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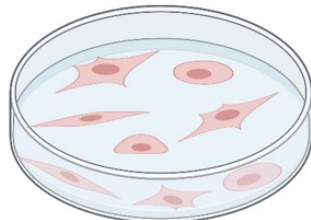
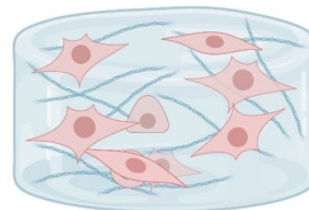
Approachies for *in vitro* exercise mimicry

Cells in 2D or 3D

Cells growing on a 2D dish

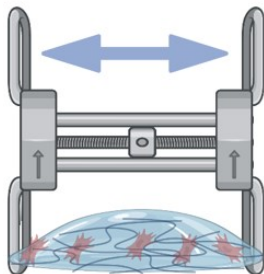


Cells growing on a 3D extracellular matrix (ECM)

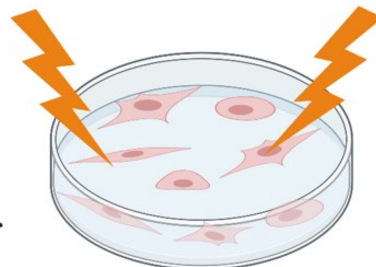


Mechanical and/ or electrical exercise mimicing stimulation

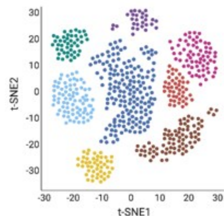
Mechanical stretching

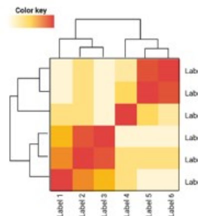


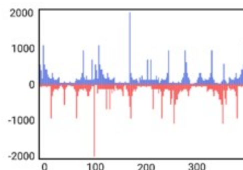
Exercise-like electrical pulse stimulation (EL-EPS)

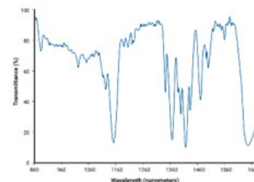


omics analyses









1 # Mimicking exercise *in vitro* – effects of myotube contractions and
2 mechanical stretch on omics

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12 **Running title**: Review – omics perspective of *in vitro* exercise mimicry

13 **Word count** (excluding abstract and references): 3220

14 **Keywords**: electrical pulse stimulation, skeletal muscle, transcriptomics, proteomics,
15 metabolomics

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20 **Abbreviations**: 2D, two-dimensional; 3D, three-dimensional; ECM, extracellular ma-
21 trix; EL-EPS, exercise-like electrical pulse stimulation; EVs, extracellular vesicles,
22 miRNA, microRNA; mRNA, messenger RNA; MS, mass spectrometry; NMR, nuclear
23 magnetic resonance; qPCR, quantitative polymerase chain reaction; ROS, reactive oxy-
24 gen species; RNA-seq, ribonucleic acid sequencing; RE, resistance exercise; SkM, skel-
25 etal muscle

26 **ABSTRACT**

27 The number of studies using skeletal muscle (SkM) cell culture models to study
28 exercise *in vitro* are rapidly expanding. Progressively, more comprehensive analysis
29 methods, such as different omics approaches including transcriptomics, proteomics and
30 metabolomics have been used to examine the intra- and extracellular molecular
31 responses to exercise mimicking stimuli in cultured myotubes. Among other techniques,
32 exercise-like electrical pulse stimulation (EL-EPS) and mechanical stretch of SkM cells
33 are the two most commonly used methods to mimic exercise *in vitro*. In this mini-
34 review we focus on these two approaches and their effects on the omics of myotubes
35 and/or cell culture media. Furthermore, besides traditional two-dimensional (2D)
36 methods, the use of three-dimensional (3D) SkM approaches are increasing in the field
37 of *in vitro* exercise mimicry. Our aim with this mini-review is to provide the reader with
38 an up-to-date overview of the 2D and 3D models and the use of omics approaches to
39 study the molecular response to exercise *in vitro*.

40 **INTRODUCTION**

41 Exercise has many beneficial health effects, but the underlying mediators that occur
42 within skeletal muscle (SkM) are poorly understood. *In vitro* models of exercise, such
43 as exercise-like electrical pulse stimulation (EL-EPS) and mechanical stretch have been
44 developed to enable exclusive examination of SkM myotubes and their intra- and
45 extracellular responses to contractions and/or passive lengthening. In this mini-review,
46 EL-EPS refers to electrically induced contractions of cultured myotubes, and
47 mechanical stretch refers to external forces applied to cultured cells to enable
48 lengthening and tension of myotubes with the aim of mimicking exercise-like responses.
49 Overall, these methods reproduce many of the key physiological (e.g., hypertrophy) and
50 molecular (e.g., transcriptional, translational, and metabolic) changes induced by
51 exercise *in vivo* (see reviews (1, 2)). Based on the rapidly growing literature, EL-EPS
52 and/or mechanical stretch of cultured myotubes seem to be effective tools for
53 investigating exercise-mimicking responses within a well-controlled environment (1, 2).
54 Additionally, other approaches, such as pharmacological compounds, have been used to
55 mimic exercise *in vitro*. However, they are not within the scope of this mini-review (for
56 review, see (3)), nor are the responses to exercise-like stimuli in myoblasts.

57 Exercise can be used to treat and prevent multiple diseases, and the effects may
58 be partially mediated by SkM-derived signaling molecules. Indeed, SkM is considered a
59 secretory organ able to produce and release signaling molecules such as proteins,
60 peptides, metabolites, and nucleic acids that can be transported within e.g., extracellular
61 vesicles (EVs) to target nearby and/or distant cells (4). Although the nomenclature of
62 these signaling molecules may differ depending on the study focus, for clarity, various
63 exercise-induced molecules derived from the contracting SkM myotubes are referred to
64 as exerkines herein (4). Although very few exerkines solely originate from SkM (5), it
65 is plausible that SkM significantly contributes to the circulating exerkine pool, given its´
66 large proportion of the whole-body mass and extensive vascularization.

67 Alongside the development of novel research methods, the number of identified
68 exerkines has expanded (6). However, the function and target tissues of many of these
69 exerkines is still unclear and warrant future investigation. Omics-methods have become
70 popular approaches to examine exerkines and exercise-induced changes in muscle
71 epigenetics, transcription, protein signaling/translation and metabolism, both *in vivo* (7)

72 and, as demonstrated in this mini-review, *in vitro*. Our aim here is to provide the reader
73 with a comprehensive overview of the recent advances of 2D and 3D *in vitro* exercise
74 models combined with various omics techniques. Firstly, we summarize *in vitro*
75 exercise omics studies and their relevance to *in vivo* exercise omics studies. Secondly,
76 we discuss the important considerations related to *in vitro* exercise studies before
77 outlining future perspectives.

78 **OMICS OF THE CONTRACTING OR MECHANICALLY STRETCHED**
79 **MYOTUBES**

80 **2D *in vitro* exercise and epigenomics**

81 Epigenetics has become a popular area of interest within molecular exercise physiology
82 given that epigenetic modifications can determine messenger RNA (mRNA) expression,
83 ultimately affecting the response to exercise. Specifically, acetylation and methylation
84 of histones as well as DNA methylation have received considerable attention (see
85 reviews (8, 9)). Despite previous research demonstrating the importance of histone
86 modifications during myogenesis in cultured SkM cells (10, 11) and in response to the
87 exercise *in vivo* (8), to our knowledge, no studies have examined the histone response to
88 exercise mimicking stimuli in cultured myotubes. DNA methylation response has been
89 investigated in mechanically stretched cultured myotubes (12). However, no studies
90 have yet investigated DNA methylation after EL-EPS in 2D/3D, nor after mechanical
91 stretch in 2D. Therefore, future studies utilizing genome-wide ´omic´ approaches to
92 investigate the epigenomic response to exercise-like stimuli in cultured myotubes (e.g.,
93 EL-EPS, mechanical stretch, or a combination of the two stimuli (13)) is required.

94 **2D *in vitro* exercise and transcriptomics**

95 Several factors including reactive oxygen species (ROS) (14), contraction frequency
96 (Hz) (14, 15), adrenaline (16), lack of long non-coding RNA taurine-upregulated gene 1
97 (*Tug1*) (17) and media glucose content (18) all regulate the transcriptome after EL-EPS
98 in C2C12 myotubes (Table 1). Regarding the exercise-like stimuli in cultured myotubes,
99 the transcriptional response has traditionally been analyzed via quantitative polymerase
100 chain reaction (qPCR) and microarrays. These methods have demonstrated that EL-EPS
101 upregulated the expression of exerkines, many of which are related to inflammatory
102 and/or cytokine signaling (19–22). However, RNA sequencing (RNA-seq) has
103 expanded the knowledge of the contraction-responsive genes in myotubes as discussed
104 below.

105 Of the individual genes, nuclear receptor subfamily 4 group A member 3
106 (*NR4A3*) has been shown to be one of the most exercise- and inactivity-responsive
107 genes in SkM across all *in vivo* acute/chronic and aerobic/resistance exercise studies

108 analyzed (23). *In vitro*, *NR3A3* mRNA expression increased after EL-EPS in primary
109 human (23) and C2C12 (17) myotubes. Moreover, *NR4A3* silencing abolished
110 contraction-induced glucose uptake and altered mRNA levels of many other exercise-
111 and inactivity-responsive genes (21). Overall, this suggests that *NR4A3* has a critical
112 role in SkM metabolism and adaptation to changes in physical activity (23). In addition
113 to *NR4A3*, amphiregulin (*Areg*) has been shown to be upregulated after EL-EPS in
114 C2C12 myotubes and in SkM of exercised mice (24) suggesting its´ role as a novel
115 exerkine.

116 Two recent studies examined the transcriptome after EL-EPS in primary human
117 myotubes (25, 26). Genes related to pro-inflammatory pathways were upregulated in
118 myotubes originating from females with polycystic ovary syndrome relative to healthy
119 controls (25). Although EL-EPS responses may be affected by donor characteristics (1,
120 3), a recent study suggests that age, sex, and EL-EPS parameters may not have
121 substantial transcriptomic and proteomic effects (26).

122 Based on RNA-seq, we (18) and others (16, 26) observed that processes related
123 to cytokine and inflammatory pathways were upregulated after EL-EPS in C2C12
124 myotubes. Local inflammation plays a role in SkM adaptation to exercise (27). After
125 acute aerobic exercise, these effects might be partly mediated by monocyte attractants,
126 such as cytokines from the cytokine families CXC and CC (23). Indeed, upregulation of
127 *Cxcl1*, *Cxcl5* (CXC family) and *Ccl2* (CC family) in response to EL-EPS has been
128 reported using transcriptomic (16, 18, 26) and targeted methods (20–22). These specific
129 cytokines have been associated with exercise-induced macrophage infiltration into SkM
130 to promote tissue regeneration and/or hypertrophy *in vivo* (5, 21, 28).

131 Although high-frequency EL-EPS (≥ 30 Hz (1)) induces greater transcriptome-
132 wide changes than low-frequency EL-EPS (≤ 5 Hz (1)) in C2C12 myotubes (14, 15), the
133 time effect cannot be ruled out. Indeed, these studies have compared the effects of low-
134 and high-frequency EL-EPS on myotube transcriptome only after short-term (≤ 8h (1))
135 stimulation. In contrast, we (18) and others (16, 26) have reported that long-term (≥ 24h
136 (1)) low-frequency EL-EPS is also sufficient to modify the transcriptome of C2C12
137 myotubes. Notably, based on pathway analysis, the processes identified after short- and
138 long-term EL-EPS seem to differ. More specifically, while short-term stimulation
139 promotes mitochondrial adaptations (15) as well as mitogen-activated protein kinase
140 (MAPK), p53, glutathione and pentose phosphate pathway upregulation (14), long-term

141 stimulation targets mostly contractility and cytokine and/or inflammatory processes (16,
142 18). Similar to the changes observed after exercise *in vivo* (23, 29), the intensity (for *in*
143 *vitro* stimulation frequency and voltage) and stimulation duration seem to have an effect
144 on the transcriptome of the contracting myotubes. However, due to the variability in
145 EL-EPS parameters employed within the literature and such few studies conducted, a
146 direct comparison of the results obtained across the different EL-EPS protocols is
147 difficult. Future research is warranted to better understand the extent to which EL-EPS
148 parameters affect the gene transcription in myotubes and the relevance for investigating
149 the transcriptomic response to exercise *in vitro*. The use of primary human myotubes
150 from different donors will allow one to decipher the inter-individual effects of exercise
151 *in vitro*.

152 **2D *in vitro* exercise and proteomics and secretome**

153 The SkM proteome and secretome have been examined after acute exercise and long-
154 term training *in vivo* (7, 30). Proteomics focuses mainly on tissue proteins, while
155 secretome, a subfield of proteomics, examines secreted signaling factors (7). The
156 secretome is typically analyzed via assessment of proteins, peptides, cytokines as well
157 as EVs and their cargo (7). Proteins have been extensively studied after exercise *in vivo*
158 (5) and EL-EPS *in vitro* (22, 31), while EV responses are restricted to exercise *in vivo*
159 (32).

160 To date, there are a number of *in vitro* studies analyzing the proteome (33, 34),
161 secretome (35) or both (26, 36) in response to EL-EPS in myotubes (Table 1). These
162 studies reported increased intracellular abundance of proteins related to ATP production
163 and that the sarcomeric Z-disc was one of the key targets of phosphorylation in
164 contracting C2C12 myotubes (33). Recently, EL-EPS increased the content of
165 cytoskeletal proteins in primary human myotubes derived from young male donors (37).
166 Additionally, phosphoproteomics identified filamin A-interacting protein 1 (FILIP1) in
167 murine C2 cells as an important regulator of filamin homeostasis, which has been
168 associated with the development of SkM cells (34). A comprehensive phosphoproteome
169 of exercise “mimetics” has been recently published *in vitro* (38). These and other post-
170 translational “epiproteomics" approaches should be used in the future also with EL-EPS.

171 Secretome analysis of contracting primary human myotube media and human
172 plasma after EL-EPS and acute endurance exercise and sprint intervals, respectively,

173 identified growth differentiation factor 15 (GDF15) as a novel exerkine targeting
174 adipose tissue (35). Another exerkine, thymosin β4, was identified from the media of
175 the contracting C2C12 myotubes after EL-EPS as well as from the circulation after *in*
176 *vivo* exercise irrespective of the exercise mode (36). Furthermore, CXC motif
177 chemokines (e.g., CXCL1, CXCL2, CXCL5 and CXCL8) and other exerkines were
178 increased in the media of primary human myotubes after EL-EPS in comparison to non-
179 stimulated controls (26). Together these proteomic findings agree with the
180 transcriptomic results discussed above showing that EL-EPS targets specifically
181 contractile machinery within the myotubes and that myotubes release similar exerkines
182 to contracting SkM *in vivo*.

183 During the last few years, EVs have gained a lot of interest as potential mediators
184 of exercise adaptations (39). The EVs, including exosomes, microvesicles, and
185 apoptotic bodies that originate from all cell types (32) can be found from all biofluids
186 and cell culture media (40). Therefore, *in vitro* exercise models could be feasible tools
187 to study the contraction-induced EV release and EV cargo exclusively from the SkM
188 myotubes. The EVs can transport different types of cargo, such as proteins, nucleic
189 acids, and lipids (32). Myotube-derived EVs and their content in response to EL-EPS or
190 mechanical stretch have remained an understudied topic. However, a recent study
191 reported that EL-EPS had no effect on EV size distribution, protein yield or surface
192 marker abundance in C2C12 myotubes (41). MicroRNAs (miRNAs) are common cargo
193 of EVs, and they are known to modify gene expression in response to exercise *in vivo*
194 (32). The most characterized SkM-derived and exercise-responsive miRNAs include
195 miR-1, miR-206, miR-133a and miR-499 (32). We have analyzed the C2C12 cell-
196 derived EVs and their miRNA content after low-frequency EL-EPS (18). We observed,
197 in agreement with the *in vivo* studies (32), that packing of miR-1-3p, and possibly miR-
198 133a-3p, into EVs was enhanced in response to myotube contractions (18). Taken
199 together, as myotube derived EVs are currently an understudied topic, more studies are
200 needed to better understand if *in vitro* models resemble this aspect of *in vivo* exercise.

201 **2D *in vitro* exercise mimetics and metabolomics**

202 Although the number of studies analyzing the metabolome after exercise *in vivo* is high
203 (see reviews (7, 42, 43)), less is known about the effects of EL-EPS on myotube and
204 media metabolome (14, 44, 45) (Table 1). We analyzed the metabolome of C2C12

myotubes and their culture media after 24-hour of low-frequency EL-EPS using nuclear magnetic resonance (^{1}H-NMR)-based approach (45). Others have used short-term low- and/or high-frequency EL-EPS protocol to examine C2C12 myotube metabolome using mass spectrometry (MS)-based approach (14, 44). We (45) and others (44) also compared the effects of media glucose content on the contraction-induced responses in the myotube and/or media metabolome. The difference between the ^{1}H-NMR and MS approaches is that ^{1}H-NMR allows identification of highly abundant molecules very quantitatively, while MS enables identification of less abundant molecules, albeit less quantitatively. Thus, as the metabolites identified by these methods differ in number and composition, comparison between these above-described studies is introductory. This clearly highlights the need for further *in vitro* EL-EPS metabolomics studies comparing and even combining these two methods.

In line with the *in vivo* observations (42, 43), we demonstrated that EL-EPS affected intermediates and amino acids associated with energy metabolism in both myotubes and their media (45). Moreover, higher glucose availability augmented many of the intra- and extracellular metabolomic changes and under low glucose conditions, glucose was almost completely depleted from the cells and the media (45). Others reported that glucose availability of the contracting C2C12 myotubes also regulated the intracellular content of adenine nucleotides and adenosine (44). More precisely, in glucose-depleted conditions, myotube contractions increased AMP content and attenuated ATP regeneration in comparison to high glucose condition (44). Further related to energy metabolism, Hoshino and colleagues showed that high-, but not low-frequency EL-EPS, affected metabolites related to carbohydrate metabolism (14). Similar to our findings (45) this research group observed that the C2C12 myotubes increase lactate production (14), thus suggesting that these glycolytic cells (46) rely heavily on glycolysis, especially during high-frequency EL-EPS.

Additionally, the volume or total number of contractions seem to be important. Low-frequency EL-EPS had only minor effects on the myotube metabolome after a short-term stimulation (14), while long-term stimulation significantly affected both the myotube and the media metabolome and small molecule content (45). Notably, we also analyzed the cell-free media metabolome to robustly estimate EL-EPS-induced metabolite uptake (e.g., glucose and serine) and release (e.g., lactate and acetate).

237 However, further studies using isotope tracers are needed to examine dynamic
238 metabolite turnover (i.e., fluxomics).

239 **3D *in vitro* exercise mimetics and omics**

240 In addition to traditional 2D cultures, 3D approaches are used to better mimic native
241 cell-cell and cell-extracellular matrix (ECM) interactions within tissues being one
242 translational step between *in vitro* and human responses alongside with *ex vivo* and *in*
243 *vivo* models. To the best of our knowledge, there are no studies on 3D bioengineered
244 SkM epigenetics, transcriptomics, or proteomics. However, contraction-induced
245 changes in the metabolome of the tissue-engineered human SkM myotubes have been
246 studied after low- and high-frequency EL-EPS (47) (Table 1). The results showed that
247 3D EL-EPS promoted glycolytic and fatty acid metabolic fluxes in primary human
248 myotubes (47). Additionally, with increasing frequency, there was a greater abundance
249 of different acylcarnitines, thus supporting the increased fatty acid metabolism observed
250 after EL-EPS (47). *In vivo*, different acylcarnitine types have been shown to increase in
251 blood after exercise (42). Similar to *in vivo* findings (48) and our observations after EL-
252 EPS in 2D (45), the abundance of intracellular amino acids, such as branched chain
253 amino acids (BCAAs), were also increased after EL-EPS in 3D muscle (47), thus
254 suggesting amino acid recycling in response to exercise-like stimulation *in vitro*.
255 To our knowledge, there are currently no studies that have undertaken
256 transcriptomic analysis after mechanical stretch in 3D SkM. However, we mechanically
257 stretched C2C12 cells and assessed mRNA expression of 37 genes previously altered
258 across the human transcriptome (49) and epigenome (50) after resistance exercise (RE)
259 (12). Interestingly, mRNA expression of 86% (32/37 genes) and 95% (35/37 genes)
260 genes analyzed *in vitro* were similar when compared to RE in human and rodent SkM,
261 respectively (12, 51). Overall, this suggests that mechanical stretch of cultured
262 myotubes alone (i.e., in absence of concentric shortening contraction) is sufficient for
263 inducing comparable mRNA expression responses to RE *in vivo*. Nonetheless, future *in*
264 *vitro* studies should consider transcriptomic analysis after mechanical stretch to identify
265 novel mechano-sensitive genes and to directly compare the results with the *in vivo*
266 transcriptome after RE. Moreover, unlike mRNA expression, targeted DNA methylation
267 changes across the majority of genes analyzed *in vitro* did not mimic the epigenetic

268 response to acute RE in humans (12) warranting further studies using omics approaches
269 with and without EL-EPS.

CONCLUSIONS AND FUTURE DIRECTIONS

Advances in cell culture models and analytical methods have opened new avenues within the field of exercise physiology. As the models and methods improve (e.g., become more affordable and user-friendly), more scientists can utilize these approaches to further expand the knowledge in the field. At the same time, the modern world encounters problems due to sedentary lifestyle. To overcome this deleterious metabolic condition, more knowledge of the molecular mechanisms of physical activity and inactivity is needed. Omics analyses of SkM after exercise *in vivo* and exercise-like stimuli *in vitro* provide a new holistic perspective of the molecular mechanisms of exercise as each have their pros and cons (Figure 1). To sum up, mostly *in vitro* models and their omics-based analyses corroborate with findings observed after exercise *in vivo*. Nevertheless, novel findings have been obtained from *in vitro* studies, such as the discovery of putative exerkines (e.g., amphiregulin and GDF15), are worth investigating further.

The future of *in vitro* exercise models looks promising. The development of *in vitro* exercise systems using bio-printing or 2D/3D organoid/tissue-on-a-chip models could provide more advanced models for studying, for example, intercellular communication, even with other cell types. Indeed, a recent study has developed a tissue-on-a-chip system to recapitulate *in vivo* metabolism (52). The 3D models also allow studies on myotube-ECM interactions, as the cells produce ECM components to support the structures. Although more methodological development is needed to affordably scale up the use of 3D models in *in vitro* exercise research, this approach could provide a more physiological environment for the SkM cells to study their omics responses to exercise-like stimuli.

It is also important to note the limitations of *in vitro* exercise models. Human and rodent muscle cell lines differ in their genetic properties, which may affect their responses to exercise-like stimulation (53). Importantly, regardless of the cell line used, myotube contractions are not visible immediately after the electrical current is applied to the media. Thus, if the stimulation time is shorter than the time to detect visible contractions (i.e., contractile components are not yet fully functional), changes in the studied parameters can possibly be caused, for example, by Ca^{2+} flux rather than contractions *per se*. Additionally, nutrition is a key regulator of metabolism *in vivo*, but

302 this aspect has remained overlooked and understudied within *in vitro* exercise studies.
303 Indeed, media composition can affect myotube epigenome, transcriptome, proteome,
304 metabolome and even extraction of the EVs. Therefore, it is important that the media
305 composition (including serum used (54)) is carefully considered and well-reported in
306 future studies. Lastly, a major limitation of the current *in vitro* exercise models is the
307 inability to apply stimuli to induce active lengthening (i.e., eccentric contractions) and
308 to mimic long-term training. The development of such approaches will provide better
309 translation to *in vivo* exercise. Furthermore, future studies should also investigate
310 questions such as does muscle activation through motor neurons and neurotransmitters
311 yield different outcomes than EL-EPS.

312 To summarize, there is a clear need for more physiologically relevant *in vitro*
313 exercise mimicking models. However, many of the current *in vitro* omics findings after
314 exercise-like stimuli are similar after *in vivo* exercise. This encourages the further use of
315 *in vitro* exercise models in skeletal muscle and exercise research individually and as
316 complementary for *in vivo* studies. The importance of SkM and exercise research in the
317 world that faces multiple issues due to passive lifestyle needs better understanding of
318 molecules that could have beneficial systemic effects. Thus, combination of omics
319 approaches could provide even broader overview of all the health benefits of exercise.

320 **AUTHOR CONTRIBUTIONS**

321 J.H.L., D.C.T., and L.Y-O., drafted the manuscript, J.H.L. and L.Y-O. prepared the
322 figures and tables. A.P.S., R.K., S.P. and J.J.H., critically reviewed and edited the
323 manuscript. All authors approved the final version of the manuscript.

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Owing space limitation, we regret omitting citations to papers that have contributed to the *in vitro* exercise mimicking field. The figures were created with BioRender.com.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

FIGURE LEGENDS

FIGURE 1. Omics approaches have become popular in *in vivo* and *in vitro* exercise research. Both study models have their pros and cons that affect the outcomes and interpretation of the results. Despite the limitations of the *in vitro* exercise mimicry, it has been shown to have similar responses as *in vivo* exercise at the transcriptional, translational, and metabolic levels. *NR4A3*, nuclear receptor subfamily 4 group A member 3; ROS, reactive oxygen species; GDF15, growth differentiation factor 15; EVs, extracellular vesicles; miRNA, microRNA; BCAAs, branched chain amino acids.

344 **REFERENCES**

345 1. **Nikolić N**, **Görgens SW**, **Thoresen GH**, **Aas V**, **Eckel J**, **Eckardt K**.
346 Electrical pulse stimulation of cultured skeletal muscle cells as a model
347 for in vitro exercise - possibilities and limitations. *Acta Physiologica* 220:
348 310–331, 2017.
349 2. **Kasper AM**, **Turner DC**, **Martin NRW**, **Sharples AP**. Mimicking exercise
350 in three-dimensional bioengineered skeletal muscle to investigate cellular
351 and molecular mechanisms of physiological adaptation. *J Cell Physiol* 233:
352 1985–1998, 2018.
353 3. **Carter S**, **Solomon TPJ**. In vitro experimental models for examining the
354 skeletal muscle cell biology of exercise: the possibilities, challenges and
355 future developments. *Pflügers Archiv-European Journal of Physiology* 471:
356 413–429, 2019.
357 4. **Safdar A**, **Saleem A**, **Tarnopolsky MA**. The potential of endurance
358 exercise-derived exosomes to treat metabolic diseases. *Nat Rev Endocrinol*
359 12: 504, 2016.
360 5. **Catoire M**, **Kersten S**. The search for exercise factors in humans. *The*
361 *FASEB Journal* 29: 1615–1628, 2015.
362 6. **Severinsen MCK**, **Pedersen BK**. Muscle–Organ Crosstalk: The Emerging
363 Roles of Myokines. *Endocr Rev* 41: 594–609, 2020.
364 7. **Hoffman NJ**. Omics and exercise: global approaches for mapping
365 exercise biological networks. *Cold Spring Harb Perspect Med* 7: a029884,
366 2017.
367 8. **McGee SL**, **Walder KR**. Exercise and the Skeletal Muscle Epigenome.
368 *Cold Spring Harb Perspect Med* 7: a029876, 2017.
369 9. **Seaborne RA**, **Sharples AP**. The Interplay Between Exercise Metabolism,
370 Epigenetics, and Skeletal Muscle Remodeling. *Exerc Sport Sci Rev* 48: 188–
371 200, 2020.
372 10. **Harada A**, **Maehara K**, **Ono Y**, **Taguchi H**, **Yoshioka K**, **Kitajima Y**, **Xie Y**,
373 **Sato Y**, **Iwasaki T**, **Nogami J**, **Okada S**, **Komatsu T**, **Semba Y**, **Takemoto**
374 **T**, **Kimura H**, **Kurumizaka H**, **Ohkawa Y**. Histone H3.3 sub-variant
375 H3mm7 is required for normal skeletal muscle regeneration. *Nat Commun*
376 9: 1400, 2018.
377 11. **Asp P**, **Blum R**, **Vethantham V**, **Parisi F**, **Micsinai M**, **Cheng J**, **Bowman**
378 **C**, **Kluger Y**, **Dynlacht BD**. Genome-wide remodeling of the epigenetic
379 landscape during myogenic differentiation. *Proceedings of the National*
380 *Academy of Sciences* 108, 2011.
381 12. **Turner DC**, **Gorski PP**, **Seaborne RA**, **Viggars M**, **Murphy M**, **Jarvis JC**,
382 **Martin NRW**, **Stewart CE**, **Sharples AP**. Mechanical loading of
383 bioengineered skeletal muscle in vitro recapitulates gene expression
384 signatures of resistance exercise in vivo. *J Cell Physiol* 236: 6534–6547, 2021.
385 13. **Kim H**, **Kim M-C**, **Asada HH**. Extracellular matrix remodelling induced
386 by alternating electrical and mechanical stimulations increases the
387 contraction of engineered skeletal muscle tissues. *Sci Rep* 9: 2732, 2019.

388 14. **Hoshino D**, **Kawata K**, **Kunida K**, **Hatano A**, **Yugi K**, **Wada T**, **Fujii M**,
389 **Sano T**, **Ito Y**, **Furuichi Y**. Trans-omic analysis reveals ROS-dependent
390 pentose phosphate pathway activation after high-frequency electrical
391 stimulation in C2C12 myotubes. *iScience* 23: 101558, 2020.
392 15. **Tamura Y**, **Kouzaki K**, **Kotani T**, **Nakazato K**. Electrically stimulated
393 contractile activity-induced transcriptomic responses and metabolic
394 remodeling in C2C12 myotubes: twitch vs. tetanic contractions. *American*
395 *Journal of Physiology-Cell Physiology* 319: C1029–C1044, 2020.
396 16. **Fukushima T**, **Takata M**, **Kato A**, **Uchida T**, **Nikawa T**, **Sakakibara I**.
397 Transcriptome Analyses of In Vitro Exercise Models by Clenbuterol
398 Supplementation or Electrical Pulse Stimulation. *Applied Sciences* 11: 10436,
399 2021.
400 17. **Trewin AJ**, **Silver J**, **Dillon HT**, **Della Gatta PA**, **Parker L**, **Hiam DS**, **Lee**
401 **YP**, **Richardson M**, **Wadley GD**, **Lamon S**. Long non-coding RNA Tug1
402 modulates mitochondrial and myogenic responses to exercise in skeletal
403 muscle. *BMC Biol* 20: 164, 2022.
404 18. **Lautaoja JH**. Muscle metabolism and intercellular crosstalk - effects of in
405 vitro exercise and changes in muscle size [Online]. University of
406 Jyväskylä: 2022. http://urn.fi/URN:ISBN:978-951-39-9084-8 [17 Nov.
407 2022].
408 19. **Chen W**, **Nyasha MR**, **Koide M**, **Tsuchiya M**, **Suzuki N**, **Hagiwara Y**,
409 **Aoki M**, **Kanzaki M**. In vitro exercise model using contractile human and
410 mouse hybrid myotubes. *Sci Rep* 9: 11914, 2019.
411 20. **Nedachi T**, **Fujita H**, **Kanzaki M**. Contractile C2C12 myotube model for
412 studying exercise-inducible responses in skeletal muscle. *Am J Physiol*
413 *Endocrinol Metab* 295, 2008.
414 21. **Miyatake S**, **Bilan PJ**, **Pillon NJ**, **Klip A**. Contracting C2C12 myotubes
415 release CCL2 in an NF-κB-dependent manner to induce monocyte
416 chemoattraction. *American Journal of Physiology-Endocrinology and*
417 *Metabolism* 310: E160–E170, 2016.
418 22. **Scheler M**, **Irmler M**, **Lehr S**, **Hartwig S**, **Staiger H**, **Al-Hasani H**,
419 **Beckers J**, **Hrabé de Angelis M**, **Häring H-U**, **Weigert C**. Cytokine
420 response of primary human myotubes in an in vitro exercise model.
421 *American Journal of Physiology-Cell Physiology* 305: C877–C886, 2013.
422 23. **Pillon NJ**, **Gabriel BM**, **Dollet L**, **Smith JAB**, **Puig LS**, **Botella J**, **Bishop**
423 **DJ**, **Krook A**, **Zierath JR**. Transcriptomic profiling of skeletal muscle
424 adaptations to exercise and inactivity. *Nat Commun* 11: 1–15, 2020.
425 24. **Lee B**, **Kim SK**, **Shin YJ**, **Son YH**, **Yang JW**, **Lee S-M**, **Yang YR**, **Lee K-P**,
426 **Kwon K-S**. Genome-wide analysis of a cellular exercise model based on
427 electrical pulse stimulation. *Sci Rep* 12: 21251, 2022.
428 25. **McIlvenna LC**, **Altıntaş A**, **Patten RK**, **McAinch AJ**, **Rodgers RJ**, **Stepto**
429 **NK**, **Barrès R**, **Moreno-Asso A**. Transforming growth factor β1 impairs
430 the transcriptomic response to contraction in myotubes from women with
431 polycystic ovary syndrome. *J Physiol* 600: 3313–3330, 2022.

432 26. **Mengeste AM**, **Nikolić N**, **Dalmao Fernandez A**, **Feng YZ**, **Nyman TA**,
433 **Kersten S**, **Haugen F**, **Kase ET**, **Aas V**, **Rustan AC**, **Thoresen GH**. Insight
434 Into the Metabolic Adaptations of Electrically Pulse-Stimulated Human
435 Myotubes Using Global Analysis of the Transcriptome and Proteome.
436 *Front Physiol* 13, 2022.
437 27. **Peake JM**, **Neubauer O**, **della Gatta PA**, **Nosaka K**. Muscle damage and
438 inflammation during recovery from exercise. *J Appl Physiol* 122: 559–570,
439 2017.
440 28. **Huh JY**. The role of exercise-induced myokines in regulating metabolism.
441 *Arch Pharm Res* 41: 14–29, 2018.
442 29. **Popov D V.**, **Makhnovskii PA**, **Shagimardanova EI**, **Gazizova GR**,
443 **Lysenko EA**, **Gusev OA**, **Vinogradova OL**. Contractile activity-specific
444 transcriptome response to acute endurance exercise and training in
445 human skeletal muscle. *American Journal of Physiology-Endocrinology and*
446 *Metabolism* 316: E605–E614, 2019.
447 30. **Burniston JG**, **Hoffman EP**. Proteomic responses of skeletal and cardiac
448 muscle to exercise. *Expert Rev Proteomics* 8: 361–377, 2011.
449 31. **Raschke S**, **Eckardt K**, **Holven KB**, **Jensen J**, **Eckel J**. Identification and
450 validation of novel contraction-regulated myokines released from
451 primary human skeletal muscle cells. *PLoS One* 8: e62008, 2013.
452 32. **Vechetti IJ**, **Valentino T**, **Mobley CB**, **McCarthy JJ**. The role of
453 extracellular vesicles in skeletal muscle and systematic adaptation to
454 exercise. *J Physiol* 599: 845–861, 2021.
455 33. **Reimann L**, **Wiese H**, **Leber Y**, **Schwäble AN**, **Fricke AL**, **Rohland A**,
456 **Knapp B**, **Peikert CD**, **Drepper F**, **van der Ven PFM**. Myofibrillar Z-discs
457 are a protein phosphorylation hot spot with protein kinase C (PKCα)
458 modulating protein dynamics. *Molecular & Cellular Proteomics* 16: 346–367,
459 2017.
460 34. **Reimann L**, **Schwäble AN**, **Fricke AL**, **Mühlhäuser WWD**, **Leber Y**,
461 **Lohanadan K**, **Puchinger MG**, **Schäuble S**, **Faessler E**, **Wiese H**,
462 **Reichenbach C**, **Knapp B**, **Peikert CD**, **Drepper F**, **Hahn U**, **Kreutz C**,
463 **van der Ven PFM**, **Radziwill G**, **Djinović-Carugo K**, **Fürst DO**,
464 **Warscheid B**. Phosphoproteomics identifies dual-site phosphorylation in
465 an extended basophilic motif regulating FILIP1-mediated degradation of
466 filamin-C. *Commun Biol* 3: 253, 2020.
467 35. **Laurens C**, **Parmar A**, **Murphy E**, **Carper D**, **Lair B**, **Maes P**, **Vion J**,
468 **Boulet N**, **Fontaine C**, **Marquès M**, **Larrouy D**, **Harant I**, **Thalamas C**,
469 **Montastier E**, **Caspar-Bauguil S**, **Bourlier V**, **Tavernier G**, **Grolleau J-L**,
470 **Bouloumié A**, **Langin D**, **Viguerie N**, **Bertile F**, **Blanc S**, **de Glisezinski I**,
471 **O'Gorman D**, **Moro C**. Growth and differentiation factor 15 is secreted by
472 skeletal muscle during exercise and promotes lipolysis in humans. *JCI*
473 *Insight* 5: e131870, 2020.
474 36. **Gonzalez-Franquesa A**, **Stocks B**, **Borg ML**, **Kuefner M**, **Dalbram E**,
475 **Nielsen TS**, **Agrawal A**, **Pankratova S**, **Chibalin A v.**, **Karlsson HKR**,
476 **Gheibi S**, **Björnholm M**, **Jørgensen NR**, **Clemmensen C**, **Hostrup M**,

477 **Treebak JT**, **Krook A**, **Zierath JR**, **Deshmukh AS**. Discovery of thymosin
478 β4 as a human exerkine and growth factor. *American Journal of Physiology-*
479 *Cell Physiology* 321: C770–C778, 2021.
480 37. **Mengeste AM**, **Nikolić N**, **Dalmao Fernandez A**, **Feng YZ**, **Nyman TA**,
481 **Kersten S**, **Haugen F**, **Kase ET**, **Aas V**, **Rustan AC**, **Thoresen GH**. Insight
482 Into the Metabolic Adaptations of Electrically Pulse-Stimulated Human
483 Myotubes Using Global Analysis of the Transcriptome and Proteome.
484 *Front Physiol* 13, 2022.
485 38. **Needham EJ**, **Humphrey SJ**, **Cooke KC**, **Fazakerley DJ**, **Duan X**, **Parker**
486 **BL**, **James DE**. Phosphoproteomics of Acute Cell Stressors Targeting
487 Exercise Signaling Networks Reveal Drug Interactions Regulating Protein
488 Secretion. *Cell Rep* 29: 1524-1538.e6, 2019.
489 39. **Trovato E**, **Di Felice V**, **Barone R**. Extracellular vesicles: delivery vehicles
490 of myokines. *Front Physiol* 10: 522, 2019.
491 40. **Safdar A**, **Tarnopolsky MA**. Exosomes as mediators of the systemic
492 adaptations to endurance exercise. *Cold Spring Harb Perspect Med* 8:
493 a029827, 2018.
494 41. **Bydak B**, **Pierdoná TM**, **Seif S**, **Sidhom K**, **Obi PO**, **Labouta HI**, **Gordon**
495 **JW**, **Saleem A**. Characterizing Extracellular Vesicles and Particles
496 Derived from Skeletal Muscle Myoblasts and Myotubes and the Effect of
497 Acute Contractile Activity [Online]. *Membranes (Basel)* 12: 464, 2022.
498 https://www.mdpi.com/2077-0375/12/5/464.
499 42. **Schranner D**, **Kastenmüller G**, **Schönfelder M**, **Römisch-Margl W**,
500 **Wackerhage H**. Metabolite Concentration Changes in Humans After a
501 Bout of Exercise: a Systematic Review of Exercise Metabolomics Studies.
502 *Sports medicine-open* 6: 11, 2020.
503 43. **Sakaguchi CA**, **Nieman DC**, **Signini EF**, **Abreu RM**, **Catai AM**.
504 Metabolomics-Based Studies Assessing Exercise-Induced Alterations of
505 the Human Metabolome: A Systematic Review. *Metabolites* 9: 164, 2019.
506 44. **Sato S**, **Dyar KA**, **Treebak JT**, **Jepsen SL**, **Ehrlich AM**, **Ashcroft SP**, **Trost**
507 **K**, **Kunzke T**, **Prade VM**, **Small L**, **Basse AL**, **Schönke M**, **Chen S**,
508 **Samad M**, **Baldi P**, **Barrès R**, **Walch A**, **Moritz T**, **Holst JJ**, **Lutter D**,
509 **Zierath JR**, **Sassone-Corsi P**. Atlas of exercise metabolism reveals time-
510 dependent signatures of metabolic homeostasis. *Cell Metab* 34: 329-345.e8,
511 2022.
512 45. **Lautaoja JH**, **O'Connell TM**, **Mäntyselkä S**, **Peräkylä J**, **Kainulainen H**,
513 **Pekkala S**, **Permi P**, **Hulmi JJ**. Higher glucose availability augments the
514 metabolic responses of the C2C12 myotubes to exercise-like electrical
515 pulse stimulation. *American Journal of Physiology-Endocrinology and*
516 *Metabolism* 321: E229–E245, 2021.
517 46. **Burch N**, **Arnold A-S**, **Summermatter S**, **Santos GBS**, **Christe M**,
518 **Boutellier U**, **Toigo M**, **Handschin C**. Electric pulse stimulation of
519 cultured murine muscle cells reproduces gene expression changes of
520 trained mouse muscle. *PLoS One* 5: e10970, 2010.

47. **Khodabukus A**, **Madden L**, **Prabhu NK**, **Koves TR**, **Jackman CP**, **Muoio DM**, **Bursac N**. Electrical stimulation increases hypertrophy and metabolic flux in tissue-engineered human skeletal muscle. *Biomaterials* 198 Elsevier: 259–269, 2019.
48. **Tipton KD**, **Hamilton DL**, **Gallagher IJ**. Assessing the Role of Muscle Protein Breakdown in Response to Nutrition and Exercise in Humans. *Sports Medicine* 48: 53–64, 2018.
49. **Turner DC**, **Seaborne RA**, **Sharples AP**. Comparative Transcriptome and Methylome Analysis in Human Skeletal Muscle Anabolism, Hypertrophy and Epigenetic Memory. *Sci Rep* 9: 4251, 2019.
50. **Seaborne RA**, **Strauss J**, **Cocks M**, **Shepherd S**, **O'Brien TD**, **van Someren KA**, **Bell PG**, **Murgatroyd C**, **Morton JP**, **Stewart CE**, **Sharples AP**. Human Skeletal Muscle Possesses an Epigenetic Memory of Hypertrophy. *Sci Rep* 8: 1898, 2018.
51. **Schmoll M**, **Unger E**, **Sutherland H**, **Haller M**, **Bijak M**, **Lanmüller H**, **Jarvis JC**. SpillOver stimulation: A novel hypertrophy model using co-contraction of the plantar-flexors to load the tibial anterior muscle in rats. *PLoS One* 13: e0207886, 2018.
52. **Ronaldson-Bouchard K**, **Teles D**, **Yeager K**, **Tavakol DN**, **Zhao Y**, **Chramiec A**, **Tagore S**, **Summers M**, **Stylianos S**, **Tamargo M**, **Lee BM**, **Halligan SP**, **Abaci EH**, **Guo Z**, **Jacków J**, **Pappalardo A**, **Shih J**, **Soni RK**, **Sonar S**, **German C**, **Christiano AM**, **Califano A**, **Hirschi KK**, **Chen CS**, **Przekwas A**, **Vunjak-Novakovic G**. A multi-organ chip with matured tissue niches linked by vascular flow. *Nat Biomed Eng* 6: 351–371, 2022.
53. **Abdelmoez AM**, **Sardón Puig L**, **Smith JAB**, **Gabriel BM**, **Savikj M**, **Dollet L**, **Chibalin A V**, **Krook A**, **Zierath JR**, **Pillon NJ**. Comparative profiling of skeletal muscle models reveals heterogeneity of transcriptome and metabolism. *American Journal of Physiology-Cell Physiology* 318: C615–C626, 2020.
54. **Allen SL**, **Elliott BT**, **Carson BP**, **Breen L**. Improving Physiological Relevance of Cell Culture: The Possibilities, Considerations and Future Directions of the Ex Vivo Co-Culture Model.

In vivo

Pros

Intact neuromuscular and myotendinous junctions

Ability to study all types of exercise

Broadly reported pre- and post-exercise changes

Broader population range studied (age, sex, etc.)

Ability to observe systemic effects

Cons

Usually low cell specificity

Poor suitability for secretome studies

Time consuming and laborious

Participant dropouts, inability to monitor all eating, activity, etc.

Universal findings

Transcriptomics

Chemokines/cytokines ↑
e.g., CXC and CC families

Exercise-responsive genes ↑
e.g., *NR4A3*

Gene expression altered *e.g.*, by nutrients and ROS

Proteomics

Exerkine secretion ↑
e.g., GDF15, thymosin β4

EV release

Exercise-responsive miRNAs in EVs ↑
e.g., miR-1

Metabolomics

Energy metabolism ↑
e.g., glucose and lactate

Amino acid recycling ↑
e.g., BCAAs

Acylcarnitines ↑

In vitro

Pros

High cell specificity, relatively easy and fast

Excellent suitability for secretome studies

Ability to control all parameters and conditions

Possibility to study direct intercellular crosstalk

Isolated test environment

Cons

Unideal cellular microenvironment

Murine cell lines more popular than primary human cell lines

Inability to study all types of exercise and long-term exercise training

Scarcely reported pre- and post-stimulation changes

TABLE 1. Overview of the different exercise-like electrical pulse stimulation (EL-EPS) parameters for investigation of *in vitro* exercise omics. The C2C12 and C2 cell lines are of murine origin, while HSkM are primary human skeletal muscle-derived cells.

Cell line	2D/3D	Duration	Stimulation parameters	Omics approach	Reference
C2C12	2D	2, 5, 15, 30 and 60 min. After 60 min + 1 h, 3 h or 6 h rest.	2 or 20 Hz, 50 V, 3 ms	Transcriptomics and metabolomics	(14)
	2D	3 h	Twitch: 2 Hz, 13 V, 2 ms Tetanic: 66 Hz, 13 V, 2 ms (5 s off, 5 s on)	Transcriptomics	(15)
	2D	3 h	66 Hz, 13 V, 2 ms, 5 s on and 5 s off	Transcriptomics	(17)
	2D	3 h	1 Hz, 30 V, 2 ms	Metabolomics	(44)
	2D	16 - 24 h	0.5 Hz, 10 - 12 V, 4 ms	Phosphoproteomics	(33)
	2D	24 h	1 or 10 pulses at 1 Hz, 20 V, 5 ms	Transcriptomics	(16)
	2D	24 h	1 Hz, 12 V, 2 ms	Transcriptomics	(18)
	2D	24 h + 1h rest	1 Hz, 11.5 V, 1 ms pulse stimulus of 2 ms duration	Transcriptomics	(24)
	2D	24 h	1 Hz, 11.5 V, 2 ms	Proteomics	(36)
	2D	24 h	1 Hz, 12 V, 2 ms	Metabolomics	(45)
HSkM	2D	3 h or 24 h	3 h: 0.5 Hz, 10 V, 24 ms 24 h: 0.1 Hz, 10 V, 2 ms	Proteomics	(35)
	2D	6 h	1 Hz, 11.5 V, 2 ms	Transcriptomics	(25)
	2D	24 h or 48 h	24 h: 0.1 Hz, 10 V, 2 ms 48 h: 1 Hz, 30 V, 2 ms	Transcriptomics and proteomics	(26)
	3D	1 h + 7 h rest, 1 week	1 Hz, 70 mA, 2 ms or 0.5 s 10 Hz pulse train every 5 s, 70 mA, 2 ms	Metabolomics	(47)
C2	2D	4 h	0.05 Hz, 10 V, 4 ms	Phosphoproteomics	(34)