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Review article

Exercise metabolism and adaptation in skeletal muscle

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Exercise metabolism and adaptation in skeletal muscle

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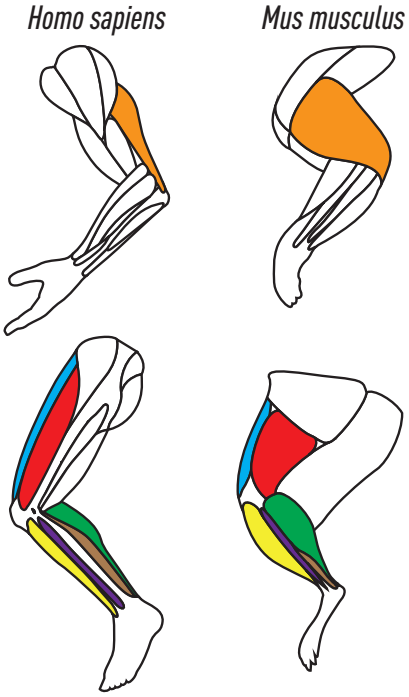
A



	Homo sapiens			Mus musculus			
	Major fibre types			Major fibre types			
	I/slow	IIA	IIX	I/slow	IIA	IIX	IIB
Triceps brachii	37.3	35.9	26.6	<1	<1	~10	~80
Rectus femoris	~35-40	~60-70		<1	7	18	74
Vastus lateralis	~35-70	~10-48	~2-33	<1	<1	6	94
Tibialis anterior	~76-84	~10-20	<2	3	9	21	67
Ex. dig. longus	~50	~50		9	14	30	47
Gastrocnemius	~24-79	~23-42	~1-24	<1	14	25	61
Soleus	78.1	21.9	<1	44	51	5	<1

B

Classic metabolic classification	Fibre type	Gene
Slow oxidative (SO)	I/slow	*Myh7*
Fast oxidative glycolytic (FOG)	IIA	*Myh2*
	IIX	*Myh1*
Fast glycolytic (FG)	IIB	*Myh4*

Motor unit activity profile

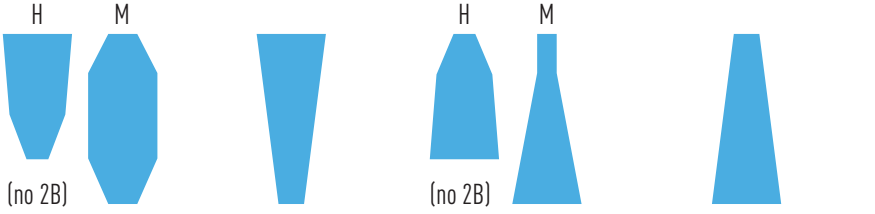
- 24 h tot. activity
- Pulse duration
- Firing freq. (Hz)

Ca^{2+} signalling

- SR and T-tubules
- Cytosol. $[Ca^{2+}]$ at rest
- Ext. cell. Ca^{2+} entry
- Ca^{2+} trans. dur.
- Contr. threshold
- Ca^{2+} released
- Ca^{2+} re-uptake

General metabolic and contractile characteristics

- Oxidative enzymes
- [Triacylglycerides]
- Capillary density
- Substrate transport
- Resistance to fatigue
- Glycolytic enzymes
- Glycogenolysis
- G3PS
- [Glycogen]
- Short. $velocity_{max}$, $Power_{max}$
- ATP consumption rate



Supplementary Figure 1: Contractile and metabolic properties of human and rodent skeletal muscle fibres[1-25].

(a) Comparative anatomy and fibre type composition of select human and mouse muscles.

The complimentary characteristics of different fibre types support specific muscle function[26]. In humans, postural (also known as tonic) muscles like the soleus contain more type I fibres, whereas phasic muscles (for example, the triceps brachii and lateral head of the rectus femoris) have a greater percentage of type II fibres. Mice and rats are often used to study muscle physiology, however there are important differences between rodent and human muscle. Notably, murine muscle has a higher proportion of type II fibres, and several limb muscles of mice and rats are mostly comprised of fast-glycolytic type IIB fibres (MyHC-IIB, encoded by *Myh4*)[27,28], which have the greatest peak power of all fibre types[27] but are not expressed in human muscle[27-31].

(b) Muscle fibre contractile and metabolic characteristics.

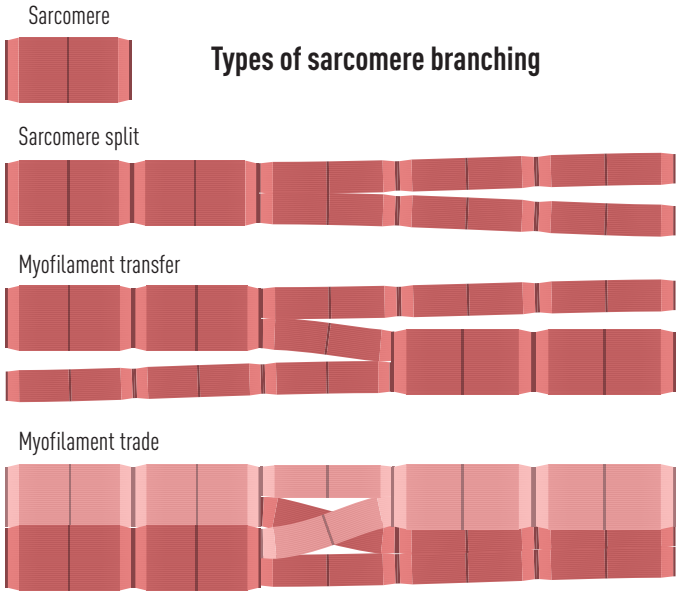
Muscle fibre types are typically classified according to their functional and metabolic properties, which largely reflect differences in upstream α-motor unit activity, and downstream intracellular calcium-handling and energy-dependent pathways. In human muscle, fast isoforms of myofibrillar and calcium-trafficking proteins are enriched in type II fibres[30,31], as are regulators of the adenylate kinase[30], glycogenolysis, and glycolysis-to-lactate production and transport reactions (including the glycerol-3-phosphate shuttle, G3PS)[30,31] to accommodate the ≥2.5-4-fold greater activity of fast-myosin ATPases[32]. Alternatively, type I fibres mostly express slower contractile isoforms[30,31] and have lower ATP consumption rates[32] that can be met through oxidative metabolism[33]. Hence, these fibres have denser capillarization[26,34,35], and higher peroxisomal[31] and mitochondrial contents[36], including enzymes of β-oxidation[30], the tricarboxylic acid cycle, and electron transport chain[30,31]. Energy depots also differ accordingly between fast-twitch and slow-twitch fibres. Whereas type II fibres store more creatine phosphate and glycogen[37], type I fibres have a greater abundance of intramyocellular lipids[36,38] (see main text Box 3). The relatively unique transcriptomes[39], proteomes[30,31], and metabolomes[33] of slow-twitch and fast-twitch fibres has enabled the generation of '-omics-based signatures, which facilitate deconvolution of approximate type I and type II fibre composition from bulk RNA analysis of human muscle biopsies[40,41]. However, the separation of type IIA and type IIX fibres using such methods is currently not possible[40,41] due to the substantial co-expression of genes[39,42] and proteins[30,43] between type II fibres. Although human and rodent fibres share a similar pattern of slow- to fast-twitch progression from type I to type IIX fibres, human fibres contract slower compared to orthologous mouse and rat fibres[27]. Furthermore, in contrast to human muscle, type IIA fibres are more oxidative than type I in rodents[28,43].

Panels in Supplementary Fig. 1b that directly compare the properties of human and mouse fibres are labelled with H (human fibres) and M (mouse fibres), respectively. All other panels illustrate differences between fibre types only, not species.

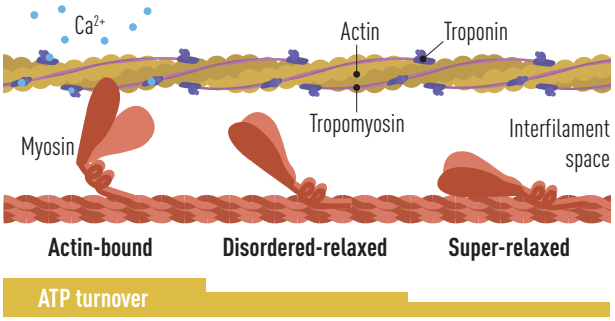
Abbreviations:

Ex. dig. longus, extensor digitorum longus; SR, sarcoplasmic reticulum; T-tubules, transverse tubules; Ext. cell. Ca^{2+} entry, extracellular Ca^{2+} entry; Ca^{2+} trans. dur., Ca^{2+} transient duration; Contr. threshold, contraction threshold; Short. $velocity_{max}$, maximum shortening velocity.

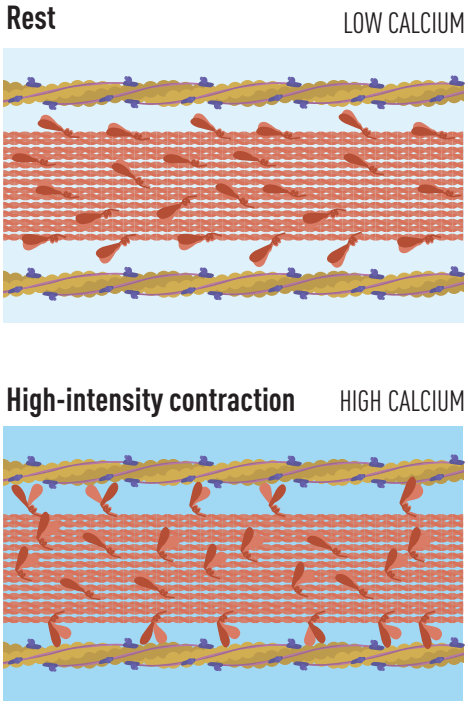
A


Sarcomere
Types of sarcomere branching
Sarcomere split
Myofilament transfer
Myofilament trade

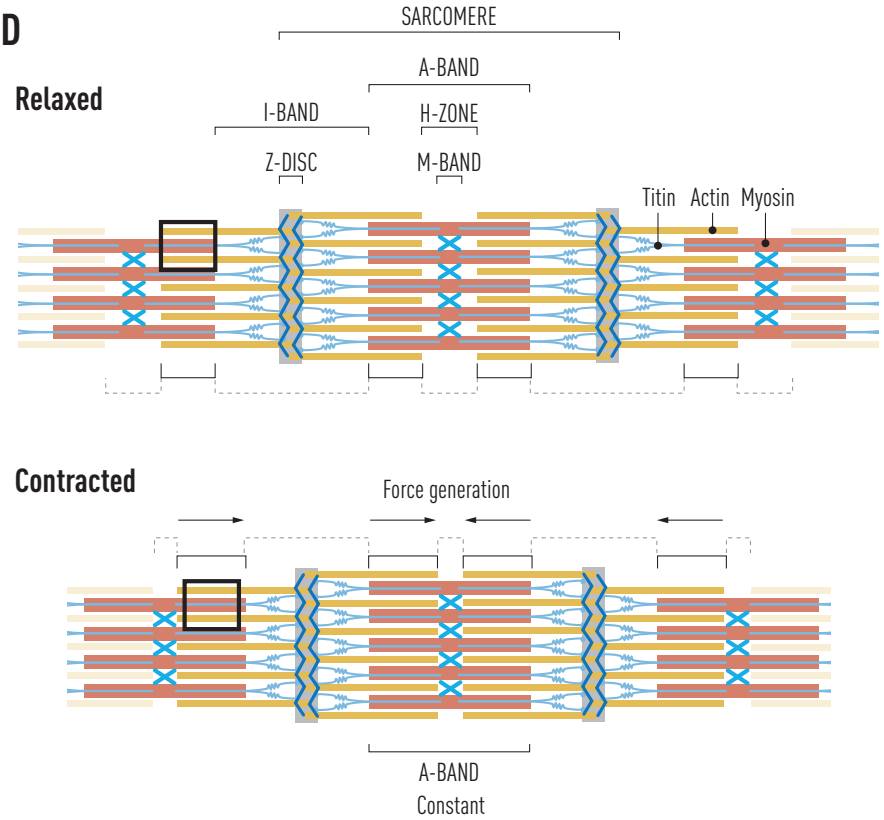
B


Myosin head states
Ca2+
Actin
Troponin
Tropomyosin
Myosin
Interfilament space
Actin-bound
Disordered-relaxed
Super-relaxed
ATP turnover

C


Rest
LOW CALCIUM
High-intensity contraction
HIGH CALCIUM

D


Relaxed
SARCOMERE
A-BAND
I-BAND
H-ZONE
Z-DISC
M-BAND
Titin
Actin
Myosin
Contracted
Force generation
A-BAND
Constant

Supplementary Figure 2: The myofibrillar matrix and skeletal muscle contraction.

(a) Three types of branching between sarcomeres.

Myofibrils form a nonlinear matrix of sarcomeres[44-46] connected through three branching subtypes[44]: (1) Sarcomere splitting, where myofilaments from one sarcomere separate into two distinct myofibrils; (2) Myofilament transfer, in which myofilaments from a single sarcomere segregate to join a distinct, adjacent sarcomere; and (3) Myofilament trade, where myofilaments are shared between two neighbouring sarcomeres. Supplementary Fig. 2a is inspired by the focused ion beam-scanning electron microscopy images in ref.[44].

(b) The conformational states of myosin heads.

Individual sarcomeres contain organised arrangements of parallel thin (actin), thick (myosin), and elastic (titin/connectin) filaments. Under basal conditions, myosin heads are maintained in disordered-relaxed or super-relaxed conformations, with slow to extremely-slow ATP turnover kinetics[47,48] that contribute to the lower relative metabolic rate of muscle at rest[48]. Disordered-relaxed myosin heads protrude into the inter-myofilament space and can readily engage in contraction. However, tropomyosin sterically prevents their association with actin at resting sarcoplasmic calcium (Ca^{2+}) levels. Conversely, myosin binding protein C (ref.[49]), regulatory light chains[48,50], and essential light chains[50] may act to anchor super-relaxed myosin to the thick filament backbone, away from actin. Super-relaxed myosins could be suppressed further by direct head-to-head contact (that is, the 'interacting-heads' motif) that inhibits myosin ATPase[50]. Supplementary Fig. 2b is modified from ref.[51].

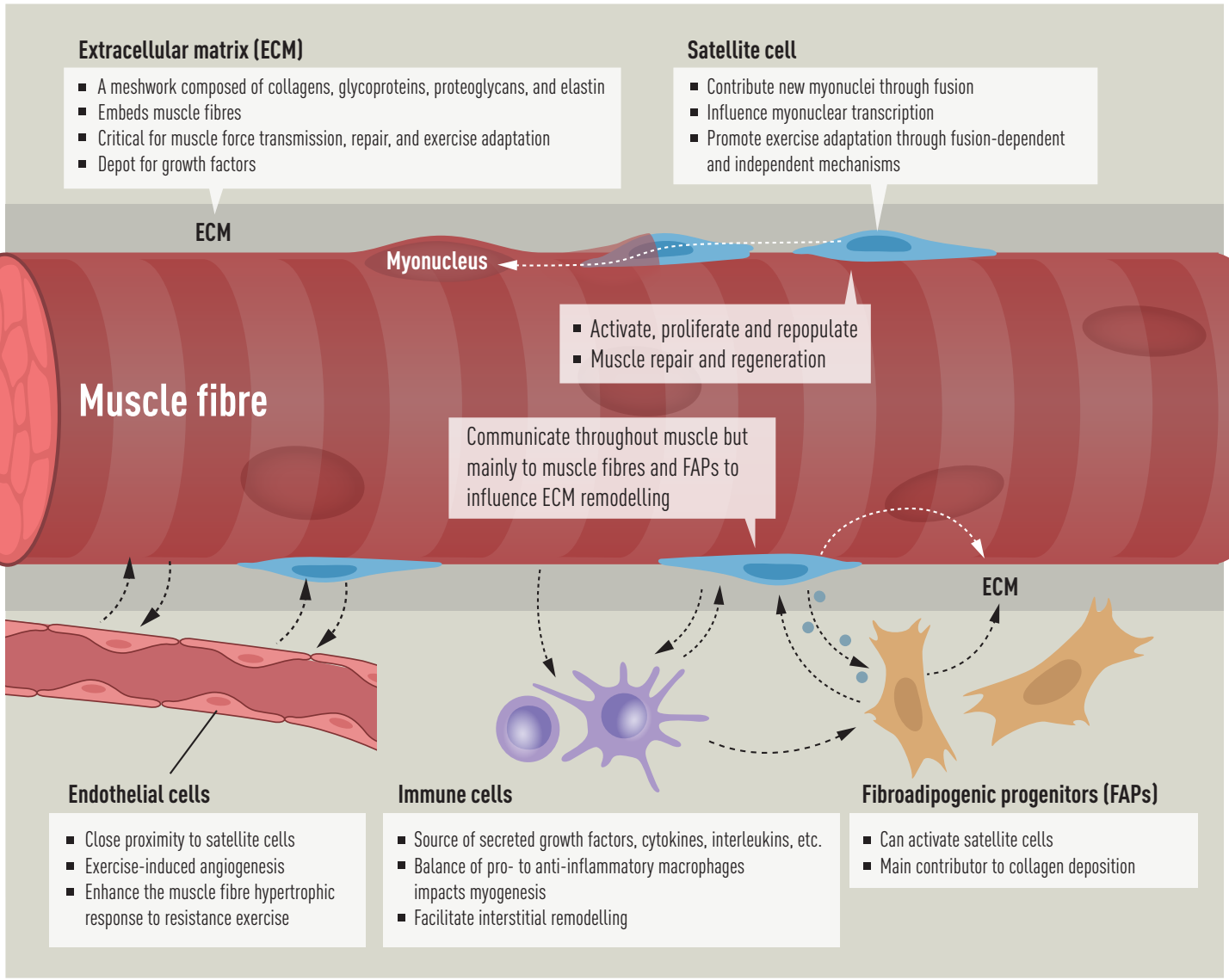
(c) Excitation-contraction coupling and myosin activation.

Upon neuromuscular transmission, action potential propagation along the sarcolemma induces a voltage-dependent alteration of dihydropyridine receptor tetrads in the transverse (T)-tubule, which allosterically opens ryanodine receptor 1 on the sarcoplasmic reticulum to stimulate Ca^{2+} release[52]. Ca^{2+} binding of troponin exposes sites on actin that permits interaction with the head domain of formerly disordered-relaxed myosin after ATP hydrolysis[53]. This initial step imposes mechanical strain on thick filaments in response to high external load and critically initiates super-relaxed myosin binding with actin[53]. Mechano-stimulation of the thick filament is necessary for maximal force production[53,54] and occurs differently depending on muscle fibre type composition[54]. The gradual step-wise activation of super-relaxed myosin in slow-twitch rat soleus muscle contrasts with the rapid thick filament activation of fast-twitch extensor digitorum longus[54], but is consistent with the metabolic characteristics of these muscles[28] and the contractile properties of their predominant fibre types[27].

Panels in (c) correspond to the areas within black boxes on adjacent sarcomeres in (d).

(d) The sliding filament model of contraction.

After strongly binding with actin, myosin undergoes a conformational change, releasing inorganic phosphate and shortening the sarcomeric I-band and H-zone regions to generate force (that is, the 'power stroke'), while A-band filaments remain relatively constant. The liberation of ADP from myosin post power stroke causes transient rigor until a new ATP molecule enters the myosin head nucleotide-binding pocket. This high-energy myosin-ATP state reduces affinity of myosin for actin and promotes dissociation of the myosin-actin crossbridge. Detached (disordered-relaxed) myosin then hydrolyses bound ATP and is again primed to interact with actin, repeating the cycle (reviewed in ref.[55]).


Extracellular matrix (ECM)
A meshwork composed of collagens, glycoproteins, proteoglycans, and elastin
Embeds muscle fibres
Critical for muscle force transmission, repair, and exercise adaptation
Depot for growth factors
Satellite cell
Contribute new myonuclei through fusion
Influence myonuclear transcription
Promote exercise adaptation through fusion-dependent and independent mechanisms
ECM
Myonucleus
Activate, proliferate and repopulate
Muscle repair and regeneration
Muscle fibre
Communicate throughout muscle but mainly to muscle fibres and FAPs to influence ECM remodelling
ECM
Endothelial cells
Close proximity to satellite cells
Exercise-induced angiogenesis
Enhance the muscle fibre hypertrophic response to resistance exercise
Immune cells
Source of secreted growth factors, cytokines, interleukins, etc.
Balance of pro- to anti-inflammatory macrophages impacts myogenesis
Facilitate interstitial remodelling
Fibroadipogenic progenitors (FAPs)
Can activate satellite cells
Main contributor to collagen deposition

Supplementary Figure 3: 'Supporting' cell types in skeletal muscle exercise adaptation.

Approximately 40-45% of all cells in human vastus lateralis muscle are interstitial[56] and communication between these mononuclear cell populations, satellite cells, and muscle fibres is essential for muscle integrity, regeneration[57], and exercise adaptation.

Resident myonuclei have a transcriptional reserve, and can sustain a degree of load-induced hypertrophy[58,59] and cell-autonomous repair[60] in the absence of satellite cells. However, the presence and fusion of satellite cells ultimately appears necessary to maximise muscle mass accrual[58,59,61] and to support functional adaptation, proprioceptive coordination[61], and overall exercise performance[58,61]. Ribosome biogenesis is greatest within the first four sessions of a 12-bout (three-week) resistance training intervention in humans[62], and a primary role of satellite cell-incorporation could be the provision of transcripts required for efficient ribosome assembly[58,63] during this time. Indeed, upregulation of translational machinery permits beneficial proteome remodelling with exercise training[64] and ablation of satellite cells results in delayed transcriptomic signatures of ribosome biogenesis, oxidative metabolism, and sarcomeric adaptations in mice[58].

Mechanical overload transiently increases the content of fibroadipogenic progenitors (FAPs) in muscle[59], possibly due to an initial chemotaxis of migrating FAPs-like adipose stromal cells from subcutaneous adipose tissue[65], followed by a wave of resident FAPs proliferation[59,65]. Ligand-receptor interactions are predicted to occur between FAPs and myogenic cells during the earlier stages of muscle differentiation[57], and the release of thrombospondin 1 (THBS1) by FAPs is at least partially responsible for satellite cell activation upon mechanical loading[59]. Activated satellite cells can signal back to FAPs[66,67], muscle fibres[68], and other cells of the microenvironment – including macrophages and endothelial cells[66] – through secreted factors, such as myomiR-206-containing extracellular vesicles[66-68]. This crosstalk helps direct appropriate transcriptional responses in recipient cells[63,66] and facilitates physiological extracellular matrix (ECM) deposition. After hypertrophic exercise training, mouse muscle deficient of satellite cells lacks enrichment for ECM remodelling genes[58] and displays irregular ECM networks[58,67], with lower proportions of densely-packed collagen[58]. Conversely, most of these maladaptive responses are mitigated when satellite cells are retained for the first 4 (ref.[66])-7 (ref.[67]) days of an eight-week synergist ablation, indicating a critical early influence of satellite cells independent of their fusion into fibres, which enables future adaptation.

During inflammatory muscle damage, temporal tissue repair appears coordinated between infiltrating immune cells (for example, macrophages) and satellite cells[69,70]. A similar interplay between macrophages and satellite cells might also compliment muscle recovery and adaptation to physical activity. Following acute resistance exercise, macrophages exist along a pro- to anti-inflammatory (M1-to-M2) immunomodulatory spectrum of sub-clusters in human muscle[71]. Conditioned media from M1 and M2 macrophages enhances in vitro myoblast proliferation and differentiation markers, respectively[72], whereas altering the M1-M2 macrophage balance impairs murine muscle regeneration in vivo, concomitant with excessive collagen accumulation[73]. After downhill running exercise[74], the production of insulin-like growth factor 1 (IGF1)[75] and tumour necrosis factor-α (TNF-α)[74] by metoerin-like (*Metrnl*)$^{+}$ macrophages stimulates satellite cell-mediated repair[75] and FAP apoptosis[74] to aid restoration of muscle morphology[74,75], function, and ECM integrity[74] in mice. Reciprocally, muscle could signal to macrophages by means of leukaemia inhibitory factor (LIF)[76] and protonated succinate[77] to promote type I collagen turnover[76] and neuromuscular adaptations[77].

Muscle capillarization is a hallmark of exercise training and a subpopulation of activating transcription factor 3/4 (*Atf3/4*)$^{+}$ endothelial cells, enriched near oxidative type I and type IIA

rodent fibres, are largely responsible for exercise-induced angiogenesis in mice[78]. Endothelial cell expansion could be triggered by apelin (APLN) exiting muscle[79] during exercise[80], and the combined effects of vascular endothelial growth factor (VEGF) and secreted phosphoprotein 1 (SSP1) produced by muscle in a peroxisome proliferator–activated receptor-γ coactivator-1α_1 (PGC-1α_1)-dependent manner[81]. Increased vascularisation may augment the hypertrophic response of muscle to resistance training[82], particularly of type II fibres in older individuals[35,71,83], through closer association of satellite cells with capillaries[84] and/or endothelial-to-fibre communication based on canonical Notch signalling[85].

In human vastus lateralis, mesenchymal, endothelial, and myogenic cells are among the most transcriptionally responsive mononuclear populations three hours after acute sprinting exercise[86]. Still, other cells residing in muscle also respond to exercise training in mice[87] (including neural cells, pericytes, smooth muscle cells, and monocytes) and the precise role of these cell types in human exercise adaptation warrants further exploration at single-cell resolution.

Supplementary boxes

Supplementary Box 1: Skeletal muscle myogenesis

Myogenesis is the transitional process of muscle differentiation from quiescent progenitors to mature muscle fibres, across the following stages: quiescence (myogenic progenitors) → activation (proliferating myoblasts) → commitment (myocytes, exited cell cycle) → fusion (myotubes) → maturation (muscle fibres). This is orchestrated by a combination of extrinsic cell-to-cell communication[57] (see Supplementary Fig. 3), secreted cues (including growth factors) from the stem cell niche, and an intrinsic transcriptional programme. Integral to the intrinsic programme is the temporal expression of paired box (PAX) transcription factors and myogenic regulatory factors (MRFs), and their cooperation with myocyte enhancer factor 2 (MEF2A, MEF2C, MEF2D), RNA-binding proteins, and non-coding RNAs, among other modifiers (reviewed in ref.[88]).

PAX3 initiates the myogenic programme in embryonic muscle progenitors[89]. However, PAX7 is the key regulator of satellite cell fate in adult muscle. Post-translational methylation[90] and acetylation[91] events activate PAX7-dependent asymmetrical satellite cell divisions, producing more committed (myogenic factor 5-positive, *Myf5*$^{+}$) daughter cells. Upregulation of the muscle-making MRFs *Myf5* and myogenic differentiation 1 (*MyoD1*) is essential for muscle formation[92]. MYF5 and MYOD1 work synergistically but diverge in function[93]. MYF5 acts earlier to specify myogenic lineage by promoting chromatin remodelling around target binding sites for MYOD1 to then robustly activate muscle-specific transcription[93]. MYOD1 also serves as an anchor protein for chromatin loops[94]. The dynamic structural and epigenetic reorganisation of chromatin enables greater three-dimensional promoter-enhancer interaction within regions of myogenic transcription factors (such as myogenin, *Myog*)[95] and myosin heavy chains (MyHC, encoded by *Myh* genes)[96,97]. MYOG is critical for establishing multinucleated fibres through downstream targets myomaker (*Mymk*) and myomixer (*Mymx*)[95], which govern early and late stages of the membrane fusion reaction, respectively[98].

Muscle fibre phenotype (see Supplementary Fig. 1b) is ultimately directed by the generally synchronous expression of specific calcium-handling, sarcomeric, and metabolic genes from myonuclei within the same fibre[39]. This is somewhat coordinated by innervation and differences in transcription factor enrichments, chromatin accessibilities[39], and *Myh* promoter-enhancer interactions[96,97] between fibre types. MYOD1 and MYOG can bind at least two enhancers of *Myh7* (MyHC-I)[97] and could act alongside fast-twitch suppressing microRNAs[99] to facilitate a predominance of slow-twitch apparatus in type I fibres[30,31]. Alternatively, fast-*Myh* expression appears regulated by competitive binding among *Myh1* (MyHC-IIX), *Myh2* (MyHC-IIA), and *Myh4* (MyHC-IIB) promoters for elements of a 38 (ref.[97])-42 (ref.[96]) kilobase intergenic super enhancer that maintains a closed conformation in slow-*Myh7*$^{+}$ myonuclei[96]. The upstream regulators of fast-*Myh* promoter-super enhancer interactions have yet to be determined, but could include the transcription factor homeobox protein SIX 1 (SIX1)[39,100] and/or the long intergenic noncoding RNA *linc-Myh*[96,100].

The current understanding of myogenesis is largely derived from animal models and further interrogation of human muscle development and regeneration will be valuable in the pursuit of strategies to improve muscle repair and combat muscle-related diseases.

Supplementary Box 2: The impact of biological sex on skeletal muscle phenotype and exercise response

Muscle mass distribution, morphology, and composition differs between sexes. Women have ~8% less total muscle than men and carry a greater proportion of that mass in their lower (~60%) versus upper (~40%) body[101]. Type II fibres can be ~17-25% bigger than type I fibres in the vastus lateralis of young adult men[34,102] but are of equivalent size in the corresponding muscle of women[102]. Men have larger motor units and neuromuscular properties may vary between sexes at a given intensity of voluntary contraction[103]. Yet, the pattern of motor unit recruitment is similar when increasing force production[103]. Thus, the greater fatigue-resistance[104] but lower strength[103,105] and power[106] of muscle groups in women might derive mostly from differences in muscle mass[101] and fibre capillary density[105], proportion[102] and size[71,102,105,107]. The vastus lateralis of women contains ~5-6% more type I fibres[102], and both type I (~14-32%) and type II (~34-70%) fibres can be markedly smaller than observed in men[71,102,105,107]. However, sex-associated divergence in substrate utilisation[108], fatigability[104], and the muscle transcriptome[109] seems to normalize with exercise training.

Most endocrine hormones elicit strong sexual dimorphism (see Supplementary Table 1), and the physiology of premenopausal women could be further influenced by fluctuations of 17β-oestradiol (E2) and progesterone (P4) across the menstrual cycle (reviewed in ref.[110]). A session of moderate-intensity endurance exercise performed in the luteal phase can rely less on carbohydrates and more on fat oxidation compared to the same exercise bout in the follicular phase[111]. Menstrual cycle-related differences in fat oxidation correlate with changes in the 17β-oestradiol-progesterone ratio between phases[112] and may also relate to 17β-oestradiol-oestrogen receptor-α (ERα) signalling in liver[113]. This 17β-oestradiol-ERα axis was shown to suppress hepatic gluconeogenesis in mice through a serine/threonine protein kinase (AKT)-forkhead box protein O1 (FOXO1)-dependent mechanism[113], which could contribute towards lower rates of plasma glucose appearance during submaximal exercise in the mid-luteal phase[110]. However, any mild effects of the menstrual cycle on moderate-intensity exercise metabolism are likely surpassed by the metabolic demands of more strenuous physical activity (see main text section 'Acute exercise metabolism in skeletal muscle' and Fig. 2). Indeed, intramuscular glycogen depletion, blood lactate, and serum non-esterified fatty acid (NEFA) responses to high-intensity interval exercise were mostly similar between menstrual cycle phases[114].

Periodizing resistance exercise so that greater training volumes are performed in the follicular (as opposed to luteal) phase might improve strength and hypertrophy outcomes[115-117]. The underlying mechanisms are unclear but follicular phase training coincides with higher muscle ERα protein abundance[118] and potentiation of 17β-oestradiol-ERα signalling in satellite cells[119] could augment myonuclear accretion to a follicular phase resistance training intervention[117]. Still, there is substantial interindividual variation in both reported menstrual cycle-associated symptoms[120] and adaptations to menstrual phase-periodized training[115]. Thus, further investigation is required before general, evidence-based recommendations in this area can be made. In the meantime, individuals should take a personalized approach to exercise training according to self-perceived readiness for physical activity. Importantly for women using monophasic second-generation oral contraceptives, these do not appear to negatively impact gains in muscle mass or strength following 12 weeks (36 sessions) of resistance exercise[121].

Supplementary tables

Supplementary Table 1: Key exercise-responsive hormones

Hormone	Effect on skeletal muscle	Impact of exercise	Circadian differences	Biological sex differences	Ageing
Growth hormone (GH) [122-128]	●Anabolic (hyperplasia and hypertrophy; stimulates muscle protein synthesis ~66%) ●Stimulates systemic (mainly hepatic) and skeletal muscle production of IGF1 ●Promotes lipid oxidation and mitochondrial biogenesis ●Increases muscle blood flow ●GH-deficient adults show reduced muscle mass, impaired exercise capacity, and increased adipose tissue	●Induced by aerobic and resistance exercise; acute transient increase according to relative intensity ●Peaks ~30 min after start of exercise, with return to baseline after ~1 h ●Exercise-induced increase retained if rest is ≥3 h between subsequent training bouts	●Pulsatile; major peak at night associated with first slow-wave sleep episode; lowest levels observed during the day	●Mean levels ~2-fold greater in women than men	●Mean levels decrease with age, ~14% per decade after ~40 years of age ●Exercise-induced release reduces with age
Insulin-like growth factor-1 (IGF1) [124,128,129]	●Anabolic (hyperplasia and hypertrophy; stimulates muscle protein synthesis ~70%) ●Anticatabolic (inhibits muscle protein breakdown ~40%) ●Increases muscle blood flow	●Acute and transient increase of muscle-derived IGF1 isoforms after resistance and endurance exercise	●Relatively stable	●Similar levels in women and men	●Mean levels decrease with age
Glucocorticoids [128,130-141] Mainly cortisol and corticosterone	●Catabolic (increases muscle protein breakdown) ●Transcriptional regulation of ubiquitin-proteasome and	●Increased at higher exercise intensities (≥60% $\dot{V}O_{2max}$) ●Circulating levels return to baseline within ~1-3 h post exercise	●Blood levels lowest around midnight (~50 nmol l^{-1}) and highest during the early-morning (~500 nmol l^{-1})	●Conflicting literature. In general, there appears to be no major differences between women and men. Heterogeneity in results may reflect	●Equivocal reports, possibly for same reasons as stated for biological sex differences ●Progressive increase in mean

	autophagy-lysosome system components via ligand-activated glucocorticoid receptors ●Can directly synchronize skeletal muscle circadian clocks through glucocorticoid response elements (GREs) in regulatory regions of several clock genes			confounders related to sleep schedules, body composition, nutrition, psychological stress, etc.	24-h levels in both women and men from ~20-80 years of age
Catecholamines 131,142-146 Adrenaline and noradrenaline	●Anticatabolic (inhibits muscle protein breakdown ~20%) ●Catabolic (stimulates glycogenolysis and lipolysis) ●Mobilization of glycogen and triacylglyceride energy stores producing lactate, glycerol, and non-esterified fatty acids (NEFA) ●Increases substrate oxidation and thermogenesis ●Increases muscle blood flow ●Inotropic	●During exercise, plasma adrenaline and noradrenaline can increase ~10-times baseline levels depending on intensity and duration ●After exercise, return to baseline occurs within minutes due to short half-life and reuptake mechanisms ●Adrenaline clearance increases ~15% during moderate exercise and decreases ~22% below baseline levels during intense exercise ●Noradrenaline increase is enhanced if heat receptors are stimulated ●Both noradrenaline and adrenaline responses are augmented if cold receptors are stimulated ●Exercise response greater in the upright, as opposed to supine, position	●Adrenaline and noradrenaline show diurnal rhythms, even under supine conditions, with increased levels during the active phase (day), and reduced levels during the rest phase (night). ●Sleep and posture both have direct effects on noradrenaline levels ●24-h noradrenaline levels oscillate between ~150-350 ρg ml^{-1} under constant recumbent conditions, and up to ~600 ρg ml^{-1} under ambulatory conditions ●24-h adrenaline levels oscillate from ~20-50 ρg ml^{-1} and are not significantly affected by posture or sleep stage	●Most studies have reported no sex differences in catecholamine response to exercise	●Adrenaline levels are similar between young and elderly adults ●Noradrenaline levels increase with age

		●Exercise response is potentiated in states of insulin deficiency (e.g., fasting, high fat diet, poorly-controlled diabetes) ●Exercise response is reduced in individuals with obesity who have increased insulin secretion ●Long-term physical training increases the size and secretory capacity of the adrenal medulla, which may improve exercise capacity			
Thyroid hormones [147-151] Thyrotropin (TSH), thyroxine (T_4), and triiodothyronine (T_3)	●Transcriptional regulation of muscle fibre type and metabolic profile by ligand-activated thyroid hormone receptors (e.g., repression of *MYH7* and stimulation of *SERCA1*, *PPARGC1A*, and *GLUT4*) ●Potentiates the effects of catecholamines ●Promotes mitochondrial biogenesis ●Increases rates of contraction and relaxation ●Increases energy consumption and heat production	●Literature is inconsistent, or contradictory (see summary on table 4 of ref.[149]) ●Overall, baseline thyroid hormone levels do not appear to be affected by chronic endurance exercise ●Exercise tolerance and performance are reduced in patients with subclinical hypothyroidism	●TSH oscillates ~2-fold; highest at night and lowest during the day ●Circulating levels of free T_4 are fairly stable. ●Circulating levels of free T_3 oscillate with low amplitude and follow TSH rhythm with ~90-min delay	●No major differences in TSH between men and women prior to menopause ●Free T_4 and T_3 blood concentrations lower in women compared to men	●Increased prevalence of subclinical thyroid disease with age ●Reduced TSH secretion during healthy ageing ●Blunted peak of circadian TSH, with ~1-1.5-h phase advance ●TSH is higher in women post-menopause versus men ●Total and free T_4 unchanged with age ●Reduced total and free T_3 with age
Androgens [152-159]	●Anabolic (concentration-dependent increase	●Short, intense exercise (e.g., sprint or resistance modalities)	●Testosterone and DHT levels oscillate ~10-20% over 24 h, with	●Levels are ~20-times higher in men than in	●In men, mean testosterone levels peak at ~20 years of age

Mainly testosterone and 5α-dihydrotestosterone (DHT)	in satellite cell number, myonuclear accretion, and muscle protein synthesis) ●Increases strength ●Transcriptional regulation mediated by ligand-activated androgen receptor	transiently increase testosterone (for ~30 min) ●Testosterone response increases in an intensity-dependent manner ●Exercise-induced increases are greater in younger compared to older men ●Prolonged submaximal exercise (e.g., endurance-type) reduces testosterone levels	peak levels in the morning and trough levels in the evening	women or children	and modestly decline until ~80 years of age, with slightly faster decline thereafter ●Reduced 24-h rhythm of serum testosterone in healthy, elderly men ●In women, mean testosterone levels peak in late adolescence, decline gradually over the next two decades, and then remain relatively stable
Oestrogens [160-164] Mainly 17β-oestradiol (E2) and oestrone (E1)	●Transcriptional regulation via ligand-activated oestrogen receptors ●Inhibits GH-dependent hepatic production of IGF1 ●Promotes mitochondrial function and protects against oxidative stress ●Promotes insulin sensitivity ●Chronically reduced levels in postmenopausal women are associated with blunted response to anabolic stimuli; response is restored to premenopausal levels under oestrogen replacement therapy	●In postmenopausal monozygotic twins, hormone replacement therapy increased maximum walking speed, vertical jump height, and thigh muscle cross-sectional area relative to the untreated twin	●24-h E2 rhythm peaks at night during follicular phase; 24-h rhythm absent during luteal phase in premenopausal women	●Levels are ~4-times higher in premenopausal women versus men	●In women, mean oestrogen levels peak at ~16 years of age and remain relatively constant up to ~40 years of age ●Levels remain chronically decreased in postmenopausal women ●In men, mean oestrogen levels peak at ~20 years of age and remain relatively stable thereafter

GLUT4, glucose transporter 4 mRNA; *MYH7*, myosin heavy chain 7 (MyHC-I) mRNA; *PPARGC1A*, peroxisome proliferator-activated receptor-γ coactivator-1α (*PGC1α*) mRNA; *SERCA1*, mRNA of the fast-twitch sarco/endoplasmic reticulum Ca^{2+}-ATPase isoform.

Supplementary Table 2: Notable studies of the human skeletal muscle transcriptomic response to exercise

Focus & Study	Participant Population	Design	Main Findings
Acute			
Endurance: Mahoney et al., 2005[165]	Healthy young men (n=14 total, n=4 for microarray)	VL biopsy 1-2 weeks pre, then 3 h, and 48 h following 75 min of high-intensity cycling	● Larger gene response at 3 h with several persistent or differential changes at 48 h ● Regulation of mitochondrial, metabolic, oxidative stress, and ion transporter genes
Resistance: Zambon et al., 2003[166]	Healthy men aged 31-51 years old (n=4)	VL biopsy 6 h and 18 h following 10 sets x 8 repetitions of unilateral knee extension, with non-exercised contralateral control	● More genes regulated at 18 h versus 6 h after resistance exercise ● Resistance exercise regulated myogenic, Interleukin-1 (IL-1), and circadian-related genes
Concurrent: Lundberg et al., 2016[167]	Moderately trained young men (n=10)	VL biopsy 3 h following resistance or resistance + endurance (i.e., concurrent) exercise	● Combined endurance and resistance exercise augmented gene expression for both muscle growth and oxidative capacity
Training			
Endurance: Timmons et al., 2005[168]	Healthy young men (n=8)	VL biopsy pre and following 6 weeks of moderate-intensity cycle ergometer training	● Extracellular matrix and calcium binding genes most enriched after training
Resistance: Raue et al., 2012[169]	Healthy young women and men (n=8 per group)	VL biopsy pre and following 12 weeks of progressive resistance training (also, an acute exercise bout in untrained and trained states)	● Modest changes to transcriptome with training ● Acute exercise in the untrained and trained state revealed a common 'signature' of adaptation
Biological Sex Differences			
Endurance: Lindholm et al., 2016[170]	Healthy young women (n=11) and men (n=12)	VL biopsy pre and following 12 weeks of endurance knee-extensor training	● Sex differences in the transcriptome at baseline ● No sex-specific responses to training
Resistance: Liu et al., 2010[171]	Healthy young women (n=8) and men (n=6) divided equally between 4 h and 24 h biopsy groups	BB biopsy 4 h and 24 h following 3 sets x 6 repetitions of several single-arm exercises, with non-exercised contralateral control. The acute bout was performed after 12 weeks of unilateral training.	● Sex differences in the transcriptome at baseline ● Transcriptional alterations more sustained in men 48 h after acute exercise ● Similar pathways regulated between men and women, but downregulation of negative regulators of mTOR signalling in men and specificity for Notch and TGFβ signalling genes in women
Ageing			
Endurance (HIT), resistance, and concurrent: Robinson et al., 2017[64]	Young (n=29) and older (n=23) women and men	VL biopsy pre and following 12 weeks of HIT (n=11 young and 8 older), resistance (n=10 young and 8 older), or concurrent (n=8 young and 7 older) training	● Age differences in the transcriptome at baseline ● HIT upregulated largest number of genes in both young and older participants ● In muscle of elderly, HIT had strongest impact on mitochondrial, muscle growth, and insulin signalling-related transcripts

Resistance: Melov et al., 2007[172]	Healthy young (n=26) and older (n=14) women and men	VL biopsy pre and following 26 weeks of resistance training in the older group	● Compared to baseline, the resistance training intervention resulted in a transcriptional signature more similar to that in muscle of young sedentary individuals
Fibre Type			
Resistance: Raue et al., 2012[169]	Healthy young and older women (n=14)	VL biopsy pre and following an acute resistance exercise bout in the untrained and trained state	● Significant response to acute resistance exercise in type IIA fibres of young, but not older women ● Limited transcriptomic response in type I fibres
Meta-Analysis			
Pillon et al., 2020[173]	66 studies that included >1100 individuals	Acute and chronic resistance, endurance, and concurrent training studies spanning different time frames, biological sexes, ages, and conditions	● *NR4A3* was among the most exercise responsive genes regardless of exercise modality ● Continually updated MetaMex online database tool (https://metamex.serve.scilifelab.se/) for publicly available analysis of gene expression with exercise

BB, biceps brachialis; VL, vastus lateralis; HIT, high-intensity interval training; mTOR, mammalian target of rapamycin; *NR4A3*, nuclear receptor subfamily 4 group A member 3 (also known as *NOR1*) mRNA. TGFβ, transforming growth factor-β. All studies determined gene expression using microarray and/or RNA-sequencing.

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