

# ALCOHOL-INDUCED REGULATION OF GENES INVOLVED IN BONE MARROW CELL OSTEOGENESIS AND ADIPOGENESIS

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**Introduction:** The action of alcohol on cells in bone and marrow could contribute to abnormal lipid metabolism (1-3). To help understand the mechanism, we determined the effect of ethanol treatment on gene expression by cells obtained from bone marrow stroma, and 3T3 NIH cells in culture. Our experiments were directed towards understanding the role of alcohol on the changes in gene expression by the cells during adipogenesis and osteogenesis.

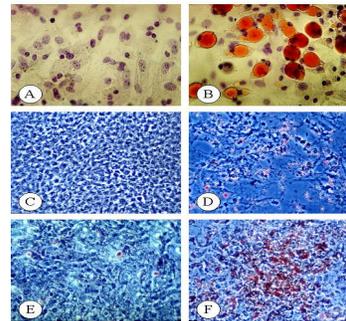
**Materials and Methods:** Bone marrow stromal cells obtained from femora of mice, a multipotential cell, D1 (4), was cloned from bone marrow and 3T3 NIH cells were used in our experiments. The cells were maintained in culture, the marrow stromal cells were treated with 0.03, 0.09, or 0.15 molar ethanol every forty-eight hours for 21 days, and D1 cells were treated with 0.09, 0.15, or 0.21 molar ethanol daily and 3T3 NIH cells were treated with 0.03, 0.06, 0.09, 0.15, or 0.21 molar ethanol daily for 14 days. The effect of ethanol on adipogenesis was examined by determining the accumulation of triglyceride vesicles within the cells by phase contrast microscopy and by staining with Sudan IV. Osteogenesis and adipogenesis were measured with RT-PCR to detect osteocalcin and PPAR $\gamma$  mRNAs in D1 cells and 3T3 NIH cells at 14 days. The level of osteocalcin in culture media from marrow stromal cells treated with 0.15 molar ethanol for 14 days was determined by radioimmunoassay. A colorimetric assay was used to determine alkaline phosphatase (ALP) activity in D1 cells treated with ethanol on days 4, 8, and 12 and in marrow stromal cells treated with 0.15 molar ethanol on days 8, 10 and 12.

**Results:** The marrow stromal cells and D1 and 3T3 NIH cells treated with ethanol accumulated triglyceride vesicles, which were small initially and increased in size with time (Figure 1). The number of adipocytes in culture increased when the cells were treated with higher concentrations of ethanol. Cultures of marrow stromal cells treated with 0.15 molar ethanol for 21 days contained 249-fold more adipocytes than the cultures without ethanol ( $p < 0.001$ ) (Figure 2). The results of RT-PCR showed that treatment with ethanol decreased expression of osteocalcin mRNA but did not change expression of PPAR $\gamma$  mRNA in D1 cells. Expression of PPAR $\gamma$  mRNA increased in 3T3 cells treated with ethanol for 14 days (Figure 3). The level of osteocalcin in culture media of marrow stromal cells treated without ethanol was 2.93-fold higher than that the cells treated with 0.15 molar ethanol for 14 days ( $p < 0.001$ ). The cells treated without ethanol expressed the highest levels of ALP activity. ALP activity in the marrow stromal cells was 2.2, 2.6, and 4.8 fold higher than in the cells treated with 0.03, 0.09, and 0.15 molar ethanol respectively ( $p < 0.001$ ) (Figure 4), and that in D1 cells was 1.7, 2.8, and 13.9 fold higher than in the cells treated with 0.09, 0.15, and 0.21 molar ethanol respectively ( $p < 0.001$ ) (Figure 5).

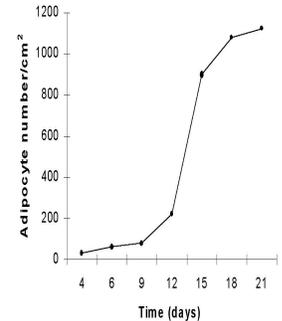
**Discussion:** In this study, D1 cells and marrow stromal cells accumulated triglyceride vesicles and showed decreased osteocalcin gene expression and ALP activity demonstrating increased adipogenesis and decreased osteogenic properties of the cells. 3T3 NIH cells accumulated triglyceride vesicles and also showed increased PPAR $\gamma$  gene expression commensurate with increased adipogenic properties in culture. Inhibition of osteocalcin gene expression without an increase in PPAR $\gamma$  mRNA suggests that adipogenesis such as increased triglyceride vesicles is brought about by the action of ethanol downstream in the fatty acid metabolism pathway. The decreased osteogenic properties and increased adipogenesis in bone and marrow cells may be a major factor contributing to alcohol-related osteonecrosis and osteoporosis.

## References:

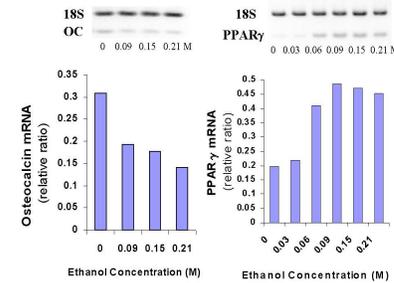
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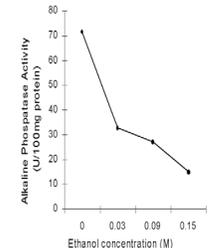
**Figure 1:** Staining with Sudan IV showing no triglyceride vesicles after treatment without ethanol in marrow stromal cells (A), D1 cells (C) and 3T3 cells (E), and accumulation of triglyceride vesicles after treatment with ethanol in marrow stromal cells (0.15 molar ethanol) (B), D1 cells (0.21 molar ethanol) (D) and 3T3 cells (0.21 molar ethanol) (F).



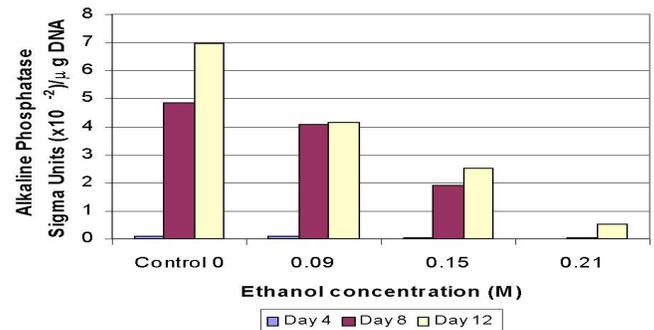
**Figure 2:** The number of adipocytes in culture of marrow stromal cells increased with time of treatment with 0.15 molar ethanol.



**Figure 3:** RT-PCR showed that treatment with ethanol decreased expression of osteocalcin in D1 cells, and increased expression of PPAR $\gamma$  mRNA in 3T3 cells.



**Figure 4:** ALP activity in marrow stromal cells decreased upon treatment with increasing concentrations of ethanol for 12 days.



**Figure 5:** ALP activity in D1 cells at 4, 8 and 12 days after treatment with ethanol. D1 cells treated without ethanol expressed high levels of ALP activity, which was 1.7, 2.8 and 13.9-fold higher than that in cells treated with 0.09, 0.15, and 0.21 molar ethanol respectively at 12 days.