# An Investigation into the Effects of Omega-3 Fatty Acids on Bone Resorption in the Female Ovariectomised Rat

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# Abstract

Estrogen deficiency results in disruption of the normal bone remodeling cycle leading to a loss of bone mineral and, in many cases, the development of osteoporosis. Various studies have demonstrated a beneficial effect of essential fatty acids (EFAs) in reducing the loss of bone density as a consequence of estrogen deficiency. The aim of the present study was to examine the specific effects of the n-3 EFA, eicosopentaenoic acid (EPA) on bone density and strength in ovariectomised female rats.

60 Sprague-Dawley rats were randomized into four groups and either ovariectomised (n=45) or sham operated (n=15). Ovariectomised animals were fed calcium adequate diets containing either corn oil (OVX control, n=15), corn oil + 0.1g/kg body weight EPA (low dose, n=15) or corn oil + 1.0g/kg body weight EPA (high dose, n=15) for a period of nine weeks. Sham rats were fed the corn oil diet as per the OVX control group. Urinary calcium and phosphate excretion, serum type 1 collagen c-telopeptide concentration, bone density, bone ash and bone breaking strength were measured. Plasma fatty acid composition and serum concentrations of 25 hydroxyvitamin $D_3$ were also determined.

Femur bone density was significantly lower in the high dose group compared to sham, OVX control and low dose EPA groups ($p<0.001$, $p=0.0096$ and $p=0.0047$ respectively). Low dose EPA supplementation had no significant effect on bone density. No significant differences in urinary calcium or phosphate concentrations, serum concentrations of type-1 collagen c-telopeptide or bone breaking strength were evident with either dose of EPA compared to unsupplemented, ovariectomised controls. EPA supplementation resulted in significant decreases in the levels of n-6 EFAs and increases in the levels of n-3 EFAs except docosahexaenoic acid in plasma lipids. Both low and high dose EPA supplementation led to significant increases in serum concentration of 25(OH) vitamin $D_3$.

In conclusion 1.0g EPA/kg body weight had a detrimental effect on bone density in ovariectomised rats. It is proposed that high intake of the highly unsaturated EPA resulted in significant lipid peroxidation. This in turn disrupted membrane structure and inhibited

intestinal calcium absorption thereby stimulating PTH-mediated bone resorption. A potential role for n-3 EFAs in the regulation of vitamin D activity is also outlined.

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| | |
|---|---|
| $1,25(OH)_2D_3$ | 1,25 dihydroxyvitamin $D_3$ |
| $25(OH)vitD_3$ | 25 hydroxyvitamin $D_3$ |
| AA | Arachidonic Acid (20:4n-6) |
| ALA | Alpha-Linolenic Acid (18:3n-3) |
| ATP | Adenosine Triphosphate |
| ATPase | Adenosine Triphosphatase |
| BGP | Bone Gla Protein (osteocalcin) |
| BMI | Body Mass Index |
| BMP | Bone Morphogenic Protein |
| BRU | Bone Remodelling Unit |
| Ca or $Ca^{2-}$ | calcium |
| cAMP | Cyclic Adenosine Monophosphate |
| Cbfa-1 | Core Binding Factor 1 |
| Cl | Chloride |
| CLA | Conjugated Linoleic Acid |
| COX | Cyclooxygenase |
| CTX | C-terminal telopeptide of type-1 collagen |
| DHA | Docosahexaenoic Acid (22:6n-3) |
| DLX-5 | Distal-less 5 transcription factor |
| DPA | Docosapentaenoic Acid (22:5n-3) |
| Dpyd | Deoxypyridinoline |
| EFA | Essential Fatty Acid |
| EGF | Erythrocyte Growth Factor |
| ELISA | Enzyme-linked Immunoassay |
| EPA | Eicosapentaenoic Acid (20:5n-3) |
| FGF | Fibroblast Growth Factor |
| g | gram |
| GLA | Gamma Linolenic Acid (18:3n-6) |

| | |
|---|---|
| gp130 | Glycoprotein 130 |
| GTPase | Guanisine Triphosphatase |
| $H^-$ | Hydrogen |
| hGH | Human Growth Hormone |
| HMG-CoA | Hydroxymethylglutaryl Coenzyme A |
| IFN | Interferon |
| IGF | Insulin-like Growth Factor |
| IGFBP | Insulin-like Growth Factor Binding Protein |
| IL | Interleukin |
| IV | intravenous |
| K or $K^+$ | Potassium |
| kg | kilogram |
| LA | Linoleic Acid (18:2n-6) |
| LT | Leukotriene |
| LTB4 | Leukotriene B4 |
| LTB5 | Leukotriene B5 |
| M-CSF | Monocyte-Macrophage Colony Stimulating Factor |
| mg | milligram |
| Mg or $Mg^{2+}$ | Magnesium |
| mL | milliliter |
| mm | millimeter |
| mMol | millimoles |
| MMPs | Matrix Metalloproteinases |
| N | Newton |
| n-3 | omega 3 |
| n-6 | omega 6 |
| n-9 | omega 9 |
| Na or $Na^+$ | Sodium |
| NF-κB | Nuclear Factor-κB |
| ng | nanogram |
| $N/mm^2$ | Newtons per square millimeter |

| | |
|---|---|
| OPG | Osteoprotegerin |
| OVX | ovariectomised |
| PDGF | Platelet-derived Growth factor |
| $PGE_2$ | Prostaglandin E2 |
| $PGE_3$ | Prostaglandin E3 |
| PKC | Protein Kinase C |
| $PO_4$ | Phosphate |
| POV | Peroxide Value |
| PPAR | Peroxisome Proliferator Activated Receptor |
| PPRE | Peroxisome Proliferator Response Element |
| PTH | Parathyroid Hormone |
| PTHrp | Parathyroid Hormone-related protein |
| PUFA | Polyunsaturated Fatty Acid |
| RANK-L | RANK ligand |
| RXR | Retinoid X Receptor |
| SD | Standard Deviation |
| SE | Standard Error |
| T3 | Triiodothyronine 3 |
| T4 | Thyroxine |
| TGF | Transforming Growth Factor |
| TNF | Tumour Necrosis Factor |
| TRAFs | Tumour Necrosis Factor Receptor-Associated Factors |
| TxB2 | Thromboxane B2 |
| VDR | Vitamin D Receptor |
| WHO | World Health Organisation |
| Yrs | Years |