In humans, nutrient deprivation and extreme endurance exercise both activate autophagy. We hypothesized that cumulating fasting and cycling exercise would potentiate activation of autophagy in skeletal muscle. Well-trained athletes were divided into control (n = 8), low-intensity (LI, n = 8), and high-intensity (HI, n = 7) exercise groups and submitted to fed and fasted sessions. Muscle biopsy samples were obtained from the vastus lateralis before, at the end, and 1 h after a 2 h LI or HI bout of exercise. Phosphorylation of ULK1(Ser317) was higher after exercise (P < 0.001). In both the fed and the fasted states, LC3bII protein level and LC3bII/I were decreased after LI and HI (P < 0.05), while p62/SQSTM1 was decreased only 1 h after HI (P < 0.05), indicating an increased autophagic flux after HI. The autophagic transcriptional program was also activated, as evidenced by the increased level of LC3b, p62/SQSTM1, GabarapL1, and Cathepsin L mRNAs observed after HI but not after LI. The increased autophagic flux after HI exercise could be due to increased AMP-activated protein kinase α (AMPKα) activity, as both AMPKα(Thr172) and ACC(Ser79) had a higher phosphorylation state after HI (P < 0.001). In summary, the most effective strategy to activate autophagy in human skeletal muscle seems to rely on exercise intensity more than diet. -Schwalm, C., Jamart, C., Benoit, N., Naslain, D., Prémont, C., Prévet, J., Van Thienen, R., Deldicque, L., Francaux, M. Activation of autophagy in human skeletal muscle is dependent on exercise intensity and AMPK activation.

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Legends to supplementary figures

**Supplementary figure S1.** Representative western blots for A) AktSer473 and AktTotal; B) AktThr308 and AktTotal; C) 4E-BP1Thr37/46 and 4E-BP1Total; D) ULK1Ser757 and ULK1Total; E) AMPKThr172 and AMPKTotal; F) ACCSer79 and ACCTotal; G) ULK1Ser317 and ULK1Total; H) LC3b I and LC3b II; I) p62/SQSTM1 and GAPDH.

**Supplementary figure S2.** Changes in A) ULK1Total, B) FoxO1/3aThr24/32 and C) FoxO3aTotal in response to concentric endurance exercise as a function of intensity, nutritional state and time. Data were collected at baseline, before, immediately after and 1 h after cycling exercise at low (55 % VO2 peak, LI) or high intensity (70 % VO2 peak, HI) or in control conditions (CTRL). D) Representative western blots for FoxO1/3aThr24/32 and FoxO3aTotal. Values are presented as the means ± SEM. $P < 0.05, $$P < 0.01$ vs control group (exercise effect); ¶$P < 0.05$ vs low intensity (intensity effect); *$P < 0.05, **P < 0.01$ vs fed (nutrition effect); #*$P < 0.05, ###$P < 0.001$ vs pre-exercise (time effect).