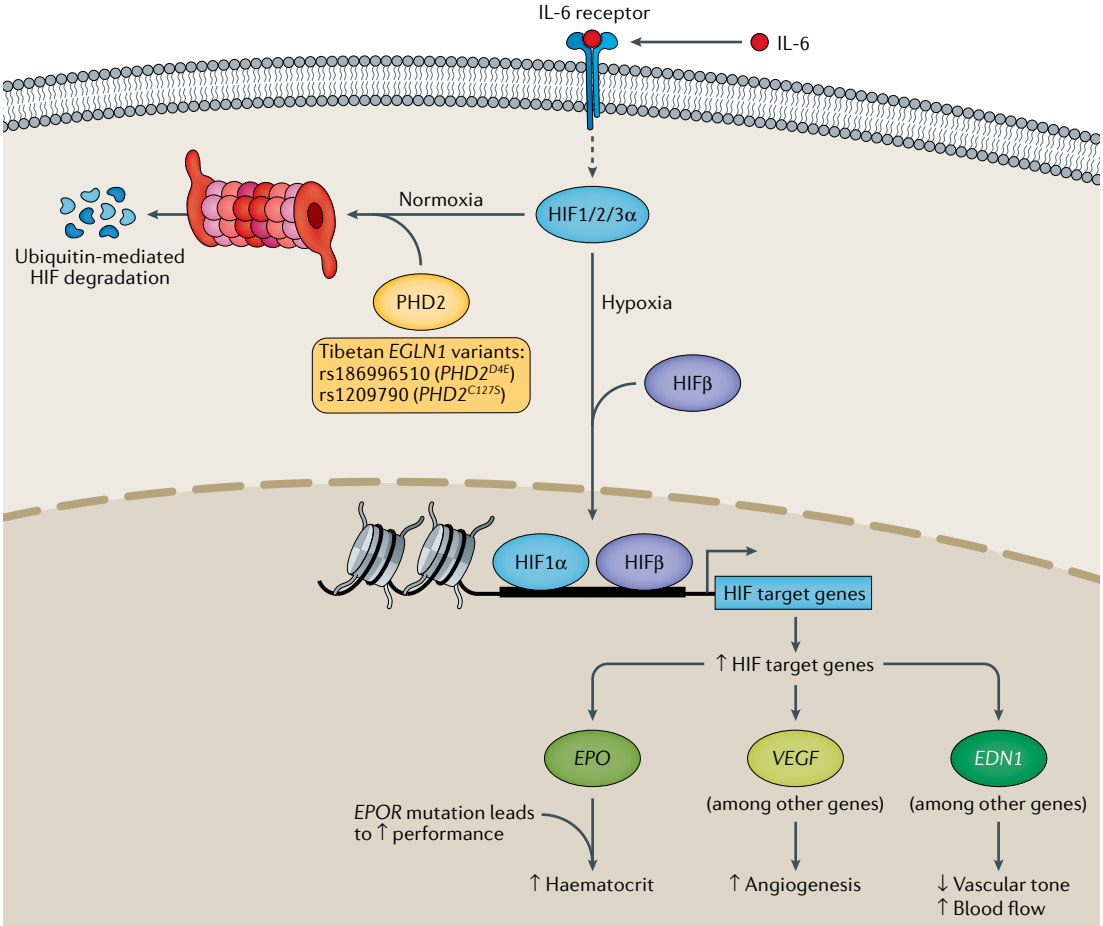


Fig. 2 | **The hypoxia-inducible factor signalling pathway affects the haematopoietic and vascular systems.** Under conditions of normal oxygenation (normoxia), prolyl hydroxylase domain-containing protein 2 (PHD2) binds to hypoxia-inducible factor (HIF) transcription factors HIF1/2/3α and leads to ubiquitin-mediated HIF degradation. Under conditions of low oxygen (hypoxia), the HIF1/2/3α transcription factors are not degraded and instead bind to HIFβ to activate HIF transcription factor family target genes, including *EPO* (erythropoietin, previously implicated in benign erythrocytosis that aided Eero Mäntyranta in Olympic cross-country skiing)[8], the vascular endothelial growth factor (VEGF) family of genes, and *EDN1* (encodes endothelin-1, a protein that regulates vascular smooth muscle tone). Separately, IL-6, as part of the acute inflammatory response associated with exercise[12], can induce HIF1/2/3α transcription factor activation. The study of high-altitude adaptation in Tibetans has identified two variants in *EGLN1* (encoding the protein PHD2) that result in constitutive degradation of HIF1/2/3α transcription factors, even under conditions of hypoxia[40,139]. The consequences of these mutations on, or their association with, human performance or endurance physical activity (EPA) are not yet known, but *EGLN1* mutations have been reported to cause erythryocytosis[140].

increases protein levels of glucose transporter type 4 (GLUT4) and numerous other transcription factors[47], which leads to increased mitochondrial density and, via activation of PPARδ (encoded by the *PPARD* gene)[48], an improved capacity for fatty acid metabolism and oxidative metabolism[49]. In addition, PGC1α acts through a HIF-independent pathway to mediate skeletal muscle angiogenesis in response to exercise[50]. Knockout of *Ppargc1a* specifically in the skeletal muscle of mice leads to a shift from type I (slow-twitch, high oxidative capacity) to type II/fast-twitch muscle fibre, with an accompanying decrease in endurance capacity and increase in muscle damage with exercise[51]. These phenotypes can be partially rescued by expression of human *PPARGC1A* in the *Ppargc1a*-knockout mice, as measured by an increase in mitochondrial gene expression and a decrease in fatiguability[49]. Subsequent analyses found that overexpression of *PPARGC1A* in skeletal muscle of mice increased the distance ran and led to a 24% increase in peak VO_2 max compared with control littermates[52]. In humans, endurance athletes had a significantly greater 'endurance genotype score' (comprising genes involved in the *PPARGC1A* transcriptional pathway) compared with controls and power athletes[53]. Separately, a missense variant of *PPARGC1A* was identified in a recent GWAS of endurance athletes but was found not to be significant after filtering for false discovery rate[13].

Taken together, mouse studies provide additional support for the role of biological pathways implicated in human performance by GWAS, notably the importance of skeletal muscle and oxygen. Genetic variation in *ACTN3* can affect the structure and function of skeletal muscle[37], and variants in genes in metabolic pathways (primarily mediated by *PPARGC1A*[42,49,50,52])

Expression quantitative trait locus
(eQTL). A genetic variant that is associated with a gene expression phenotype.

affect the degree to which gene expression, and ultimately oxygen delivery and mitochondrial function, in muscle are affected by exercise. HIF family pathway signalling has been implicated in EPA[38], and several genes in this pathway (including *IL6* and *EDN1*) have been identified in GWAS of VO_2 max[12,13]. These results stress the importance of red blood cell volume and oxygen-carrying capacity in determining peak performance potential. However, the relatively small number of convincing candidate genes identified to date via various approaches also underlines the urgent need for larger, better powered studies of EPA (see REF.[54] for a review of GWAS and candidate gene studies up until 2019).

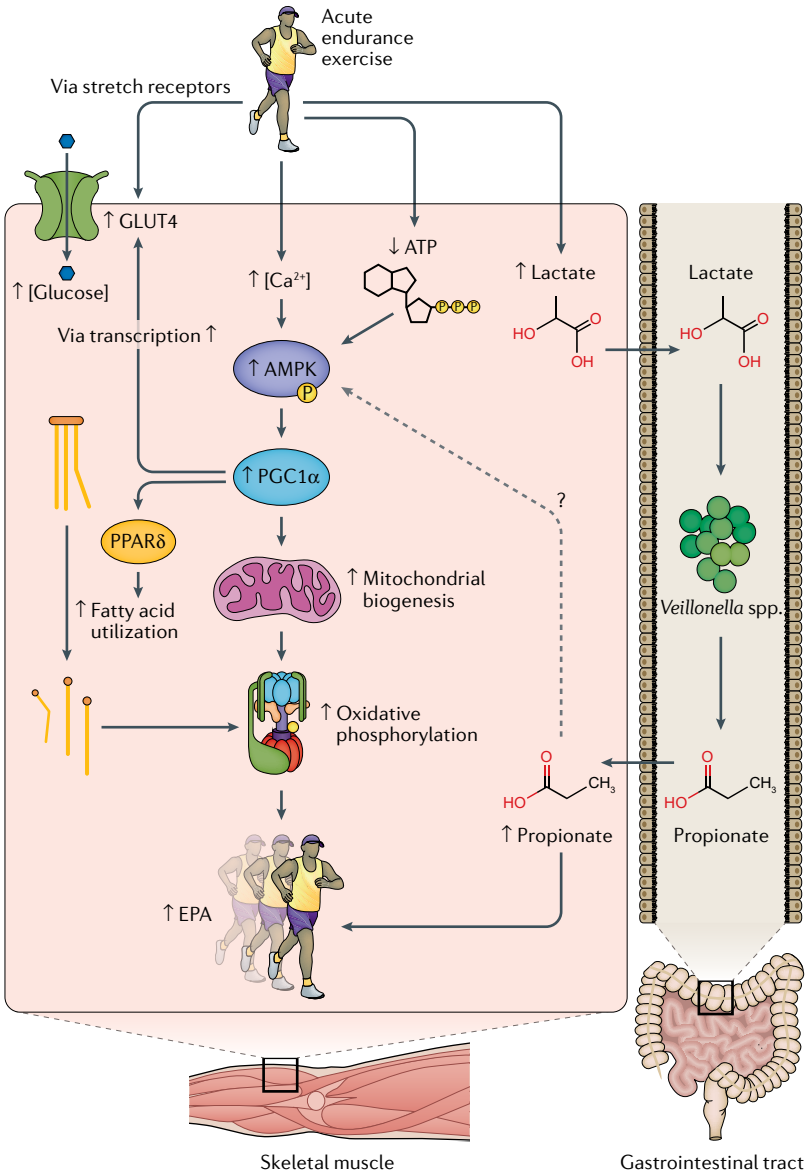


Fig. 3 | **Metabolic changes with acute exercise converge on PGC1α in skeletal muscle.** In skeletal muscle, intense exercise leads to activation of glucose transporter type 4 (GLUT4, the most common glucose transporter in skeletal muscle) and intracellular accumulation of AMP, and Ca^{2+}. AMP and Ca^{2+} are sensed by AMP kinase (AMPK), which in turn activates a signalling cascade centred on peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α), the so-called 'master regulator' of mitochondrial biogenesis. PGC1α activation results in increased *GLUT4* transcription and a metabolic switch in skeletal muscle cells, which leads to increased oxidative phosphorylation, in part via PPARδ-mediated increases in fatty acid utilization. These metabolic changes cause a switch from type II to type I (oxidative) muscle fibres with greater endurance performance. Additionally, recent longitudinal multi-omic data[109] support a potential role for host–microbiome interactions in this response to acute exercise: *Veillonella* spp. in the gut convert lactate into propionate, which, if taken up by skeletal muscle cells, may contribute to enhanced endurance physical activity (EPA) via AMPK activation[45,111].

Genetics of endurance-related phenotypes

Given the difficulty of recruiting the necessary populations to perform a sufficiently powered GWAS on VO_2 max or elite endurance athlete status, focus has shifted to discrete intermediate phenotypes that contribute to EPA. These GWAS have enriched our understanding of EPA by increasing the number of known loci involved in relevant human traits associated with human performance (TABLE 1).

Genetic studies of mechanical strength. Although mechanical strength is traditionally thought of as an RPA-related phenotype, nearly all forms of endurance performance (such as cycling, rowing and cross-country skiing) incorporate elements of resistance or muscular strength[36]. Hence, the genes that influence mechanical strength are likely to also affect EPA. Hand-grip dynamometry is frequently used as a proxy for overall mechanical strength because it is easy to implement, is strongly correlated with formal dynamometry[55], is reliable[56] and has prognostic value[57]. Hand-grip strength is strongly heritable (approximately 20–65%)[58,59] and has been studied in four GWAS to date[59–62]. The first GWAS did not yield any replicated associations at nominal significance[60], while the second study identified one replicated SNV[61] that was not validated in larger, subsequent studies[59,62]. The two most recent GWAS leveraged data from the UK Biobank and other consortia and consequently had significantly greater statistical power: the first GWAS ($n = 195{,}180$) identified 12 SNVs associated with hand-grip strength that were replicated in a formal two-stage analysis[62]; the second GWAS ($n = 334{,}925$), which used partially overlapping data with the first GWAS and adjusted hand-grip strength for body size, identified 64 replicating loci[59], eight of which had been previously reported[62]. Interestingly, expression quantitative trait locus (eQTL) analyses of these 64 loci showed strong enrichment for neuronal function rather than skeletal muscle[59]. This finding is consistent with long-known observations that muscular strength is dependent on both muscle size and/or composition and the ability of the brain to recruit muscle units for contraction[63]. In addition, a restricted polygenic score based on significant SNVs for hand-grip strength was inversely correlated with body fat, leptin and measures of insulin resistance. Mendelian randomization using this restricted polygenic score for hand-grip strength found potentially causal protective effects against ischaemic heart disease and atrial fibrillation[59]. These data suggest that genetically determined muscle strength, mediated by both the nervous system and peripheral muscle, is protective against obesity and cardiometabolic disease in addition to its effects on EPA and overall human performance.

Cardiac output
The amount of blood the heart pumps through the circulatory system (measured in litres per minute and estimated by multiplying the heart rate by stroke volume). Cardiac output is one of the primary contributors to VO_2 max.

Genetics of cardiac-related phenotypes. VO_2 max is strongly influenced by maximal cardiac output (FIG. 1). During exercise, increases in VO_2 are driven by increases in cardiac output (via increased stroke volume and heart rate) and oxygen extraction at the tissue level[64]. Noninvasive cardiac imaging, such as echocardiography or MRI, offers a wealth of data on the structure and function of the heart. Numerous small GWAS have been performed on echocardiography measures[65–67], but with few replicating signals. However, by combining data from 30 cohorts ($n = 32,212$), four loci associated with measures of cardiac function (*CDKN1A*, *SLC35F1*, *MTSS1* and *ATXN2*)[68] were identified and later replicated in independent data[69]. Separately, analysis of data from Biobank Japan ($n = 19,516$) identified one locus (*SMARCB1*)[70] that replicated in subsequent analyses[69]. Numerous GWAS have been performed using cardiac MRI data from UK Biobank[69,71,72]. In the first pass analysis ($n = 16,923$), four loci for cardiac function (*TTN*, *BAG3*, *SH2B3* and *CLCNKA*)[71] were identified and later replicated[69]. A meta-analysis of UK Biobank and other consortia data ($n = 26,638$) identified an additional signal, in *TMEM40* (REF.[72]). The largest analysis to date ($n = 36,041$) validated these five loci and identified an additional 21 loci associated with cardiac systolic function[69]. Interestingly, several of the newly identified loci for cardiac function overlap with genes implicated in cardiomyopathy (such as *BAG3* and *TTN*). A recent meta-analysis demonstrated a correlation between genetically determined cardiac function and cardiomyopathy risk — suggesting that variants that are adaptive in early life may contribute to cardiomyopathy risk later in life[30].

Autonomic and endocrine signals increase heart rate with exercise and therefore increase cardiac output. Slower heart rate, reflecting improved parasympathetic tone, correlates with fitness and is strongly protective against cardiac death[73]. Both the acute[74] and long-term[75,76] effects of exercise on heart rate increase are strongly heritable (approximately 32% and 34%, respectively). The genetics of long-term heart rate changes with exercise have not been investigated outside the HERITAGE study[77], and hence, no replicated signals exist for this phenotype[78]. By contrast, two recent large-scale studies from the UK Biobank have provided a wealth of knowledge on genetic loci that affect acute heart rate increase and recovery with submaximal exercise[10,11]. Both studies used the same release of UK Biobank data, and hence had similar sample sizes ($n \sim 60,000$) and studied similar phenotypes[10,11]. For the acute increase in heart rate with exercise, three signals were found in both studies: *SNCAIP*, *POP4* and *MCTP2* (REFS[10,11]). By comparison, eight signals were found in both studies for heart rate recovery after exercise: *CHRM2*, *SYT10*, *CNTN3*, *PAX2*, *CAV2*, *MED13L*, *RNF220* and *NDUFA11* (REFS[10,11]). Of these signals, *SYT10* and *RNF220* were also associated with resting heart rate[79]. These 11 loci can be broadly categorized into three groups based on gene ontology: neural development and/or lifespan, cardiac development and ion channel function[78]. Among these 11 genes, only one (*CHRM2*) was previously identified in candidate gene studies. *CHRM2* encodes M2R, the primary muscarinic acetylcholine receptor in the heart; its parasympathetic activation causes decreased heart rate and reduced cardiac contractility[80]. Two other SNVs in the *CHRM2* region have been associated with decreased heart rate recovery after exercise and increased risk of cardiovascular death[81]. These findings, in combination with the numerous genes identified with neural and/or cardiac development, highlight the multifactorial control of heart rate response in optimizing EPA.

Genetics of vascular adaptations to exercise. Both systolic and diastolic blood pressure change with acute exercise in untrained and trained individuals[75]. With RPA, both systolic and diastolic blood pressure can increase dramatically, with blood pressures of >300 mmHg systolic and >200 mmHg diastolic documented in highly trained weightlifters[82]. By contrast, endurance exercise generally elicits increases of ~30–80 mmHg and ~6–14 mmHg in systolic and diastolic blood pressure, respectively[83]. Interestingly, the magnitude of increase is lessened for both systolic and diastolic blood pressure during acute exercise in trained endurance athletes, reflecting improved vascular function[83]. This decrease in vascular resistance helps to increase cardiac output by reducing the outflow resistance. Both acute (~25%)[74] and long-term (~22%)[75,76] blood pressure changes with acute and long-term endurance training are heritable. Although large-scale GWAS have been performed for resting blood pressure[84], no GWAS with replicated signals have been reported for the response of blood pressure to exercise.

Genetics of erythrocytosis in response to exercise. Long-term endurance training leads to significant expansion of plasma volume and red blood cell mass (up to 40%), resulting in increased VO_2 max[85]. Several studies suggest that this increase is mediated by the HIF pathway and erythropoietin[86] (FIG. 2). In addition, replicated studies of endurance athletes have also identified *NFIA-AS2* as a mediator of erythropoiesis, with carriers of the variant genotype having higher haemoglobin mass[25,29]. Separately, studies of HFE^{H63D} have found it to be associated with endurance performance; this variant causes a mild form of haemochromatosis, with increased total body iron levels theorized to increase erythropoiesis and hence oxygen-carrying capacity[87]. However, while baseline red blood cell traits have been studied thoroughly[88–90] and several erythropoiesis-related genes have been linked with endurance capacity[25,87], no GWAS have been performed on the phenotype of change in red blood cell traits in response to exercise[91].

Taken together, targeted studies of specific intermediate phenotypes that affect VO_2 max have identified numerous genes and pathways involved in human performance, highlighting the role of the nervous system, skeletal muscle and cardiovascular systems. In particular, loci involved in muscle contraction and heart rate response to exercise are both enriched for brain-expressed genes, emphasizing the importance of organized muscle recruitment (for hand-grip strength)[59] and autonomic regulation (for heart rate response)[10,11] in optimizing human performance. By contrast, studies

Antagonistic pleiotropy
Occurs when a locus or variant has a beneficial effect on one trait but confers deleterious effects on a separate trait.

Epigenetic modifications
Chemical alterations to DNA (most commonly by methylation) or DNA-associated histones (via acetylation, phosphorylation or methylation) that result in heritable changes to gene expression without changing the DNA sequence.

of cardiac function have highlighted numerous genes that are possible examples of antagonistic pleiotropy: although *TTN* and *BAG3* may increase cardiac function during the reproductive years, there may be a later trade-off whereby they confer increased long-term risk of cardiomyopathies[92]. Further study of these genes within the specific context of EPA will be needed to validate their effects on overall human performance.

Multi-omic changes with exercise

Multi-omic approaches offer a different route to investigate the underlying pathways through which exercise exerts its beneficial effects. Technologies now exist to measure epigenetic modifications and expression of mRNA, protein, metabolites and lipids on a genome-wide scale. Unlike GWAS, which focus on fixed DNA associations with outcomes, multi-omic measures are influenced by both underlying genetics and environmental exposure; hence, they offer insight into gene–environment and time-dependent interactions. These studies complement GWAS and candidate gene studies by identifying the complex networks that a single gene (such as *PPARGC1A*) can regulate in response to acute exercise.

Epigenetic changes with acute and chronic endurance exercise. Epigenetic modifications regulate gene expression, often in response to environmental stimuli. For example, DNA hypermethylation is associated with gene silencing[93], whereas histone acetylation is associated with gene expression. Hence, the study of epigenetic changes in response to exercise will shed light on gene regulatory pathways and, ultimately, phenotypic adaptation with chronic endurance training[94]. However, the specific epigenetic modifications elicited by exercise are not well elucidated[14]. To our knowledge, changes in histone modifications at specific genes after either an acute or chronic endurance exercise intervention have not yet been studied in humans; one small study ($n = 9$) showed that a single session of acute exercise led to a global increase in histone H3 acetylation, but the specific chromatin regions and/or genes affected were not studied[95]. By contrast, a single session of acute exercise led to a global reduction in DNA methylation[96] (which is associated with gene activation), with long-term endurance training causing DNA hypomethylation specifically at enhancers and regulatory (but not promoter) regions[97]. In sedentary young adults, one 20-min exercise session led to DNA hypomethylation of the promoter regions of *PPARGC1A* (FIG. 4), *TFAM* (a gene activated by *PPARGC1A* that protects mtDNA to promote mitochondrial biogenesis[98]), *PDK4* (another gene activated by *PPARGC1A*, which increases fatty acid metabolism[99]) and *MEF2A* (encodes a transcription factor that modulates cardiac remodelling with exercise training[100])[96]. Chronic endurance training results in more heterogeneous effects on DNA methylation, with hundreds[101] to thousands[97] of genes being differentially methylated compared with non-endurance-trained controls. Moreover, cross-sectional comparisons of elderly versus young patients showed increased genome-wide DNA methylation in elderly skeletal muscle, with physical activity possibly preventing this hypermethylation[102]. The genes identified by these studies highlight pathways primarily related to increased oxidative phosphorylation, glucose intake and/or metabolism, and lipid metabolism, with concurrent decreases in expression of specific myofibril genes that likely reflect muscle fibre type switching[96,97,101]. These global findings support the individual and specific pathways that were previously identified by exercise metabolism, candidate gene, GWAS and animal model studies.

Transcriptome changes with acute and chronic endurance exercise. In comparison with exercise-related effects on the epigenome, changes to the skeletal muscle transcriptome in response to acute and chronic exercise interventions have been well studied (FIG. 4). Two recent meta-analyses, conducted on data from MetaMEx and ExtraMeta, showed convergent results[103,104]. Both analyses confirmed previous observations that *PPARGC1A* expression increased with acute exercise but remained unaltered in fit and trained endurance athletes, suggesting that *PPARGC1A* initiates transcriptional changes transiently in response to acute (but not chronic) exercise[103,104]. In the MetaMEx meta-analysis, in which 66 human skeletal muscle transcriptome studies were integrated[103], genes involved in inflammation were upregulated in inactive participants, but expression of genes related to lipid metabolism and mitochondrial oxidative respiration decreased[103]. By contrast, trained endurance athletes had significantly increased expression of genes related to lipid metabolism and mitochondrial oxidative respiration[103]. The ExtraMeta meta-analysis combined subject-level data from 43 studies of skeletal muscle and adjusted for the effects of potential confounders and/or mediators (such as, age, sex, time post-exercise and training type) and statistical biases associated with inclusion of smaller studies in meta-analyses. This approach identified transcriptomic signals specific to older age and biological sex. In particular, older individuals had a more prominent inflammatory response with acute endurance activity, and women had greater upregulation in genes related to histone modification and chromatin organization. In addition, time series analyses identified four different gene networks based on time trajectory: the early gene network increased expression of genes related to blood vessel development and cellular stress response, whereas the early-to-mid gene network increased expression of *FOXO*-mediated genes including those involved in striated muscle adaptation. Two late gene networks were also identified: one increased immune cell activation and regulation, while the other decreased expression of genes related to fatty acid metabolism and mitochondrial protein import[104].

In a recent integrative study, transcriptome and molecular profiling of various intermediate phenotypes (mitochondrial content, muscle fibre composition and cross-sectional muscle area) were combined in fit and trained (>15 years training) endurance athletes, resistance athletes and healthy controls[105]. Transcriptome profiling successfully differentiated fit and trained endurance athletes from controls, whereas the profiles

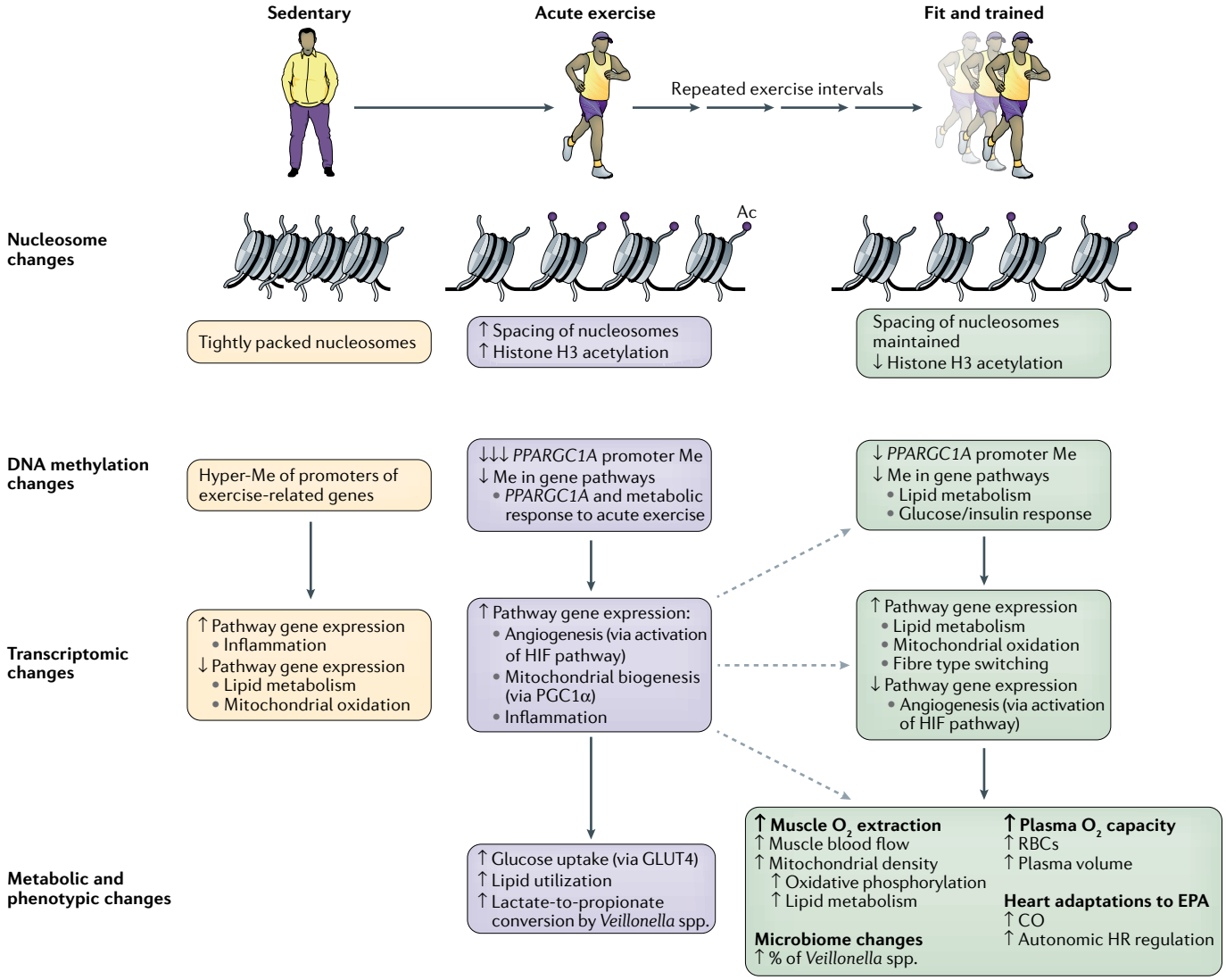


Fig. 4 | **Summary of molecular changes with acute exercise and long-term endurance training.** Numerous molecular changes occur in different activity states: sedentary, acute exercise and the fit and trained endurance athlete. At baseline, DNA for genes related to the body's response to exercise are hypermethylated (hyper-Me) and gene expression is silenced, leading to a pro-inflammatory state with lower capacity for lipid and glucose oxidative metabolism. With acute exercise there is global hypomethylation and upregulation of gene pathways regulated by PGC1α and HIF1α, leading to improved glucose uptake and lipid metabolism. Finally, fit and trained endurance athletes have upregulation of pathways related to lipid metabolism and/or mitochondrial oxidation and genes related to type II to type I fibre switching. These epigenetic and gene expression changes lead to a final phenotype of optimized endurance performance activity (EPA) in the trained athlete. Ac, histone H3 acetylation; CO, cardiac output; HIF, hypoxia-inducible factor family of transcription factors; HR, heart rate; Me, DNA methylation; RBCs, red blood cells.

for resistance athletes and healthy controls were not significantly different from each other. In fit and trained endurance athletes, there was a chronic upregulation of genes for mitochondrial oxidative respiration (with associated increases in carbohydrate and lipid metabolism), inflammatory cell migration and type I/oxidative muscle fibre switching[105]. Although male and female participants had differing gene regulatory changes with acute endurance exercise[104], the differences between female and male endurance athletes became much less apparent with long-term endurance training[105], compared with prior studies of a 3-month exercise intervention[39]. Specifically, with long-term endurance training, male athletes initially upregulate genes related to lipid oxidation whereas female athletes upregulate genes related to carbohydrate metabolism; however, both ultimately converge on a common final phenotype with increased lipid and carbohydrate metabolism capabilities, increased type I muscle fibres, and cardiac and vascular adaptations, hence increasing their VO_2 max and EPA[105] (FIG. 1).

Longitudinal multi-omic studies of exercise. The regulation of the body's response to exercise is variable, depending on the type of activity (endurance versus resistance), length and/or intensity of exercise, chronicity (for example, acute versus fit and trained), and the

subject's underlying physiology (such as the presence of insulin resistance)[103–106]. To begin to unravel this complicated molecular choreography, longitudinal multi-omic studies have been proposed, and one such recent study has demonstrated the ability of this approach to identify exercise-related molecular patterns in gene expression and protein levels[106]. In this study, 36 participants and 14 controls were recruited, and blood samples were collected at baseline and then at 2, 15, 30 and 60 min after acute endurance activity. Transcriptomic, proteomic, metabolomic and lipidomic analyses identified numerous patterns. Early multi-omic changes in response to acute endurance exercise involved processes such as energy metabolism, oxidative stress and immune response, whereas later changes involved the energy homeostasis and tissue repair and/or remodelling pathways. In addition, a transient spike in inflammation-related proteins was identified at 15 min of recovery, which was positively correlated with increase in VO_2 max. IL-6 was identified as one of the candidate regulators of this immune response after acute endurance activity. Despite the novel insights and potential demonstrated by this study for longitudinal multi-omic analyses of acute endurance exercise[106], there are numerous limitations to consider: this study had a relatively small sample size; only peripheral blood was drawn for multi-omics analyses; and, because it was performed in humans[106], numerous tissues relevant to endurance physical activity (such as, heart and/or aorta, liver and nerve)[107] could not be assessed.

To address these and other limitations of human-specific multi-omic studies, the Molecular Transducers of Physical Activity Consortium (MoTrPAC)[107] was established through an NIH Common Fund programme. MoTrPAC complements the international Athlome Project Consortium[108], and has both animal and human components, with an overarching goal of defining the molecular map associated with physical activity[107]. In the animal component of MoTrPAC, rats ($n = 820$) underwent either a single bout of endurance exercise or chronic endurance training before biospecimen collection (19 tissue types) and multi-omic analyte measurements. The study design allows for time-variant analyses and collection of tissues (such as brain, heart and arteries) that cannot be accessed in human volunteers[107]. In the prospective human component, sedentary adult participants ($n \sim 1{,}900$) will undergo a bout of acute exercise and have baseline VO_2 max and body composition assessments and biospecimen (blood/plasma, skeletal muscle and adipose) collection before being randomized to EPA training, RPA training or an untrained control group. After 12 weeks of training, exercise-related phenotypes will be reassessed and another set of biospecimens collected. In addition, both endurance-trained and resistance-trained highly active individuals will undergo acute testing and sample collection[107]. For both the preclinical and clinical arms, biospecimens will be collected at multiple time points in relation to exercise, with baseline genetics, transcriptome, proteome, metabolome and lipidome measured. Finally, there is a smaller, separate study of paediatric participants included within the MoTrPAC study[107]. Once completed, MoTrPAC will be among the most comprehensive public resources monitoring multi-omic changes with acute and chronic EPA versus RPA. Moreover, the substudy of highly active individuals within MoTrPAC, in combination with the ongoing Exercise at the Limit—Inherited Traits of Endurance (ELITE) study has the potential to identify rare variants with large effect sizes on human performance.

Analysis of endurance athletes links the microbiome to exercise metabolism. A recent multi-omic study of the microbiome of elite endurance athletes found that there was an increase in *Veillonella* spp. (bacteria that ferment lactate to the short-chain fatty acid propionate) in athletes with endurance training[109]. Pre- and post-exercise assessments of microbiomes found a post-endurance exercise enrichment in genes aiding *Veillonella* spp. in converting lactate into propionate. The same study demonstrated in mice that radiolabelled lactate could cross into the lumen of the gastrointestinal tract. Interestingly, both faecal transplants of *Veillonella atypica* and propionate supplementation in mice resulted in improved endurance capacity on treadmill testing[109]. These microbiome results suggest a novel link between the microbiome and exercise metabolism, whereby lactate — often misconstrued as a by-product of anaerobic metabolism[110] — is converted into a molecule with the potential to improve endurance performance activity[109]. However, the mechanism through which *V. atypica* (and/or other species) or propionate supplementation lead to increased endurance capacity is not yet known. One proposed hypothesis is that propionate activates AMPK, similar to butyrate[45], hence activating the PGC1α pathway of mitochondrial biogenesis[111] (FIG. 3). Further work will be required to elucidate the molecular mechanisms of this interaction between the microbiome and exercise metabolism.

Taken together, multi-omic studies of the effects of exercise converge on several themes. First, there is a key role for *PPARGC1A* in the numerous physiological adaptations that occur with acute exercise[96,97,101,103,104]. Epigenetic and transcriptomic data align with findings from gene discovery studies and, when combined with recent microbiome data[109], offer a possible integrative pathway through which endurance capacity (via AMPK and PGC1α) is affected by host–microbiome interactions[111]. Second, both transcriptomic[103,104] and longitudinal multi-omic data[106] provide evidence for upregulation of inflammatory signals with inactivity and ageing[112]. Although there is a transient increase in inflammatory markers with acute exercise, this is hypothesized to be necessary for immune cell migration to active muscles for later tissue repair and/or remodelling[106] (which may, in conjunction with its role in HIF pathways, explain the prior finding of *IL6* as a candidate gene for VO_2 max[13]). Finally, with the fit and trained endurance athlete, the primary changes seem to be metabolic, with an upregulation in genes related to mitochondrial oxidative respiration (with accompanying changes in carbohydrate and/or lipid metabolism) and muscle fibre type switching to type I/slow twitch[105]. These findings complement and extend our

understanding of the genetic pathways involved in physiological adaptation to exercise and training.

Conclusions and future perspectives

The advent of modern civilization has led to greatly increased human lifespans. However, as humans have shifted away from our evolutionary history[92] of endurance activity optimization[1,2] to largely sedentary lifestyles, a resultant epidemic in cardiometabolic, neuropsychiatric and musculoskeletal diseases (among many others, including cancer) has emerged, limiting the healthspan of the general population[5]. Innumerable studies have shown that all forms of exercise, and particularly endurance exercise, confer benefits across all domains of health and function[6].

Integration of the recent work highlighted in this Review identifies genetic pathways that may underlie the beneficial effects of endurance exercise. GWAS of VO_2 max have identified a common nonsense variant in *ACTN3* (REFS[12,13]) that causes increased type I muscle fibre formation and hence, improved EPA[35,37]. Larger GWAS of targeted phenotypes affecting VO_2 max[10,11,59,79] highlight the role of the brain and heart in mediating exercise performance[63]. In addition, these GWAS emphasize the possible antagonistic pleiotropy[92] that exists for cardiac-related genes (such as *TTN* and *BAG3*)[30,69,71,72]. Finally, longitudinal multi-omic analyses of the body's response to exercise provides evidence of the role of inflammation and of gene expression changes in pathways related to erythrocytosis (*HIF1A*) and oxidative metabolism (*PPARGC1A*), which ultimately result in switching from type II/fast-twitch to type I/slow-twitch muscle fibres[105,106].

Given the benefits of exercise and the advances in our understanding of their molecular underpinnings, there has been interest in developing pharmacological agents to target these pathways. GW501516, an AMPK pathway modulator (FIG. 3), is perhaps the best-known exercise mimetic, as it was abused by numerous Russian athletes, resulting in the ban of more than 100 athletes from the 2016 Olympics[48]. Despite demonstrated efficacy in animal models, pharmaceutical companies have abandoned its development owing to the unintended side effect of tumour growth[48]. These anecdotes underscore

Table 2 | **Integration of findings at each level of multi-omics, highlighting gaps in current knowledge**

Domain	Current research	Future research needs
Genetics		
EPA and/or VO_2 max	GWAS and mouse studies: *ACTN3* Mouse and metabolism studies: *PPARD*, *PPARGC1A*, *HIF1A*	Gold standard (larger) GWAS Study of elite athletes to identify rare variants Study of structural and mitochondrial variation
Performance-related traits	Excellent GWAS for: hand-grip strength, cardiac function, heart rate response to exercise	GWAS for erythropoiesis from exercise GWAS of blood pressure response to exercise
Epigenetics		
DNA modification	Moderate evidence for global DNA hypomethylation with acute exercise Specific hypomethylation of exercise-response genes (*HIF1A*)	High-throughput ChIP-Seq studies of epigenetic marks Study of epigenetic modifications other than DNA methylation (such as histone acetylation) Role of methylation and/or biological ageing in response to acute exercise and endurance training
Histone modification	Possible histone H3 deacetylation with exercise	–
MicroRNA	–	–
Transcriptomics		
mRNA expression levels	Excellent public resources (such as MetaMex and ExtraMeta) Confirmation that *PPARGC1A* and *HIF1A* expression increases with acute exercise, but not in trained and fit endurance athletes	Standardized time point collection protocols Analysis of the response of additional tissue to exercise (such as adipose and liver tissue)
Proteomics, lipidomics and metabolomics		
Non-targeted approaches	Increased inflammatory signals/ muscle repair Changes to energy metabolism (more lipid utilization)	Randomization design Longitudinal time point collection

GWAS, genome-wide association study; ChIP-Seq, chromatin immunoprecipitation followed by sequencing.

the immense complexity of exercise: despite the proven health benefits of exercise, we still understand very little of the underlying molecular mechanisms and when they exert their effects[107].

Moving forward in exercise genomics, we believe there are several key areas that require attention (TABLE 2). First, the data produced for most analyses have been lacking in depth — this is most apparent for GWAS of VO_2 max and endurance performance[12], where sample sizes are ~10–1,000× smaller than comparable studies for type 2 diabetes[113]. Much larger sample sizes and an overall 'gold standard' GWAS of VO_2 max is needed to identify non-canonical candidate genes that may also affect performance, which can then be followed up with laboratory experimentation to tease apart mechanisms of action. Similarly, there have not been large studies examining the association of rare, structural and mitochondrial genomic variation with performance, all of which may account for some of the missing heritability observed for complex traits[114]. Secondly, there is some evidence that genetic ancestry might affect performance — for example, marathon runners of East African ancestry dominate the competition[108]. Furthermore, most studies have focused on men, despite there being strong evidence of differential endurance performance by biological sex[115]. Hence, we argue for targeted inclusion of non-European ancestry and female participants in the larger genetic studies outlined above. This diversity is of particular importance as we move towards the implementation of polygenic scores (and other multi-omic measures of risk) in the clinical setting, which have been shown to underperform in non-European populations[116]. Finally, given the immense complexity of exercise physiology and the multi-omic changes that occur with training, additional studies like MoTrPAC are needed[107]. Specifically, other studies should aim to replicate the randomization, standardized longitudinal time points and multiple tissue-type collection presented by MoTrPAC[107]. We strongly advocate for additional multinational, publicly funded projects with similar design to better tease apart the molecular mechanisms through which exercise exerts its beneficial effects. Such studies have the potential to unravel the complex pathways that underlie the protective effects of endurance exercise and to identify molecular targets for intervention, hence improving the quality and quantity of life of people around the world.

Published online 14 September 2021

1. Carrier, D. R. et al. The energetic paradox of human running and hominid evolution [and comments and reply]. *Curr. Anthropol.* **25**, 483–495 (1984).
2. Bramble, D. M. & Lieberman, D. E. Endurance running and the evolution of *Homo*. *Nature* **432**, 345–352 (2004).
This article popularized the 'evolution for endurance' hypothesis first proposed by Carrier et al. (1984).
3. Crittenden A. N. & Marlowe F. W. in *Attachment Reconsidered: Culture, Mind, and Society* (eds Quinn N. & Mageo J. M.) (Palgrave Macmillan, 2013) Book Series of the Society for Psychological Anthropology.
4. Schnohr, P., O'Keefe, J. H., Marott, J. L., Lange, P. & Jensen, G. B. Dose of jogging and long-term mortality: the Copenhagen City Heart Study. *J. Am. Coll. Cardiol.* **65**, 411–419 (2015).
5. Booth, F. W., Roberts, C. K., Thyfault, J. P., Ruegsegger, G. N. & Toedebusch, R. G. Role of inactivity in chronic diseases: evolutionary insight and pathophysiological mechanisms. *Physiol. Rev.* **97**, 1351–1402 (2017).
6. Lieberman, D. E. Is exercise really medicine? An evolutionary perspective. *Curr. Sport. Med. Rep.* **14**, 313–319 (2015).
7. Bouchard, C. et al. Familial aggregation of$\dot{V}$o 2 max response to exercise training: results from the HERITAGE family study. *J. Appl. Physiol.* **87**, 1003–1008 (1999).
8. de la Chapelle, A., Träskelin, A. L. & Juvonen, E. Truncated erythropoietin receptor causes dominantly inherited benign human erythrocytosis. *Proc. Natl Acad. Sci. USA* **90**, 4495–4499 (1993).
This article describes a study of the family of Eero Mäntyranta that identified a familial mutation that caused benign erythrocytosis and hence, improved VO_2 max.
9. Sarzynski, M. A. & Bouchard, C. World-class athletic performance and genetic endowment. *Nat. Metab.* **2**, 796–798 (2020).
10. Verweij, N., Vegte, Y. J. V. D. & Harst, P. V. D. Genetic study links components of the autonomous nervous system to heart-rate profile during exercise. *Nat. Commun.* **9**, 898 (2018).
11. Ramírez, J. et al. Thirty loci identified for heart rate response to exercise and recovery implicate autonomic nervous system. *Nat. Commun.* **9**, 1947 (2018).
12. Bye, A. et al. Identification of novel genetic variants associated with cardiorespiratory fitness. *Prog. Cardiovasc. Dis.* **63**, 341–349 (2020).
This article describes the largest GWAS to date of VO_2 max; it specifically highlights the role of an *ACTN3* nonsense variant in optimizing EPA.
13. Harvey, N. R. et al. Genetic variants associated with exercise performance in both moderately trained and highly trained individuals. *Mol. Genet. Genomics* **295**, 515–523 (2020).
14. Jacques, M. et al. Epigenetic changes in healthy human skeletal muscle following exercise – a systematic review. *Epigenetics* **14**, 633–648 (2019).
This excellent systematic review summarizes the evidence for epigenetic changes that occur with acute exercise.
15. Liu, X., Platt, C. & Rosenzweig, A. The role of microRNAs in the cardiac response to exercise. *Cold Spring Harb. Perspect. Med.* **7**, a029850 (2017).
16. Bouchard, C. et al. Aims, design, and measurement protocol. *Med. Sci. Sports Exerc.* **27**, 721 (1995).
This article describes the original HERITAGE study, which laid the foundation for exercise genomics.
17. Bouchard, C. et al. Familial resemblance for VO2max in the sedentary state: the HERITAGE family study. *Med. Sci. Sports Exerc.* **30**, 252–258 (1998).
18. Eynon, N., Morãn, M., Birk, R. & Lucia, A. The champions' mitochondria: is it genetically determined? A review on mitochondrial DNA and elite athletic performance. *Physiol. Genomics* **43**, 789–798 (2011).
19. Stefano, E., Marsigliante, S., Vetrugno, C. & Muscella, A. Is mitochondrial DNA profiling predictive for athletic performance? *Mitochondrion* **47**, 125–138 (2019).
20. Wone, B. W. M., Yim, W. C., Schutz, H., Meek, T. H. & Garland, T. Mitochondrial haplotypes are not associated with mice selectively bred for high voluntary wheel running. *Mitochondrion* **46**, 134–139 (2019).
21. Harvey, N. R. et al. Investigating the influence of mtDNA and nuclear encoded mitochondrial variants on high intensity interval training outcomes. *Sci. Rep.* **10**, 11089 (2020).
22. Timmons, J. A. et al. Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans. *J. Appl. Physiol.* **108**, 1487–1496 (2010).
23. Bouchard, C. et al. Genomic predictors of the maximal O2 uptake response to standardized exercise training programs. *J. Appl. Physiol.* **110**, 1160–1170 (2011).
24. Ghosh, S. et al. Integrative pathway analysis of a genome-wide association study of $\dot{V}$O2max response to exercise training. *J. Appl. Physiol.* **115**, 1343–1359 (2013).
25. Ahmetov, I. et al. Genome-wide association study identifies three novel genetic markers associated with elite endurance performance. *Biol. Sport.* **32**, 3–9 (2015).
26. Rankinen, T. et al. No evidence of a common DNA variant profile specific to world class endurance athletes. *PLoS ONE* **11**, e0147330 (2016).
Although a null GWAS on the 'case status' of elite endurance athletes, this study remains the largest and most multi-ethnic genetic study of variation affecting endurance performance.
27. Al-Khelaifi, F. et al. Genome-wide association study reveals a novel association between MYBPC3 gene polymorphism, endurance athlete status, aerobic capacity and steroid metabolism. *Front. Genet.* **11**, 595 (2020).
28. Papadimitriou, I. D. et al. No association between ACTN3 R577X and ACE I/D polymorphisms and endurance running times in 698 Caucasian athletes. *BMC Genomics* **19**, 13 (2018).
29. Malczewska-Lenczowska, J. et al. HIF-1α and NFIA-AS2 polymorphisms as potential determinants of total hemoglobin mass in endurance athletes. *J. Strength Cond. Res.* https://doi.org/10.1519/JSC.0000000000003686 (2020).
30. Tadros, R. et al. Shared genetic pathways contribute to risk of hypertrophic and dilated cardiomyopathies with opposite directions of effect. *Nat. Genet.* **53**, 128–134 (2021).
This article describes a large GWAS of both cardiac function and dilated versus hypertrophic cardiomyopathy risk, which highlights the possibility of antagonist pleiotropy for genes that aid cardiac function during reproductive ages and then cause elevated risk later in life.
31. Kim, D. S., Gloyn, A. L. & Knowles, J. W. Genetics of type 2 diabetes: opportunities for precision medicine: JACC focus seminar. *J. Am. Coll. Cardiol.* **78**, 496–512 (2021).
32. Nezhad, F. Y. et al. Genes whose gain or loss-of-function increases endurance performance in mice: a systematic literature review. *Front. Physiol.* **10**, 262 (2019).
33. Seto, J. T. et al. ACTN3 genotype influences muscle performance through the regulation of calcineurin signaling. *J. Clin. Invest.* **123**, 4255–4263 (2013).
34. North, K. N. et al. A common nonsense mutation results in α-actinin-3 deficiency in the general population. *Nat. Genet.* **21**, 353–354 (1999).
35. Yang, N. et al. ACTN3 genotype is associated with human elite athletic performance. *Am. J. Hum. Genet.* **73**, 627–631 (2003).
36. Coso, J. D. et al. More than a 'speed gene': ACTN3 R577X genotype, trainability, muscle damage, and the risk for injuries. *Eur. J. Appl. Physiol.* **119**, 49–60 (2019).
This study describes a mouse knockout model of *ACTN3* that demonstrates fibre switching and enhanced endurance performance.

37. MacArthur, D. G. et al. Loss of ACTN3 gene function alters mouse muscle metabolism and shows evidence of positive selection in humans. *Nat. Genet.* **39**, 1261–1265 (2007).
38. Lindholm, M. E. & Rundqvist, H. Skeletal muscle hypoxia-inducible factor-1 and exercise. *Exp. Physiol.* **101**, 28–32 (2016).
39. Lindholm, M. E. et al. Negative regulation of HIF in skeletal muscle of elite endurance athletes: a tentative mechanism promoting oxidative metabolism. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**, R248–R255 (2014).
40. Lorenzo, F. R. et al. A genetic mechanism for Tibetan high-altitude adaptation. *Nat. Genet.* **46**, 951–956 (2014).
41. Mason, S. D. et al. Loss of skeletal muscle HIF-1α results in altered exercise endurance. *PLoS Biol.* **2**, e288 (2004).
42. Wu, Z. et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* **98**, 115–124 (1999).
43. Kamei, Y. et al. PPARγ coactivator 1β/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. *Proc. Natl Acad. Sci. USA* **100**, 12378–12383 (2003).
44. Arany, Z. et al. Transcriptional coactivator PGC-1α controls the energy state and contractile function of cardiac muscle. *Cell Metab.* **1**, 259–271 (2005).
45. Gao, Z. et al. Butyrate Improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58**, 1509–1517 (2009).
46. Lantier, L. et al. AMPK controls exercise endurance, mitochondrial oxidative capacity, and skeletal muscle integrity. *FASEB J.* **28**, 3211–3224 (2014).
47. Michael, L. F. et al. Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc. Natl Acad. Sci. USA* **98**, 3820–3825 (2001).
48. Fan, W. & Evans, R. M. Exercise mimetics: impact on health and performance. *Cell Metab.* **25**, 242–247 (2017).
49. Lin, J. et al. Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* **418**, 797–801 (2002).
50. Arany, Z. et al. HIF-independent regulation of VEGF and angiogenesis by the transcriptional coactivator PGC-1α. *Nature* **451**, 1008–1012 (2008).
51. Handschin, C. et al. Skeletal muscle fiber-type switching, exercise intolerance, and myopathy in PGC-1α muscle-specific knock-out animals. *J. Biol. Chem.* **282**, 30014–30021 (2007).
52. Calvo, J. A. et al. Muscle-specific expression of PPARγ coactivator-1α improves exercise performance and increases peak oxygen uptake. *J. Appl. Physiol.* **104**, 1304–1312 (2008).
53. Eynon, N., Ruiz, J. R., Meckel, Y., Morán, M. & Lucia, A. Mitochondrial biogenesis related endurance genotype score and sports performance in athletes. *Mitochondrion* **11**, 64–69 (2011).
54. Semenova, E. A., Fuku, N. & Ahmetov, I. I. in *Sports, Exercise, and Nutritional Genomics* 73–104 (Elsevier, 2019)
55. Persson, H. C., Danielsson, A. & Sunnerhagen, K. S. A cross sectional study of upper extremity strength ten days after a stroke; relationship between patient-reported and objective measures. *BMC Neurol.* **15**, 178 (2015).
56. Bohannon, R. W. Test-retest reliability of measurements of hand-grip strength obtained by dynamometry from older adults: a systematic review of research in the pubmed database. *J. Frailty Aging* **6**, 83–87 (2017).
57. Leong, D. P. et al. Prognostic value of grip strength: findings from the Prospective Urban Rural Epidemiology (PURE) study. *Lancet* **386**, 266–273 (2015).
58. Reed, T., Fabsitz, R. R., Selby, J. V. & Carmelli, D. Genetic influences and grip strength norms in the NHLBI twin study males aged 59–69. *Ann. Hum. Biol.* **18**, 425–432 (2009).
59. Tikkanen, E. et al. Biological insights into muscular strength: genetic findings in the UK biobank. *Sci. Rep.* **8**, 6451 (2018).
This article reports the largest GWAS to date of hand-grip strength that provides evidence for brain–muscle association.
60. Chan, J. P. L. et al. Genetics of hand grip strength in mid to late life. *Age* **37**, 3 (2015).
61. Matteini, A. M. et al. GWAS analysis of handgrip and lower body strength in older adults in the CHARGE consortium. *Aging Cell* **15**, 792–800 (2016).
62. Willems, S. M. et al. Large-scale GWAS identifies multiple loci for hand grip strength providing biological insights into muscular fitness. *Nat. Commun.* **8**, 16015 (2017).
63. SALE, D. G. Neural adaptation to resistance training. *Med. Sci. Sports Exerc.* **20**, S135 (1988).
64. Higginbotham, M. B. et al. Regulation of stroke volume during submaximal and maximal upright exercise in normal man. *Circ. Res.* **58**, 281–291 (2018).
65. Vasan, R. S. et al. Genome-wide association of echocardiographic dimensions, brachial artery endothelial function and treadmill exercise responses in the Framingham Heart Study. *BMC Med. Genet.* **8**, S2 (2007).
66. Vasan, R. S. et al. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. *JAMA* **302**, 168–178 (2009).
67. Fox, E. R. et al. Genome-wide association study of cardiac structure and systolic function in African Americans. *Circ. Cardiovasc. Genet.* **6**, 37–46 (2018).
68. Wild, P. S. et al. Large-scale genome-wide analysis identifies genetic variants associated with cardiac structure and function. *J. Clin. Invest.* **127**, 1798–1812 (2017).
69. Pirruccello, J. P. et al. Analysis of cardiac magnetic resonance imaging in 36,000 individuals yields genetic insights into dilated cardiomyopathy. *Nat. Commun.* **11**, 2254 (2020).
70. Kanai, M. et al. Genetic analysis of quantitative traits in the Japanese population links cell types to complex human diseases. *Nat. Genet.* **50**, 390–400 (2018).
71. Aung, N. et al. Genome-wide analysis of left ventricular image-derived phenotypes identifies fourteen loci associated with cardiac morphogenesis and heart failure development. *Circulation* **140**, 1318–1330 (2019).
72. Choquet, H. et al. Meta-analysis of 26 638 individuals identifies two genetic loci associated with left ventricular ejection fraction. *Circ. Genom. Precis. Med.* **13**, e002804 (2020).
73. Rovere, M. T. L. et al. Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction. *Lancet* **351**, 478–484 (1998).
74. Ingelsson, E. et al. Heritability, linkage, and genetic associations of exercise treadmill test responses. *Circulation* **115**, 2917–2924 (2007).
75. RICE, T. et al. Heritability of HR and BP response to exercise training in the HERITAGE family study. *Med. Sci. Sports Exerc.* **34**, 972–979 (2002).
76. An, P. et al. Familial aggregation of exercise heart rate and blood pressure in response to 20 weeks of endurance training: the HERITAGE family study. *Int. J. Sports Med.* **24**, 57–62 (2003).
77. Rankinen, T. et al. Heritability of submaximal exercise heart rate response to exercise training is accounted for by nine SNPs. *J. Appl. Physiol.* **112**, 892–897 (2012).
78. van de Vegte, Y. J., Tegegne, B. S., Verweij, N., Snieder, H. & van der Harst, P. Genetics and the heart rate response to exercise. *Cell Mol. Life Sci.* **76**, 2391–2409 (2019).
79. Eppinga, R. N. et al. Identification of genomic loci associated with resting heart rate and shared genetic predictors with all-cause mortality. *Nat. Genet.* **48**, 1557–1563 (2016).
80. Brodde, O. E. & Michel, M. C. Adrenergic and muscarinic receptors in the human heart. *Pharmacol. Rev.* **51**, 651–690 (1999).
81. Hautala, A. J. et al. Acetylcholine receptor M2 gene variants, heart rate recovery, and risk of cardiac death after an acute myocardial infarction. *Ann. Med.* **41**, 197–207 (2009).
82. MacDougall, J. D., Tuxen, D., Sale, D. G., Moroz, J. R. & Sutton, J. R. Arterial blood pressure response to heavy resistance exercise. *J. Appl. Physiol.* **58**, 785–790 (1985).
83. WILMORE, J. H. et al. Heart rate and blood pressure changes with endurance training: the HERITAGE family study. *Med. Sci. Sport. Exer* **33**, 107–116 (2001).
84. Surendran, P. et al. Discovery of rare variants associated with blood pressure regulation through meta-analysis of 1.3 million individuals. *Nat. Genet.* **52**, 1314–1332 (2020).
85. Heinicke, K. et al. Blood volume and hemoglobin mass in elite athletes of different disciplines. *Int. J. Sports Med.* **22**, 504–512 (2001).
86. Lundby, C., Gassmann, M. & Pilegaard, H. Regular endurance training reduces the exercise induced HIF-1α and HIF-2α mRNA expression in human skeletal muscle in normoxic conditions. *Eur. J. Appl. Physiol.* **96**, 363–369 (2006).
87. Semenova, E. A. et al. The association of HFE gene H63D polymorphism with endurance athlete status and aerobic capacity: novel findings and a meta-analysis. *Eur. J. Appl. Physiol.* **120**, 665–673 (2020).
88. Ganesh, S. K. et al. Multiple loci influence erythrocyte phenotypes in the CHARGE consortium. *Nat. Genet.* **41**, 1191–1198 (2009).
89. Vuckovic, D. et al. The polygenic and monogenic basis of blood traits and diseases. *Cell* **182**, 1214–1231.e11 (2020).
90. Chen, M.-H. et al. Trans-ethnic and ancestry-specific blood-cell genetics in 746,667 individuals from 5 global populations. *Cell* **182**, 1198–1213.e14 (2020).
91. Sarzynski, M. A., Ghosh, S. & Bouchard, C. Genomic and transcriptomic predictors of response levels to endurance exercise training. *J. Physiol.* **595**, 2931–2939 (2017).
92. Benton, M. L. et al. The influence of evolutionary history on human health and disease. *Nat. Rev. Genet.* **22**, 269–283 (2021).
This article is an excellent recent review of the role of evolution on gene selection, and how prior evolutionary pressures may affect disease risk in the modern era without selection pressures.
93. Bird, A. P. & Wolffe, A. P. Methylation-induced repression — belts, braces, and chromatin. *Cell* **99**, 451–454 (1999).
94. McGee, S. L. & Hargreaves, M. Epigenetics and exercise. *Trends Endocrinol. Metab.* **30**, 636–645 (2019).
95. McGee, S. L., Fairlie, E., Garnham, A. P. & Hargreaves, M. Exercise-induced histone modifications in human skeletal muscle. *J. Physiol.* **587**, 5951–5958 (2009).
96. Barrès, R. et al. Acute exercise remodels promoter methylation in human skeletal muscle. *Cell Metab.* **15**, 405–411 (2012).
This seminal study provides much of the evidence for changes in DNA methylation after exercise.
97. Lindholm, M. E. et al. An integrative analysis reveals coordinated reprogramming of the epigenome and the transcriptome in human skeletal muscle after training. *Epigenetics* **9**, 1557–1569 (2015).
98. Theilen, N. T., Kunkel, G. H. & Tyagi, S. C. The role of exercise and TFAM in preventing skeletal muscle atrophy. *J. Cell Physiol.* **232**, 2348–2358 (2017).
99. Pettersen, I. K. N. et al. Upregulated PDK4 expression is a sensitive marker of increased fatty acid oxidation. *Mitochondrion* **49**, 97–110 (2019).
100. Medrano, J. L. & Naya, F. J. The transcription factor MEF2A fine-tunes gene expression in the atrial and ventricular chambers of the adult heart. *J. Biol. Chem.* **292**, 20975–20988 (2017).
101. Nitert, M. D. et al. Impact of an exercise intervention on DNA methylation in skeletal muscle from first-degree relatives of patients with type 2 diabetes. *Diabetes* **61**, 3322–3332 (2012).
102. Turner, D. C. et al. DNA methylation across the genome in aged human skeletal muscle tissue and muscle-derived cells: the role of HOX genes and physical activity. *Sci. Rep.* **10**, 15360 (2020).
103. Pillon, N. J. et al. Transcriptomic profiling of skeletal muscle adaptations to exercise and inactivity. *Nat. Commun.* **11**, 470 (2020).
104. Amar, D. et al. Time trajectories in the transcriptomic response to exercise — a meta-analysis. *Nat. Commun.* **12**, 3471 (2021).
This article accompanies the online ExtraMeta (https://extrameta.org) resource, and highlights the changes to gene expression that occur with endurance exercise.
105. Chapman, M. A. et al. Skeletal muscle transcriptomic comparison between long-term trained and untrained men and women. *Cell Rep.* **31**, 107808 (2020).
This study compares the transcriptome and molecular and physiological phenotypes of fit and trained endurance athletes with resistance athletes or untrained individuals.
106. Contrepois, K. et al. Molecular choreography of acute exercise. *Cell* **181**, 1112–1130.e16 (2020).
This article compares the transcriptome and proteome of fit and trained endurance athletes with resistance athletes and untrained individuals.
107. Sanford, J. A. et al. Molecular transducers of physical activity consortium (MoTrPAC): mapping the dynamic responses to exercise. *Cell* **181**, 1464–1474 (2020).
108. Pitsiladis, Y. P. et al. Athlome project consortium: a concerted effort to discover genomic and other

"omic" markers of athletic performance. *Physiol. Genomics* **48**, 183–190 (2016).
109. Scheiman, J. et al. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nat. Med.* **25**, 1104–1109 (2019).
This article describes a longitudinal study with accompanying mouse evidence for the role of the microbiome in exercise performance.
110. Rabinowitz, J. D. & Enerbäck, S. Lactate: the ugly duckling of energy metabolism. *Nat. Metab.* **2**, 566–571 (2020).
111. Frampton, J., Murphy, K. G., Frost, G. & Chambers, E. S. Short-chain fatty acids as potential regulators of skeletal muscle metabolism and function. *Nat. Metab.* **2**, 840–848 (2020).
112. Schaum, N. et al. Ageing hallmarks exhibit organ-specific temporal signatures. *Nature* **583**, 596–602 (2020).
113. Vujkovic, M. et al. Discovery of 318 new risk loci for type 2 diabetes and related vascular outcomes among 1.4 million participants in a multi-ancestry meta-analysis. *Nat. Genet.* **52**, 680–691 (2020).
114. Eichler, E. E. et al. Missing heritability and strategies for finding the underlying causes of complex disease. *Nat. Rev. Genet.* **11**, 446–450 (2010).
115. Landen, S. et al. Genetic and epigenetic sex-specific adaptations to endurance exercise. *Epigenetics* **14**, 523–535 (2019).
116. Dikilitas, O. et al. Predictive utility of polygenic risk scores for coronary heart disease in three major racial and ethnic groups. *Am. J. Hum. Genet.* **106**, 707–716 (2020).
117. Fishman, D. et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. *J. Clin. Invest.* **102**, 1369–1376 (1998).
118. Illig, T. et al. Significant association of the interleukin-6 gene polymorphisms C-174G and A-598G with Type 2 Diabetes. *J. Clin. Endocrinol. Metab.* **89**, 5053–5058 (2004).
119. Clément, K. et al. Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N. Engl. J. Med.* **333**, 352–354 (1995).
120. Paré, G. et al. Genetic analysis of 103 candidate genes for coronary artery disease and associated phenotypes in a founder population reveals a new association between endothelin-1 and high-density lipoprotein cholesterol. *Am. J. Hum. Genet.* **80**, 673–682 (2007).
121. Wang, Q. et al. Positional cloning of a novel potassium channel gene: KVLQT1 mutations cause cardiac arrhythmias. *Nat. Genet.* **12**, 17–23 (1996).
122. Chen, Y.-H. et al. KCNQ1 gain-of-function mutation in familial atrial fibrillation. *Science* **299**, 251–254 (2003).
123. Watkins, H. et al. Mutations in the cardiac myosin binding protein-C gene on chromosome 11 cause familial hypertrophic cardiomyopathy. *Nat. Genet.* **11**, 434–437 (1995).
124. Tannahill, G. M. et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* **496**, 238–242 (2013).
125. Tomita, S. et al. Defective brain development in mice lacking the Hif-1α gene in neural cells. *Mol. Cell Biol.* **23**, 6739–6749 (2003).
126. Wang, Y.-X. et al. Regulation of muscle fiber type and running endurance by PPARδ. *PLoS Biol.* **2**, e294 (2004).
127. Narkar, V. A. et al. AMPK and PPARδ agonists are exercise mimetics. *Cell* **134**, 405–415 (2008).
128. Patten, I. S. et al. Cardiac angiogenic imbalance leads to peripartum cardiomyopathy. *Nature* **485**, 333–338 (2012).
129. Kong, X. et al. IRF4 is a key thermogenic transcriptional partner of PGC-1α. *Cell* **158**, 69–83 (2014).
130. Egan, M. F. et al. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* **112**, 257–269 (2003).
131. Hall, D., Dhilla, A., Charalambous, A., Gogos, J. A. & Karayiorgou, M. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am. J. Hum. Genet.* **73**, 370–376 (2003).
132. Soliman, F. et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. *Science* **327**, 863–866 (2010).
133. Zhao, C. et al. Charcot-Marie-Tooth disease type 2A caused by mutation in a microtubule motor KIF1Bβ. *Cell* **105**, 587–597 (2001).
134. Gerull, B. et al. Mutations of TTN, encoding the giant muscle filament titin, cause familial dilated cardiomyopathy. *Nat. Genet.* **30**, 201–204 (2002).
135. Norton, N. et al. Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. *Am. J. Hum. Genet.* **88**, 273–282 (2011).
136. Wang, J. C. et al. Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. *Hum. Mol. Genet.* **13**, 1903–1911 (2004).
137. Luo, X. et al. CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case–control structured association study. *Hum. Mol. Genet.* **14**, 2421–2434 (2005).
138. Ma, P. et al. The ubiquitin ligase RNF220 enhances canonical Wnt signaling through USP7-mediated deubiquitination of β-catenin. *Mol. Cell Biol.* **34**, 4355–4366 (2014).
139. Song, D. et al. Tibetan PHD2, an allele with loss-of-function properties. *Proc. Natl Acad. Sci. USA* **117**, 12230–12238 (2020).
140. Percy, M. J. et al. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. *Proc. Natl Acad. Sci. USA* **103**, 654–659 (2006).
141. Wood, B. & Collard, M. The human genus. *Science* **284**, 65–71 (1999).
142. Alello, L. & Dean, C. in *An Introduction to Human Evolutionary Anatomy* Ch. 15 (Elsevier, 2002) https://doi.org/10.1016/b978-0-08-057100-3.50019-5.
143. Kram, R. & Taylor, C. R. Energetics of running: a new perspective. *Nature* **346**, 265–267 (1990).
144. Lieberman, D. E. Human locomotion and heat loss: an evolutionary perspective. *Compr. Physiol.* **5**, 99–117 (2014).
145. Kamberov, Y. G. et al. Comparative evidence for the independent evolution of hair and sweat gland traits in primates. *J. Hum. Evol.* **125**, 99–105 (2018).
146. Baccaredda-Boy, A. A note on the cutaneous arterial vessels of some primates. *J. Vasc. Res.* **1**, 209–212 (1964).
147. Robertshaw, D. Sweat and heat exchange in man and other mammals. *J. Hum. Evol.* **14**, 63–73 (1985).
148. Shave, R. E. et al. Selection of endurance capabilities and the trade-off between pressure and volume in the evolution of the human heart. *Proc. Natl Acad. Sci. USA* **116**, 19905–19910 (2019).
149. Meyer, H. V. et al. Genetic and functional insights into the fractal structure of the heart. *Nature* **584**, 589–594 (2020).
150. Minetti, A. E. Efficiency of equine express postal systems. *Nature* **426**, 785–786 (2003).
151. Lieberman, D. E. et al. Foot strike patterns and collision forces in habitually barefoot versus shod runners. *Nature* **463**, 531–535 (2010).
152. Schmidt-Nielsen, K., Bretz, W. L. & Taylor, C. R. Panting in dogs: unidirectional air flow over evaporative surfaces. *Science* **169**, 1102–1104 (1970).
153. Taylor, C. & Rowntree, V. Temperature regulation and heat balance in running cheetahs: a strategy for sprinters? *Am. J. Physiol.* **224**, 848–851 (1973).
154. Liebenberg, L. P. Hunting by modern hunter-gatherers. *Curr. Anthropol.* **47**, 1017–1026 (2006).

Acknowledgements
The authors thank J. W. Knowles, M. E. Lindholm and C. M. Mattsson for their feedback on drafts of this article. D.S.K. thanks L. Han, N. Liu, M. Roy-O'Reilly and G. Salzman for excellent patient care, allowing for the time needed to write this article. D.S.K. also thanks J. K. Rathkey for stimulating conversations on biomechanical efficiency and exercise metabolism.

Author contributions
E.A.A. and D.S.K. wrote the article. All authors researched data for the article, had substantial contribution to the discussion of the content, and reviewed and edited the manuscript before submission.

Competing interests
M.T.W. reports grants and personal fees from Verily, Myokardia and ArrayBio, and consultancy fees from BioTelemetry, outside the submitted work. E.A.A. reports advisory board fees from Apple and Foresite Labs. E.A.A. has ownership interest in Nuevocor, DeepCell and Personalis, outside the submitted work. E.A.A. declares ownership interest in SVEXA, and is a board member of AstraZeneca. D.S.K. declares no competing interests.

Peer review information
Nature Reviews Genetics thanks I. Ahmetov, N. Fuku and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

RELATED LINKS
Athlome Project Consortium: http://www.athlomeconsortium.org/
ExtraMeta: http://www.extrameta.org
Exercise at the Limit — Inherited Traits of Endurance: https://med.stanford.edu/elite.html
MetaMEx: http://www.metamex.eu
Molecular Transducers of Physical Activity Consortium: https://motrpac-data.org