Abstract #2700

Vitamin D Enhances Adipose Stem Cell Proliferation and Increases Adipocyte Metabolism

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Introduction:

Variable and significant post-operative reabsorption of fat grafts remains a significant drawback for restoring soft tissue volume. We have previously demonstrated that systemic administration of calcitriol or Vitamin D3 (VD3) after grafting enhanced human adipose retention in a xenograft murine model (6 unique tissue donors). Further, VD3 significantly increased adipose stromal cell (ASC) viability in a dose-response relationship when lipoaspirate was cultured for 1 week *ex vivo* (7 unique donors). We hypothesize that VD3 improves fat grafting outcomes by inducing a metabolic switch toward anerobic glycolysis in lieu of aerobic oxidative phosphorylation. This hypothesis is based on the observation that activation of membrane vitamin D receptors increases intracellular calcium, limiting mitochondrial ATP synthesis. To test this, we treated adipose stem cells and stem cell-differentiated adipocytes *in vitro* with log doses of VD3. Measured outcomes included proliferation, survival in hypoxia, oxygen consumption and metabolic phenotype.

Methods:

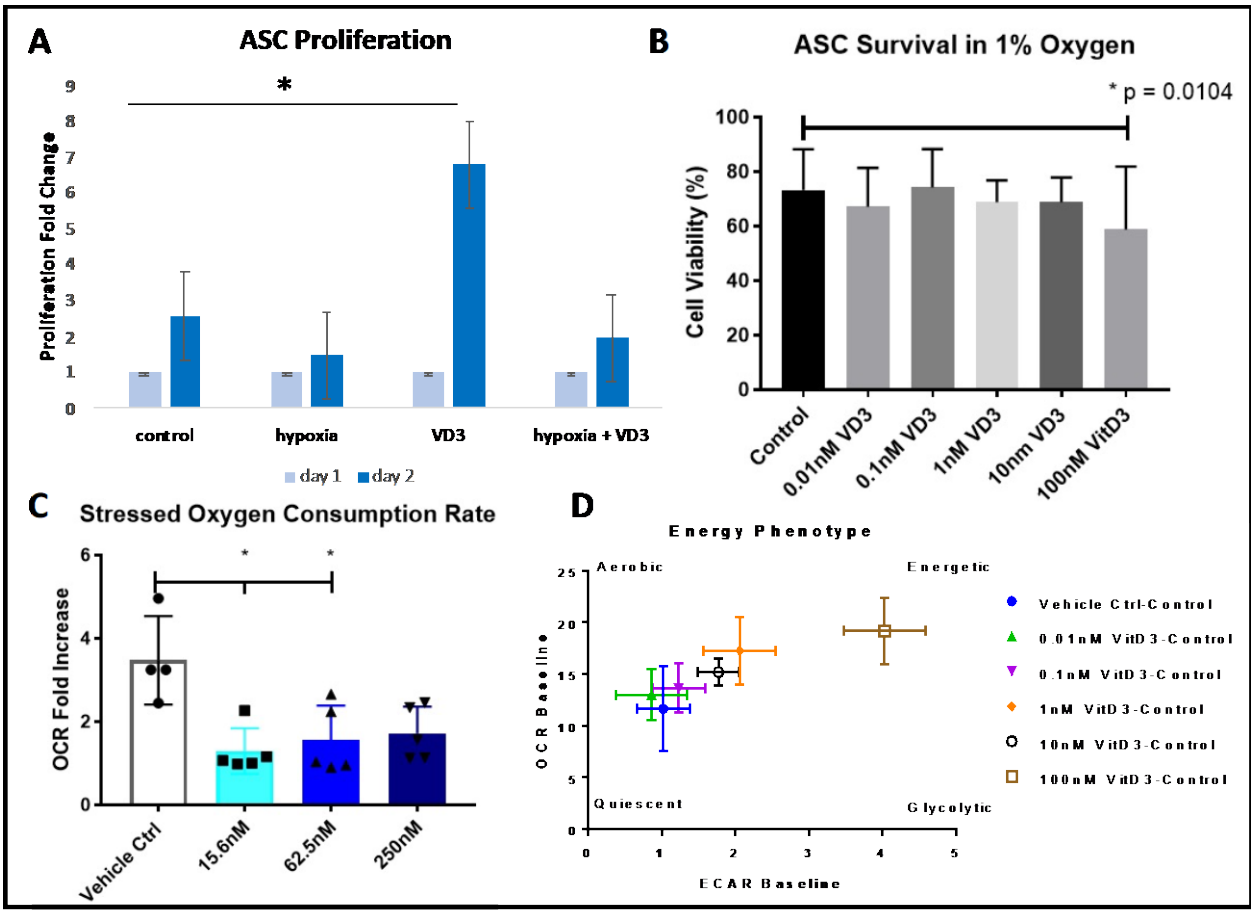
Stromal vascular cells were obtained from enzymatically digested adipose excised during elective surgical procedures and obtained under IRB exemption. Culture expanded stromal cells (ASCs) were plated in 96well plates and incubated with increasing doses of VD3. Proliferation was measured over 7 days using an MTT assay. ASCs were further cultured under 1% O_2 using hypoxia chambers and survival was measured with calcein AM and propidium iodide. Finally, oxygen consumption and metabolism of ASCs and *in vitro* differentiated adipocytes were measured using a seahorse bioanalyzer (Agilent).

Results:

In vitro, ASCs cultured in ambient oxygen in presence of VD3 had significantly increased proliferation compared to controls ($p<0.05$). Interestingly, VD3 did not increase ASC survival or proliferation in the presence of hypoxia, counter to our hypothesis. Low concentrations of VD3 significantly decreased oxygen consumption rate following stress induction with mitochondrial inhibitors oligomycin and FCCP, while high concentrations (250nM) did not significantly alter metabolism. Finally, VD3 significantly increased adipocyte metabolism with a dose response relationship, also counter to our hypothesis (**Figure One**).

Conclusion:

In this study, we investigated the effects of VD3 on adipose stromal cell and adipocyte metabolism, testing the underlying hypothesis that VD3 suppresses aerobic metabolism in favor of aerobic glycolysis. Our results did not support this hypothesis and in fact, showed that VD3 activated stromal cells, significantly increasing cell proliferation and decreasing survival under severe hypoxia. Our results further showed that VD3 increased the energetic profile of adipocytes. Future studies will investigate alternative hypotheses to explain why VD3 improves fat graft retention *in vivo*.


A
ASC Proliferation
*
Proliferation Fold Change
9
8
7
6
5
4
3
2
1
0
control
hypoxia
VD3
hypoxia + VD3
day 1
day 2
B
ASC Survival in 1% Oxygen
* p = 0.0104
Cell Viability (%)
100
80
60
40
20
0
Control
0.01nM VD3
0.1nM VD3
1nM VD3
10nm VD3
100nM VitD3
C
Stressed Oxygen Consumption Rate
OCR Fold Increase
6
4
2
0
*
*
Vehicle Ctrl
15.6nM
62.5nM
250nM
D
Energy Phenotype
Aerobic
Energetic
Quiescent
Glycolytic
OCR Baseline
ECAR Baseline
25
20
15
10
5
0
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1
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3
4
5
Vehicle Ctrl-Control
0.01nM VitD3-Control
0.1nM VitD3-Control
1nM VitD3-Control
10nM VitD3-Control
100nM VitD3-Control