Mitochondrial dynamics including mitotic fusion/fission (e.g., Dynamin-related protein 1 (Drp1) and Fission 1 (Fis1)) and fusion (e.g., Mitofusin 2) regulates mitochondrial homeostasis. Defects in mitochondrial dynamics are suggested to contribute to skeletal muscle mitochondrial dysfunction and insulin resistance associated with obesity and Type 2 Diabetes. Roux-en-Y gastric bypass (RYGB) surgery markedly improves metabolic health as indicated by enhanced substrate oxidation and insulin action in skeletal muscle. However, the underlying cellular mechanisms responsible for these improvements are not clear and could possibly be due to the improvement of mitochondrial dynamics. **PURPOSE:** The purpose of this study was to determine whether RYGB surgery improves mitochondrial dynamics proteins in primary human myotubes derived from severely obese humans. **METHODS:** Primary human skeletal muscle cells were isolated from muscle biopsies obtained from six lean subjects (BMI = 23.4 ± 0.6 kg/m²) and six RYGB patients prior to, 1-month and 7-months after surgery (BMI = 50.2 ± 2.0, 43.2 ± 2.8 and 35.7 ± 2.2 kg/m², respectively) and were differentiated to myotubes. On day 7 of differentiation, myotubes were harvested for immunoblot analysis in order to assess the expressions of mitophagy dynamics proteins. **RESULTS:** Before surgery, Drp1 Ser616 phosphorylation and Fis1 protein expression were significantly higher in primary myotubes derived from severely obese patients when compared to lean controls (41% and 26%, respectively, P < 0.05). While there were no significant improvements at 1-month post-surgery, Drp1 Ser616 phosphorylation and Fis1 protein expression were significantly decreased in primary myotubes from severely obese humans at 7-months post-surgery (P vs. 7-months pre: 0.046 ± 0.004 vs. 0.035 ± 0.003; 0.023 ± 0.008 vs. 0.014 ± 0.003 AU; respectively, P < 0.05), and not statistically different from lean controls. However, MFN2 protein expression did not change in primary myotubes derived from severely obese patients at any timepoint post-surgery in comparison to pre-surgery. **CONCLUSION:** These data suggest that RYGB surgery reduces obesity-induced rise in mitochondrial fission, but not fusion, protein expression in primary human myotubes derived from severely obese humans.

**Purpose:** The mitogen-activated protein kinases (MAPKs) represent a central signaling pathway in the regulation of skeletal muscle function. It is also known that MAPKs are required to promote mitochondrial biogenesis in skeletal muscles. Mitochondrial dysfunction underlies numerous diseases including those of skeletal muscle. The MAPKs are negatively regulated by MAPK phosphatases (MKPs). We have demonstrated that MKP-5 regulates regenerative myogenesis and rescues muscle degeneration by inactivating and dephosphorylating both p38 MAPK and JNK. However, the physiological and molecular roles of MKP-5 in regenerative myogenesis and progression of skeletal muscle degeneration have remained unclear. We tested the central hypothesis that MKP-5 regulates mitochondrial function and thus contributes to enhanced myogenesis and regeneration in mice lacking MKP-5. **Methods:** To test our hypothesis, we induced skeletal muscle damage by cardiotonic (CTX) injection into both mkp-5−/− and mkp-5+/- mice. Mitochondrial respiratory function in permeabilized muscle fibers was assessed in regenerating skeletal muscles from mkp-5−/− and mkp-5+/- mice. Mitochondrial biogenesis was determined by quantitative PCR for mtDNA. The amount of mitochondrial DNA (mtDNA) copy number was also quantified by qRT-PCR. **Results:** Our data show that MKP-5-deficient mice exhibited 49% enhanced ADP-stimulated mitochondrial respiratory function in regenerative skeletal muscle compared with mkp-5−/− mice (P<0.05). Furthermore, expression of genes associated with mitochondrial biogenesis such as PGC1-α, NRF-1, TIM23, and subunits of complex I were significantly increased in regenerating skeletal muscles of animals lacking MKP-5. The amount of mitochondrial DNA copy number was also significantly increased in mkp-5−/− mice, compared with mkp-5+/- mice (P<0.001). **Conclusions:** Collectively, these results demonstrate that MKP-5 negatively regulates mitochondrial function and biogenesis in skeletal muscle during myogenesis and regeneration.