The Role of Vegetables in the Maintenance of Acid-Base Balance and Bone Structure

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Abstract

Metabolic acidosis may over time lead to osteoporosis by causing a release of calcium and other mineral phases from bone. The regular consumption of fruits and vegetables is believed to be associated with higher bone mineral density. In the last ten years various population-based studies have found positive effects of fruit and vegetable intakes on bone health. The aim of the present study was to investigate the effects of broccoli, onion, and potato on bone density and strength in male rats.

Forty male Sprague-Dawley rats were randomized into four equal groups of ten each. The animals were fed either a base, broccoli, onion, or potato diet for a period of eight weeks. The apparent percentage calcium and phosphorus retained, the serum type 1 collagen C-telopeptide concentration, bone density and bone strength, and the urinary parameters i.e. ammonia, creatinine, urea, specific gravity and osmolality were determined.

The groups on onion and potato diets had significantly higher apparent percentage calcium retained (over the balance period) than the group on control diet ($p=0.021$ and 0.008 respectively). Apparent percentage calcium retained was also significantly higher in the group on potato diet compared to the group on broccoli diet ($p=0.037$). There were no significant differences between groups for percentage phosphorus retained on ANOVA. However, the discriminant analysis (multivariate

method) showed that the group on the broccoli diet retained significantly more phosphate over the balance period compared to the other groups. The urinary ammonia excretion (over the balance period) was significantly lower in the group on broccoli diet than in the groups on base and potato diets (p=0.040 and 0.055 respectively). As for the urinary urea excretion over the balance period, the group on base diet had significantly higher urea excretion than the groups on onion and potato diets (p=0.002 and p=0.000 respectively). Urinary urea excretion (over the balance period) was also significantly higher in the group on broccoli diet compared to the groups on onion and potato diets (p=0.005 and 0.000 respectively). The differences between groups for the volume of urine produced over the balance period were also significant i.e. the group on broccoli diet produced significantly more urine than the groups on base, onion, and potato diets (p=0.011, p=0.008, and p=0.001 respectively). However, there were no significant differences between groups for urinary specific gravity, osmolality, and creatinine, and bone density, bone breaking strength, and serum type 1 collagen C-telopeptide concentrations on ANOVA.

In conclusion 1g of broccoli per day significantly reduced urinary ammonia excretion and increased apparent percentage phosphorus retained whereas 1g of onion or potato per day significantly increased apparent percentage calcium retained in growing male rats. The decrease in urinary ammonia excretion was most likely due to the buffering of metabolic acids by the bases present in broccoli resulting in decreased ammonia production and secretion. Similarly the increased apparent percentage phosphorus retained (in the group on broccoli diet) may be due to the bases present in broccoli that may have buffered metabolic acids thereby reducing the need for phosphate buffering. This increased apparent percentage phosphorus retained may also be due to a high pH which is known to enhance renal phosphate uptake. The buffering of metabolic acids by the bases present in onion and potato may have reduced the need for calcium buffering resulting in higher apparent percentage calcium retained in the groups on onion and potato diets. Thus broccoli, onion, and potato intake may protect against the bone depleting effects of an acidogenic diet and may also have the potential to increase bone mineral density.

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μg	=	Microgram
$1,25\ (OH)_2D_3$	=	1,25- dihydroxy vitamin D_3 (calcitriol)
25- hydrocholecalciferol	=	Calcidiol
ANOVA	=	Analysis of Variance
ATPase	=	Adenosine Triphosphatase
Blast	=	Osteoblast
BMC	=	Bone Mineral Content
BMD	=	Bone Mineral Density
BMP	=	Bone Morphogenetic Protein
BRU	=	Bone Remodeling Unit
Ca^{2+} or Ca	=	Calcium
$Ca_{10}\ (PO_4)_6\ (OH)_2$	=	Calcium Hydroxyapatite
CAMS	=	Cell Adhesion Molecules
Cbl	=	Casitas B-lineage lymphoma
Cl	=	Chloride
Clast	=	Osteoclast
CO_2	=	Carbon Dioxide
CSF-1	=	Colony Stimulating Factor 1
CSF-2	=	Colony Stimulating Factor 2
CTX	=	C-terminal telopeptide of type- 1 collagen

DASH	=	Dietary Approaches to Stopping Hypertension
DEXA	=	Dual Energy X-ray Absorptiometry
E1	=	Estrone
E2	=	Estradiol
ECF	=	Extracellular Fluid
ECM	=	Extracellular Matrix
EDTA	=	Ethylene Diamine Tetra-Acetic Acid
ELISA	=	Enzyme-Linked Immunosorbent Assay
FGFs	=	Fibroblast Growth Factors
g	=	grams
G	=	gauge
GAGs	=	Glycosaminoglycans
Gla	=	γ- carboxyglutamate
$GluCONH_2$	=	Glutamine
GluCOO-	=	Glutamate
GPCS	=	γ-L-glutamyl-trans-S-1-propenyl-L-cysteine sulphoxide
H^+	=	Hydrogen Ion
HCl	=	Hydrochloric Acid
HCO_3^-	=	Bicarbonate
H_2CO_3	=	Carbonic Acid
HPO_4^{-2}	=	Monohydrogen Phosphate

H_2PO_4	=	Dihydrogen Phosphate
H_3PO_4	=	Phosphoric Acid
H_2SO_4	=	Sulphuric Acid
IHH	=	Indian Hedgehog
J	=	Joule
JKN	=	c- Jun N- terminal kinase
K^+	=	Potassium Ion
kg	=	Kilogram
kV	=	Kilovolt
M	=	Mole
Max	=	Maximum
M- CSF	=	Macrophage Colony Stimulating Factor
mg	=	milligram
mL	=	millilitre
mm	=	millimeter
mmol/L	=	millimoles per litre
N	=	Newton
Na or Na^+	=	Sodium
$NaHCO_3$	=	Sodium Bicarbonate
Na^+ - K^+ = ATPase	=	Sodium Potassium Adenosine Triphosphatase
Na_2SO_4	=	Sodium Sulphate
NDOs	=	Non-Digestible Oligosaccharides
NF – kB	=	Nuclear Factor kappa B

ng	=	nanogram
nm	=	nanometer
NH_3	=	Ammonia
NH_4^+	=	Ammonium Ion
NH_4Cl	=	Ammonium Chloride
OPG	=	Osteoprotegerin
P	=	Phosphorus
pH	=	Potential of Hydrogen
PO_4^{3-}	=	Phosphate
PBM	=	Peak Bone Mass
PBS	=	Phosphate Buffered Saline
PGE_2	=	Prostaglandin E_2
PTH	=	Parathyroid Hormone
PTH- rp	=	Parathyroid Hormone- related protein
RANK	=	Receptor Activator of the Nuclear Factor kappa B
RANKL	=	Receptor Activator of the Nuclear Factor kappa B ligand
rpm	=	revolutions per minute
SAPK	=	Stress Activated Protein Kinases
SE	=	Standard Error
TRAFs	=	Tumour Necrosis Factor Receptor-Associated Factors

TNF	=	Tumour Necrosis Factor
VEGF	=	Vascular Endothelial Growth Factor
WHO	=	World Health Organization
Wt	=	Weight (g)
Yrs	=	Years

THE ROLE OF VEGETABLES IN THE MAINTENANCE OF ACID-BASE BALANCE AND BONE STRUCTURE

A. General Introduction

Bones are generally viewed as inert. They perform unseen and this makes it easy for us to forget how important and valuable they actually are. However, bones are not inert as they really are dynamic, living tissues. They are continually being remodeled and rebuilt (Blair et al., 2002).

In order to live an active lifestyle, it is critical to form and maintain strong, healthy bones. The skeletal system performs the following functions:

- Support the body and protect the internal organs
- Allow movements from sitting, standing, and walking, to running, lifting and carrying
- Anchor muscles
- Store minerals e.g. calcium and phosphorus
- Form blood cells.

Problems of the skeletal system are for most of us simply a fact of life and their impact on our health, vitality and mobility can be enormous, causing great problems. Diseases of the bone, in particular osteoporosis affect about one-half of all women and one-fourth of all men who are over the age of 50 (Christenson, 1997). Osteoporosis is a complex, 2 shows the modifiable and non-modifiable factors that influence bone health. Amongst these, nutrition is an important modifiable factor in the development and maintenance of bone mass. Nutrition is also important in the prevention and treatment of osteoporosis (Ilich and Kerstetter, 2000) because many compounds present in food have biological effects within our bodies. Also, diet and disease are intimately associated. Nutrition is amenable to change as it is an exogenous factor. A nutritional approach is far more popular with osteoporosis sufferers than drug intervention because of poor long-term compliance rates associated with the latter (New, 1999).

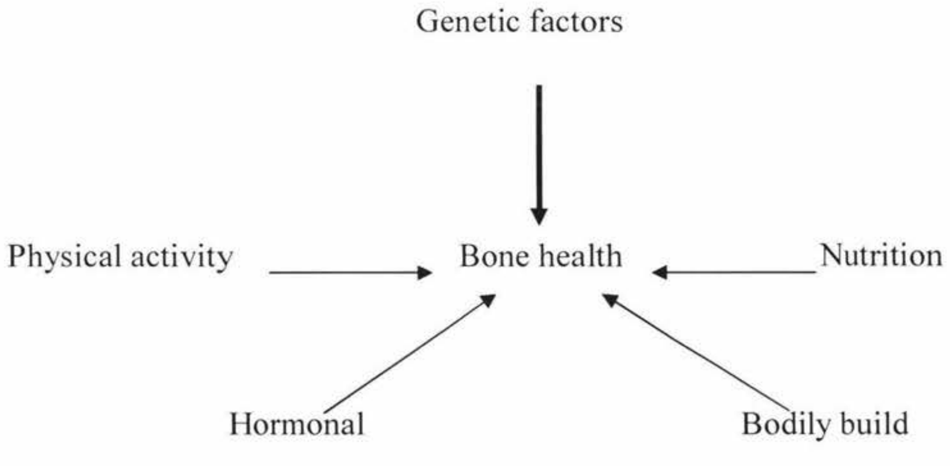

Figure 2. Modifiable v. non-modifiable factors influencing bone health. (From New, 2002).

The factors that determine post-menopausal bone mass are both peak bone mass (PBM) attained early in life and the rate of bone mass loss later in life. PBM is the mass of bony tissue present at the end of skeletal maturation and it is a major determinant of skeletal health throughout life

(Bachrach et al., 1999) i.e. the higher the maximum bone density and strength the lower will be the risk of osteoporosis. The age at which PBM is attained is not certain. However, estimates of attainment of peak total-body bone mineral density and bone mineral content range from age 18 years to the 20s (Teegarden et al., 1999). PBM is determined by gender, and as mentioned earlier by a combination of endogenous (genetic, hormonal) and environmental (nutrition, exercise) factors (Heaney, 1996).

Estrogen is required for the attainment of maximal peak bone mass in both sexes. However, the additional action of testosterone on stimulating periosteal apposition accounts for the larger size of the adult male skeleton and thicker cortical bone. Thus, men normally have greater PBM and bone density than women (Wahlqvist and Wattanapenpaiboon, 2002). Estrogen also plays a major role in maintaining bone mass and estrogen deficiency is the major cause of accelerated age-related bone loss in both sexes (Riggs et al., 2002).

In both men and women a slow loss of bone mass begins around the ages of 35 to 40 (Wahlqvist and Wattanapenpaiboon, 2002). Women however experience accelerated bone loss during the menopausal and post-menopausal period (Bord et al., 2001). This loss occurs over and above the slower rate and continues for around 5 to 10 years after menopause. Subsequently the rate of loss declines so that by age 60 it again matches that of men. The lifetime bone loss in women is up to 45-50% of bone mass whereas in men it is 20-30%. Refer Figure 3 to view the lifetime alterations in skeletal mass in both men and women.

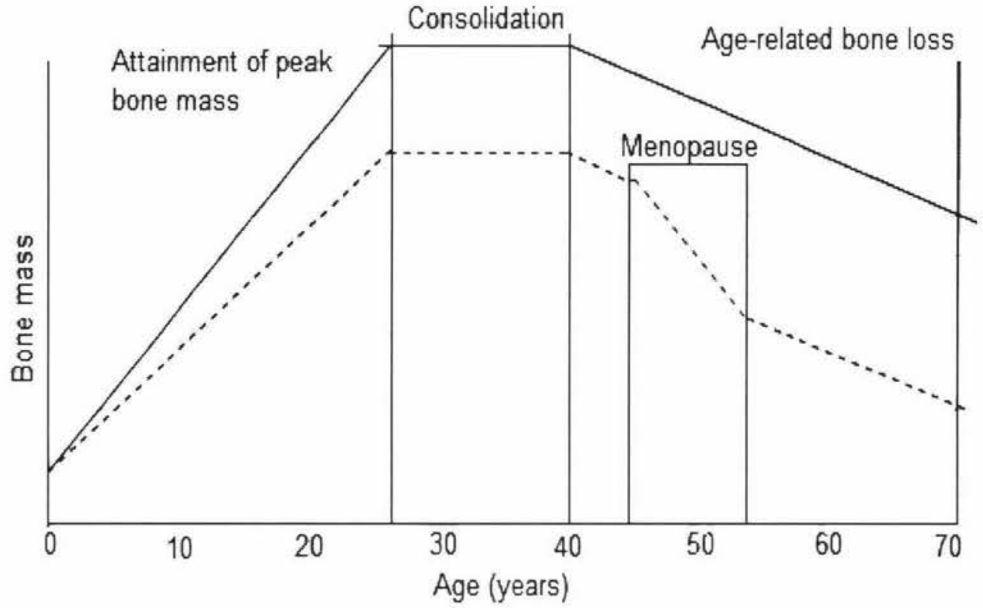

Figure 3. Alterations in skeletal mass in men (-) and women (--) throughout the life cycle. (From New, 2002).

The median age of populations is increasing. For instance, in New Zealand, during the time period 1950-52 survival to age 60 and older was about 76% and 82%, respectively for both males and females. It increased to about 84% for males and 90% for females during the time period 1990-1992, and it is projected to rise to about 89% and 94%, for both males and females respectively in 2011. In 2031, it will be about 90% for males and 95% for females (Zodzekar, 1994). As a consequence of this it is predicted that osteoporotic fractures will reach epidemic proportions (Ilich and Kerstetter, 2000). Osteoporosis is already a significant public health problem. In 1990 there were 1.66 million osteoporotic fractures throughout the world and this figure is projected to rise to 6.26 million by 2050 (World Health Organization, 1994). The estimated lifetime risks of fracture for women with osteoporosis is >40%. In contrast, the risk of fracture for men is only 13% (Kanis and the WHO Study Group, 1994). It has been estimated that between 25% and 50% of Caucasian women and 5-10% of Caucasian men will probably suffer an osteoporosis-related fracture at some time in their lives (Melton and Riggs, 1983).

The normal Western diet is high in protein, salt and refined, processed foods. This diet when combined with a sedentary lifestyle may contribute to the increasing incidence of osteoporosis in the elderly (Maurer et al., 2003). Thus, initiating appropriate healthy behaviors such as eating enough fruits and vegetables early in life and continuing them throughout life may prevent osteoporosis and osteoporosis-related fractures (Tylavsky et al., 2004).

Our understanding of nutritional influences on bone health is limited. This is because most studies regarding the influence of nutrition on bone health have focused on the importance of dietary calcium. High fruit and vegetable intakes may also have beneficial effects on bone (Tylavsky et al., 2004; New et al., 2000; Muhlbauer and Li, 1999) as they contain amino acids and base precursors that may provide a natural source of base to buffer the acid produced by other dietary compounds such as protein-rich foods, grains and cereals (New, 2002). Another study found that there is an association between several nutrients present in fruit and vegetables (i.e.

potassium, magnesium, β-carotene and vitamin C) and bone health (New et al., 2000). Fruits and vegetables also contain antioxidants e.g. vitamin C, carotenoids, flavonoids, and other phenolics that protect the body against reactive oxygen species (Williamson and Manach, 2005). Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species. Thus, the antioxidants that are found in fruits and vegetables may positively affect bone health.

The overall renal function of humans declines as they become older and this affects their ability to excrete acid (Frassetto et al., 1996). Hence humans develop progressively increasing blood acidity as they grow older (Frassetto and Sebastian, 1996). The skeleton (alkaline bone mineral) may thus be used to buffer the acid.

A variety of population-based studies have demonstrated a beneficial effect of fruit and vegetable/potassium intake on indices of bone health (New, 2002). This beneficial effect occurs in all age groups i.e. in young boys and girls, premenopausal women, perimenopausal women, postmenopausal women, and elderly men and women (New, 2002).

B. Reason for the Study

There is evidence of a positive link between the intake of fruit and vegetables and bone health (Tucker et al., 1999; New et al., 2000). Further investigations are warranted to determine the relations found between the nutrients present in fruit and vegetables, bone mineral density (BMD), and bone turnover. Current available evidence is insufficient to generate recommendations as to the amounts of fruit and vegetables that are necessary to produce a positive effect in humans (Muhlbauer et al., 2002) mainly as it is unclear which nutrients are producing the effect.

Fruits and vegetables contain large amounts of base-producing organic acid salts (potassium and magnesium may be part of them) that are believed to buffer noncarbonic metabolic acids generated from cystine- and methionine-rich dietary proteins (Tucker et al., 1999). Potassium and magnesium are present in a variety of whole, unrefined foods, including fruits and vegetables (Remer and Manz, 1995). If these bases were absent then the metabolic acids would be buffered by bone mineral which will lead to bone dissolution (Tucker et al., 1999; New et al., 2000). However, Muhlbauer et al (2002) reported that the inhibitory effect of foodstuffs of vegetable origin on bone resorption is not mediated by their base excess but possibly by pharmacologically active compound(s). Thus, it is necessary to determine how vegetables affect bone health.

C. Vegetables included in the Study

In the present study we included onion, broccoli, and potato. Onion was included in the study as a positive control because it has been reported to have a positive effect on bone mineral content (BMC). Muhlbauer and Li (1999) found that when male rats were fed 1g dry onion per rat per day for four weeks their BMC increased (significant) by 17.7 ± 6.4% ($p<0.05$; $n=6$), mean cortical thickness increased by 14.8 ± 7.6%, and BMD increased by 13.5 ± 3.1% ($p<0.05$) relative to controls. Onions contain significant levels of potassium, calcium, phosphorus, carotenoids, and vitamin C, all of which have been positively linked to bone health.

Broccoli was included in the study as it has also been shown to significantly inhibit bone resorption in rats at a dose of 1g per rat per day (Muhlbauer and Li, 1999). Broccoli is high in carotenoids, and is a rich source of vitamin K, potassium, calcium, phosphorus, and vitamin C.

Potatoes were included as a negative control on the basis that potatoes consumed at a dose of 1g per rat per day did not significantly inhibit bone resorption in the above quoted study.

D. Objectives

The specific objective of this project was to evaluate the effect of vegetables (broccoli, onion, and potato) in male rats on:

- Bone density
- Bone strength
- Markers of bone metabolism
- Calcium balance
- Urinary buffering

Bone density is a measure of bone strength. When minerals fill the bone matrix they make it dense. Levels of serum C-terminal fragment (CTX) were measured because it is a specific marker of osteoclastic activity i.e. bones resorption. Calcium and phosphorus balance were done to determine the amount of calcium and phosphorus retained respectively. The acid load of urine was determined by measuring the urinary buffers ammonia and phosphate. The urinary ammonia excretion is increased as urine becomes more acidic.

E. Hypothesis

The regular consumption of vegetables (broccoli and onion) will increase BMD and affect acid base balance in the body.