OSTEOCALCIN DOES NOT INCREASE INSULIN SENSITIVITY OR MITOCHONDRIAL BIOGENESIS IN PALMITATE TREATED C2C12 MYOTUBES

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ABSTRACT

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. Conclusion: 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.

RESULTS

- No change was seen in PTP1B content or IRS-1 activation with either OC, PA, or combination treatment (Fig 1).
- OC treatment resulted in a significant increase in Akt activation in PA treated cells (Fig 1).
- GLUT4 content decreased in OC and PA treated cells compared to control (Fig 2).
- A non-significant increase in AS160 was observed in both PA treated conditions (Fig 2).
- No change was observed in PGC-1α and citrate synthase with all treatments (Fig 3).
- In conclusion, OC and PA treatment has limited effect on insulin signaling. However, both OC and PA decrease GLUT4 content, thus possibly decreasing glucose uptake.

INTRODUCTION

- Osteocalcin (OC) is produced by osteoblasts and has been shown to increase expression of Insulin in β-cells and Adiponecin in adipose cells [1].
- OC treatment partially blunted elevations in body weight and fat pad mass while improving glucose tolerance and insulin sensitivity in mice fed a high fat diet [2, 3].
- Gene expression for Pgc1a increased in mice fed a high-fat diet treated and injected with OC compared to vehicle [2].
- Daily injections of OC increased energy expenditure in mice fed a high-fat diet [3].
- The purpose of this work was to determine whether a pharmaceutical dose of OC in PA treated cells increases expression of markers of insulin signaling.

METHODS

Mouse myotubes (C2C12) were treated with palmitate-BSA (2mM) with or without osteocalcin (100ng/ml) for 24 hours. Protein content or phosphorylation of multiple regulators of insulin signaling were quantified via western blot. Target protein expression was normalized to β-actin or total target protein content for phosphorylated protein. Data was analyzed by a one-way ANOVA with significance determined by p < 0.05 and indicated by *.

Fig 1 Osteocalcin and palmitate increase Akt activation. Protein Tyrosine Phosphatase B (PTP1B) on left, insulin receptor substrate 1 (IRS-1) - activation (indicated by pIRS-1/IRS), and protein kinase B (Akt) - activation (indicated by pAkt) normalized to total Akt (pAkt/Akt) from C2C12 myotubes treated as described in methods.

Fig 2 Osteocalcin and Palmitate does not alter GLUT4 receptors or AS160. GLUT4 on left and Akt substrate 160 (AS160) expression from C2C12 myotubes treated as described in methods.

Fig 3 Osteocalcin and Palmitate do not change markers of mitochondrial biogenesis. Peroxisome proliferator-activate receptor γ co-activator 1α (PGC-1α) on left and citrate synthase expression from C2C12 myotubes treated as described in methods.

REFERENCES