The effect of a milk lipid fraction on bone properties of growing female rats and the growth and function of bone cells

A thesis presented in partial fulfilment of the requirements for

the degree of

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in

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New Zealand

Zhongling Cao

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Abstract

Objective: To investigate the effects of a milk lipid fraction (MLF) on the accruel of bone mass in growing rats by evaluating the effects of MLF on growth and bone parameters such as bone mineral content (BMC), bone mineral density (BMD) and biomechanical properties in growing rats and the effects of MLF on the development and activity of bone cells, including osteoblasts and osteoclasts.

Methods: There were one hundred and eight 3-month old female Sprague-Dawley rats were randomly allocated into three groups: a control group (n=48), a low-dose MLF group (n=30) fed with 250 mg/rat/day of MLF, and a high-dose MLF group (n=30) fed with 500 mg/rat/day of MLF. Forty-five rats (n=15 for each group) were selected to terminate at month 3 for biomechanical testing while sixty-three continued into a second arm of the trial after ovariectomy. Body composition and bone parameters of animals (n=108) were measured *in vivo* by Dual Energy X-Ray Absorptiometry (DEXA) at baseline and week 12 of the study. Length and diaphyseal width and thickness of the left femur were measured. The three-point bending test was used to evaluate the biomechanical characteristics of the left femur of rat. The effect of MLF on proliferation, differentiation and mineralization of murine osteoblasts were investigated using the osteoblastic cell line MC3T3-E1. The cells were cultured with 0-1,000 μg/ml or 0-100 μg/ml MLF for 5, 9 and 24 days, respectively for proliferation, differentiation and mineralization. Cell proliferation was determined using the methyl-thiazolyl tetrazolium (MTT) assay. The differentiation of osteoblasts was detected using the alkaline phosphatase (ALP) activity assay. Mineralized nodules were examined using an Alizarin red histochemistry assay. The effect of MLF on RANKL-induced osteoclastogenesis was evaluated in the murine monocytic cell line RAW264.7. The cells were cultured with 0-100 μg/ml MLF for five days. Osteoclastogenesis was determined using the tartrate-resistant acid phosphatase (TRAP) staining assay and counting numbers of TRAP-positive multinucleated cells.

Results: Rats fed with the high-dose MLF diet had a significant increase in body lean mass compared with those fed with the control and low-dose MLF diets. The high-dose MLF group also had significant gains in BMC at the femur and in BMD at

the femur and lumbar spine compared with the control group. There were no significant differences in dimensional and biomechanical results among groups. The MLF significantly increased the proliferation of MC3T3-E1 at 0.1, 1.0 and 100 μg/ml. There was a dose-dependent, but not significant increase in the differentiation of osteoblasts cultured with MLF for 9 days. After 24-days of cell culture, the MLF at the low concentrations of 0.1 and 1.0 μg/ml led to non-significant increase in calcium deposition by the differentiated osteoblasts. MLF at 10 and 100 μg/ml significantly inhibited calcium nodule formation. RANKL-stimulated osteoclastogenesis was significantly increased in RAW 264.7 cells cultured with the MLF at concentrations up to 10 μg/ml.

Conclusion: These results indicated that oral administration of MLF to the growing rats improved bone accrual and has a favourable effect on achievement of peak bone mass. The MLF increased the proliferation of MC3T3-E1 pre-osteoblast cell line, but there was no effect on osteoblast differentiation and the higher concentration of MLF may have inhibited the function of mature osteoblast. Additionally, the MLF stimulated osteoclastogenesis from RAW264.7 cells. Further studies are required to investigate some of the contradictory findings presented in this report.

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Abbreviations

$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D_3
AA	Arachidonic acid
ALA	α-linolenic acid
ALP	Alkaline phosphatase
BA	Bone area
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMP	Bone morphogenic protein
BMU	Basic multicellular unit
BSA	Bovine serum albumin
Cbfa1	Core-binding factor α1
CLA	Conjugated linoleic acid
COX	Cyclooxygenases
CV	Coefficients of variation
DEXA	Dual energy X-ray absorptiometry
DHA	Docosahexaenoic acid
DMSO	Dimethyl sulfoxide
DPA	Docosapentaenoic acid
DPA	Docosapentaenoic acid
Dpyd	Deoxypyridinoline
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
ER	Estrogen receptor
ERK	Extracellular regulated kinase
FBS	Fetal bovine serum
FOS	Framingham Osteoporosis Study
GPR	G protein-coupled receptor
HDAC	Histone deacetylase
Hyp	Hydroxyproline
IGF-1	Insulin-like growth factor-1

Ihh	Indian hedgehog
IL	Interleukin
iNOS	inducible nitric oxide synthase
LA	Linoleic acid
LOX	Lipooxygenase
LPS	Lipopolysaccharide
LT	Leukotriene
MAPK	Mitogen-activated protein kinases
MBP	Milk basic protein
M-CSF	Macrophage-colony stimulating factor
MLF	Milk lipid fraction
MSC	Mesenchymal stem cell
MTT	Methyl-thiazolyl tetrazolium
MUFA	Monounsaturated fatty acid
MyD88	Myeloid differentiation factor 88
NF- κB	Nuclear factor -κB
NO	Nitric oxide
NTx	N-telopeptides
OPG	Osteoprotegerin
OSX	Osterix
PBS	Phosphate-buffered saline
PGE_2	Prostaglandin E_2
p-NPP	p-nitrophenyl phosphate
PPARs	Peroxisome proliferator-activated receptors
PTH	Parathyroid hormone
Pyd	Pyridinoline
QC	Quality control
RANK	Receptor activator of nuclear factor -κB
RANKL	Receptor activator of nuclear factor -κB ligand
RCT	Randomised controlled trial
SFA	Saturated fatty acid
STAT3	Signal transducer and activator of transcription 3
T_3	Triiodothyronine

T_4	Thyroxine
TBS	Tris buffered saline
TFA	Trans-fatty acid
TGF-β	Transforming growth factor-β
TLR4	Toll-like receptor 4
TNF	Tumour necrosis factor
VA	Vaccenic acid
WHO	World Health Organization