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1 **Stimuli and sensors that initiate skeletal muscle hypertrophy**
2 **following resistance exercise**

3

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21

22 Abbreviated running title: Hypertrophy stimuli & sensors

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28

29 **Abstract**

30 One of the most striking adaptations to exercise is the skeletal muscle hypertrophy that
31 occurs in response to resistance exercise. A large body of work shows that a mTORC1-
32 mediated increase of muscle protein synthesis is the key, but not sole, mechanism by which
33 resistance exercise causes muscle hypertrophy. Whilst much of the hypertrophy signaling
34 cascade has been identified, the initiating, resistance exercise-induced and hypertrophy-
35 stimulating stimuli have remained elusive. For the purpose of this review, we define an
36 initiating, resistance exercise-induced and hypertrophy-stimulating signal as “**hypertrophy**
37 **stimulus**”, and the sensor of such a signal as “**hypertrophy sensor**”. In this review we
38 discuss our current knowledge of specific mechanical stimuli, damage/injury-associated and
39 metabolic stress-associated triggers as potential hypertrophy stimuli. Mechanical signals are
40 the prime hypertrophy stimuli candidates and a Filamin-C-BAG3-dependent regulation of
41 mTORC1, Hippo and autophagy signalling is a plausible albeit still incompletely
42 characterised hypertrophy sensor. Other candidate mechanosensing mechanisms are
43 nuclear deformation-initiated signalling or several mechanisms related to costameres, which
44 are the functional equivalents of focal adhesions in other cells. Whilst exercise-induced
45 muscle damage is probably not essential for hypertrophy, it is still unclear whether and how
46 such muscle damage could augment a hypertrophic response. Interventions that combine
47 blood flow restriction and especially low load resistance exercise suggest that resistance
48 exercise-regulated metabolites could be hypertrophy stimuli but this is based on indirect
49 evidence and metabolite candidates are poorly characterised.

50

51

52 Introduction

53 Adequate muscle mass and strength are not only important for sporting performance but
54 these attributes also are associated with good health and longevity (25, 162). For example, a
55 recent analysis of the data of half a million people demonstrated that low grip strength is
56 associated with a higher all-cause and disease-specific mortality as well as disease
57 incidence for several major diseases (20). The key intervention to induce muscular
58 hypertrophy and to make us stronger is resistance exercise in combination with nutrition. The
59 current recommendation is for individuals to train with \approx 40-80% of their 1 repetition maximum
60 (1RM, i.e. the maximal weight that we can lift once) for hypertrophy, with loads $>$ 60% to
61 increase maximal strength (135). Additionally, exercisers should perform multiple sets, rest
62 for $>$ 2 min in-between sets and consume a diet that contains at least 1.6 g of protein per kg
63 body weight per day (101).

64
65 With respect to the muscle protein synthesis and the hypertrophic response to resistance
66 exercise, the mechanistic target of rapamycin (the key mTOR containing complex is
67 abbreviated as mTORC1) is a downstream hypertrophy signaling “hub” that controls protein
68 synthesis (15, 94, 117). This is supported by extensive experimental evidence including
69 research showing that mTORC1 blockade with rapamycin prevents or reduces the increase
70 of muscle protein synthesis and/or muscle size after resistance exercise in humans (33) and
71 in rodents (83) or when muscle is overloaded through synergist ablation (15, 53). Other
72 signaling pathways and genes (94, 155) also regulate muscle size but their specific
73 contribution to resistance exercise-induced muscle hypertrophy is incompletely understood.

74
75 Whilst many studies have identified molecules and molecular mechanisms that regulate
76 muscle mass, one key question has remained largely unanswered. It is: “What are the
77 initiating **hypertrophy stimuli** that trigger hypertrophic signal transduction and skeletal
78 muscle fiber hypertrophy in response to resistance exercise and what are their **sensors**?”
79 Here, we define “**hypertrophy stimulus**” as a “first-in-line”, initiating stimulus that is of a
80 sufficient magnitude and duration to trigger a skeletal muscle hypertrophic response to
81 resistance exercise. Additionally, we define “**hypertrophy sensor**” as a sensor that senses
82 hypertrophy stimuli. This definition means that hypertrophy regulators such as IGF-1 or its
83 MGF splice variant are not hypertrophy stimuli because their expression change after
84 resistance exercise (56) must be preceded by signaling events that alter their expression.
85 Therefore, hypertrophy regulators such as IGF-1 are not “first-in-line”, initiating hypertrophy
86 stimuli. So why are hypertrophy stimuli important? No matter how we vary resistance
87 exercise variables such as load, repetitions or sets, it is the hypertrophy stimuli that will
88 induce hypertrophic signal transduction and the resultant hypertrophy. Thus if we would

89 know the actual hypertrophy stimuli then we could measure them with the goal of identifying
90 interventions that maximally induce these signals.

91
92 The aim of this review is to summarize our current understanding of candidate hypertrophy
93 stimuli and sensors in three sections. First, we will discuss evidence that mechanical signals
94 can act as hypertrophy stimuli after resistance exercise. In the second and third sections we
95 will review evidence that exercise-induced muscle damage and metabolic signals,
96 respectively, can trigger or augment a muscle hypertrophic response to resistance exercise.
97 We aim to reconcile differences wherever possible and we will end with a statement of
98 research directions.

99

100 **Is mechanical load a hypertrophy stimulus?**

101 Several reviews already discuss how mechanical stimuli could trigger a skeletal muscle
102 hypertrophic response (18, 67, 127). Here we provide an update with a focus on mechanical
103 stimuli of muscle hypertrophy and their sensors. Mechanical signals are arguably the most
104 intuitive hypertrophy stimuli. This is based on three lines of indirect evidence. First, muscles
105 atrophy when mechanical load is reduced through limb immobilization (e.g. (122), reviewed
106 by (6)]. This suggests that a “normal” mechanical loading pattern is essential for baseline
107 muscle mass. Second, Alfred Goldberg (51) and others have mechanically overloaded
108 muscles such as the plantaris in rodents through the ablation of plantar flexor synergists, or
109 cast-induced stretch. Because the overloaded muscles hypertrophied in a range of
110 experimental conditions, the researchers concluded that mechanical overload is sufficient for
111 skeletal muscle hypertrophy (reviewed in (57)). The issue with these studies is that the
112 models used do not only alter mechanical load but additionally a host of other, potentially
113 confounding variables such as metabolism, or cause damage. Third, mechanical load is also
114 the key candidate hypertrophy stimulus that links human resistance exercise to skeletal
115 muscle hypertrophy. This is because high forces distinguish hypertrophy-inducing resistance
116 exercise from low load endurance exercise that triggers little or no hypertrophy. However, as
117 we will address later, mechanical loading does not need to be excessive for muscle
118 hypertrophy stimulation. Loads as low as $\approx 30\%$ of the 1RM seem sufficient to trigger a near
119 maximal hypertrophic response (2).

120

121 The importance of mechanical load for muscle growth was demonstrated in a study where
122 either young (24 ± 6 years) or older (70 ± 5 years) males completed similar work (i.e. the
123 force x time-under-tension product) of leg extensor exercise at 20-90% of the 1RM. This
124 study showed greater muscle protein synthesis (labelled the fractional synthetic rate, FSR) at
125 higher loads peaking between 60 and 90% of the 1RM (84). A caveat to these findings is that,

126 in an effort to equate workload, participants did not exercise to failure, especially when using
127 lighter loads. To study the effect of different loads on muscle hypertrophy whilst training to
128 failure, Lasevicius et al exercised subjects for 12 weeks using leg extension and elbow
129 extension with one leg or arm at 20% 1RM and then either 40%, 60% or 80% with the
130 opposite leg or arm (86). This study showed that resistance training of at least 40% of the
131 1RM to failure caused a similar amount of hypertrophy as the higher load conditions. This
132 finding is in line with a meta-analysis that concluded that lower load ($\leq 60\%$ 1RM) resistance
133 training causes a similar degree of hypertrophy as higher load ($>60\%$) resistance training
134 (135). In untrained individuals even submaximal aerobic training (i.e. low mechanical load
135 exercise) (77), or very low loads (16% of the 1RM) can increase muscle protein synthesis
136 somewhat (4). In summary, a large amount of mainly indirect evidence suggests that
137 mechanical load is a key hypertrophy stimulus associated with resistance exercise. However,
138 the actual loads do not need to be excessive as loads of $\approx 30\%$ of 1RM seem sufficient to
139 trigger near maximal hypertrophic gains.

140

141 **Candidate molecular sensors that are capable of sensing mechanical load in skeletal**
142 **muscle.** Life on Earth evolved in an environment where a gravity of 9.8 m s^{-2} mechanically
143 loaded organisms. It is therefore no wonder that living beings and their cells have not only
144 evolved mechanical structures such as the muscles, the skeleton and cytoskeleton to
145 withstand or overcome the pull of gravity, but also a plethora of sensors that detect
146 mechanical stimuli. Such mechanosensors not only help cells to adapt to the direct force of a
147 muscle fiber contraction, but also to adapt to more indirect mechanical signals such as shear
148 stress, deformation, compression, and the stiffness of the extracellular matrix that surrounds
149 each cell (18, 49, 145). In this section we discuss several types of candidate
150 mechanosensors that allow muscle fibers to sense mechanical signals during and after
151 resistance exercise, and trigger hypertrophic signaling and skeletal muscle hypertrophy.

152

153 **Mechanosensors within the skeletal muscle force transduction system.** Skeletal muscle
154 fibers are unique because they generate much higher forces than non-muscle cells. Single,
155 skinned human type I and IIa muscle fibers have been reported to generate forces of
156 532 ± 208 and $549 \pm 262 \mu\text{N}$, respectively (81), with each myosin contributing $\approx 6 \text{ pN}$ (120).
157 Non-muscle cells can also produce force through their actin-cytoskeleton but the forces are
158 lower. For example, fibroblasts have been reported to produce forces of $16 \pm 7 \mu\text{N}$ per cell
159 (79). Whilst these force values are just examples, they demonstrate that striated muscle
160 fibers are unique in their high force-generating ability.

161

162 The forces generated by the sarcomeres of a muscle fiber are transmitted to tendons and
163 bones via two force-transducing systems:

- 164 1) Forces are transmitted longitudinally from one end of a muscle fiber to the other end.
- 165 2) Forces are additionally transmitted laterally from the sarcomere through the muscle fiber
166 membrane (sarcolemma) to the extracellular matrix (141) via costameres (73) which are
167 the focal adhesion equivalent in muscle fibers.

168

169 There are several candidate mechanosensors in the skeletal muscle force transduction
170 systems. For a true hypertrophy-triggering mechanosensor, a mechanism must exist by
171 which force modifies the mechanosensor to trigger an early signaling response that then
172 initiates hypertrophic signaling and muscle hypertrophy. Here we discuss costameres, titin
173 and filamin-C-Bag3 signaling as potential mechanosensors in the force transmission systems
174 of muscle fibers.

175

176 **Costamere-related mechanosensors.** Historically, mechanical stimuli became a research
177 focus when researchers discovered in the 1950s that cancer cells can grow on soft agar
178 without anchorage whereas most non-cancer cells cannot. Researchers then discovered
179 from the 1970s onwards that cells anchor the extracellular matrix through focal adhesion
180 complexes that include proteins such as vinculin, talin and integrins as well as kinases
181 including focal adhesion kinase or integrin-linked kinase (Ilk). Focal adhesions not only
182 anchor cells on a substrate but also connect the exterior mechanically to the cytoskeleton
183 and can sense and trigger adaptations to mechanical stimuli (72, 145).

184

185 Costameres are the functional equivalent of focal adhesions in skeletal muscle. They are Z-
186 disc associated structures of muscle fibers that are related to focal adhesions of other cells.
187 Costameres connect the cytoskeleton to the extracellular matrix and also transmit force
188 laterally from the sarcomere to the extracellular matrix. There are two costamere complexes
189 which are the dystrophin-glycoprotein complex and the vinculin-talin-integrin complex.
190 Costameres are clearly essential for normal muscle function as the mutation of costamere
191 genes such as the dystrophin-encoding *DMD* gene often result in severe muscle diseases
192 such as Duchenne muscular dystrophy (73). Given that these complexes function to anchor
193 muscle fibers on the extracellular matrix to transmit force laterally, can they potentially
194 function as sensors that sense mechanical stimuli? Is there evidence that costamere-
195 associated proteins are hypertrophy sensors?

196

197 In skeletal muscle, focal adhesion kinase (the protein is abbreviated as FAK and encoded by
198 the gene *PTK2*) is a nonreceptor tyrosine kinase that moves to focal adhesions upon the

199 adhesion of a cell to a substrate (54). In cultured C2C12 myotubes, IGF1 can increase FAK
200 Tyr397 autophosphorylation and FAK is required for IGF1-induced hypertrophy and Tsc2,
201 mTOR and S6K1 signalling (28). However, it is unclear whether and how FAK itself is
202 activated by mechanical load during resistance exercise and whether there is a mechanical
203 hypertrophy sensor that can activate FAK. At the moment there is no compelling evidence
204 that FAK is directly activated by mechanical load during resistance exercise because unlike
205 filamin-C or titin, FAK does not appear to have a mechano-activated protein domain.
206 Moreover, 4 sets of 10 repetitions of resistance exercise did not affect activity-related FAK
207 Tyr576/577 phosphorylation 6 h after exercise in fasted and fed individuals (50). However,
208 phosphorylated FAK Tyr397 was increased 60-90 minutes post eccentric exercise when
209 compared to concentric bout exclusively at the distal site of the vastus lateralis muscle (43).
210 Generally, whilst FAK might help to regulate muscle size, there is no evidence yet that FAK
211 is linked to a mechanosensor that senses mechanical load as a hypertrophy stimulus during
212 resistance exercise.

213

214 Focal adhesions are associated with phosphatidic acid-generating enzymes, such as
215 phospholipases. Recently, it has been shown that mechanical stimuli in the form of
216 attachment to either a soft or stiff substrate promote the conversion of phosphatidylinositol
217 4,5-bisphosphate (PIP₂) to phosphatidic acid. This synthesis of phosphatidic acid was
218 catalyzed by phospholipase C γ 1 (PLC γ 1) and activated the Hippo pathway effectors Yap
219 (Yes-associated protein 1, gene *Yap1*) and its paralogue Taz (gene *Wwtr1*) (98). Yap and
220 Taz are mechanosensitive (34) transcriptional co-factors that regulate gene expression
221 mainly by co-activating Tead1-4 transcription factors. Yap and Taz regulate muscle
222 differentiation, satellite cell function (157), are affected by many exercise-associated stimuli
223 (47) and increased Yap activity in muscle fibers can cause hypertrophy (52, 159). Whilst
224 these papers suggest no link to mTORC1 and even demonstrate that Yap can cause
225 hypertrophy with rapamycin treatment (52), there are known links between Yap and
226 mTORC1. Yap has been reported to suppress the mTORC1 inhibitor Pten (151) and to
227 induce the expression of *Slc7a5* and *Slc3a2* that encode the Lat1 amino acid transporter (58).
228 Whilst Pten expression does not decrease in the vastus lateralis 2.5 h and 5 h after human
229 resistance exercise (156) and in synergist-ablated, hypertrophying plantaris muscle (21), the
230 expression of the Lat1-encoding genes *Slc7a5* and *Slc3a2* as well as of other Yap targets
231 such as *Ankrd1* increases in both situations. Collectively this suggests a scenario where
232 mechanical load, via an as yet unknown sensor, increases phosphatidic acid to activate Yap
233 and Taz. Yap and Taz then increase the abundance of Lat1 which would sensitize the
234 mechanically loaded muscle to leucine stimulation of mTORC1. However, phosphatidic acid
235 not only modulates Hippo signalling but importantly for muscle, it can also activate mTORC1

236 (68), which is the primary regulator of muscle protein synthesis. Indeed, hypertrophy-
237 inducing eccentric contractions increased the concentration of phosphatidic acid for up to 60
238 minutes in tibialis anterior muscles (109). Moreover, inhibition of phosphatidic acid synthesis
239 by butanol prevents the phosphorylation of mTORC1 activity markers, suggesting that
240 phosphatidic acid is a mediator of eccentric exercise-induced hypertrophic signalling (109).
241 Whilst the Hornberger group first identified Z-disc-linked phospholipase D (PlD) as a
242 phosphatidic acid-synthesizing enzyme (i.e. phosphatidic generating-enzymes are not only
243 located in focal adhesions), they later identified a reaction catalyzed by diacylglycerol kinase
244 ξ (Dgk ξ) as another source of phosphatidic acid in mechanically loaded muscle (164).
245 Collectively, these studies suggest that mechanical stimuli can activate phospholipases to
246 synthesize phosphatidic acid which in turn can activate mTORC1 and the Hippo effectors
247 Yap and Taz. However, whilst these studies elucidate key signalling mechanisms in-between
248 the mechanical stimulus and hypertrophy-mediating pathways, neither study identifies the
249 actual mechanosensor. To identify the actual, phosphatidic acid synthesis-stimulating
250 mechanosensor is a key task for future research in this area.

251
252 Integrins are another protein group that are part of costameres. Specifically, the $\alpha_7\beta_1$ -integrin
253 isoform (encoded by the gene *Itga7*) has been linked to muscle size as $\alpha_7\beta_1$ -integrin
254 overexpressing mice have larger muscle fibers and increase muscle fiber size after eccentric
255 exercise training when compared to wild-type mice. Also mTOR and its downstream target
256 p70S6k are more phosphorylated at activity-related residues at rest and after eccentric
257 exercise in $\alpha_7\beta_1$ -integrin overexpressing mice (167), suggesting that $\alpha_7\beta_1$ -integrin might help
258 to activate mTORC1 signaling in response to exercise. However, it is unknown whether and
259 how $\alpha_7\beta_1$ -integrin is activated by a mechanical hypertrophy stimulus during resistance
260 exercise, and how $\alpha_7\beta_1$ -integrin then activates mTORC1 and other signaling proteins that
261 cause the muscle fiber to hypertrophy.

262
263 Costamere-based mechanosensors may also sense two additional types of mechanical
264 stimuli that have been discussed as hypertrophic triggers in the more applied literature. The
265 first stimulus is muscle fiber swelling, which is known as the “pump” by exercisers. The
266 second potential mechanical stimulus is a change in the stiffness of the extracellular matrix
267 as a result of resistance exercise. We will briefly discuss these two potential mechanical
268 stimuli here. Resistance exercise results in a temporary perception frequently described as a
269 “pump,” which is interpreted as muscle fiber swelling (134). Moreover, exercise-induced
270 muscle damage (EIMD) can also lead to muscle swelling (116), although the associated
271 edema from EIMD can last far longer than the “pump”. Whilst little definite evidence exists for
272 actual muscle fiber swelling (i.e. a swelling of the muscle fiber and not of the interstitium)

273 after resistance exercise, at least the whole muscle can swell as a result of single-bout of
274 resistance exercise (39). In primary rat myotubes, swelling brought about by culture in a
275 hypoosmotic culture medium increases glutamine uptake by 71% when compared to isotonic
276 culture medium. This is dependent on integrins and the cytoskeleton, as integrin or
277 cytoskeleton inhibitors prevent this effect (90). Together these data suggest that
278 differentiated muscle can respond to cell swelling with increased glutamine uptake and that
279 this depends on integrin or cytoskeletal loading. Such glutamine intake is potentially
280 important, as it is a requirement for the uptake of protein synthesis-stimulating essential
281 amino acids such as leucine (105). However, it is unknown whether the duration and extent
282 of swelling is sufficient to load the cytoskeleton and that such cytoskeletal loading does not
283 only induce glutamine uptake but also protein synthesis for up to three days post resistance
284 exercise (99). Muscle swelling also occurs up to several days after exercise-induced muscle
285 damage (166) at a time when muscle protein synthesis should have returned to baseline (99).
286 Given that costameres are the sites where the cytoskeleton connects to the extracellular
287 matrix and where mechanical signals can be sensed, it seems likely that any fiber swelling
288 exerts a strain on costameres which then could trigger the hypertrophy response.

289
290

291 **Titin (gene: *Ttn*).** Titin is a giant protein that is essential for muscle function and human
292 health as mutations in the titin-encoding *Ttn* gene cause various human genetic diseases
293 including myopathies (130). Titin spans half a sarcomere, from the Z-disc at the end of a
294 sarcomere to the M-line in the middle (82). The I-band-spanning portion of titin is elastic and
295 contributes to the elasticity of a passively stretched muscle. The M-line portion of titin
296 contains a stretch-activated kinase. The kinase within the titin protein is activated when a
297 stretch pulls several amino acids out of a so-called ATP-binding pocket, allowing ATP to bind.
298 ATP binding then causes titin to tyrosine-phosphorylate itself, which in turn activates the
299 kinase within the titin protein (124). Because of its stretch-activated kinase and association
300 with numerous other proteins, titin has been proposed to be an exercise-related
301 mechanosensor (82). Using our terminology, mechanical load would be the hypertrophy
302 stimulus and titin the hypertrophy sensor.

303

304 So what is the evidence for titin being a mechanical hypertrophy sensor? There are two
305 points to consider. First, titin lies parallel to the force-generating actin-myosin proteins. This
306 means if myosin and actin generate force and shorten a muscle fiber then titin will go slack.
307 Consequently, the forces within a titin molecule should actually decrease rather than
308 increase during a concentric contraction. Thus, titin cannot be a true force sensor in this
309 situation. However, at longer muscle lengths titin forces increase and titin unfolds (62) and so

310 this might activate titin kinase and trigger downstream signaling events. Related to this,
311 resistance training at longer muscle lengths may cause a greater hypertrophy when
312 compared to resistance training with shorter muscle length (96, 107).

313

314 Second, whilst many signaling interactions have been reported for titin (82) there is not yet a
315 convincing link between titin and mTORC1 signaling, which is the primary mediator of the
316 muscle hypertrophy response to resistance exercise (see above). However, some titin
317 signaling interactions are related to protein turnover through Murf1/2-proteasome and
318 autophagy signalling and thus could regulate some aspects of muscle hypertrophy (82). In
319 conclusion, whilst titin is a mechanosensitive skeletal muscle protein with a kinase domain it
320 seems unlikely that it is the major mechanical hypertrophy sensor during standard resistance
321 exercise, except perhaps at long muscle lengths.

322

323 **Filamin-C Bag3 (genes: *FlnC* and *Bag3*).** Bag3 and filamin-C are proteins important for
324 muscle function as mutations of these proteins cause severe myofibrillar myopathies (137).
325 Filamin-C and Bag3 localize to the Z-disc in human muscle (153). Here, we discuss evidence
326 that filamin-C and Bag3 form mechanosensor complex that is capable of activating mTORC1,
327 the Hippo effector YAP1 and autophagy (see **Figure 1**). Filamins are mechanosensitive,
328 actin-crosslinking molecules. In skeletal muscle, filamin-C is the major filamin located at the
329 Z-disc (137). Filamins form V-shaped homodimers and forces of $\approx 5\text{-}20$ pN deform the so-
330 called domain pair 20-21 (128). One myosin head generates a force of 6 pN (120) and thus
331 actin-linked filamins should deform if sufficient myosin heads pull on the actin to which the
332 filamins are attached.

333

334 In addition, filamins bind multiple proteins including the androgen receptor (112), which
335 influences muscle size (71), and the Z-disc linked protein Bag3 (153) which has been
336 proposed to sense the mechanical loading of filamin (152). However, how a mechanically
337 loaded filamin dimer activates Bag3 is still unclear. Assuming that mechanically loaded
338 filamin-C can activate Bag3, how could Bag3 trigger a hypertrophic signaling response?
339 BAG3 connects through its WW domain (WW stands for the two tryptophanes that are
340 separated by ≈ 20 amino acids (142)) to proline-rich motifs (e.g. PPxY motifs) of other
341 proteins to potentially regulate three muscle hypertrophy-associated functions:

342 1) **mTORC1 signaling.** The WW domain of Bag3 binds the proline-rich motif of the
343 mTORC1 inhibitor tuberous sclerosis 1 (TSC1). So the hypertrophy-inducing mechanism
344 might be that Bag3 sequesters TSC1 away from mTORC1, resulting in mTORC1
345 activation and increased protein synthesis in response to mechanical loading (75).

346 2) **Hippo signaling.** Bag3 sequesters through its WW domain proteins such as LATS1 and
347 AMOTL1 which normally inhibit the Hippo effector YAP (152). As a consequence, YAP
348 will be more active in mechanically loaded muscle, which is relevant for muscle size
349 because increased YAP activity in muscle fibers can elicit muscle fiber hypertrophy (52,
350 160).

351 3) **Autophagy.** Bag3 binds synaptopodin-2 (Synpo2) to regulate chaperone-assisted
352 selective autophagy (CASA) of damaged Z-disc proteins (7, 154). This might contribute to
353 the increased autophagy (61) and rate of protein breakdown seen after resistance
354 exercise (149), a process that may be important in full and functional muscle hypertrophy.

355
356 Phosphoproteomic studies have shown that both Filamin-C and Bag3 change their
357 phosphorylation after high intensity exercise in human muscle (65) and after maximal
358 intensity stimulation of mouse skeletal muscle (121). This suggests that Filamin-C and Bag3
359 are additionally targeted by currently unknown kinases and phosphatases that might further
360 help to regulate Bag3 activity in a contracting skeletal muscle.

361
362 The aforementioned Bag3-focussed hypertrophy stimulus-sensing mechanisms are
363 illustrated in **Figure 1**.

364
365

366 **Please insert Figure 1 here.**

367
368 In summary, a filamin-Bag3-mTORC1/YAP/autophagy signaling cascade is a plausible but
369 far from completely characterised mechanism by which mechanical loading during resistance
370 exercise could stimulate hypertrophic signaling and skeletal muscle hypertrophy. However,
371 whilst physiological, mechanical forces will probably deform a filamin homodimer, it is unclear
372 how this then activates Bag3 and other hypertrophic signaling. Also the kinases and
373 phosphatases that phosphorylate and dephosphorylate Filamin-C and Bag3 during exercise
374 are currently unknown and it remains unclear as to how such phosphorylation affects
375 Filamin-C and Bag3 function and muscle size. This is clearly another important area for
376 future research.

377
378 **Nuclear deformation and signal transduction.** In muscle fibers, myonuclei are surrounded
379 by thick tubulin filaments (17) and by intermediate desmin filaments (126). These filaments
380 not only anchor myonuclei to the cytoskeleton but also expose them to forces when the
381 cytoskeleton is loaded (8) either by a passive stretch, by an active contraction or by muscle
382 fiber swelling. For example, when muscle fibers are passively stretched, myonuclei

383 subsequently deform (113). Intriguingly, such nuclear deformation has recently been
384 identified as a mechanism by which mechanical load causes the Hippo effector Yap and
385 potentially other proteins to translocate from the cytosol to the nucleus (37). Given that
386 increased YAP activity can induce muscle fiber hypertrophy (52, 160), this might be a
387 mechanism by which mechanical loading could contribute to skeletal muscle growth.
388 Together the above filamin-Bag3-YAP and nuclear deforming-YAP-mTORC1 signaling
389 cascades are plausible mechanisms by which a mechanical hypertrophy stimulus could be
390 sensed and trigger hypertrophy signaling. However, there are two caveats to this hypothesis.
391 First, YAP-induced muscular hypertrophy is comparatively small and seems to be
392 independent of mTORC1 as it can occur when mTORC1 is blocked with rapamycin (52).
393 Second, myonuclear deformation has so far only been demonstrated for passive stretch
394 (113), and not for an active, shortening contraction. Nevertheless, proteins that sense nuclei
395 deformation to activate Hippo signaling should be characterized in the future.

396
397 Another type of mechanosensor are stretch-activated ion channels encoded by the genes
398 *PIEZO1* and *PIEZO2*. Spangenburg and McBride demonstrated that broad, non-specific
399 inhibition of stretch-activated ion channels in rats *in vivo* with streptomycin or gadolinium
400 could attenuate the load-induced activation of mTORC1 (140). However, the expression of
401 *PIEZO1/2* stretch-activated ion channels is among the lowest in human skeletal muscle when
402 compared to other tissues (www.gtexportal.org, see (97)) and so the effect might not depend
403 on the inhibition of PIEZO1/2 channels in skeletal muscle. For that reason we do not discuss
404 stretch-activated ion channels further.

405
406 In summary, there are several plausible but far from completely characterised mechanisms
407 by which mechanical hypertrophy stimuli could activate mechanical hypertrophy sensors
408 after a bout of resistance exercise. It may well be that there are several mechanical
409 hypertrophy stimuli (e.g. the contraction force, loading of the cytoskeleton and the
410 mechanical properties of the extracellular matrix) and sensors as has previously been
411 proposed by the Hornberger group (45). To date no mechanism is fully characterised as
412 either the mechanosensing mechanism or the link to mTORC1 or other hypertrophy-
413 regulating signaling proteins is incompletely described in skeletal muscle. Research into such
414 mechanisms is further hampered by the fact that the knockout of putative mechanosensors
415 such as Bag3 not only abolishes a potential hypertrophy response to resistance exercise, but
416 often leads to severe myopathies and dystrophies. This means that researchers can in many
417 cases not use global knock out animal models to test whether these proteins are essential for
418 the hypertrophy response to exercise.

419

420 **Is exercise-induced muscle damage a hypertrophy stimulus?**

421 The possible role of exercise-induced muscle damage (EIMD) as a hypertrophy stimulus has
422 been discussed and studied since it was proposed in the 1990's (29, 38, 132). EIMD is
423 damage that is triggered when individuals engage in new types of exercise, especially
424 lengthening or eccentric contractions conducted with a large range of motion (115, 132).
425 However, there is usually little EIMD when already resistance-trained individuals lift weights
426 due to the "repeated bout effect". EIMD is associated with microscopic, structural changes
427 such as Z-line streaming in skeletal muscle myofibrils. This is then usually followed by a local
428 inflammatory response, disturbed Ca^{2+} regulation, activation of protein breakdown and
429 increased levels of proteins such as creatine kinase in the blood that escape or are secreted
430 from damaged muscle fibers (23, 76, 115). In their review, Hyldahl and Hubal (2014) propose
431 a continuum of skeletal muscle fiber damage after eccentric exercise that spans possible
432 adaptive cell signaling responses to pervasive membrane damage and tissue necrosis as the
433 most severe form of EIMD (69).

434

435 **Evidence from human studies for EIMD as a hypertrophy stimulus.** Although some
436 authors have endeavored to test whether EIMD contributes to muscle hypertrophy, the
437 results of these interventions are difficult to interpret. This is because the manipulation of
438 resistance training parameters to alter EIMD can also directly affect muscle mass, not just
439 EIMD. Therefore it is difficult to separate the effect of EIMD on muscle hypertrophy from the
440 effect of the confounding factors. For instance, training at long muscle lengths (i.e. the
441 stretched position) is not only associated with a greater magnitude of EIMD (11, 115) but
442 also possibly with increased muscle hypertrophy when compared to exercising with short
443 muscle lengths, at least in some muscles (14, 107) . However, this may not be due to EIMD
444 but due to the larger force production at longer fascicle lengths (40). Similarly, eccentric
445 muscle actions not only increase EIMD but also cause a slightly larger hypertrophic response
446 than concentric muscle action (32, 136). Again, it is unclear whether this is due to a higher
447 dose of an EIMD-associated hypertrophy stimulus after eccentric exercise (100) or simply
448 due to a confounding factor such as increased training load (36, 100). Collectively some
449 studies suggest a connection between EIMD and muscle hypertrophy but this could be due
450 to confounding factors.

451

452 In contrast, other studies show that the extent of muscle damage does not correlate with
453 muscle protein synthesis (48) or the magnitude of hypertrophy. Severe EIMD does not give
454 any further benefit on hypertrophy but rather attenuates it (42). Flann et al compared muscle
455 hypertrophy of naïve and pre-trained group with the same cumulative workload. The pre-
456 trained group did not experience EIMD as judged by plasma creatine kinase levels and

457 muscle soreness but increased muscle strength and volume at the same magnitude as the
458 naïve group suggesting that EIMD is not essential for hypertrophy (41). However, in an effort
459 to reduce EIMD, the pre-trained group performed an additional three weeks of resistance
460 training, which may have confounded results.

461
462 A final argument against EIMD as a hypertrophy factor is that EIMD also occurs after
463 exercise that does not typically induce hypertrophy. For example EIMD occurs after
464 endurance exercise with an eccentric component such as marathon running (63) but damage
465 in these situations alone does not seem to cause muscle hypertrophy. If anything, marathon
466 running decreases muscle fiber size (150). However, these data are again difficult to interpret
467 as endurance athletes may have a low trainability for muscle hypertrophy and their long-
468 duration exercise, combined with low energy availability, may excessively activate AMPK and
469 thereby inhibit mTORC1 (70) for long periods. In summary, it is difficult to conclude based on
470 indirect human studies whether and how EIMD contributes to muscle hypertrophy. The key
471 reason for this is that it is virtually impossible to separate direct EIMD stimuli from
472 confounding stimuli that co-occur with EIMD.

473
474 **Muscle damage or increased regeneration alone may induce muscle hypertrophy.** The
475 regeneration of skeletal muscle after injury is a (re-) growth process but can injury per se
476 promote muscle fiber hypertrophy? In mice, severe injury of mouse tibialis anterior muscles
477 e.g. through cardiotoxin injection results in larger, but fewer muscle fibers when compared to
478 uninjured fibers (59), suggesting that injury alone is sufficient to trigger the hypertrophy of
479 some muscle fibers. A caveat is that we do not know whether the larger fibers are
480 hypertrophied, regenerated fibers or whether these are new but muscle fibers that are larger
481 than the previous muscle fibers. There is some evidence that injured muscle fibers and their
482 satellite cells can contribute to hypertrophy as transplanting muscle fiber-associated satellite
483 cells into a recipient muscle whilst inducing injury results in a near-lifelong muscle
484 hypertrophy (55). Together, these data suggest that injury alone and the combination of
485 injury and more satellite cells can lead to the development of larger muscle fibers or induce
486 muscle fiber hypertrophy.

487
488 **Satellite cells, EIMD and muscle hypertrophy.** Satellite cells are the resident stem cells of
489 skeletal muscle (131) and add nuclei to adult muscle fibers after resistance training (24).
490 Although non-damaging exercise can activate satellite cells to proliferate (27), satellite cell
491 activation and proliferation is larger after exercise that induces EIMD (26). In humans,
492 individuals that responded with greater hypertrophy to a resistance training programme also
493 added more myonuclei, presumably derived mainly from satellite cells, than individuals that

494 responded less with less hypertrophy to the same training programme (118). This suggests
495 that the ability of satellite cells to proliferate and to add new myonuclei to muscle fibers might
496 limit muscle hypertrophy. However, satellite cells may expand especially in response to
497 EIMD to have a role in muscle repair and less so to increase myonuclei when muscle
498 actually hypertrophies, at least in the early stages of muscle growth (30).

499

500 The causal role of satellite cells on muscle hypertrophy has been investigated in mice. It
501 seems that the initial hypertrophy in response to mechanical overload can occur in wildtype
502 and satellite cell-depleted muscles (95, 103). However, the initial hypertrophy cannot be
503 maintained for months when satellite cells are depleted (46). Other research suggests that
504 satellite cells are also required for the initial hypertrophy at the muscle fiber level (35).
505 Collectively these studies show that satellite cells are essential for full skeletal muscle
506 hypertrophy over time and that satellite cell numbers and myonuclei increase after resistance
507 training. It is not, however known whether EIMD is essential in the long run to induce satellite
508 cells to proliferate and in turn trigger a muscle hypertrophic response to resistance training.

509

510 However, our main question in the present review is not whether satellite cells are essential
511 for hypertrophy but: How do hypertrophy stimuli activate satellite cells in the first step and
512 how do activated satellite cells cause muscle fiber hypertrophy in a second step? According
513 to our definition, the EIMD-related hypertrophy stimulus would be the repeated mechanical
514 load that causes muscle damage in a susceptible muscle. A damage-associated stimulus
515 would then activate satellite cells in the first step. There are too many possible stimuli
516 activating satellite cells to be effectively covered in this review. Currently the strongest
517 candidate pathway to activate quiescent satellite cells to proliferate following injury as well as
518 after exercise or mechanical stretching is the nitric oxide-metalloproteinase-hepatocyte
519 growth factor (HGF) pathway (147). Whether these stimuli activate satellite cells in a context
520 of resistance exercise bout especially after EIMD is unknown.

521

522 **Other potential EIMD-associated hypertrophy stimuli and their sensors.** EIMD is
523 associated with potential hypertrophy stimuli such as amino acids that result from protein
524 breakdown or factors linked to the immune and inflammatory response to EIMD and to
525 satellite cells. As a consequence of EIMD, inflammatory cells enter muscles and produce
526 substances including myokines such as IL-6 that have been reported to be able to both
527 increase (138) or decrease muscle size (10) in different contexts. The inflammatory response
528 to EIMD is thought to also induce cyclooxygenase production, which may aid hypertrophy as
529 non-steroidal anti-inflammatory drugs (NSAID, which target cyclooxygenase) blunt
530 hypertrophy following regimented resistance training (88). There is also evidence that

531 reactive oxygen species (ROS) promote hypertrophy, as antioxidant supplementation can
532 blunt hypertrophic signaling (114) and reduce the magnitude of exercise-induced muscle
533 hypertrophy (12). However, even if IL-6 and ROS can influence muscle size, they are clearly
534 middlemen in the hypertrophic process, as there must be upstream hypertrophy stimuli and
535 sensors that increase their concentration in response to resistance exercise. Moreover, ROS
536 are not only induced by EIMD but also by endurance running (125), which does not typically
537 cause hypertrophy. In summary, the evidence suggesting that EIMD is associated with
538 hypertrophy is mostly indirect, some is contradictory, and putative mechanisms and sensors
539 are incompletely characterised.

540

541 **Is metabolic stress a hypertrophy stimulus?**

542 We have already mentioned that mechanical forces are probably the most important
543 hypertrophy stimuli. When mechanical forces are absent or reduced, other signals typically
544 only have small effects on muscle size. For example, when post-operative brace-immobilized
545 knee surgery patients intermittently occluded their thighs, their muscles atrophied by $\approx 7\%$
546 within 14 days which was significantly less than the $\approx 15\%$ atrophy seen in the no occlusion
547 controls (146). This experiment suggests that potential occlusion-related hypertrophic stimuli
548 cannot compensate for the loss of mechanical loading but that they can limit atrophy.
549 However, when combining vascular occlusion with dynamic muscular contractions, marked
550 hypertrophy invariably occurs, even when employing relatively light loads or no external
551 loads at all (1, 89). In these training regimes, the vascular occlusion increases metabolic
552 stress as judged by the drop in phosphocreatine (PCr) and pH (143). Similarly, muscles
553 hypertrophy more if resistance training with relatively heavy load is conducted under
554 intermittent hypoxia versus normoxia (85, 93, 106). The fact that blood flow restriction and
555 hypoxia affect metabolism has led some researchers to suggest that metabolic stress-
556 associated signals such as metabolites (i.e. molecules involved in metabolism that are
557 typically below ≈ 1500 Da) may have an anabolic effect and contribute to muscle hypertrophy
558 (133). An alternative proposal is that “*metabolites simply augment muscle activation and*
559 *cause the mechanotransduction cascade in a larger proportion of muscle fibers*” (31). This is
560 another way of saying that some fibers fatigue during contraction, which is linked to changes
561 in metabolite concentrations such as a drop of phosphocreatine or increase of lactate. As a
562 consequence, additional fibers need to be recruited to sustain force output and these
563 additional fibers are then additionally exposed to hypertrophy stimuli. However, recent work
564 found that the addition of blood flow restriction training to a traditional resistance training
565 program preferentially enhanced type 1 fiber cross sectional area in a cohort of elite
566 powerlifters (13). This seemingly refutes the hypothesis that the hypertrophic effects of blood
567 flow restriction training are simply a function of increased high-threshold motor unit

568 recruitment, and raise the possibility that the associated metabolite accumulation may induce
569 anabolism via other mechanisms. Henceforth, we discuss the potential role of metabolites as
570 hypertrophy stimuli.

571

572 **Metabolic stress.** Metabolic stress can be defined as the changes in energy metabolism
573 and metabolites that occur during non-steady state muscle contractions. Non-steady state
574 contractions are contractions where not all of the hydrolyzed ATP can be resynthesized by
575 oxidative phosphorylation alone. As a consequence, the concentration of PCr will
576 continuously decline as PCr resynthesizes ADP to ATP via the Lohmann reaction
577 ($\text{PCr} + \text{ADP} \leftrightarrow \text{ATP} + \text{creatine}$). Moreover, the lactate concentration will rise and the pH will drop
578 as ATP is additionally resynthesized through glycolysis. Thus, a low PCr concentration, a
579 high lactate concentration and a low pH are biomarkers for metabolic stress. In relation to
580 these metabolites, blood flow restriction will not change the rate of ATP hydrolysis but it will
581 reduce oxygen delivery and oxidative ATP resynthesis, which requires greater PCr
582 breakdown and a higher rate of glycolysis in active muscle fibers (143, 144).

583

584 **Metabolic stress during resistance exercise versus other types of exercise.** The higher
585 the exercise load, the more ATP will be hydrolyzed per second and the faster PCr, lactate
586 and the pH will change. Thus, during high intensity resistance exercise, the PCr
587 concentration and the pH will drop more per second than during low load resistance exercise
588 (143, 144, 158). However, as metabolic stress either causes fatigue or is associated with it
589 (5), metabolic stress will be higher at the end of a longer duration set with low loads because
590 we can lift a lower load with a more fatigued muscle than during a shorter duration set with
591 high loads as we can only lift a high load if fatigue and metabolic stress are low.

592

593 The logic that a set with lower loads to exhaustion will cause more metabolic stress than a
594 set with heavy loads is supported by experimental data. In a biopsy study, Tesch et al
595 measured intramuscular PCr and other metabolites in the vastus lateralis before and after
596 several sets of ≈ 10 repetition leg muscle contractions to failure in trained bodybuilders.
597 Intramuscular PCr decreased from 21.3 ± 3.7 mmol/kg pre exercise to 10.9 ± 2.5 mmol/kg (51%
598 of pre-exercise) after the last set of exercise (148), suggesting moderate metabolic stress. In
599 contrast, during intermittent resistance exercise with 25% of the 1RM which is suboptimal for
600 hypertrophy, PCr decreased to $17 \pm 12\%$ of the pre-exercise concentration in adult women
601 and $18 \pm 16\%$ of the pre-exercise concentration in adult men, respectively (74), suggesting
602 high metabolic stress. Similarly, PCr decreased from 15.8 ± 1.7 mmol/kg to 1.7 ± 0.4 (11% of
603 pre-exercise) after a 400 m run (64). Collectively this shows that metabolic stress is typically

604 greater during non-steady state exercise with intensities that are sub-optimal for hypertrophy
605 (86) than during “classic” ≈ 10 repetition resistance training in trained individuals.

606

607 **Metabolites that have anabolic signaling properties.** Metabolic stress is a vague concept
608 given that ≈ 2700 metabolic enzymes catalyze ≈ 900 metabolic reactions (129) and that ≈ 4000
609 metabolites can be detected in human serum alone (123). So given the plethora of
610 metabolites, are there any metabolites or other metabolic stress-related factors that can act
611 as hypertrophy stimuli? Are there any metabolites that can be considered to be hypertrophy
612 stimuli according to our definition?

613

614 Lactate is a key biomarker for metabolic stress, used as a biomarker for performance, and
615 one of the most studied exercise metabolites. There is some evidence that lactate may affect
616 muscle differentiation and have some anabolic effects (104). In the most extensive study to
617 date, lactate affected the expression of regulators of muscle differentiation in vitro. Also the
618 authors found that a combination of a 30 min low-intensity running training program together
619 with a dose of lactate and caffeine increased muscle mass and hypertrophic signaling in rats
620 (111). It is not possible to conclude, however, how much of the hypertrophy was due to
621 lactate. Other studies suggest that skeletal muscle may sense changes in extracellular
622 lactate. For instance work from George Brooks’ lab demonstrated that when 20 mM lactate
623 caused L6 rat myotubes to express lactate-related genes (60), but this did not show that
624 lactate is a hypertrophy-stimulus. More recently, Ohno et al found that 20 mM lactate was
625 able to induce anabolic signaling and hypertrophy in C2C12 cells, possibly in a GPR81
626 dependent manner (110). This suggests that extracellular lactate can initiate signaling events
627 through membrane-bound receptors in skeletal muscle. Whilst these data indicate that
628 lactate may be a modifier of muscle signaling and hypertrophy, lactate concentrations are
629 typically highest during exercise that is suboptimal for hypertrophy such as a 400 m run.

630

631 Another anabolism-related energy metabolite is α -ketoglutarate which is not only a citrate
632 cycle metabolite but also a nitrogen scavenger (163). Long term supplementation for 9
633 weeks of the drinking water with 2% α -ketoglutarate resulted in significant gastrocnemius
634 skeletal muscle hypertrophy and increased markers of mTORC1 activity (19), suggesting that
635 α -ketoglutarate could stimulate muscle hypertrophy. In contrast, however, L-arginine α -
636 ketoglutarate supplementation did not increase strength measures such as the 1 RM after a
637 resistance training program in humans when compared to placebo control (161).

638

639 Other anabolic metabolites are phosphatidic acid and lysophosphatidic acid, which can
640 activate mTORC1 (68, 139) and Hippo (165) signaling, respectively. We have already

641 discussed in the mechanotransduction section that hypertrophy-inducing eccentric
642 contractions increase the phosphatidic acid concentrations in tibialis anterior muscles (109).

643

644 Another potential source of hypertrophy-inducing metabolites is from muscle protein
645 breakdown. The activation of skeletal muscle protein synthesis by resistance exercise seems
646 to be correlated to the activation of skeletal muscle protein breakdown (119). Cell based
647 experiments demonstrate that simply increasing the intracellular concentration of key amino
648 acids like leucine by as little as 7% is sufficient for half maximal activation of mTORC1 (22).
649 Additionally, a single bout of resistance exercise in rodents causes an approximate 25%
650 increase in the intramuscular leucine concentration (91). It is theorized that this increase in
651 intracellular leucine, possibly from protein breakdown, is sensed by the amino acid sensor
652 mVPS34 leading to mTORC1 activation (91). However, feeding 40 g of protein can almost
653 triple the intracellular leucine content in human skeletal muscle (92) and it seems unlikely
654 that the small transient changes in intramuscular leucine as a result of resistance exercise
655 make a major contribution to the hypertrophy response to resistance exercise

656

657 In addition to metabolites, metabolic enzymes might also be involved in hypertrophy
658 signaling, too. Researchers found in HEK293 cells and fibroblasts that the glycolytic enzyme
659 glyceraldehyde-3-phosphate dehydrogenase (GAPDH) binds Rheb and inhibit mTORC1
660 signaling. However, when glycolytic flux is high as would be at the end of a set of resistance
661 exercise then GAPDH no longer inhibited mTORC1 and cells grow (87). In this scenario, the
662 signals that activate glycolytic enzymes such as phosphorylase and phosphofruktokinase
663 would be the hypertrophy stimuli and the enzymes would be their sensors. This shows a
664 plausible mechanism by which a signal related to glycolytic flux might act as a hypertrophy
665 stimulus capable of activating mTORC1 and skeletal muscle hypertrophy. These and further
666 links between metabolism, muscle mass and regeneration have recently been reviewed (78).

667

668 **Studies that do not support energy stress being a hypertrophy stimulus.** During
669 evolution, mechanisms evolved that reduce protein synthesis and cell growth when there is
670 metabolic stress. For example, when the metabolic stress-mimicking AMPK activator AICAR
671 was given to rats then muscle protein synthesis reduced significantly to 55% of the protein
672 synthesis measured in control rats (16). Soon after the Guan group demonstrated that the
673 metabolic stress sensor AMPK inhibited mTORC1 via TSC2 (70). Consistent with this, the
674 synergist-ablated plantaris hypertrophied more in AMPK α 1 knockout than wildtype control
675 mice suggesting that energy-stress activation of AMPK can blunt hypertrophy at least in
676 some hypertrophy models (102). However, whilst prolonged metabolic stress might work
677 through such mechanisms to explain reduced muscle hypertrophy during concurrent

678 endurance and resistance training (9), it is unclear whether these studies explain what
679 happens during short term metabolic stress during acute resistance exercise, which might
680 exert its effect via different metabolites and signaling molecules.

681

682 **Overall summary, conclusions and directions for future research**

683 Whilst there is a large amount of mainly indirect evidence about hypertrophy stimuli and their
684 sensors, this evidence is often difficult to interpret and as a consequence many questions
685 remain. Mechanical stimuli stand out as the most likely and most potent hypertrophy stimuli
686 and several potential mechanosensing mechanisms have been partially characterised. To us,
687 a key question is whether muscle fibers, which are the cells that produce the highest forces,
688 have their own specific mechanosensing system in addition to the generic focal adhesions
689 (i.e. costameres in muscle) that sense the mechanical environment of most cells. The Z-disc
690 is a prime striated muscle-specific candidate site for muscle-specific force sensing. Z-discs
691 are not only directly exposed to the forces generated by sarcomeres but Z-discs additionally
692 transmit these forces longitudinally and laterally via costameres (44). Moreover, the Z-disc
693 becomes a signalling hub when muscles contract with high intensity and generate large
694 forces. This is supported by the results of a recent phosphoproteomic study which reported
695 that the majority of Z-disc proteins robustly alter their phosphorylation in response to maximal
696 intensity contractions of mouse muscles. In particular, the Z-disc localized kinases obscurin
697 and Speg change their phosphorylation and the Z-disc localized Filamin-Bag3 complex
698 proteins are also phosphorylated (121, 153). Thus future studies should seek to answer the
699 question “Is it mainly the Z-disc or the costameres where mechanical hypertrophy stimuli are
700 sensed and transduced after resistance exercise?”

701

702 Data suggesting and supporting EIMD or metabolic stress-related hypertrophy stimuli are
703 mostly indirect and the related molecular mechanisms are poorly understood. Moreover,
704 growth can occur in the relative absence of either of these putative signals, lending further
705 support for the hypothesis that mechanical stimuli are the primary hypertrophy stimuli. That
706 said, research indicates that both EIMD and metabolic stress regulate multiple factors
707 involved in the hypertrophic process, and a sound rationale exists whereby their resistance
708 training-induced manifestation may contribute to hypertrophic adaptations. If so, it remains to
709 be determined whether these factors are additive to mechanically-derived signaling or
710 perhaps redundant provided a given level of mechanical force is achieved. Moreover, if these
711 signals are indeed additive, it remains to be determined whether an upper threshold exists
712 beyond which no further growth-related benefits are realized. In particular, any hypertrophic
713 effects of EIMD would almost certainly follow a hermetic curve, with benefits seen only up to
714 a given point and they ultimately inhibit hypertrophy when EIMD is excessive. To this point, a

715 high degree of EIMD impairs a muscle's force-producing capacity, which in turn interferes
716 with an individual's ability to train as well as negatively impacting recovery (80, 108). Thus,
717 there may be a "sweet spot" whereby a combination of mechanical, metabolic, and damage-
718 related signals interact synergistically to promote a maximal hypertrophic response.

719

720 Finally, how to proceed towards the long-term goal of identifying all major hypertrophy stimuli
721 and their sensors? It is clear that the leading researchers must move beyond indirect
722 association studies as there are just too many confounding variables to draw valid
723 conclusions. Force, metabolism and EIMD are all linked and it seems impossible to vary only
724 one of these variables during resistance exercise without varying the others and so such
725 studies are never fully conclusive. One key experiment is to assess whether a putative
726 hypertrophy sensor is essential for the muscle hypertrophy adaptation to resistance exercise.
727 To test for this, the gene that encodes the sensor needs to be knocked out or inhibited
728 pharmacologically to evaluate whether this prevents adaptation to exercise. However, the
729 problem with this approach is that putative hypertrophy sensors such as Bag3 are essential
730 for normal muscle function (66). Hence, their global knock out typically causes a myopathy or
731 dystrophy, which limits the usefulness of such models for studying their role in hypertrophy
732 signaling. Here, more sophisticated transgenic animal models are needed. Strategies could
733 involve targeting the transgenesis to skeletal muscle only, making it inducible and modulating
734 solely those sites of a protein that are likely mediators of the hypertrophy-sensing function.
735 But even a highly targeted transgenesis may cause problems, as mechanosensors may
736 already be essential for normal muscle function. This is a major challenge for researchers in
737 this area. Another strategy to identify the hypertrophy sensor is based on the knowledge that
738 any hypertrophy sensing protein must physically interact with the proteins that mediate
739 hypertrophy further downstream. Here, interaction proteomic studies in resting and
740 resistance trained skeletal muscle could provide some answers (3). For example,
741 researchers could co-immunoprecipitate mTORC1 protein complexes in resting and
742 resistance exercise-trained muscle to see via mass spectrometry analysis what proteins
743 interact with mTORC1 under load or metabolic stress when compared to rest. This might
744 reveal either the hypertrophy sensor itself or intermediate proteins that connect a
745 hypertrophy sensor to mTORC1 and other downstream hypertrophy mediators. Whilst this
746 sounds feasible, it will be a difficult experiment in reality as the interpretation of interaction
747 proteomic experiments is typically hampered by many false positive results.

748

749 In summary, conclusively identifying major hypertrophy stimuli and their sensors is clearly
750 one of the big remaining questions in exercise physiology. However, experimentally this is
751 difficult to achieve, which explains why there is still a large amount of uncertainty despite

752 many studies. We hope that this review helps to update on the status quo and to stimulate
753 future research in this area.

754

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1275 **Figure legends**

1276

1277 **Figure 1.** Schematic overview over how Filamin-C and Bag3 might trigger muscle
1278 hypertrophy in response to resistance exercise (see text for references). 1 Filamin is a Z-
1279 disc-linked protein that binds to actin and becomes deformed in response to mechanical load.
1280 2 Filamin is linked to Bag3 and both Filamin and Bag3 become phosphorylated by unknown
1281 kinases during intense muscle contractions. 3 Bag3 has a WW domain through which is can
1282 bind and sequester proteins with proline-rich PPXY domains including Tsc1, a mTORC1
1283 inhibitor. 4 Bag3 can also sequester inhibitors of the Hippo effector Yap such as Lats1,
1284 Amotl1, and Amotl2. Alternatively, YAP might be important into myonuclei as a result of
1285 nuclear deformation as has been demonstrated in non-muscle cells. Such Yap activation
1286 could be relevant for hypertrophy as YAP can induce the gene that encodes the Lat1 leucine
1287 transporter. 5 Finally, Bag3 also binds to Synpo2 which regulates chaperone-assisted
1288 selective autophaghy (CASA) which regulates the degradation of damaged Z-disc proteins.

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