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

A thesis presented in partial fulfillment of the requirements for the degree of

Master of Science
In
Nutritional Science

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Raewyn Carol Poulsen
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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, eicosapentaenoic 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 D3 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 D3.

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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<td>1,25(OH)$_2$D$_3$</td>
<td>1,25 dihydroxyvitamin D$_3$</td>
</tr>
<tr>
<td>25(OH)vitD$_3$</td>
<td>25 hydroxyvitamin D$_3$</td>
</tr>
<tr>
<td>AA</td>
<td>Arachidonic Acid (20:4n-6)</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-Linolenic Acid (18:3n-3)</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>ATPase</td>
<td>Adenosine Triphosphatase</td>
</tr>
<tr>
<td>BGP</td>
<td>Bone Gla Protein (osteocalcin)</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BMP</td>
<td>Bone Morphogenic Protein</td>
</tr>
<tr>
<td>BRU</td>
<td>Bone Remodelling Unit</td>
</tr>
<tr>
<td>Ca or Ca$^{2-}$</td>
<td>calcium</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic Adenosine Monophosphate</td>
</tr>
<tr>
<td>Cbfa-1</td>
<td>Core Binding Factor 1</td>
</tr>
<tr>
<td>CI</td>
<td>Chloride</td>
</tr>
<tr>
<td>CLA</td>
<td>Conjugated Linoleic Acid</td>
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<td>COX</td>
<td>Cyclooxygenase</td>
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<td>CTX</td>
<td>C-terminal telopeptide of type-1 collagen</td>
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<td>DPA</td>
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<td>Dpyd</td>
<td>Deoxypyridinoline</td>
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<td>EFA</td>
<td>Essential Fatty Acid</td>
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<td>EGF</td>
<td>Erythrocyte Growth Factor</td>
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<td>Fibroblast Growth Factor</td>
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<td>g</td>
<td>gram</td>
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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 Mg2+  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-kB  Nuclear Factor-kB
ng  nanogram
N/mm²  Newtons per square millimeter
OPG  Osteoprotegerin
OVX  ovariectomised
PDGF  Platelet-derived Growth factor
PGE₂  Prostaglandin E₂
PGE₃  Prostaglandin E₃
PKC  Protein Kinase C
PO₄  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