Physical exercise increases autophagic signaling through ULK1 in human skeletal muscle

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Møller AB, Vendelbo MH, Christensen B, Clasen BF, Bak AM, Jørgensen JO, Møller N, Jessen N. Physical exercise increases autophagic signaling through ULK1 in human skeletal muscle. J Appl Physiol 118: 971–979, 2015. First published February 12, 2015; doi:10.1152/japplphysiol.01116.2014.—Data from transgenic animal models suggest that exercise-induced autophagy is critical for adaptation to physical training, and that Unc-51 like kinase-1 (ULK1) serves as an important regulator of autophagy. Phosphorylation of ULK1 at Ser555 stimulates autophagy, whereas phosphorylation at Ser757 is inhibitory. To determine whether exercise regulates ULK1 phosphorylation in humans in vivo in a nutrient-dependent manner, we examined skeletal muscle biopsies from healthy humans after 1-h cycling exercise at 50% maximal O2 uptake on two occasions: 1) during a 36-h fast, and 2) during continuous glucose infusion at 0.2 kg/h. Physical exercise increased ULK1 phosphorylation at Ser555 and decreased lipidation of light chain 3B. ULK1 phosphorylation at Ser555 correlated positively with AMP-activated protein kinase-α Thr172 phosphorylation and negatively with light chain 3B lipidation. ULK1 phosphorylation at Ser757 was not affected by exercise. Fasting increased ULK1 and p62 protein expression, but did not affect exercise-induced ULK1 phosphorylation. These data demonstrate that autophagy signaling is activated in human skeletal muscle after 60 min of exercise, independently of nutritional status, and suggest that initiation of autophagy constitutes an important physiological response to exercise in humans.

autophagy signaling; exercise training; skeletal muscle; nutritional status; ULK1; human

Autophagy is a cellular recycling process that contributes to cellular homeostasis by delivering cytoplasmic proteins and organelles to the lysosomes for degradation, thereby providing free amino acids for de novo protein synthesis, oxidation, gluconeogenesis, and ketogenesis (3). Increased autophagy has been observed in several physiological and pathological conditions, such as fasting, atrophy, and following physical exercise (7, 15, 22, 24), and the metabolic adaptations to exercise training rely on normally regulated autophagy in mice (9, 18). Conversely, impaired autophagy causes accumulation of misfolded proteins and dysfunctional organelles, which are characteristic features of certain myopathies (6, 19, 26).

Unc-51 like kinase-1 (ULK1) plays a critical role in initiation of autophagy (31). The activity of ULK1 is regulated by site-specific phosphorylations (30). In vitro, ULK1 activity is negatively regulated by mammalian target of rapamycin (mTOR) complex 1 (mTORC1) through phosphorylation at Ser757 and positively regulated by AMP-activated protein kinase (AMPK) through phosphorylation at Ser177 and Ser255 (2, 4, 14). mTORC1 is a nutrient and hormone-sensitive enzyme that exerts translational control, and AMPK is an energy-sensitive enzyme that is activated when the AMP-to-ATP ratio increases (12, 13, 32). Thus ULK1 represents a potential regulatory link that enables rapid adaptation in skeletal muscle by linking energy and nutrient sensing to autophagic control.

During autophagy, light chain 3B (LC3B) is converted to LC3BII through lipidation and becomes associated with the autophagic vacuole (3). The mechanism by which ULK1 controls autophagy remains to be completely understood, but ULK1 deficiency is known to impede LC3B conversion to LC3BII (14). In mice, LC3B is lipidated in response to running exercise, and this is accompanied by increased ULK1 phosphorylation at Ser317 and Ser555 and decreased phosphorylation at Ser757 (23). In humans, autophagy signaling through mTORC1/ULK1 increases in skeletal muscle during prolonged fasting, and this translates into increased LC3B lipidation (27). This suggests that signaling through mTORC1/ULK1 is dependent on nutritional status in human skeletal muscle. Exercise may also increase autophagic signaling in human skeletal muscle, because LC3B lipidation is increased in ultraendurance athletes after a 24-h treadmill run (11). These subjects had access to food during exercise, but the degree to which mTORC1/ULK1 signaling was affected under these conditions was not determined.

The aim of the present study was to investigate ULK1 signaling in response to physical exercise under physiological conditions. To further isolate the effects of exercise, the subjects were examined during fasting and during glucose infusion to imitate a prandial state. We hypothesized that physical exercise increases ULK1 signaling, and that this response is augmented during fasting.

Materials and Methods

Subjects. Eight healthy, young, recreationally active men participated in a randomized crossover study after verbal and written information and consent. The study was approved by the Ethics Committee System of Central Region Denmark (j.no. 20090026), and performed in accordance to the Declaration of Helsinki. Before completion of the experimental protocol, the subjects completed an incremental test of maximal oxygen uptake (VO2 max) on a Jaeger ER800 bicycle ergometer. Expired air was collected breath by breath and the rate of oxygen uptake and carbon dioxide release was
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Protocol

![Protocol](https://via.placeholder.com/150)

Fig. 1. Schematic presentation of the study design. Subjects completed two single bouts of cycling exercise at 50% maximal O$_2$ uptake (V$_{O2,max}$). One bout was performed with continuous glucose infusion (Glucose infusion day), and the other bout was performed during 36-h fasting (Fasting day). The order of the 2 experimental days was randomized and separated by approximately 1 mo. Skeletal muscle biopsies and blood were sampled 1 h before exercise initiation, immediately after exercise termination, and 30 min into the recovery period.

<table>
<thead>
<tr>
<th>Time</th>
<th>Exercise 50% V$_{O2,max}$</th>
<th>Glucose infusion day</th>
<th>Fasting day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td>12 h fast</td>
<td>0.2 g/kg/h</td>
<td>36 h fast</td>
</tr>
<tr>
<td>Glucose infusion day</td>
<td>0.2 g/kg/h</td>
<td>1.0 g/kg/h</td>
<td></td>
</tr>
<tr>
<td>Fasting day</td>
<td></td>
<td>0.2 g/kg/h</td>
<td></td>
</tr>
</tbody>
</table>

Determined by an automated gas exchange analyzer (Oxycon Delta, Erich Jaeger, Germany). The V$_{O2,max}$ for each subject was used to determine the intensity corresponding to 50% of V$_{O2,max}$. Protocol. A schematic presentation of the protocol is depicted in Fig. 1. At both experimental days, each participant completed a single bout of cycling exercise at 50% V$_{O2,max}$ for 60 min or until fatigue, in concomitance with a continuous glucose infusion after an overnight fast (to mimic the prandial state) or following a 36-h fasting period. The subjects were instructed not to participate in physical activities for at least 72 h before completion of the experimental protocol. The single bouts were separated by approximately 1 mo, and the first single bout was completed approximately 1 wk after the V$_{O2,max}$ test. Skeletal muscle biopsies and blood were sampled 1 h before initiation of exercise, immediately after exercise, and 30 min into the recovery period. The biopsies were obtained alternately from the right and left m. vastus lateralis using a Bergström needle, frozen in liquid nitrogen, and stored at −80°C until analyses. The glucose infusion was started after sampling of the first biopsy with a rate of 0.2 g·kg$^{-1}$·h$^{-1}$. The rate of glucose infusion was increased to 1 g·kg$^{-1}$·h$^{-1}$ during exercise and lowered to 0.2 g·kg$^{-1}$·h$^{-1}$ in the postexercise recovery period. Only tap water was allowed during the fasting period.

**Protein extraction and Western blot analysis.** Frozen muscle biopsies were homogenized in ice-cold lysis buffer (50 mM HEPES, 137 mM NaCl, 10 mM Na$_2$PO$_4$, 10 mM NaF, 2 mM EDTA, 1 mM MgCl$_2$, 1 mM CaCl$_2$, 2 mM Na$_3$VO$_4$, 1% (vol/vol) NP-40, 10% (vol/vol) glycerol, 3 mg/ml aprotinin, 5 mg/ml leupeptin, 0.5 µg/ml pepstatin, 10 µg/ml antipain, 1.5 mg/ml benzamidine, 100 µM 4-(-2-aminoethyl)-benzenesulfonyl fluoride, pH 7.4) using a Precellys homogenizer (Bertin Technologies). Insoluble materials were removed by centrifugation at 14,000 g for 20 min at 4°C. Protein concentration of the supernatant was determined using a Bradford assay (BioRad). Samples were adjusted to equal concentrations with milli-Q water, denatured by mixing with 4 × Laemmli’s buffer, and heating at 95°C for 5 min. Equal amounts of protein were separated by SDS-PAGE using the BioRad Criterion system, and proteins were electro blotted onto polyvinylidene difluoride membranes (BioRad). Control for equal loading was performed using the Stain-Free technology (8). Membranes were blocked for 2 h in a 2% bovine serum albumin solution (Sigma-Aldrich) and incubated overnight with primary antibodies. For all targets except ULK1, the membranes were initially incubated in phosphor-specific antibodies, after detection the antibodies were stripped off using a buffer containing 6 M GnHCl, 0.2% NP-40, 10 mM DTT, and 20 mM Tris-HCl (pH 7.5), and the membranes were reblocked and reincubated in antibodies against total protein. Due to difficulties with stripping the phosphorylated ULK1 antibodies off the membranes, data on ULK1 protein expression and phosphorylation were determined on nonstripped membranes. Antibodies against Akt-2 (cat. no. 3063), mTOR (cat. no. 2972), ULK1 (cat. no. 4773s), LC3B (cat. no. 3868s), Beclin-1 (cat. no. 3738), γ-aminobutyric acid receptor-associated protein (GABARAP) (cat. no. 13733s), autophagy gene-5 (Atg-5) (cat. no. 12994s), and phosphor-specific antibodies against Akt (Ser$^{743}$), mTOR (Ser$^{2444}$, cat. no. 2971s), acetyl-CoA carboxylase (ACC) (Ser$^{79}$, cat. no. 3661), ULK1 (Ser$^{555}$, cat. no. 5869), ULK1 (Ser$^{757}$, cat. no. 6888s), ULK1 (Ser$^{317}$, cat. no. 6887), AMPK-α (Thr$^{172}$, cat. no. 2531) were purchased from Cell Signaling Technology. Antibody against AMPK-α-pan (cat. no. 07-181) was purchased from Millipore. Antibody against SQSTM1/p62 (cat. no. ab56416) was purchased from Abcam (Cambridge, UK). Total ACC protein expression was detected using horseradish peroxidase-linked streptavidin (R&D Systems). After incubation in primary antibodies, the membranes were incubated 1 h with horseradish peroxidase-conjugated secondary antibody (Amersham). Proteins were visualized by chemiluminescence (Pierce Supersignal West Dura, Thermo Scientific) and quantified with the ChemiDoc MP imaging system (BioRad). Protein Plus Precision All Blue standards were used as a marker of molecular weight (BioRad).

**Blood analysis.** Plasma glucose was measured in duplicate on a Beckman Glucoanalyzer (Beckman Instruments) immediately after collection. Serum samples were frozen and stored at −20°C, and insulin was analyzed using a time-resolved fluorimmunassay (AutoDELFI; PerkinElmer, Turku, Finland). Free fatty acids (FFA) were analyzed by a commercial kit (Wako Chemicals, Neuss, Germany).

**Statistics.** The effects of exercise and nutritional state and their interactions on dependent variables (protein expression and phosphorylation, etc.) were analyzed using a mixed-effect two-way ANOVA with repeated measurements. When a significant interaction or main effect was observed, linear comparison analysis was used to evaluate differences within and between individual conditions. Pearson correlations and simple linear regressions were used to analyze associations between protein expression and phosphorylation levels. Data are presented as means ± SE, unless otherwise indicated. Normal distribution was assumed following evaluation of QQ-plots, and data not considered as normally distributed were log transformed and subsequently reevaluated before normal distribution was assumed. Data were analyzed in Stata (Stata 12.1, StataCorp LP, College Station, TX), and graphs were designed in SigmaPlot (SigmaPlot 11.0, Systat Software).

**RESULTS**

**Subject characteristics.** Subject characteristics are presented in Table 1. Subjects achieved a median V$_{O2,max}$ of 4,082 ml/min, ranging from 3,146 to 4,732 ml/min. All participants completed 60-min exercise at both experimental days, apart from one subject who fatigued after 45 min of exercise at the fasting day.

**Blood glucose and FFA levels.** An interaction between exercise and nutrient state was observed for plasma glucose and FFA levels (Table 2). Multiple comparisons revealed the following: plasma glucose decreased by −1.0 mmol/l after 36-h fast compared with overnight fast and remained at this level after exercise and 30 min into the recovery period.

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/cm$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>27 (24–35)</td>
<td>180 (168–186)</td>
<td>78 (72–88)</td>
<td>24 (23–29)</td>
</tr>
</tbody>
</table>

Values are presented as median and range (minimum and maximum); n, no. of subjects. BMI, body mass index.
Table 2. Glucose, FFA, and insulin levels in the blood

<table>
<thead>
<tr>
<th>Glucose Infusion Day</th>
<th>36-h Fast Day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Glucose, mM</strong></td>
<td></td>
</tr>
<tr>
<td>-60 min</td>
<td>5.26 ± 0.12*</td>
</tr>
<tr>
<td>60 min</td>
<td>7.78 ± 0.76#</td>
</tr>
<tr>
<td>90 min</td>
<td>5.35 ± 0.35</td>
</tr>
<tr>
<td>FFA, mM</td>
<td></td>
</tr>
<tr>
<td>-60 min</td>
<td>0.36 ± 0.07</td>
</tr>
<tr>
<td>60 min</td>
<td>0.18 ± 0.04#</td>
</tr>
<tr>
<td>90 min</td>
<td>0.12 ± 0.02#</td>
</tr>
<tr>
<td>Insulin†, pM</td>
<td></td>
</tr>
<tr>
<td>-60 min</td>
<td>60.7 ± 7.1</td>
</tr>
<tr>
<td>60 min</td>
<td>39.7 ± 4.1</td>
</tr>
<tr>
<td>120 min</td>
<td>42.0 ± 4.3</td>
</tr>
</tbody>
</table>

Values are means ± SE. FFA, free fatty acid. *Significant difference between days, P < 0.05. †Significantly different from -60 min within day, P < 0.05. ‡Main effect of exercise and nutrient state, P < 0.05. #Significantly different from overnight fast, but did not change in response to exercise (Fig. 2A). ULK1 phosphorylation at Ser555 increased after exercise, while the increase in ULK1 Ser2448 did not reach statistical significance. The ratios of p-ULK1 Ser555/ULK1 and p-ULK1 Ser757/ULK1 were lower during prolonged fasting due to increased ULK1 expression (Fig. 2, B and C).

AMPK-α phosphorylation at Thr172 increased threefold after exercise and remained elevated 30 min into the recovery period (Fig. 3A). ACC Ser79 phosphorylation increased in response to exercise, and the effect persisted 30 min into the recovery period (Fig. 3B). Akt Ser473 and mTOR Ser2448 phosphorlyations increased after exercise during glucose infusion (Fig. 3, C and D). Akt Ser473 phosphorylation was elevated immediately after exercise and remained at this level 30 min into the recovery period, whereas mTOR Ser2448 phosphorylation was elevated 30 min into the recovery. There were no change in the expression of AMPK-α, ACC, Akt-2, or mTOR.

The ratio of LC3BII to LC3BI decreased after exercise (Fig. 3E) on both examination days. This was driven by significantly lowered LC3BII protein expression following exercise, whereas LC3BII levels remained unchanged. The expression of p62 was increased 1.2-fold during fasting compared with glucose infusion (Fig. 3F), whereas the expression of Beclin-1, GABARAP, and Atg-5 remained unchanged (Fig. 4, A–C).
ULK1 Ser<sup>555</sup> phosphorylation correlates with AMPK-α Thr<sup>172</sup> phosphorylation and LC3B lipidation. As shown in Fig. 5A, AMPK-α Thr<sup>172</sup> phosphorylation and ULK1 Ser<sup>555</sup> phosphorylation correlated positively, and, furthermore, ULK1 phosphorylation at Ser<sup>555</sup> was negatively correlated to LC3BII/LC3BI (Fig. 5B). No correlation was found between ULK1 Ser<sup>757</sup> phosphorylation and LC3B lipidation.

**DISCUSSION**

In the present study, we demonstrate that short-term aerobic exercise activates autophagic signaling through ULK1 in human skeletal muscle, independently of nutrient background. ULK1 Ser<sup>555</sup> and AMPK-α Thr<sup>172</sup> phosphorylation increased after exercise under both fasting and prandial condi-

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**Fig. 3. Expression and phosphorylation of proteins involved up- and downstream of ULK1. A** and **B**: AMP-activated protein kinase (AMPK)-α phosphorylation at Thr<sup>172</sup> and phosphorylation of acetyl-CoA carboxylase (ACC) at Ser<sup>79</sup>, respectively, increased after exercise. **C** and **D**: increased mammalian target of rapamycin (mTOR) Ser<sup>2448</sup> and Akt Ser<sup>473</sup> phosphorylation, respectively, were observed after exercise at the glucose infusion day only. The ratio of light chain 3BII (LC3BII) to LC3BI was decreased after exercise (**E**), while p62 protein expression was increased at the fasting day compared with the glucose infusion day (**F**). Values are means ± SE. P values indicate interactions or main effects of nutritional state and exercise based on two-way repeated-measures ANOVA. *Post hoc test for main effect of time showed significant difference from preexercise (−60 min). †Post hoc test for interaction showed significant difference from preexercise (−60 min) at glucose infusion day. #Post hoc test for interaction showed significant difference between nutritional states. **G**: Western blots representing the time course effects for expression and phosphorylation of p-AMPK-α Thr<sup>172</sup>, AMPK-α-pan, p-ACC Ser<sup>79</sup>, ACC, pAkt Ser<sup>473</sup>, Akt-2, p-mTOR Ser<sup>2448</sup>, mTOR, LC3B and p62. Based on the applied molecular standards, approximated molecular weights are indicated on the right.
tions. Exercise is a well-known activator of AMPK (5, 29), and our results add to this by demonstrating that up to 36 h of fasting do not augment exercise-induced AMPK activation. Exercise activates an array of intracellular enzymes in skeletal muscle, and our results do not allow us to make strong mechanistic conclusions. However, in vivo studies demonstrating increased muscular autophagy after exercise accumulates (10, 11, 18, 23), and data from studies in cultured cells, demonstrate that AMPK-\(H\)9251 possesses the ability to phosphor-
ylate ULK1 at Ser555 (2, 14). The increase in AMPK-\(H\)9251 Thr172 and ULK1 Ser555 phosphorylation was positively correlated, indicating that AMPK-\(H\)9251 acts as the upstream kinase for ULK1 at Ser555 in human skeletal muscle. This confirms that previous findings obtained in mice (23) can be translated to human skeletal muscle. Thus our results point to AMPK-\(H\)9251 signaling as an activator of exercise-induced autophagy in human skeletal muscle.

The association between increased ULK1 Ser\(\text{555}^\text{S}\) phosphorylation and decreased LC3B lipidation observed in the present study indicates that exercise-induced autophagy is mediated through ULK1. This finding opposes recent observations in humans where LC3B lipidation increased after 24-h ultraendurance running (11). These findings, however, are not necessarily contradictory, but may reflect differences in the duration of exercise before biopsy sampling. LC3II itself is degraded when the autophagosome fuses with the lysosome (20), and our data could reflect increased presence of LC3BII in the lysosomal lumen and thus flux through the autophagy-lysosome

Fig. 4. Expression of autophagy related proteins. Beclin-1 (A), \(\gamma\)-aminobutyric acid receptor-associated protein (GABARAP; B), and autophagy gene-5 (Atg-5; C) protein expression did not change in response to exercise or fasting. Values are means \(\pm\) SE. **D**: Western blots representing the time course effects for expression of Beclin-1, GABARAP, and Atg-5. Based on the applied molecular standards, approximated molecular masses are indicated on the right.

Fig. 5. Exercise- and fasting-induced changes in ULK1 Ser\(\text{555}^\text{S}\) phosphorylation correlate with AMPK-\(\alpha\) Thr\(\text{172}^\text{S}\) phosphorylation and LC3B lipidation. **A**: a positive correlation between AMPK-\(\alpha\) phosphorylation at Thr\(\text{172}^\text{S}\) and phosphorylation of ULK1 at Ser\(\text{555}^\text{S}\) was observed. **B**: moreover, ULK1 Ser\(\text{555}^\text{S}\) phosphorylation was negatively correlated to LC3B lipidation. Data are presented relative to basal levels (time \(=\) 60 min) at the glucose infusion day.
system. We did not observe any changes in p62 protein expression after 1 h of moderate-intensity exercise. p62 is degraded in the autophagic process, and inhibition of autophagy leads to p62 accumulation (16). Thus our results may reflect a steady-state situation where p62 production equals p62 degradation, but they do not allow us to conclude whether or not autophagic flux is altered. It is possible that increased p62 protein production equalizes increased p62 degradation during exercise. Moreover, p62 protein levels have recently been reported not always to be inversely correlated with autophagic activity (25). The initial decrease in p62 protein expression in response to 2-h starvation in cultured cells is followed by total restoration within the next 2–6 h (25). These findings demonstrate that the expression of autophagy markers depends on temporal factors, and the diverse effects of 1-h moderate-intensity cycling vs. 24-h ultraendurance running on LC3B lipidation could be due to differences in the duration of exercise before biopsy sampling.

We did not see an effect of nutritional background on exercise-induced ULK1 Ser\textsuperscript{555} phosphorylation. In cultured cells, mTOR phosphorylates ULK1 at Ser\textsuperscript{757}, and this disrupts the interaction between ULK1 and AMPK (14). Translated to human skeletal muscle, it would mean that mTOR only would inhibit ULK1 Ser\textsuperscript{555} phosphorylation when mTOR is activated before or during the exercise bout. We did observe increased mTOR phosphorylation during glucose infusion, but this was not until 30 min into the recovery period, and not immediately after exercise. Studies in cultured cells have shown that AMPK inhibits mTOR activity at several levels downstream of Akt (13). Inhibition of mTOR by AMPK during exercise could, therefore, explain why mTOR phosphorylation did not increase until the recovery period, despite increased Akt phosphorylation immediately after exercise during glucose infusion. Thus initiation of autophagy through ULK1 is independent of high systemic glucose levels, and this indicates that autophagy during exercise is not initiated due to lack of energy substrates for oxidation. Instead, exercise-induced autophagy could serve to enable de novo protein synthesis and adaptation to exercise training. However, autophagy is a flux pathway, and our data on autophagy signaling could be affected by unknown inhibitory signals. Future studies should aim to develop a valid marker of autophagic flow in human tissues to improve our understanding of the upstream signaling events.

A borderline significant increase in ULK1 phosphorylation at Ser\textsuperscript{757} was observed in response to exercise (P = 0.07 for main effect of exercise). As seen in Fig. 2C, ULK1 Ser\textsuperscript{757} phosphorylation seems to increase within the first 30 min of the recovery period, and this increase appears more pronounced during glucose infusion. Insulin activates mTORC1 in human skeletal muscle (1), and it is likely that the elevated ULK1 Ser\textsuperscript{757} phosphorylation in the recovery period is mediated by mTORC1. However, ULK1 phosphorylation at Ser\textsuperscript{757} tends to increase after exercise during fasting without elevated insulin level. mTORC1 activity has previously been shown to increase gradually in the first 5 h of the recovery period after exercise (28), and elevated ULK1 Ser\textsuperscript{757} phosphorylation could be due to contraction-induced mTORC1 signaling. Further elaboration on this issue requires sampling of more biopsies, which introduce problems with repeated biopsy sampling, as observed in a recent study (21). It could be speculated that contraction-induced signaling through mTORC1/ULK1 serves as a mechanism by which the initial catabolic response to exercise switches to an anabolic response later in the recovery period.

p62 and ULK1 protein expressions increased after 36 h of fasting compared with an overnight fast. This result expands our recent finding after 72 h of fasting (27) and suggests that p62 protein levels increase already in the period between 12 and 36 h of fasting. Increased ULK1 protein levels have not previously been documented during fasting. In a previous fasting study, our laboratory did observe an ~20% increase in ULK1 protein expression after 72 h of fasting, but the increase did not reach statistical significance (P = 0.12) (27). However, our previous investigation may have been prone to type II errors because of limited biopsy material available, and our present data suggest that ULK1 expression is indeed increased during prolonged fasting. Studies in mice indicate a pivotal role of autophagy for surviving periods of nutrient shortage by releasing energy substrates for the TCA cycle (17). Increased expression of autophagy-related genes could, therefore, support increased reliance on amino acids as energy source during prolonged fasting in humans. However, using amino acid tracers to quantify protein turnover, our laboratory has previously demonstrated that skeletal muscle protein breakdown is unaffected during 72-h fasting (27), and we did not observe any changes in Beclin-1, GABARAP, and Atg-5 protein expression, all of which are important members of the autophagic machinery. It, therefore, remains speculative whether or not upregulation of the autophagic machinery during prolonged fasting serves to cover energy demands.

In conclusion, a single exercise bout of moderate intensity initiates autophagy signaling through ULK1 in human skeletal muscle. Activation of ULK1 by phosphorylation at Ser\textsuperscript{555} is likely due to increased AMPK-\(\alpha\) activity, and exercise-induced phosphorylation of AMPK-\(\alpha\) and ULK1 is independent of ambient glucose levels. We hypothesize that autophagy functions as an integrative response to exercise and serves to enable de novo protein synthesis and facilitate adaptation to a trained state.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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