

Mechanical Stimulation of Mesenchymal Stem Cell Differentiation to Enhance Bone Formation:

Are Osteoblasts the Only Way to a Better Skeleton?

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INTRODUCTION

While the anabolic influence of low magnitude mechanical signals (LMMS) to bone has been reported, there are a range of hypotheses regarding the mechanosensory elements which regulate the response, mostly focusing on the resident bone cell population.¹ Herein we propose that mesenchymal stem cells (MSCs) in the marrow cavity sense and respond to LMMS by increasing proliferation, making a key contribution to the increase in bone quantity. MSCs are a population of adult stem cells defined by the ability to self-renew and differentiate into cells that form mesodermal tissues such as osteoblasts and adipocytes. The development of obesity and osteoporosis can thus be tied back to the same progenitor cell, linked by an inverse and interdependence of osteoblast and adipocyte production. The deflection of stem cell differentiation into bone, rather than fat highlights the potential of LMMS as an intervention and prevention strategy for both diseases², based on a "precursor strategy" that capitalizes on the *in vivo* plasticity of MSCs.

ANIMAL MODEL

- Male C57BL/6J mice, starting at 7wks age, fed a high fat (45% kcal fat) diet.
- LMMS mice = 15min/d of a 90Hz, 0.2g acceleration, 6wks, 5 d/wk.
- CON animals were placed on an inactive platform.
- Animals euthanized after 6wk of treatment and bone marrow cells (BMCs) extracted from the femurs and tibias.

METHODS

BMCs were stained with appropriate antibodies and flow cytometry data was collected using a Becton Dickinson FACScalibur. Total RNA was extracted from BMCs and real time PCR was performed with a one-step kit (Qiagen) on a Roche Lightcycler, or by Bar Harbor Biotechnology on their Osteoporosis StellARray.

LMMS INCREASES NUMBER OF STEM CELLS

Measurements using antibodies against Stem Cell Antigen-1 (Sca-1) and Preadipocyte factor (Pref-1) indicate that in dietary induced obesity (DIO), 6wks of LMMS significantly increases the overall stem cell population relative to CON. Looking at all Sca-1 positive cells, we observe a 37.2% (p=0.028) increase in LMMS stem cell numbers over sham handled CON animals and a 46.1% (p=0.022) increase in specifically MSCs (Fig 1).

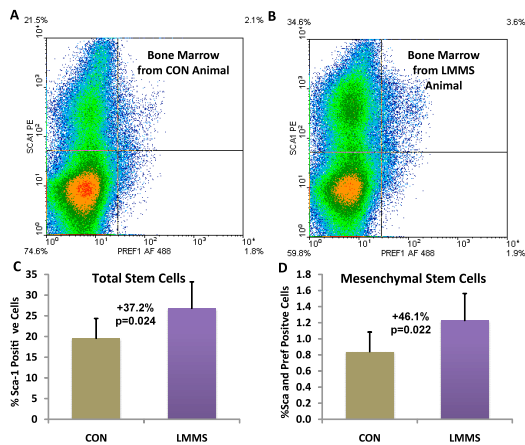


Fig 1. LMMS increased the number of stem cells. Representative dot plots from flow cytometry demonstrated the ability of low magnitude mechanical signals (LMMS) to increase the number of overall stem cells (Sca-1+) and specifically MSCs (Sca-1+, Pref-1+) for a CON animal (A) and a LMMS treated animal (B). The actual increases in cell number were calculated as % positive cells/total cells for the cell fraction showing highest intensity staining (C,D).

LMMS STIMULATES MSCS TO PREFERENTIALLY DIFFERENTIATE INTO OSTEOPROGENITORS

The potential of LMMS promotion of MSC proliferation to also translate into increased bone formation is seen by the increase in osteoprogenitors, where 6wks of LMMS increases this population by 29.9% (p=0.23, Fig 2A,B).

This increase in osteogenic potential is also seen *in vitro*, where BMCs harvested from either LMMS (7 days) or CON animals were induced to differentiate towards bone with osteogenic media. Cells from LMMS exposed animals show a 43.1% increase in bone formation as seen with von Kossa staining, even as the only stimulation the cells had received occurred *in vivo*, before cell harvest.

The "cost" of this increase in bone potential comes at the expense of the differentiation of fat cells. Pref-1 positive cells are regarded as negative regulators of mature adipocyte differentiation, and an increase (+18.5%, p=0.25) in this population indicates that in parallel, increased osteogenesis and decreased adipogenesis result from LMMS (Fig 2C).

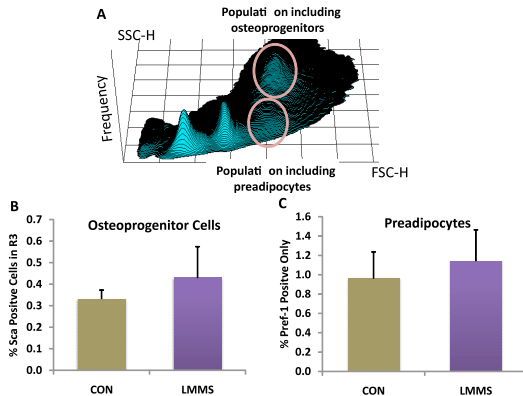


Fig 2. Osteoprogenitor cells were identified as Sca-1(+) cells, residing in the region highlighted as high forward (FSC) and side (SSC) and were 29.9% (p=0.233) more abundant in the bone marrow of LMMS treated animals (B). The preadipocyte population identified as Pref-1(+), Sca-1(-) demonstrated a trend (+18.5% in LMMS, p=0.250) towards increase between the CON and LMMS groups (C).

MOLECULAR ENVIRONMENT IN BONE MARROW IS BIASED TOWARDS OSTEOGENESIS

The cellular level trend indicating that the balance of differentiation is shifted towards osteogenesis in the marrow environment in the LMMS animals is confirmed by gene expression data. As evidenced in the bone marrow isolated from animals after 6 weeks of LMMS stimulation the transcription of Runx2 saw an upregulation of 72.5% (p=0.021) relative to CON. In these same LMMS animals, expression of PPARγ was 26.9% (p=0.042) lower than CON animals (Fig 3).

Real time PCR data with the Osteoporosis StellARray (Bar Harbor Biotech) show a one fold increase (p=0.01) in expression of estrogen related receptor (Esrra), and 2.6 fold (p=0.04) decrease in sclerostin (Sost). Both these genes, in addition to the trends listed in Fig 3, show the increase in bone formation seen in our studies exhibit expression profiles involving members of the Wnt and beta-estradiol signaling pathways.

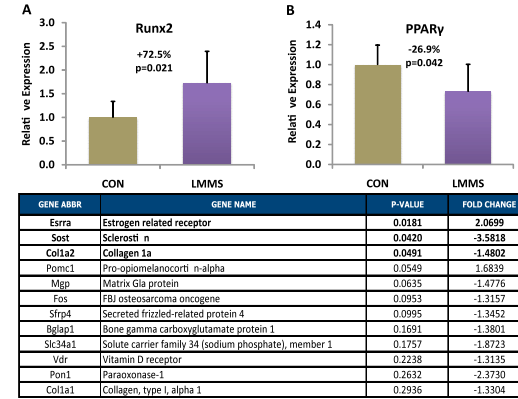


Fig 3. Animals subject to 6wk LMMS indicated a significant upregulation of the key osteogenic gene Runx2 (A) and downregulation of the key adipogenic gene PPARγ (B). Estrogen related receptor (Esrra) expression is significantly up, while sclerostin (Sost) is down (C). Additional genes involved in bone regulation shown trends towards up- or down-regulation supporting the increased osteogenesis of LMMS animals.

CONCLUSION

While the resident bone cell population is affected by mechanical stimulation, we posit that in addition, bone formation may also be regulated at the precursor stage. We show that LMMS is able to increase the proliferation of MSCs, and reduce adipogenesis while promoting osteogenesis. The increase in MSC number and osteogenic potential has been confirmed by increased mineralization *in vitro*, and more significantly increased bone formation *in vivo* (Fig 4).

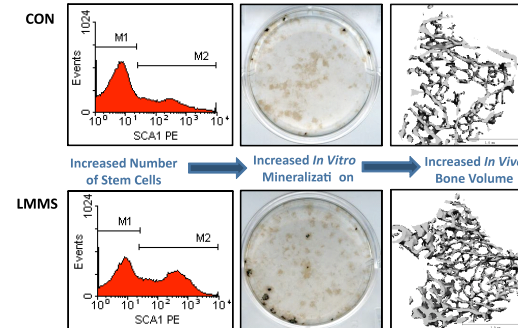


Fig 4. Increase in number and osteoblastic potential of MSC is reinforced *in vitro*, where bone marrow harvested from LMMS animals persist towards osteoblasts, expressing increased mineralization, without further mechanical stimulation. *In vivo*, the enhancement is seen in increased bone volume fraction of LMMS animals.

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