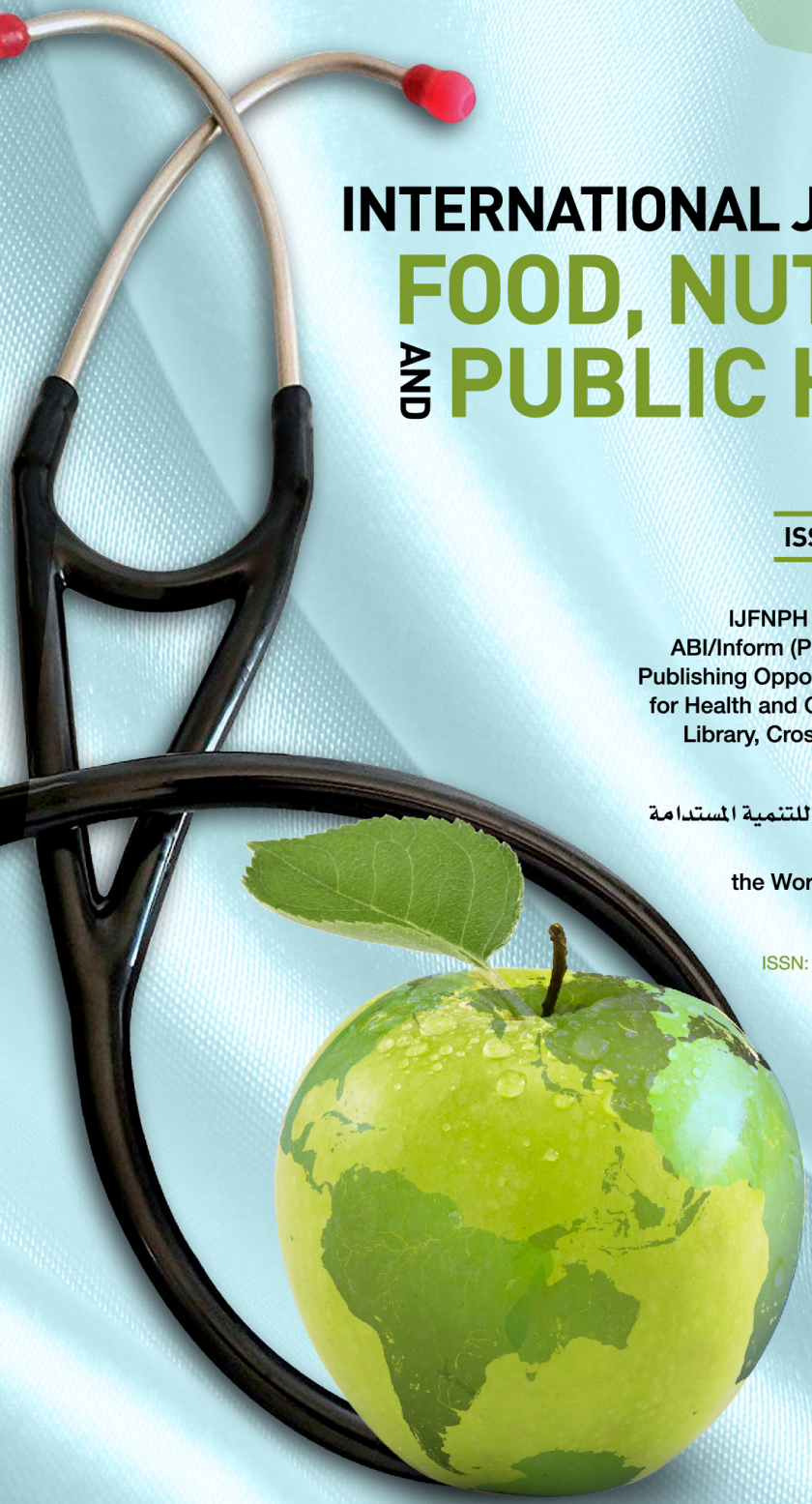

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Natto and Vitamin K2:

A promising nutraceutical aid for bone maintenance

WASD
WORLD ASSOCIATION FOR SUSTAINABLE DEVELOPMENT

Vanessa Kitching
School of Life Sciences
University of Westminster
115 New Cavendish Street
London W1W 6UW, United Kingdom

Corresponding author: Vanessa Kitching
e-mail: vanessakitching@hotmail.co.uk

Ihab Tewfik
School of Life Sciences
University of Westminster
115 New Cavendish Street
London W1W 6UW, United Kingdom

NATTO

Vitamin K2

Abstract

Purpose

The aim of this case study was to investigate the association between vitamin K_2 via intake from Natto (Japanese fermented soybeans) and its effect on bone mineral density (BMD) in humans. Epidemiological data focused on individuals between the age of 20-35 in southern England using ultrasound densitometry.

Methods

Six participants (males *n*=2; females *n*=4) were recruited on a 6-week case study intervention. BMD was determined via a SONOST 3000 bone densitometer on the calcaneus pre- and post-intervention. Natto intake was adjusted to 3x18g per/week with targeted nutritional guidance. Recipe preparation and feedback questionnaires were provided to capture data on dietary preferences, physiological changes and Natto characteristics.

Findings

BMD increased following the frequent consumption of Natto (T-scores ranging from 1.5-0.09g/cm^2). The variability between the participants' BMD T-scores pre- and post-Natto consumption supressed by -53.5%, despite the insignificant P-value (0.059).

Conclusion

Natto has potential benefits for BMD and could play a role in vascular calcification management via vitamin K-dependant proteins. Therefore, Natto has an opportunity to become integrated within western culture and through additional evidence-based nutritional guidance.

Keywords

Vitamin K_2, Natto, Osteocalcin, MGP, Vascular Calcification, BMD, CVD, VK_2, Calcium, MK-7

Introduction

The current crisis in public health

By the year 2050, there could be a total of 6.3 million hip fractures worldwide. With over 200 million people currently suffering with osteoporosis, it is clear that this is creating apprehension for public health (Garcia-Gomez and Vilahur, 2020).

Osteoporosis shares health and mortality risk factors with cardiovascular disease (CVD). Globally, CVD is the cause of 17.9 million fatalities per year. These chronic disorders place a burden on public health and medical institutions whilst also causing an international and economic strain (Gallacher and Shah, 2020). Regrettably, modifiable risk factors, such as diet, remain an issue within developed countries (Ganji et al., 2019; Brady, 2020). Despite promising advances in medical treatments over the latter half of the 20th century, there is still rising concern about these diseases. Therefore, it is vital that further treatments are considered and reviewed in order to tackle the current pandemic.

The complexity of calcium

Calcium is an essential mineral that has been discussed for centuries within the world of science, health and epidemiology. Whilst there have been many reasons to discuss calcium, research primarily focuses on calcium's fundamental role in bone metabolism and density (Liu et al., 2019; Balk et al., 2017; Lamberg-Allardt and Kemi, 2017). Reduced bone mineral density (BMD) occurs due to a failure to meet Dietary Recommended Intakes (DRI) for calcium; this encourages debilitating conditions such as osteoporosis (Hasirci and Hasirci, 2018, pp.140-131). Osteoporosis is an incurable malady affecting 3.5 million of the UK population. Fractures cost the NHS around £4.4 billion each year (Blackie, 2020), with hip fractures accounting for 1,150 fatalities monthly. More hospital beds are occupied by patients as a result of hip fractures, when compared to those suffering from heart attacks, breast cancer or diabetes (International Longevity Centre UK, 2010).

Orthopaedic remedies, such as supplements and food, require an abundance of calcium in order to assist fractures and CVD (Salisbury and Terrell, 2019). The *American Journal of Clinical Nutrition* declared that dietary calcium reduces CVD risks due to the nature of dairy's biochemical complexity (Kong et al., 2017). At the time of writing, however, there has been strong evidence to suggest the same statement does not apply to calcium sourced through pharmaceuticals (Tankeu et al., 2017). A link between calcium supplements and an increased risk of cardiovascular events and conditions (atherosclerosis) were discovered in women by Bolland et al. (2011). This emphasises the importance of care during nutritional supplementation.

Table 1: Tolerable Upper Intake Levels for Calcium

Age	Male	Female	Pregnant	Lactating
0-6 months	200mg	200mg		
7-12 months	260mg	260mg		
1-3 years	700mg	700mg		
4-8 years	1000mg	1000mg		
9-13 years	1,300mg	1,300mg		
14-18 years	1,300mg	1,300mg	1,300mg	1,300mg
19-50 years	1,000mg	1,000mg	1,000mg	1,000mg
51-70 years	1,000mg	1,200mg		
71+ years	1,200mg	1,200mg		

Source: National Institutes of Health, 2020, para. 5

Table 1 illustrates the maximum recommended intake of calcium for an individual per day. Calcium intake greater than 1,400mg/day may increase rates of coronary heart disease (CHD) – a type of CVD (Michaëlsson et al., 2013). CHD is currently the primary cause of death worldwide (WHO, 2018) (see Figure 1).

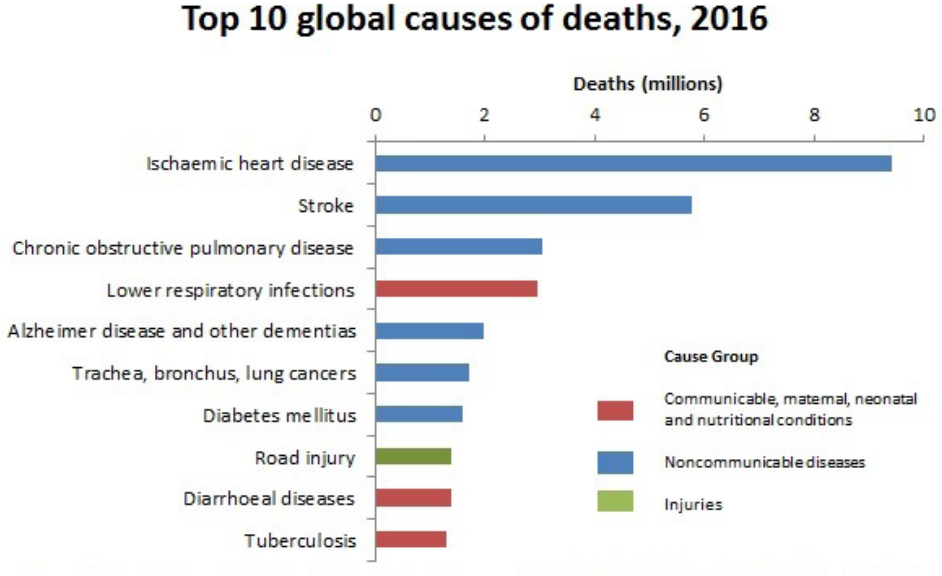
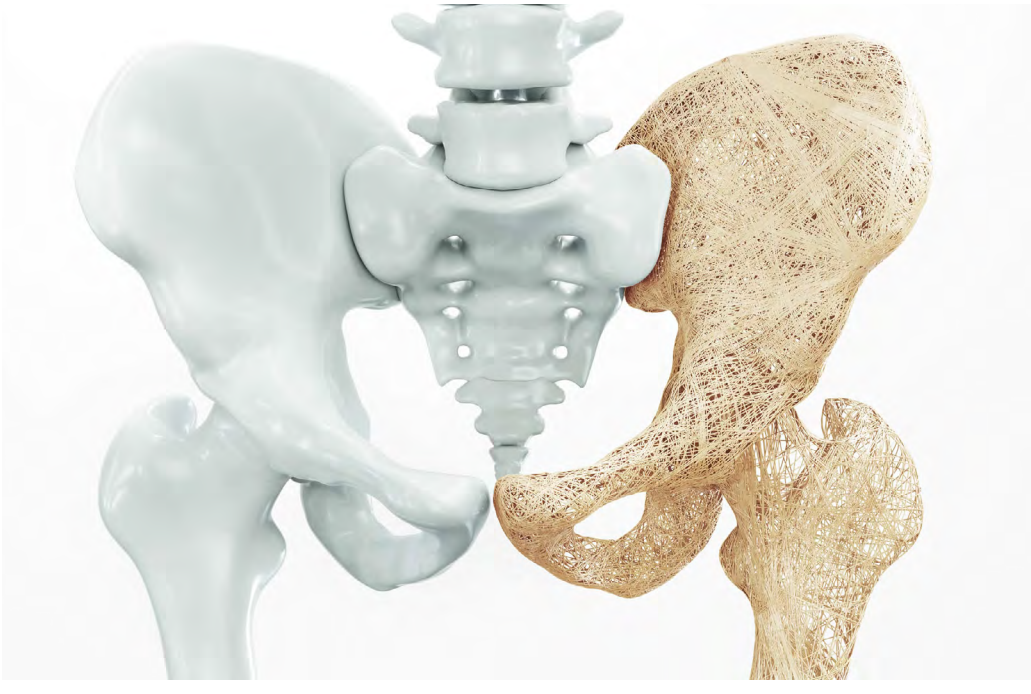


Figure 1: Top ten global mortality risks in 2016

Source: World Health Organization, 2018



It is clear that new methodologies are vital to tackle CVD and its threat to the global economy and public health (Zipes et al., 2018, p.8).

Clinical research has considered coronary arterial calcification (CAC) to be a benign process; however, current research illustrates CAC to be an established predictive biomarker of atherosclerosis, CHD and mortality. At the cell level, calcium supplementation may alter phenotypes within vascular smooth muscle; this heightens the opportunity for vascular calcification (VC). This leads scientists to further focus on how to control and manage the biological mechanisms of calcium.

Bone-vasculature axis

A relationship between low BMD and VC has been investigated for many years regarding public health (Hulbert et al., 2020). VC is a complex pathological calcification process that results in the aberrant deposition of calcium phosphate compounds, transferred to the intima within vascular smooth muscle cells (VSMC – Table 2) (Anderson et al., 2016).

Table 2: A summary of differences regarding intimal and medial vascular calcification

	Intimal Calcification	Medial Calcification
Risk Factors Molecular Mechanisms	Dyslipidaemia, hypercholesterolemia, smoking, age, diabetes, hypertension	Aging, diabetes, renal failure, osteoporosis, hypertension
	Lipid accumulation, foam cell accumulation, inflammatory cell filtration, inflammation, oxidative stress, apoptosis	Transdifferentiating of endothelial cells or VSMCs into bone-like cells (osteoblast chondrocyte and osteoclast-like cells); altered calcium, phosphate and Vitamin D metabolism; loss of calcification inhibitors (matrix Gla protein, pyrophosphate)
Consequences Complications	Plaque formation; lumen stenosis; plaque calcification; altered plaque stability	Arterial stiffening, increased pulse pressure, elevated pulse wave velocity
	Ischemia, infarction	Systolic hypertension, left ventricular hypertrophy

Source: Kovacic et al., 2011

Intimal and medial calcification generally co-exist in individuals with low BMD or signs of osteoporosis and/or CVDs (Aikawa, 2016; Osawa et al., 2016; Raggi, 2017).

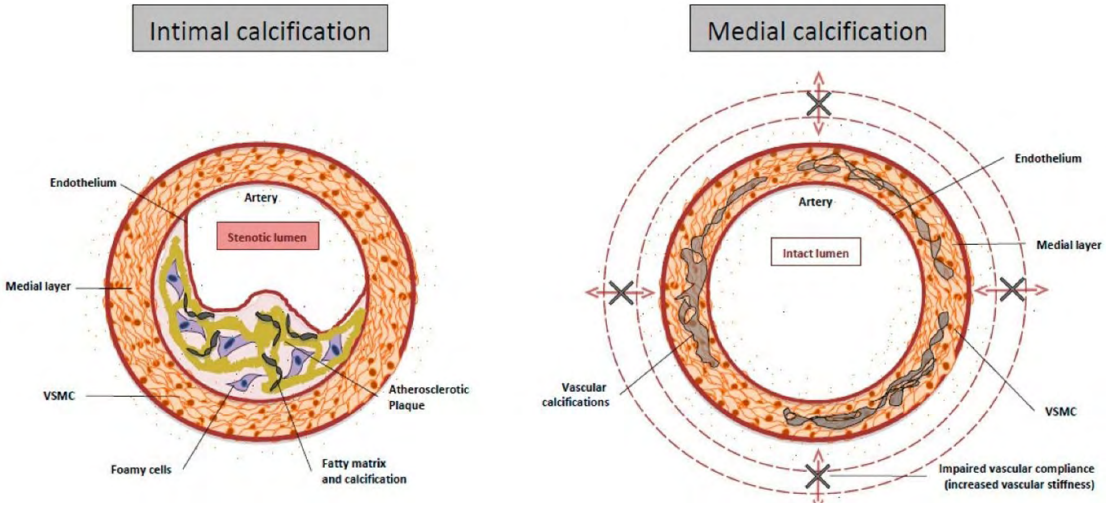


Figure 2: Schematic representation of intimal and medial calcification. Left ventricular hypertrophy; VSMC: Vascular smooth muscle cells

Source: Cozzolino et al., 2019

Intimal and medial VSMCs maintain cardiovascular management (Figure 2). VSMCs are responsible for the maintenance of phenotypes (osteoblasts), adjusting phenotypes as a feedback response to cues (such as fractures), suppressing contractile amino acids, resize proliferation and restoring arterial extra-cellular matrix (ECM) to assist migration. The alterations from contractile to osteo-phenotypes are characterised via the progressions of calcified vesicles, suppressed biological minerals and expressed calcification within the matrix. This process removes VSMC markers, replacing them with

osteochondrogenic markers that are secreted by osteoblasts such as Osteocalcin (OC). OC is the most abundant protein in bone; however, its dependency relies on the molecule Vitamin K to fulfil its biochemical duties (Durham et al., 2018).

Vitamin K_2 – an essential nutrient

Vitamin K is an essential fat-soluble nutrient responsible for the ɣ-carboxylation of Vitamin K-Dependant Proteins (VKDP) such as OC and Matrix Gla Protein (MGP).

VKDPs regulate bone preservation and control VC: therefore low amounts of VKDPs may cause the onset of osteoporosis, VC and increase cardiovascular risk (Namba et al., 2017).

There are common molecular and cellular mechanisms of skeletal metabolism and vascular biology that link to chronic conditions such as osteoporosis and CHD.

OC regulates bone and vascular mineralisation, essential for building bones (Moser and van der Eerden, 2019). MGP is a secretory protein present in a variety of tissues, such as the heart and arterial walls. MGP's primary role is to inhibit calcification; however, this can only be activated with Vitamin K (Bjorklund et al., 2020). Vitamin K is present in two forms: Vitamin K_1 (VK_1 - phylloquinone) and Vitamin K_2 (VK2 - menaquinone). VK_1 assists with blood coagulation while VK_2 has multiple benefits to human health, including bone maintenance. VK_2 has several subtypes that may differ due to the length of the isoprenoid chain of the molecule (Table 3).

Table 3: Characteristics and Sources of Vitamin K types – sourced from The Journal of Nutrition and Metabolism

Type of Vitamin K	VKDP	Function in the human body	Sources of vitamin
Vitamin K_2, menaquinone-4 (MK-4)	Osteocalcin Matrix-Gla	Synthesised in bone. Synthesised in cartilage and in blood vessel walls. It is involved in calcium transport, preventing calcium deposition in the lining of blood vessel walls, and helps improve bone density via binding calcium to the bones matrix. Short chain form with a shorter half-life.	Animal-based foods. Synthesis by bacteria in the intestinal tract (however, synthesised MK-4 is bound to the membranes of bacteria in the gut and very little is absorbed in humans). Over-the-counter (OTC) supplements.
Vitamin K_2, menaquinone-7 (MK-7)		As for MK-4 Long chain form with longer half-life	Fermented foods (Natto), some cheese. Extracted from Natto (fermented soy) as an OTC supplement.

Source: Schwalfenberg, 2017

VK_2 has become a primary focus in clinical research and is widely used in food and pharmaceutical industries to manage VC, CHD and BMD. In 2008, a Japanese population study revealed that Japan's eastern regions disclosed fewer incidences of hip fractures in comparison to the West (Yaegashi et al., 2008, pp.219-225). The key component that emerged from this study was a food called Natto (fermented soybeans).

Natto – a potential superfood

A great deal of research has since appeared, emphasising the relationship between Natto, VK_2 and the reduced risk of fractures and/or osteoporosis. In conjunction with this epiphany, a current Japanese population study highlighted that consuming 7.3g/day of Natto reduces blood pressure and aids anticoagulant activity, thus decreasing CVD mortality risk (Nagata et al., 2017). However, it must be noted that this study observed a similar trend via alternate soy products.

Natto originates from the eastern traditions of Japanese cuisine. Although Natto has been declared a superfood with an abundance of VK_2, it is not typically consumed in the western world due to cultural inhibition (Vermeer et al., 2018). This delicacy is notorious for being highly objectionable, which may have prevented the global integration process, thus affecting the potential benefits Natto could have on public health.

While the UK is burdened with chronic conditions such as osteoporosis, VC and CHD, it is self-evident that calcium remains a necessity. Focus on the metabolism of calcium supplementation is vital to manage concerns. With the right dietary guidance, Natto (MK-7) could potentially resolve the apprehension behind calcium's liability. A convincing systemic review has stated that VK_2 prevents "fractures in vertebra by 60%, hip fractures by 77% and nonvertebral fractures by 81%" (Schwalfenberg, 2017).

Despite the amount of research that is available, it must be noted that effects of Natto/VK_2 on bone status has not been fully elucidated. Further understanding on calcium, VK_2 and its biochemical nature is currently vital to support the UK and public health (Levis and Lagari, 2012).

Hypothesis

Frequent consumption of Natto reduces arterial calcification and improves bone density in adults (aged 20-35) living in the South East of the United Kingdom.

Aim

To investigate the association between vitamin K_2 intake from Natto and BMD in humans. Natto is the only known dietary source rich in VK_2 (MK-7).

The study aimed to adapt this Japanese delicacy towards the Western palate to aid its adoption into Western diets. The results will determine whether Natto will have an optimal and favourable effect on BMD within young adults.

Objectives

- To create westernised Natto recipes;
- To develop educational tools for nutritional preparation of Natto (hardcopy/website);
- To evaluate the effectiveness of Natto on BMD and VC improvements via ultrasonic methodology.

Methods

Study protocol

This study employed the gathering of participants over a 6-month period prior to the experiment. This was to assess potential health complications of the participants, complete recipes and paperwork, while allowing participants the time to prepare for the study. The study observed the changes of BMD pre- and post-experimental Natto consumption. The data used statistical models for calculating BMD progression through SPSS software (paired t-test).

Participants

The study recruited six healthy participants (Males n=2, Females n=4) who did not have any diseases known to affect bone metabolism or blood coagulation. All participants resided in East Sussex (England); this was because of geographical restrictions within the recruitment process. Participants' age varied from 28-31 years. Ethnic segregation was not a co-factor; however, this study recruited a total of two ethnicities: White English (n=5) and Asian (n=1, Thai decent).

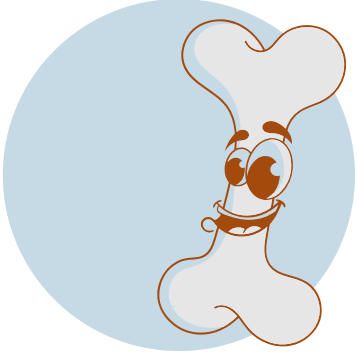
Pertinent risk factors

Medical Briefing: Before entering the laboratory, subjects were briefed on Health and Safety codes, protocol, concerns and questions. Table 4 shows all forms the participants were asked to complete.

Table 4: Experimental forms completed

Form	Purpose
Nutritional information/ COSSH (control of substances hazardous to health)	To manage the health surveillance, prevent exposure to risk factors, control safety and welfare regulations from exposure, maintain optimal testing standards and provision.
Consent form	To protect the agreement of participation within the study.
Information leaflet	To advertise the study and highlight the current pandemic with regards to public health.
Questionnaire	To collect data pre- and post-examination, compare results and interpret feedback.
Risk assessment form	To identify hazards and potential risk factors pre- and post-examination.
Attendance register (signed)	To record the participants' attendance.
Personal information sheet (PIS)	To inform potential participants of the motivation behind the study, procedures they will endure, the department background, what is expected within the experiment and sources of information with contact information.

Source: Author's own data



Ethical Considerations

Week 1: To ensure the safety and ethical considerations of the participants, maintaining optimal performance stability, forms, laboratory rules and regulations were covered during private face-to-face interviews with each participant pre-examination. This ensured a good rapport with confidentiality between the participant and the researcher.

Week 6: To gather personal feedback and thank the participants for taking part in the research, private face-to-face meetings with concluding questionnaires were conducted in Week 6.

Medical Conditions: The attention of all participants was drawn to any medical conditions and/or medications that may cause potential harm or may interfere with Vitamin K_2 absorption within the study. This information was also placed within the PIS. These included the following:

- Antibiotics (10+ days);
- Direct Oral Anticoagulants (DOACs - blood thinning medications): Warfarin;
- Apixaban, Heparin, Dabigatran, Edoxaban, Fondaparinux, and Rivaroxaban;
- Pacemaker;
- Pregnancy;
- Allergies: Soybean/unfermented soy products (stable class 1 food allergen).

Associated practitioners read the "SONOST 3000 User's Manual" prior to use. The study protocol was approved by the Ethical Committee of Westminster University. All risk assessments were updated when required.

Bone measure procedure

Duration: 6 Weeks

Equipment

Table 5: Equipment used throughout BMD screening

Equipment	Quantity	Description of use
SONOST 3000 Densitometer	1	To measure bone density via minimal ultrasonic radiation.
Ultrasound gel (250ml bottle)	2	To assist radiation via SONOST 3000.
Alcohol wipes	1	Used to sanitise participant and equipment.
Printer paper (receipt is printed after BMD is calculated. Paper thickness = 0.07mm)	1	To print out data post-scanning procedure.
Blue nitrile gloves	2	Sanitary safety precaution.
Blue paper roll	1	Used to remove ultrasound gel post-screening.
Forms (COSHH/Risk assessment form, Nutritional information form, consent form, PIS)	1	For ethical purposes.
Pen	1	To write any notes pre and post BMD collection.

Source: Author's own data

Definition of Parameter Terminology

Screening diagnostics of disorders and state of bone mass were taken via ultrasonic densitometry (Tables 5 and 6).

Table 6: Detailed explanation of SONOST 3000 test processes

SOS (speed of sound)	Velocity of quantitative ultrasound (QUS) waves through the bone; these reflect bone mineral density.
BQI (Bone Quality Index)	Generally, SOS is in proportion to the temperature, and Broadband Ultrasound Attenuation (BUA) is in inverse proportion to the temperature. These correlation coefficients are (α, β) combined as shown below. This parameter, which is represented as a constant number, compensates the precision error from the temperature.
The relationship between age and BQI	BQI = α x SOS + β x BUA
T-score	The view of probability. T-Score represents the patient's BQI above or below a reference "Young adult" mean.

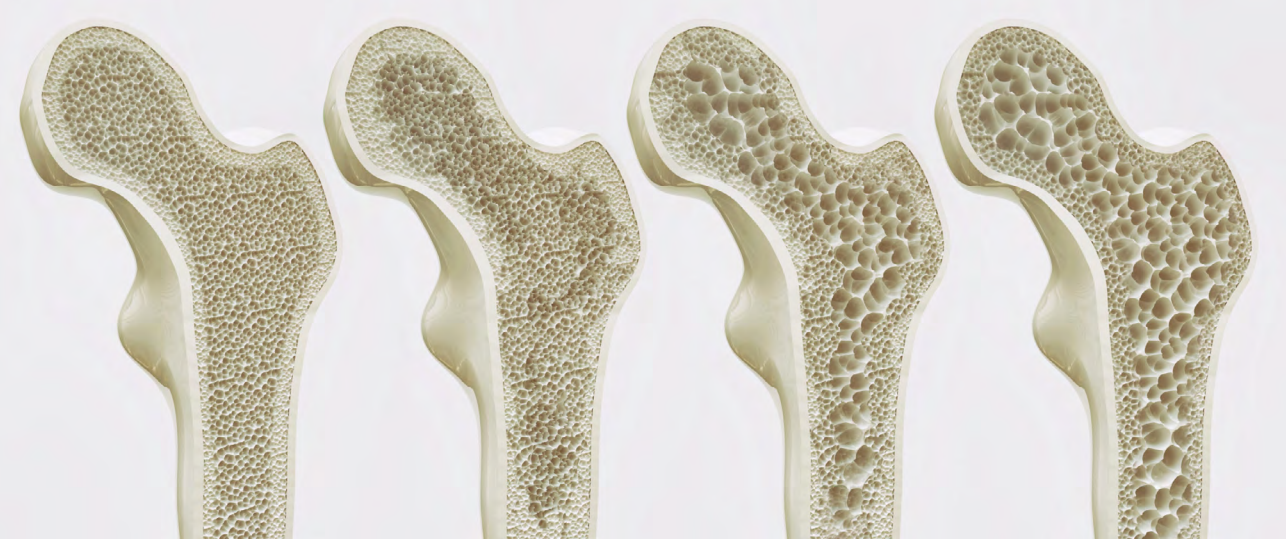
Source: OsteoSys Co., Ltd, no date

BMD was compared with established normative values to evaluate the participants' BMD T-score. A T-score at 0 standard deviation (SD) suggests the BMD is a normal measure for a healthy young adult. A T-score SD < -1 highlights low BMD. All data retrieved via the study were analysed via the standards of the National Institutes of Health – Osteoporosis and Related Bone Diseases National Resource Center (Table 7).

Table 7: World Health Organization definitions based on bone density levels

Health Status	Definition of bone density scoring
Normal	Bone density is within 1 SD (+1 or –1) of the young adult mean.
Low bone mass	Bone density is between 1 and 2.5 SD below the young adult mean (–1 to –2.5 SD).
Osteoporosis	Bone density is 2.5 SD or more below the young adult mean (–2.5 SD or lower).
Severe (established) osteoporosis	Bone density is more than 2.5 SD below the young adult mean, and there have been one or more osteoporotic fractures.

Source: National Institutes of Health Osteoporosis and Related Bone Diseases National Resource Center, 2018



Baseline measures

A verbal and practical BMD screening process was rendered prior to scanning with each participant. Once the participant was comfortable, methods featured in the 'SONOST 3000 Manual' were performed as shown in Figure 3 below.

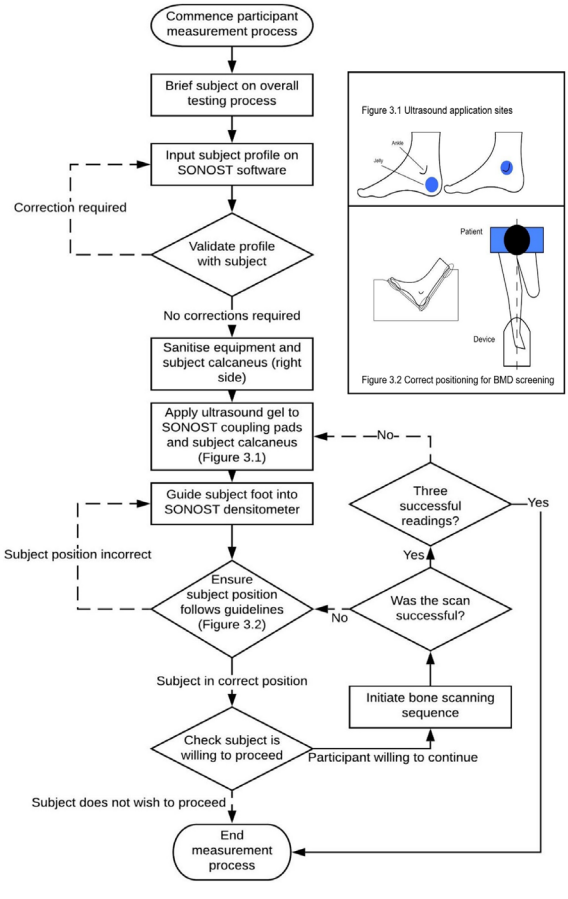


Figure 3: Demonstrates the process of BMD collection. Screened and completed via use of SONOST 3000 Bone Densitometer

Source: Author's own data

Consent

Written informed consent for all the study procedures was obtained from each subject prior to any experimental procedures.

Natto Ingestion

Consumption

Natto consumption per participant was 18g x 3 times per week (non-consecutive days). Participants were asked not to revisit their dietary habits over the six week period (including supplements).

Recipes

Three Natto recipes were provided for each participant to assist with their Natto consumption. Each recipe contained 18g of Natto and was tailored towards a westernised diet. The recipes were:

- Baked Haddock and Natto Basil Bean Salad;
- Peanut-Banana Natto on toast;
- Natto Rice and Tuna Salad.

The recipes proposed nutritional alternatives for those with dietary requirements while remaining healthy and persisting with VK_2 absorption co-factors. Recipes were provided in hardcopy and available online at www.vk-nutrition.com.

The PIS stated that the study was open to participants to create a westernised recipe of their own, with the following constraints. The meal must remain healthy and include one fat-soluble ingredient to maintain optimal VK_2 absorption. Photographic evidence was advised, sent via email or through the mobile application, WhatsApp.

Preserving nutritional value

Natto quality is important to ensure each subject consumed the optimal amount of VK_2 throughout the study. Frishnatto was selected as the source of Natto; this ws due to its nutritional values (see Table 8). To prevent loss of nutritional values, the Natto remained frozen during transportation to the following locations:

- East Sussex storage;
- Westminster University;
- Participant's home.

The Natto was dispatched within a stable polystyrene box filled with frozen ice packs. Natto remained frozen and stored in the participant's freezer and was to be thawed the day before consumption. One packet lasted approximately one week (thawed).

Table 8: Frischnatto Packet Nutritional Information [100g]

Speciality	Organic, gluten free and suitable for vegans
Energy (kJ)	1000kJ
Energy (kcal) Fat of which:	239kcal
saturated	6.9grams
mono-unsaturates	1.34grams
poly-unsaturates	1.53grams
	4.03grams
Carbohydrates of which are:	15.2grams
• Sugars	0.8grams
Fibre	7.79grams
Protein	15grams
Salt	1.1gram
Vitamin C	<0.005milligrams
Phosphorus	349milligrams
Iron	2.2milligrams

Source: Frischnatto, no date

Follow-up data collections

Week 1: PIS, recipes, the leaflet and the week 1 questionnaire were issued to each participant within a personalised folder. This questionnaire focused on first impressions towards Natto consumption as well as feedback on their experience.

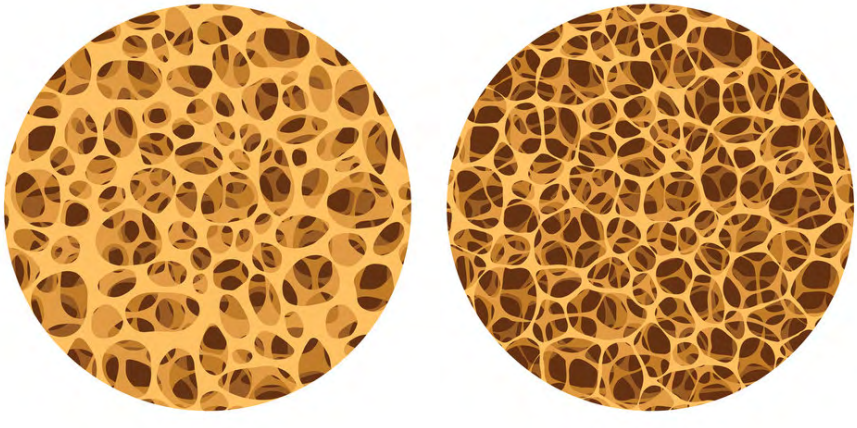
Check-ups: The researcher made weekly independent contact with subjects. This was as a follow-up to check on their health and progress, whilst providing weekly facts relating to Natto or VK_2.

Week 6: The measurement process was recited to each subject. Participants left with a certificate after completing a final questionnaire, focusing on their six weeks within the study and personal feedback.

Results

Demographic characteristics

There was no habitual Natto consumption prior to the study for each participant. All participants took part in moderate to intense exercise regimes, 3-5 times per week. All were descendants from at least one white British parent. Participant 5 was 50% Thai and grew up in Hong Kong until the age of 10; this did not warrant exclusion from the study as an outlier. Five of the six participants were in full time employment. Of the five employed, two worked in an office-based environment, the remaining three being employed in an active work environment. Two of the participants declared that there was a family history of osteoporosis. One participant described from memory a grandparent's appearance that could be associated to the disease, although this was not diagnosed. The parent of the same participant was recently diagnosed with osteopenia. One of the participants consumes daily calcium supplements. To reduce disruption to participants' living patterns, supplementation was allowed to continue.



Evaluating algorithms for calculating bone density progression

Bone measures

Baseline bone density data were used as a reference model. Progressions within bone density added predictive values for regression of arterial calcification within each participant.

Table 9: Bone densitometer data – Weeks 1 and 6 (05/02/2020-18/03/2020)

Participant	Gender (M/F)	Age (dd/mm/yyyy)	Ethnicity	BMD T-score WK1	Result WK1	BMD T-score WK6	Result WK6
1	M	06/07/1990	White British	-1.85	Osteopenia	-1.76	Osteopenia
2	M	23/09/1988	White British	-0.50	Normal	0.00	Normal
3	F	17/11/1990	White British	-0.90	Normal	-0.36	Normal
4	F	15/08/1991	Asian British	-1.17	Osteopenia	-1.00	Normal
5	F	16/05/1991	White British	2.00	Normal	3.50	Normal
6	F	18/10/1988	White British	-0.50	Normal	-0.23	Normal

Source: Author's own data

Osteopenia appeared among 33.33% (n=2) of participants, including the youngest at 29 years (participant 4). Males represented the lowest data with an average of t=-1.18 in comparison to the females with t=-0.14. Participant 5 significantly increased BMD, higher than the remainder of the study group, t=1.50. Participant 5 was not considered an outlier, due to the nature of the individual's age, ethnicity, lifestyle and diet, which balanced alongside the other constituents.

The Effect of Natto Ingestion on BMD - extreme values and conclusive data

There is a large scale of variability between participants' BMD t-scores prior to Natto consumption (M=-0.49, SD=1.32) (Table 9). Week 6 data suggest the variables were significantly supressed by -53.5% (M=0.03, SD=1.82). This implies that Natto consumption may have initiated a sympathetic response leading to the increase in BMD within the six week period, thus highlighting a positive response to VK_2 ingestion.

Figure 4 shows that the ingestion of Natto (18g) increased BMD remarkably. Subject 5 significantly increased their BMD (t=1.5); however, this did not cause an undue influence of extreme values.

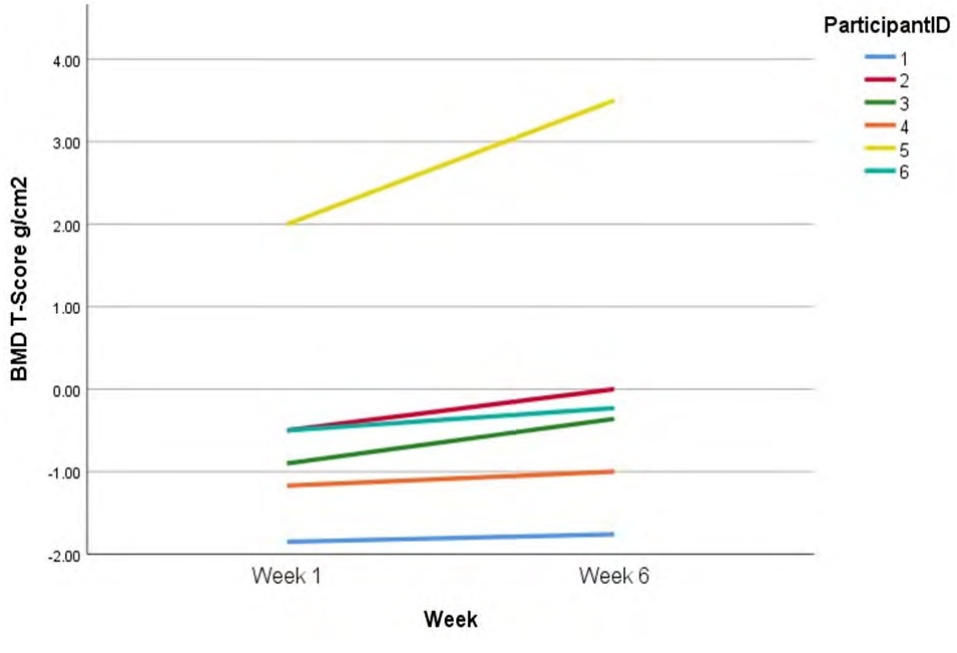


Figure 4: Line graph displaying positive correlation between bone mass density before and after Natto consumption per participant

Source: Author's own data

The BMD did not display skewed distribution and remains within acceptable ranges, displaying steady progression; this suggests that the data are plausible. Most participants continued within the lower boundary of the 'normal' BMD classification (t=-1 – t=0). Participant 1, despite continued results of osteopenia, displayed a minor increase. All the participants' BMD t-scores increased. Although the BMD of Participants 1 and 4 contained the lowest values, there was a positive outcome for Participant 4. Participant 4's BMD rose by t=0.23 advancing their status from Osteopenia to Normal range. Participant 1 increased by 0.09, remaining with Osteopenia, increasing the least out of the study. The ranking order of the participants' results did not change throughout the six weeks, yet significant changes in BMD for Participants 2, 3 and 6 emerged. Participant 5 remained significantly higher than the rest of the participants, t=3.50, increasing 1.5 in total, more than any other participant. The males remained the gender with the lowest average BMD, t=-0.88 (+0.07) whilst the females averaged at t=-0.48 (+0.19).

Table 10: Paired Samples Statistics of Weeks 1 and 6

	Mean	N	Std. Deviation	Std. Error Mean
Pair 1 Week 1 BMD	-.4867	6	1.31760	0.53791
Week 6 BMD	.0250	6	1.81804	0.74221

Source: Author's own data

Over the course of six weeks, the BMD mean value increased, signifying a positive overall BMD result (Table 10). However, the p-value between weeks 1 and 6 resulted in 0.059. This insignificance may have occurred due to the width of participants included.

Evidence of Natto consumption and questionnaire binary data

Participants sent photographic images of their recipes via WhatsApp. The ingredients and foundation of their recipes differed due to factors such as lifestyle, diet and ethnicity (Table 11).

Table 11: Recipes designed via participants

	RECIPE	SOURCE
1	Natto & Greek plain yoghurt and peanut butter (sometimes honey)	Homemade
2	Natto & slimming world recipe – 5% lean mincemeat, tomatoes, sweetcorn, carrots, broccoli, smoked paprika, onions, beans, basmati rice, Worcestershire sauce (brand – Lea & Perrins)	Slimming World
3	Natto in homemade scotch eggs, Jamie Oliver recipe	Jamie Oliver available from: https://www.jamieoliver.com/recipes/eggsrecipes/proper-scotch-eggs-with-lovelyscottish-cheese-and-pickle/
4	Natto & avocado, cannelloni beans, cress, dill, coriander, red pepper, rapeseed oil, cubes of feta, white chai seeds, raw lemon juice, salt and pepper.	Homemade

Source: Author's own data

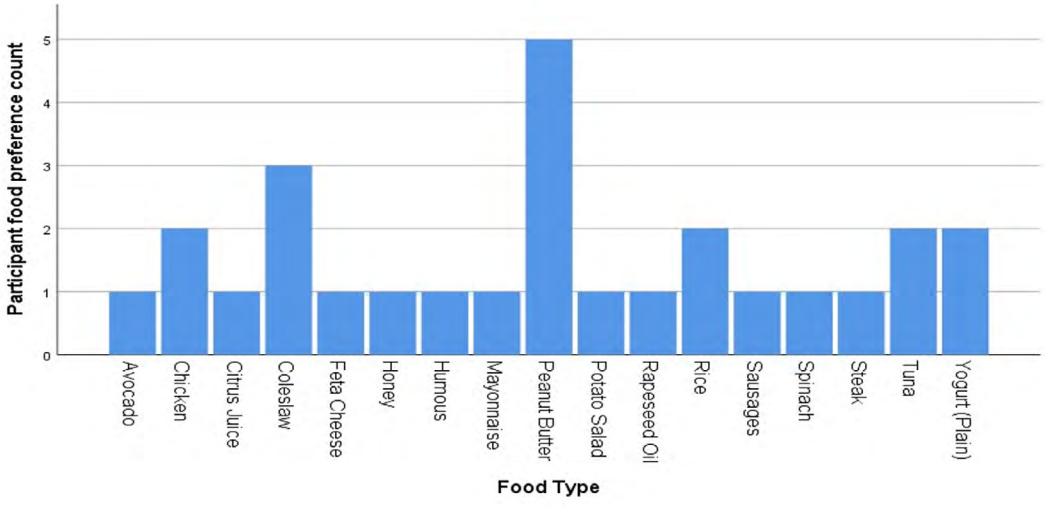


Figure 5: Keyword analysis of captured food type suggestions via participants

Source: Author's own data

Peaks within Figure 5 indicate common foods suggested by participants via the final feedback questionnaire. The study found peanut butter to be highly favourable in combination with Natto (*n*=5). This was followed by coleslaw (*n*=3), which was suggested by 50% of the participants. Chicken, tuna, rice and yoghurt were equally favoured by 33.33% (*n*=2) of participants.

Participants were asked which recipe, issued by the study, they preferred. Two of the three suggested recipes were consumed within the study and equally favoured: Tuna Natto Salad and Peanut Butter Natto Toast.

Table 12: Polarised feedback on Natto recognition

Questions	Answers	
	Yes	No
Had you heard of Natto prior this study?	0	6
Do you think Natto could be applied to a westernised diet?	6	0
Did you get used to the smell of Natto throughout the study?	2	4
Would you consume Natto again?	6	0
Did you get used to the texture of Natto throughout the study?	4	2
Did you get used to the taste of Natto throughout the study?	4	2

Source: Author's own data

Table 12 displays feedback collected via the final questionnaire. None of the participants were aware of Natto prior to the study, however, all participants agreed Natto could migrate into a westernised diet. All the participants agreed they would consume Natto again, despite 66.67% of participants disliking Natto's texture. All the participants preferred Natto at a chilled temperature.

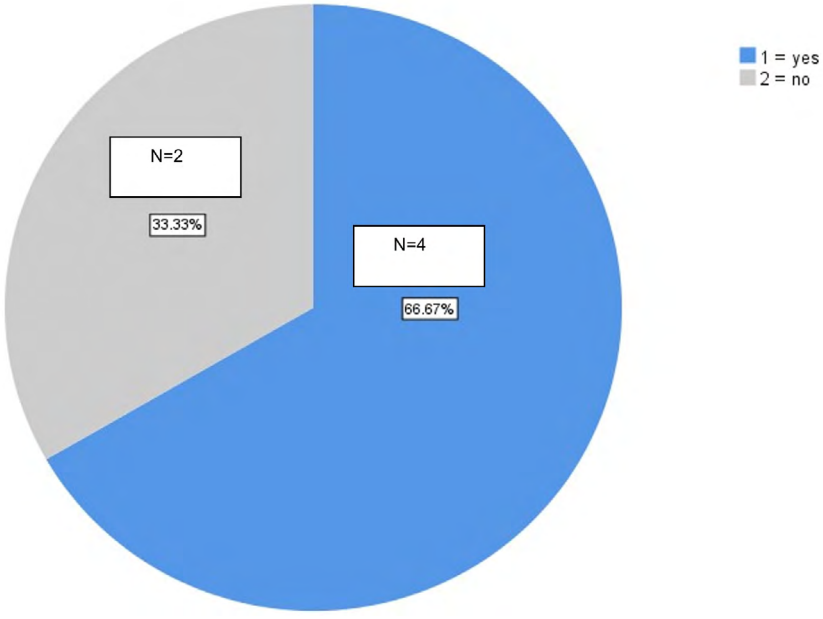


Figure 6: Pie chart illustrating the evolution of Natto's texture and taste via patient feedback

Source: Author's own data

Data from two questionnaires, mid- and post-study, revealed that participants displayed decreased levels of hostility towards Natto. The questionnaires included hedonic scales to evaluate the participants' adaptability towards the characteristics of Natto. Figure 6 shows that four of the participants agreed that the texture and taste of Natto became tolerable during consumption. On the contrary, the smell remained unpleasant to most throughout the entirety of the study.

Natto was set to be consumed three days a week, 18g (1Tbs) per intake. Three of the participants correctly consumed Natto at the recommended quantity. Participants 2 and 6 incorrectly measured their Natto; as a result, their intake was reduced from 18g to 10g (1tsp) three times per week. This was due to a communication error. The consumption of Natto appeared too arduous for Participant 4 as they could only manage to consume 18g of Natto 2-3 times a week, admittedly due to work commitments.

Participants expressed positive sentiments towards Natto, reporting increased knowledge of VK_2 and its benefits. This was encouraging behaviour, likely to have a positive effect towards their likelihood of adoption.

Discussion

Natto's influence on bone mass progression

Osteoporosis is a public health concern further exposing risk of fractures and VC. VK_2, in the form of MK-7, aids these issues via inhibition of VKDPs. This study aimed to demonstrate that post-Natto oral ingestion, MK-7 is effective in catalysing OC carboxylation (Figure 7), therefore increasing BMD and supressing VC.

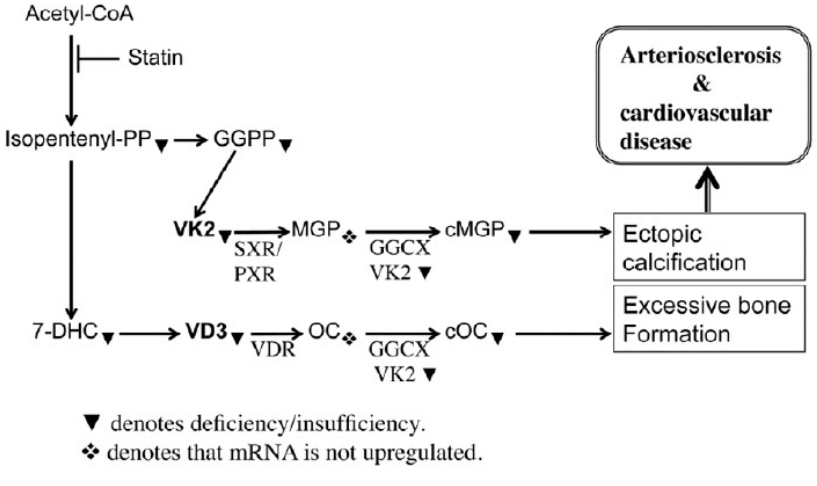


Figure 7: Biochemical pathways of VK_2, OC and MGP

Source: Hashimoto and Okuyama, 2017

Following VK-dependant carboxylation, OC develops an affinity for calcium ions and incorporates them into the hydroxyapatite crystals deposited within the bone matrix (Al-Suhaimi and Al-Jafary, 2020).

In conjunction with these processes, the study discovered that in order to achieve optimal BMD, Natto had to be consumed in specific, calculated quantities (3 x 18g per week). The final feedback questionnaire distinguished between the participant and their overall Natto consumption. The study revealed compelling results, stating those who consumed the correct amount of Natto per week achieved greater BMD than those who failed to do so. A total of three participants consumed 18g Natto three days per week. Two of these subjects significantly increased their BMD above the rest; however, the remaining participant may have a family history of osteoporosis, leaving the study to consider genetic handicaps. These data conclude the study, suggesting weekly exposure to Natto equates optimal effects on BMD.

Westernising Natto

This study aimed to reveal whether Natto could be incorporated into western culture. This was achieved through a problem-solving approach.

Natto is a traditional Japanese delicacy derived from fermented soy products cultured with Bacillus Subtilis. A local favourite within Japanese culture, with the average Japanese individual consuming 41 x 50g packs per year (Kusaka et al., 2016; Park et al., 2012). The potency and viscous texture limits Natto's adoptability. To tackle these issues, recipes were developed to guide the participants through its unique properties.

It was important to design appetising recipes for the participants, featuring typical western ingredients. Research on food sources and culinary techniques enabled the researcher to remove the unique odour emitted by Natto. Yoshiyama et al. (2019) revealed cumin, cinnamon and hot temperatures may increase the potency of Natto. At the same time, coriander, basil, pepper and cold temperatures reduced Natto's aroma significantly, thus clarifying the positive feedback towards the Tuna Salad Natto recipe. Furthermore, the stringy texture of Natto can be perceived as a drawback to its use (Weng et al., 2017). Participants were advised to use minimal amounts of oil to reduce the sticky composition. This discovery was made during the recipe design process, prior to the start of the experiment. In addition, Natto was accompanied with fat soluble foods such as oils to maximise the absorption of MK-7.

The Japanese typically consume Natto with egg, mustard, rice, soy sauce and onions (Hu et al., 2017). To maximise westernisation, participants were encouraged to avoid Asian culinary techniques, food, recipes and apply Natto to their everyday diet. The study recognised a relationship between both cultures. Three participants expressed how coleslaw complimented Natto. Coleslaw contains horseradish, which shares properties similar to that of mustard, thus highlighting the dietary link between both cultures and their palate towards Natto consumption. Peanut butter was also revealed as a popular counterpart to Natto. A claim could be made towards its salty taste, a characteristic shared with the eastern condiment, soy sauce.

Digestive health

Research on modern uses and intake of Natto is in its infancy. In addition to bone and cardiovascular health, historical research has shown that Natto aids digestive health (Kosaza, 1989). Female participants (*n*=3) disclosed during the study that bowel habits became regular and stool texture improved post-Natto consumption. This was highly beneficial for Participant 5, who is burdened with chronic constipation. On average, many participants' bowel movements occurred 30 minutes post-ingestion. This correlates with a momentous 2-week study where consumption of 50g/day of Natto (VK-2) reduced constipation and contributed to Bifidobacterium cells within the faeces (Dimidi et al., 2019). This may be due to an abundance of fibre that develops during fermentation periods (Wickramasinghe, 2017).

Participants' characteristics and their correlation with BMD

This study aimed to analyse bone mineralisation over six weeks. It was therefore vital to select the right participants to observe a significant change within a brief period. Skeletal health may decline over time due to diet, health, lifestyle and age (Tieland et al., 2015). Research states that 25%-50% of peak adulthood BMD is assembled during adolescence and that lumbar BMD plateaus after the age of 30-39yrs. Therefore, participants were aged 20-35yrs (Cheng et al., 2016; Khosla, 2013). Two of the participants declared a family history of osteoporosis. It could have been assumed that both these individuals may have been genetically disadvantaged; however, these participants had the greatest BMD readings within the data. In this case, osteoporosis is not necessarily a genetic burden and it could be due to modifiable factors.

Asians have been reported to have fewer fractures than Caucasians, however Asians have also been reported to have a genetically lower BMD overall (Kaur et al., 2019). Although this research suggests a divide towards ethnicity and bone status, this was not considered an outlier as Participant 4 was descendant from English and Thai ancestry.

Epidemiological studies have reported significant associations between sedentary lifestyles and the risk of osteoporotic fractures. It is generally known that physical activity has a constructive, long-lasting impact on bone health (Tønnesen et al., 2016). As every participant within this study undertakes moderate to high intensity physical activity 3-5 days per week, all study members were included and not perceived as outliers. All participants are members of the David Lloyd Club in Eastbourne, East Sussex, UK.

Practical aspects and BMD influencers

A bone densitometer may reveal the positive correlation of BMD to Natto consumption over a 6-week period that was due to the properties of VK_2. Natto consumption inhibited OC and MGP to remove excess calcium within the bodies' soft tissues and transport it to the bone matrix. However, this form of testing is not based on fact, which may result in incredulity from other peers.

VK_2 levels can be tested via phlebotomy, a direct and effective method of identifying VC and OC levels. The magnitude of OC carboxylation has been recognised as a sensitive and suitable biomarker for distinguishing VK_2 status within the body. It has been reported that new bioavailable OC is typically deposited within the bones; however, 10-30% of circulating OC appears within soft tissues as unOC in healthy individuals (Moser and van der Eerden, 2019). Low levels of OC signify low VC, equalling sufficient amounts of VK_2 within the body. If the blood provides high levels of OC, this can allow health professionals to assume higher rates of VC, a case of subclinical deficiency of VK_2 in bone tissue. For future research, this technique could be a favourable methodology to distinguish the relationship between VK_2, bone and cardiovascular health. An ELISA kit was not used for this research as it was not within the project's budget.

While the study outlines the biological and clinical potential for Natto and VK_2 on bone health, this study was aware of other factors that may have contributed to the increase in BMD. Biological mechanisms play an important role in bone metabolism and nutritional intake. Participant 3 was a coeliac and therefore suffered a collapsed microvilli within their digestive system. This autoimmune disease reduces the ability to absorb nutrients efficiently and may have impacted their ability to absorb the same amount of VK_2 as the other participants (Zuvarox and Belletieri, 2020). Participant 6, whose BMD increased by 0.27g/cm2, regularly consumes calcium supplements. As the participant has ingested the supplement for years and it was important to maintain their normal lifestyle habits as much as possible, this was not deemed an outlier.

Limitations and Considerations

Case studies are an essential stage in scientific research, providing data that test recruitment strategies, feasibility, data patterns and trends, leading science to further research (Leon et al., 2011). However, case study data are not often considered authentic in epidemiology due to limitations such as sample size and the possibility of making inaccurate predictions. Reported findings on two males who provided the lowest mean BMD T-score may have restricted outcomes. Therefore, this study can only suggest the significant role of Natto towards public health. Additional research with statistical sample size calculation will be required to conclude these data.

Participants had the responsibility of controlling their Natto consumption over the 6-week intervention. This could bring potential errors to the study, for instance, if the participants did not consume Natto as often as scheduled, were not truthful about their consumption of Natto, or dishonest about what was consumed with Natto. This could have interfered with the absorption levels of VK_2.

Dietary sources are not always effective for treating large populations. Supplementation is recognised as an efficient and simple way to manage clinical health. Therefore, it is no consternation that calcium supplementation appears to be on the rise (FSA, 2018).

Conclusions

This successful 6-week intervention of VK_2 administration (3 x 18g/week) improved all participants' BMD. The mechanisms by which VK_2 may exert voids on vessel disturbance could not be observed directly via bone densitometry. However, the results are auspicious, concluding Natto's potential as a superfood for western culture. The positive result was produced in what could have been deemed an implausible task throughout a brief period; strengthening the case study's outcome. Furthermore, the data correlate alongside trustworthy and current research discussing the biological benefits granted via Natto consumption; assisting clinical treatment of osteoporosis and CVD. Future research is warranted to circumvent any confounding factors, such as longevity and low participation.

In this light, Natto successfully migrated into western culture with ease. This was confidently confirmed as all participants adapted to the characteristics and obstacles that Natto yields.

All reported findings will need to be evaluated using a larger and more diverse population.

Conflict of Interest

The researchers declare no conflict of interest.

Authors' Contributions

Vanessa Kitching designed and carried out the study. This included data collection, data analysis and creating the manuscript. Ihab Tewfik assisted Vanessa with the study protocol, design and the approval of the final manuscript.

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Biographical notes

Vanessa Kitching has a First-Class Honours Degree in Human Nutrition from the University of Westminster. She is a registered Associate Nutritionist at the Association for Nutrition (AfN). Vanessa is currently enrolled on an honorary contract with Great Ormond Street Hospital (GOSH) as an Information Specialist and Dietetics Assistant. This is where she became involved with a steering group, focusing their research on nutritional therapy in paediatric intensive care (PICU).

Ihab Tewfik is a Registered Nutritionist (Public Health) who has expertise in planning, implementing and evaluating sustainable nutrition-sensitive intervention programmes at the population level. Ihab has developed an independent academic research career that underpins the pivotal role of nutrition science in modulating complications of global chronic diseases through tailored functional recipes (TFRs). In addition to his PhD from London South Bank University, Ihab holds Master of Public Health (MPH) and Doctorate of Public Health (DrPH) from Nutrition Department, University of Alexandria. Dr Tewfik's research theme: "Local Food for Global Health". The ultimate strategy of this research theme is to optimise tailored functional recipes (TFRs)/model meals to modulate global chronic disease. These functional food recipes are not limited to the elimination of malnourishment, but extend to the design and engineering of food that transcends disease prevention by improving availability of micronutrients, increasing biological functions and promoting sustainable health.