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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

At Massey University, Turitea,
Palmerston North,
New Zealand

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2004

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.

Acknowledgements

I wish to express my sincere appreciation to the following people who have not only made the development and presentation of this thesis possible but also enjoyable:

- My supervisor, Assoc. Prof. Marlena Kruger who so freely gave her time and energy and shared her expertise.

- The Human Nutrition Cluster staff who willingly shared their wealth of knowledge, sacrificed their time and provided friendship and support throughout the year. In particular:
 - Mrs Gabrielle (Gabby) Plimmer for her patient teaching
 - Mrs Kim Wylie who willingly tackled all the tedious jobs
 - Mrs Anne Broomfield who I'll be forever grateful to for helping with the DEXA scans
 - Mrs Chris Booth who could always be counted on for anything, anytime, anywhere.
 - Mr Jan Steenkamp whose surgical skills and willingness for early morning starts helped ensure we kept to schedule.

- Dr Phil Pearce and the team at the Massey Nutrition Lab who helped with the analytical work.

- Mr Michael Agnew of AgResearch Limited, Hamilton, New Zealand who conducted the plasma fatty acid analysis.

- Mr Karl Geiringer of Brainfats Biotechnology Limited, Auckland, New Zealand who kindly supplied the eicosapentaenoic acid free of charge.

Finally I would like to thank Massey University who provided financial support in the form of a Masterate Scholarship and a MURF grant.

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List of Abbreviations

1,25(OH) ₂ D 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 (<i>osteocalcin</i>)
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
TxB ₂	Thromboxane B2
VDR	Vitamin D Receptor
WHO	World Health Organisation
Yrs	Years