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Long chain polyunsaturated fatty acids and their possible interaction with phytoestrogens: Impact on bone and bone cell function in vivo and in vitro

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

Doctor of Philosophy
in
Biochemistry

at Massey University, Palmerston North,
New Zealand.

Raewyn Carol Poulsen

2007
Abstract

Inflammation is a major contributor to postmenopausal bone loss. Various long chain polyunsaturated fatty acids (LCPUFAs), particularly those of the n-3 family, are known to have anti-inflammatory activity and may have a role in minimising postmenopausal bone loss. The objectives of this thesis were to determine whether some LCPUFAs have greater bone-protective effects than others; to identify some of the mechanisms of action of LCPUFAs in bone and to explore the possibility that combined treatment with LCPUFAs and phytoestrogens offers greater bone-protective effects than either treatment alone. Using the ovariectomised rat model for postmenopausal bone loss, the relative effectiveness of eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and gamma-linolenic acid (GLA, 18:3n-6) in minimising bone loss post-ovariectomy was investigated. GLA exacerbated bone loss post ovariectomy. In vitro, treatment of MC3T3-E1/4 osteoblast-like cells with GLA resulted in greater membrane-bound RANKL expression suggesting a possible stimulatory effect of GLA on osteoclastogenesis and osteoclast activity. EPA had no effect on overall bone mass in vivo. DHA significantly ameliorated ovariectomy-induced bone loss possibly by increasing plasma IGF-1 concentration, modulating vitamin D metabolism and, as observed in a second study, by increasing the concentration of gamma-carboxylated osteocalcin. In vitro both EPA and DHA reduced the prostaglandin E2 (PGE2)-induced increase in membrane-bound RANKL expression in MC3T3-E1/4 osteoblast-like cells. However as RANKL-independent pathways are believed to be largely responsible for the ovariectomy-induced increase in osteoclastogenesis in vivo, inhibition of RANKL expression may not significantly contribute to the prevention of ovariectomy-induced bone loss. In a second study in ovariectomised rats, combined treatment with DHA and 17β-oestradiol was associated with significantly higher femur bone mineral content than either treatment alone. However, no beneficial effects of combined treatment with DHA and either of the phytoestrogens genistein or daidzein, on bone mass were apparent. In vitro, co-treatment of TNF-α - exposed MC3T3-E1/4 cells with DHA and 17β-oestradiol was associated with a higher cell number compared to either treatment alone indicating a protective effect of combined treatment against the cytotoxic and/or anti-proliferative effects of TNF-α. In contrast, combined treatment of MC3T3-E1/4 cells with DHA and genistein, but not daidzein, was associated with significantly lower cell number than either treatment alone. As genistein, but not
daidzein, is a tyrosine kinase inhibitor, this may indicate that DHA requires tyrosine kinase activity for its protective effect on cell number in TNF-α-exposed osteoblasts. Whether DHA itself is bioactive in bone cells or whether lipid mediators formed from DHA are responsible for the observed bone-protective effects is unknown. Using lipid mediator lipidomic analysis, the presence of DHA-derived lipid mediators in bone marrow in quantities known to be physiologically significant in other tissues was confirmed. Further research into the effects of these lipid mediators in bone and confirmation of the mechanisms of action of DHA in bone cells is required. This thesis demonstrates that consumption of DHA provides some protection against ovariectomy-induced bone loss in vivo and mitigates the effects of inflammation on RANKL signalling and osteoblast cell number in vitro. The bone-protective effects of DHA are complemented by co-treatment with 17β-oestradiol but may be inhibited by co-treatment with the phytoestrogens daidzein or genistein.
Acknowledgements

This thesis would not have been possible without the guidance, support and encouragement of my three fantastic supervisors. I am sincerely grateful to Professor Marlena Kruger, my chief supervisor, for always being available any day, any time, for finding money from nowhere and for generally making the impossible possible. Special thanks to Distinguished Professor Paul Moughan for his expert advice and encouragement particularly with manuscript preparation and for always knowing the right person to ask for any problem. My grateful thanks to Dr Fran Wolber for all her time and assistance with the in vitro work and for always having an open door.

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<td>12-HEPE</td>
<td>12-hydroxy-eicosapentaenoic acid</td>
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<td>12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid</td>
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<td>BRU</td>
<td>Bone Remodelling Unit</td>
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Gamma-linolenic acid

High performance liquid chromatography

Hormone Replacement Therapy

Interferon

Insulin-like growth factor

Interleukin

Indomethacin

Inducible nitric oxide synthase

Kilogram

Litre

Linoleic acid

Liquid chromatography tandem mass spectrometry

Long chain polyunsaturated fatty acid

Lipoxygenase

Low density lipoprotein receptor related proteins 5/6

Lumbar spine

Leukotriene B4

Lipoxin A4, 5S,6R,15S-trihydroxy-7E,9E,13E,11Z-eicosatetraenoic acid

Lipoxin B4, 5S,14R,15S-trihydroxyl-7E,9E,13E11Z-eicosatetraenoic acid

Mitogen activated protein kinase

Macrophage colony stimulating factor

Minimum essential media

Millilitre

Millimetre

Matrix metalloproteinase

Mole

Messenger ribonucleic acid

Nicotinamide adenine dinucleotide phosphate

Nuclear factor kappa B

Nitric oxide

PD1 generated in neural systems

o-desmethylangolensin

17β-oestradiol

Osteoprotegerin

Osterix

Ovariectomised


Platelet derived growth factor

Phycoerythrin

Prostaglandin

Peroxisome proliferator-activator receptors

Peripheral Quantitative Computed Tomography
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>RANK</td>
<td>Receptor activator of nuclear factor kappa B</td>
</tr>
<tr>
<td>RANKL</td>
<td>Receptor activator of nuclear factor kappa B ligand</td>
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<tr>
<td>RBC</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>RIA</td>
<td>Radio immunoassay</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
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<tr>
<td>Rv</td>
<td>Resolvin, resolution phase interaction product</td>
</tr>
<tr>
<td>RvD1</td>
<td>7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid;</td>
</tr>
<tr>
<td>RvE1</td>
<td>5S,12R,18R-trihydroxy-eicosa-6Z,8E,10E,14Z,16E-pentaenoic acid</td>
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<tr>
<td>RvE2</td>
<td>5S,18(R/S)-dihydroxy-eicosapentaenoic acid</td>
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<tr>
<td>RXR</td>
<td>Retinoid X receptor</td>
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<tr>
<td>s.c.</td>
<td>Sub-cutaneous</td>
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<tr>
<td>Sham</td>
<td>Sham-operated</td>
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<tr>
<td>TGF</td>
<td>Transforming Growth Factor</td>
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<tr>
<td>TNF</td>
<td>Tumour necrosis factor</td>
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<tr>
<td>TRAFs</td>
<td>Tumour necrosis factor receptor activated factors</td>
</tr>
<tr>
<td>TRAP</td>
<td>Tartrate resistant acid phosphatase</td>
</tr>
<tr>
<td>Tx</td>
<td>Thromboxane</td>
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<tr>
<td>Wnt</td>
<td>Wingless type</td>
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