either 1000 IU vitamin $D_3 \cdot d^{-1}$ (n=73) or placebo (n=76) for 12-weeks. At baseline, weeks 4, 8 and 12 (post-training) subjects provided saliva samples (passive drool) to determine secretory immunoglobulin A secretion rates (SIgA-SR) by indirect ELISA. The incidence of URTIs was assessed by administering a survey at weeks 4, 8 and 12. Serum vitamin D status (25(OH)D) was measured by radioimmunoassay. Longitudinal linear models were created using a simple-effects model to estimate symptoms. To determine whether supplementation altered SIgA-SR during training, a two-way repeated measures ANOVA was used. **RESULTS:** The proportion of recruits reporting URTI symptoms at any time during training was 72%. Baseline SIgA-SR were similar between placebo (65.4 ± 52.0 $\mu g \cdot min^{-1}$) and vitamin D groups (51.9 ± 41.9 $\mu g \cdot min^{-1}$). The relative changes in SIgA-SR were significantly greater with vitamin D supplementation at weeks 4 (5.1 ± 29.8%) and 8 (12.3 ± 31.0%) compared to placebo at the same time points (week 4; -6.5 ± 22.9% and week 8; 1.3 ± 22.9%), $p = 0.001$. Baseline 25(OH)D was significantly lower during winter (59.2 ± 22.5 $nmol \cdot L^{-1}$) compared to summer (80.4 ± 21.0 $nmol \cdot L^{-1}$), $p < 0.001$. When accounting for treatment, season and sex, there was no association between 25(OH)D and reported URTIs. **CONCLUSION:** We report that a high proportion of Marine Corps recruits experience URTIs during 12-weeks of basic military training, and although daily vitamin D supplementation led to a modest increase in SIgA-SR, this did not result in a reduction in the incidence of reported URTIs. Supported by the Defense Health Program. The views expressed are those of the authors and do not reflect the official position of the Uniformed Services University, United States Army, or United States Department of Defense.

A-50 Free Communication/Poster - **Muscle and Mitochondria**

Wednesday, May 31, 2017, 7:30 AM - 12:30 PM
Room: Hall F

382 Board #203 May 31 11:00 AM - 12:30 PM

Alterations of Mitochondrial Dynamics Proteins in Primary Human Myotubes Following Roux-en-Y Gastric Bypass Surgery

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Mitochondrial dynamics including mitochondrial 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 mitochondria 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/m^2) 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/m^2, respectively) and were differentiated to myotubes. On day 7 of differentiation, myotubes were harvested for immunoblot analysis in order to assess the expressions of mitochondria dynamics proteins. **RESULTS:** Before surgery, Drp1 Ser^{616} 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 Ser^{616} phosphorylation and Fis1 protein expression were significantly decreased in primary myotubes from severely obese humans at 7-months post-surgery (Pre vs. 7-months post: 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.

383 Board #204 May 31 11:00 AM - 12:30 PM

Osteocalcin Does Not Increase Insulin Sensitivity or Mitochondrial Biogenesis in Palmitate Treated C2C12 Myotubes

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PURPOSE: Osteocalcin (OC) is a bone matrix protein that has been shown to regulate systemic glucose homeostasis and increase mitochondrial mass in mice fed a high-fat diet, however the mechanisms by which OC stimulates metabolic adaptations in lipid overloaded muscle remain underexplored. This study examined the effects of OC on regulators of insulin signaling, glucose handling, and mitochondrial biogenesis *in vitro* using palmitate treated C2C12 myotubes.
METHODS: C2C12 myotubes were treated with control media, or media containing undercarboxylated OC (100ng/ml) both with and without 2mM palmitate-BSA conjugate (PA+OC and PA, respectively) for 24 hours. Insulin signaling (IRS-1, pIRS-1, Akt, pAkt, and PTP1B), glucose handling (GLUT-4 and AS160) and mitochondrial biogenesis (PGC-1α and Citrate Synthase) were measured via western blot. One-way ANOVAs with Tukey's post-hoc tests performed to determine between treatment differences.
RESULTS: IRS phosphorylation and PTP1B protein content remained unchanged. Surprisingly, phosphorylation of Akt significantly increased (52% ± 33%) with PA+OC compared to OC. Additionally, GLUT4 content decreased significantly in all treatments (≥50%) compared to control with no differences between the treatments. GLUT4 regulator AS160 was significantly elevated (300% ± 158%) following PA+OC compared to OC. No changes in PGC-1α or Citrate Synthase protein content were observed.
CONCLUSIONS: Overall, treatment with OC was unable to improve markers of insulin signaling and mitochondrial biogenesis in palmitate-treated C2C12 myotubes. Moreover, GLUT4 content and possibly translocation may be negatively affected by OC treatment in PA-treated cells.

384 Board #205 May 31 11:00 AM - 12:30 PM

MKP-5 Establishes Skeletal Muscle Metabolic Quiescence by Negatively Regulating MAPK-dependent Mitochondrial Function

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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 cardiotoxin (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 mRNA. 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, Tfam, 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.