Effect of calcium and vitamin D fortified milk on bone markers and vitamin D status of active, premenopausal women in Palmerston North, New Zealand

Rifana Cholidah

2013

Effect of calcium and vitamin D fortified milk on bone markers and vitamin D status of active, premenopausal women in Palmerston North, New Zealand

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

Master of Science

in

Human Nutrition

at Massey University, Manawatu, Palmerston North, New Zealand.

Rifana Cholidah

2013

Statement of originality

"I hereby declare that this thesis is my own word and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which a substantial extent has been accepted for the qualification of any other degree or diploma of a university or other institution of higher learning, except where due acknowledgement is made in the acknowledgements".

Signed …….

Date …….

Abstract

Objective: To evaluate the effects of 12 weeks supplementation with calcium and vitamin D fortified milk on vitamin D status and bone turnover markers; osteocalcin and C-telopeptide of type 1 collagen, of active, healthy premenopausal women aged 30-45 years old in Palmerston North, New Zealand.

Methods: The study was a repeated measure design. Forty three premenopausal women were recruited. Participants received two daily servings (2 x 30 g) of fortified milk (1200 mg/d calcium and 10 µg/d vitamin D) over 12 weeks. Anthropometric characteristics were measured for screening by body mass index and bone density measurement. Dietary information was collected using an estimated 3-day food record and a food frequency questionnaire. Blood samples were taken for a screening blood test and to measure plasma 25(OH)D_3, interleukin-6, and bone turnover markers; C telopeptide of type I collagen (CTx) and osteocalcin. Usual physical activity levels were estimated using the SPARC short-form New Zealand Physical Activity Questionnaires in face-to-face interviews and were objectively measured using accelerometers in a self-selected group of 25 women.

Results: A significant increase in plasma 25(OH)D_3 was found (from 69.75 ± 15.87 nmol/L at baseline to 87.83 ± 19.06 at week 12, mean ± standard deviation; *p*-value <.0001). There were significant reductions in the levels of CTx (0.31 ± 0.12 to 0.21 ± 0.09 µg/L, *p*-value <.0001) and osteocalcin (22.63 ± 6.64 to 19.64 ± 6.25 µg/L, *p*-value 0.0003). Dietary calcium intake was 1013 ± 367 mg/day and vitamin D intake was 3.9 ± 2.1 µg/day. The duration of physical activity in the questionnaire and accelerometer were 115 ± 74 and 415 ± 319 (light physical activity), 208 ± 225 and 289 ± 143 (moderate physical activity) and 126 ± 130 and 59 ± 61 minutes (vigorous physical activity) respectively.

Conclusion: Calcium and vitamin D fortified milk supplementation improved vitamin D status and decreased bone turnover markers in active premenopausal women aged 30-45 years old over a period of 12 weeks.

Acknowledgements

First and foremost, I would very much like to thank Allah, the gracious God, for giving me the strength, integrity and guidance during my study period at Massey University, New Zealand.

I would very much like to thank the following people, without whom this thesis would not have been possible. For their endless support, patience, guidance and encouragement, a special acknowledgment must be given to my supervisors, Professor Marlena Kruger and Dr Jasmine Thomson.

I would also like to express my deepest appreciation to NZAID for enabling me to pursue academic advancement, and Fonterra Brands Singapore Ltd for funding and facilitating the study. I would also like to acknowledge all the participants, who took part in this study.

I am most grateful to Mrs Chris Booth and Mrs Maria-Tine Biersteker for their generous time and help in the Human Nutrition Unit. I would like to thank Dr Fran Wolber and Ms Michelle McGrath for their assistance in the Human Nutrition Laboratory. I have been truly blessed to have Dr Janet Weber as one of my lecturer, for her guidance and support. Thank you to Assoc. Prof. Patrick C.H. Morel for his help and knowledge with statistical analysis.

My heartfelt thanks also go to pak Nadrun's family, pak Jatnika's family, mbak Uri, pak Bambang's family, mbak Nina, Anne, Anwaar, and my other friends here at New Zealand and Indonesia, your warm friendship, moral support and help have lightened my burden of this though journey.

Most importantly, my deepest thanks to my beloved husband, Bayu Sasongko, and our little angels; Lia, Sa'id and Salman, you have been my source of strength and love throughout the darkest and happiest moments in my life. Thank you, Bapak, Ibu, kak Utman, Vanti and to all my family, for your love, support and pray, I am forever indebted to you.

“Then, which of the favours of your Lord will you deny?”

(Holy Quran, Ar-Rahman:13)

Table of Contents

List of figures

List of tables

Abbreviations

$1,25(OH)_2D_3$	1,25-dihydroxyvitamin D_3
$25(OH)D_3$	25-hydroxyvitamin D_3
AI	Adequate intake
AMDR	Acceptable macronutrient distribution ranges
ANZFA	Australia New Zealand Food Authority
B-ALP	Bone-specific alkaline phosphatase
BGP	Bone gla protein
BMC	Bone mineral content
BMD	Bone mineral density
BMI	Body mass index
BMUs	Bone Multicellular Units
BTMs	Bone turnover markers
CTx	Cross-linked C telopeptide of type I collagen
CV	Coefficient of variation
CYP	Cytochrome
D_2	Ergocalciferol
D_3	Cholecalciferol
DEXA	Dual energy X-ray absorptiometry
EAR	Estimated average requirement
FFQ	Food frequency questionnaire
GM-CSF	Granulocyte macrophage colony-stimulating factor
HRP	Horseradish peroxidase
ID-LC-MS/MS	Isotope-dilution liquid chromatography–tandem mass spectrometry
IFN-ɣ	Interferon-ɣ
IL	Interleukin
LPA	Light physical activity
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage colony-stimulating factor
MGP	Matrix ɣ-carboxylic acid (gla) protein
MPA	Moderate physical activity
NCPs	Noncollagenous proteins

NHANES	National Health and Nutrition Examination Survey
NRV	Nutrient reference value
NRVANZ	Nutrient Reference Values for Australia and New Zealand
NTx	N-telopeptide of type I collagen
NZPAQ	New Zealand Physical Activity Questionnaires
NZEO	New Zealand European and Others
OC	Osteocalcin
OPG	Osteoprotegerin
PA	Physical activity
PBM	Peak bone mass
PGE_2	Prostaglandin E_2
PGs	Prostaglandins
PICP	C-propeptide of type I collagen
PTH	Parathyroid hormone
QUS	Quantitative ultrasound
RANK	Receptor activator of nuclear factor kB
RANKL	Receptor activator of nuclear factor kB ligand
RCT	Randomized controlled trial
RDI	Recommended dietary intake
SPARC	Sport and Recreation New Zealand
T_3	Triiodothyroxine
T_4	Tetraiodothyronine
TH	Thyroid hormone
TMB	Tetramethylbenzidin
TNF	Tumor necrosis factor
TSA	Tyramide signal amplification
UL	Upper level of intake
VPA	Vigorous physical activity

Introduction

Age-related bone loss affects all genders, nationalities and ethnicities (Halioua & Anderson, 1989; Li et al., 2012). Females are more affected by age-related bone loss than males due to bone loss after the onset of menopause (Mazess & Barden, 1991), and females also have a smaller skeletal mass than men which contributes to higher architectural damage (Seeman, 2002).

Evidence shows that adequate lifetime calcium intake and physical activity habits of healthy premenopausal women aged 20 to 50 years can increase their peak bone mass (Halioua & Anderson, 1989). A meta-analysis of thirty-three well-designed studies reported an overall positive correlation between calcium intake and bone mass in premenopausal females and the findings were consistent across different study designs (Welten et al., 1995). However, a more recent 12-year prospective study involving 77,761 females aged 34-59 years reported no evidence that higher calcium and milk intakes reduced fracture incidence (Feskanich et al., 1997). In children and adolescents, studies of calcium supplementation indicated a positive correlation between high calcium intake and higher bone mass (Cadogan et al., 1997; Johnston et al., 1992). In adolescent females, a randomized controlled trial evaluating the effect of 18 months milk supplementation showed a significant increase in bone mineral content and bone mineral density. However, no significant effect of milk supplementation was found on markers of bone turnover (Cadogan et al., 1997).

Few studies have been carried out to evaluate the effect of milk supplementation on bone turnover markers, particularly of premenopausal women. In a study it was found that milk supplementation significantly increased vitamin D status over 24 months (Du et al., 2004). A similar result was also reported in a short-term study of postmenopausal women (Kruger et al., 2010). In a short-term study of 82 premenopausal women, it was found that fortified milk supplementation resulted in a significant reduction of bone turnover markers (Kruger et al., 2006).

Studies of physical activity (PA) reported that physical activity benefits bone health, bone mass and bone status in children, adolescents and premenopausal women (Slemenda et al., 1991; Janz et al., 2010; Baxter-Jones et al., 2008; Kanders et al., 1988; Heinonen et al., 2012). The beneficial impacts of physical activity during adolescence remain sustained into young adulthood (Baxter-Jones et al., 2008). However, in premenopausal females, the benefits were not sustained once the exercise discontinued (Heinonen et al., 2012).

Factors such as lifestyle (physical activity), intakes of calcium and vitamin D, and sex hormone status influence development of peak bone mass as well as on going bone health (Cooper & Eastell, 1993; Heaney & Weaver, 2003; Hind & Burrows, 2007; Sakuma et al, 2007). As nutrition is essential for bone health, several other vitamins and minerals have also been identified to have significant impact on bone health and metabolism (Bonjour et al., 2010; Cashman, 2002). However, to date, limited milk supplementation studies have been performed to evaluate vitamin D status and bone turnover markers in physically active premenopausal women.