NMR Metabolomic Analysis of Osteoblasts Pre and Post Differentiation

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Introduction

This research is relevant to 18 million Americans, primarily the elderly, who suffer from osteopenia (loss of bone mass) and osteoporosis. The other targeted population is astronauts, in low-earth orbit, who lose 1 to 3 % of their bone mass per month (1). The osteoblastic cell line, MC3T3 serves as a model system to investigate potential mechanisms of osteopenia.

Methods and Results

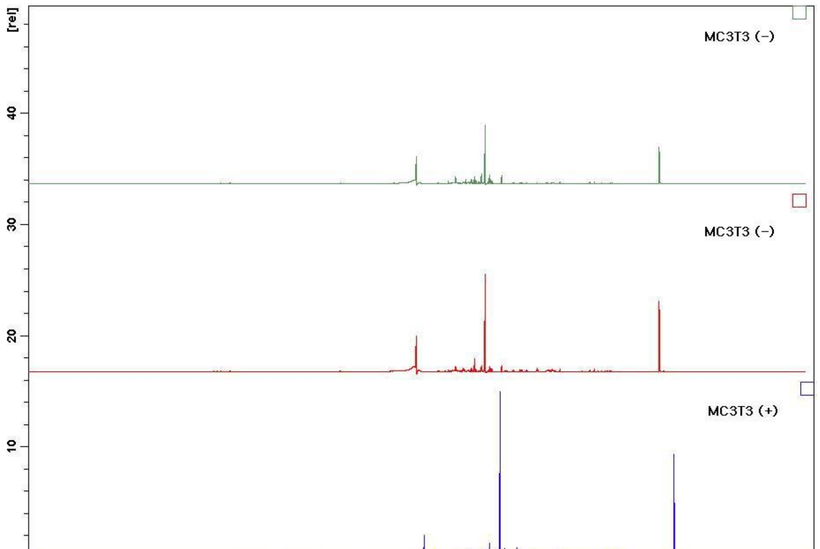


Figure 1 (a). ^{1}H-1DNMR spectra

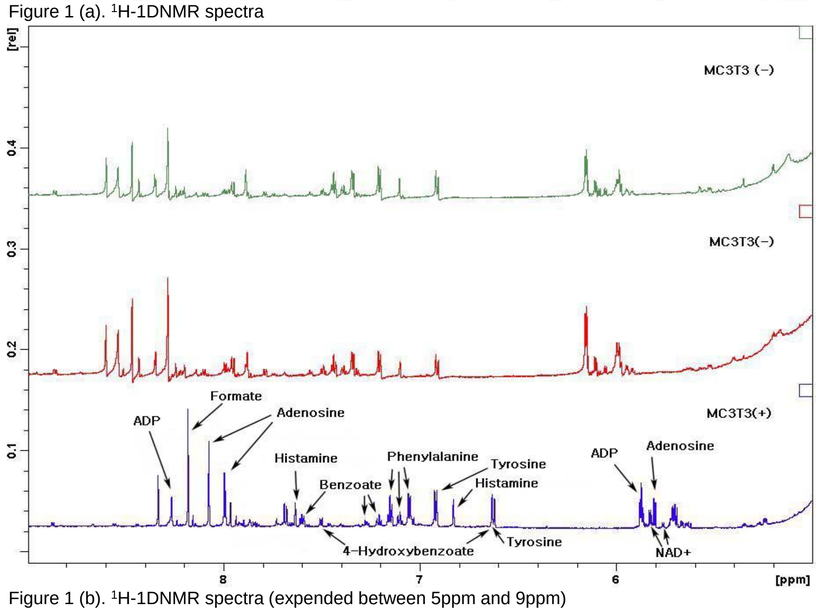
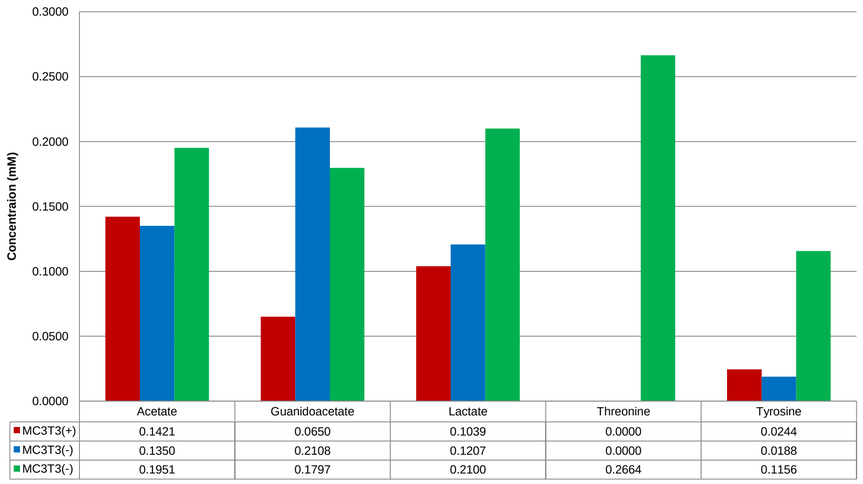


Figure 1 (b). ^{1}H-1DNMR spectra (expended between 5ppm and 9ppm)

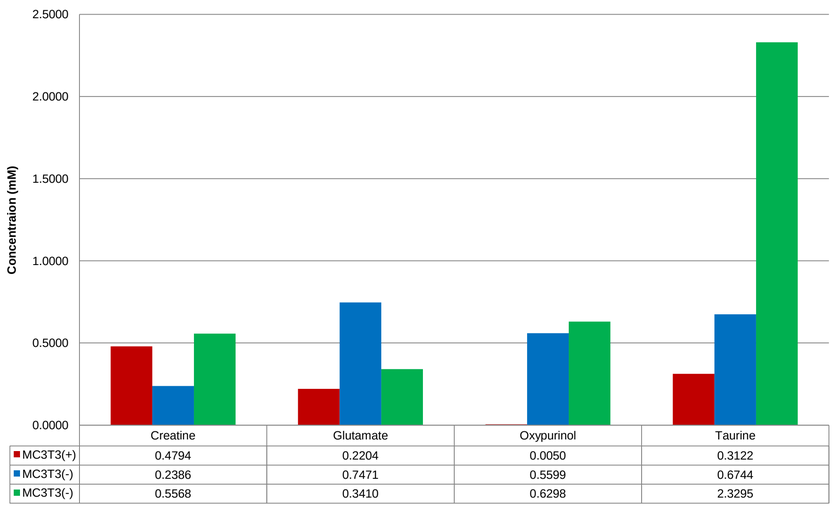
Observed Metabolites in MC3T3(+) and MC3T3(-) Mineralization Samples

1,3-Dimethylurate	Acetate	Beta-alanine	Glycine	Methylamine	Pyruvate	Tyrosine
3-Hydroxyisovalerate	Adenosine	Betaine	Guanidoacetate	Myo-inositol	Succinate	Valine
3-Methylxanthine	ADP	Creatine	Histamine	NAD+	Tartrate	Xanthine
4-Hydroxybenzoate	Alanine	Ethylene glycol	Lactate	O-phosphocholine	Taurine	Xanthosine
4-Pyridoxate	Alloisoleucine	Formate	Maleate	Oxypurinol	Threonine	
5,6-Dihydrothymine	Aspartate	Galactonate	Malonate	Phenylalanine	Trimethylamine	
Acetamide	Benzoate	Glutamate	Methanol	Pi-Methylhistidine	Trimethylamine N-oxide	



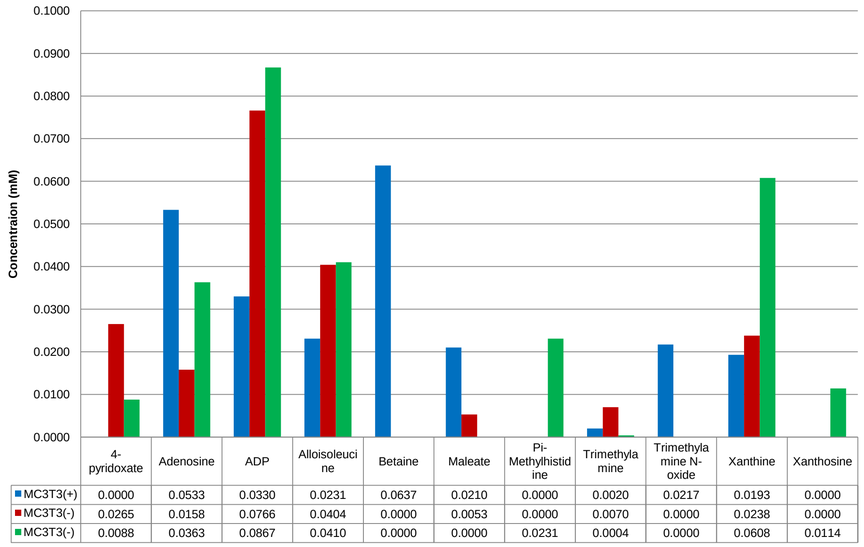
	Acetate	Guanidoacetate	Lactate	Threonine	Tyrosine
MC3T3(+)	0.1421	0.0650	0.1039	0.0000	0.0244
MC3T3(-)	0.1350	0.2108	0.1207	0.0000	0.0188
MC3T3(-)	0.1951	0.1797	0.2100	0.2664	0.1156

Figure 2(a)



	Creatine	Glutamate	Oxypurinol	Taurine
MC3T3(+)	0.4794	0.2204	0.0050	0.3122
MC3T3(-)	0.2386	0.7471	0.5599	0.6744
MC3T3(-)	0.5568	0.3410	0.6298	2.3295

Figure 2(b)



	4-pyridoxate	Adenosine	ADP	Alloisoleucine	Betaine	Maleate	Pi-Methylhistidine	Trimethylamine	Trimethylamine N-oxide	Xanthine	Xanthosine
MC3T3(+)	0.0000	0.0533	0.0330	0.0231	0.0637	0.0210	0.0000	0.0020	0.0217	0.0193	0.0000
MC3T3(-)	0.0265	0.0158	0.0766	0.0404	0.0000	0.0053	0.0000	0.0070	0.0000	0.0238	0.0000
MC3T3(-)	0.0088	0.0363	0.0867	0.0410	0.0000	0.0000	0.0231	0.0004	0.0000	0.0608	0.0114

Figure 2 (c).

- ❖^{1}H-1DNMR (700MHz) spectra of water soluble extracts from MC3T3 Osteoblastic cells were obtained.
- ❖Two osteoblast populations were studied – one cultured in cell media with the addition of ascorbic acid and the other population cultured in cell media without the addition of ascorbic. Ascorbic acid induces osteoblast cells to differentiate resulting in the production of bone matrix.
- ❖The metabolomic signature of osteoblast cells, pre and post differentiation, were compared through ^{1}H-1D NMR spectroscopy .
- ❖^{1}H NMR Spectra was analyzed by Chenomx 6.1 software. Chenomx deconvolves the chemical shifts of an NMR spectrum and assigns them to known metabolite signatures. This allows the identification of roughly 100 to 200 metabolites.

Conclusions

- ❖There are some differences in metabolite levels between pre and post osteoblast differentiation.
- ❖These results may aid understanding mechanisms of bone matrix related to bone loss.
- ❖Analysis is ongoing, though the ^{1}H-2D-TOCSY NMR spectroscopy will be used in future experiments for cells that are magnetically levitated to observe changes in cell metabolomics when gravitational loading is reduced or eliminated.
- ❖ Further work is necessary to determine normalize all the samples to compare sample to sample.

References

1. Tilton, F.E., Degioanni, J.J., Schneider,V.S., *Long-term follow-up of Skylab bone demineralization,* Aviat. Space. Environ. Med., 51(11),1209-1213, 1980.

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