Study Design and Protocol

An Evaluation of a Milk-Based Nutritional Supplement to Effect a Positive Change in Bone Health in Post-Menopausal Women at Risk of Osteoporosis.

In a novel approach to the timing of nutrient ingestion, the proposed nutrient intervention seeks to modify (reduce) the rate of bone resorption and promote the rate of bone formation to the benefit of bone health in this at risk population.

2. Study design: A block randomised, cross-over design of 24h rates of bone turnover in healthy, post-menopausal women with osteopenia receiving either a milk-based protein supplement (MBPS) or isoenergetic (maltodextran) placebo control (PLACEBO).
3. Procedures:
Subjects: Post-menopausal women with osteopenia as determined by site-specific BMD (DXA) diagnosed and screened by a clinician (Dr Manjula Hettiarachchi) and for dietary intake of calcium and Vit D by a clinical dietitian (Dr Catherine Norton).
Subject screening (clinical examination, 1h) and dietary intake of calcium and Vit D (by food frequency questionnaire, 1h) will precede the experimental protocol.

Experimental protocol and data collection: Supplemenary Material contains a Flowchart
Subjects will attend for a 2 day overnight residence in the National Altitude Training Centre, Kilmurray Village. This facility is equipped to conduct residential human trials and has on-site individual bedroom accommodation subjects & researchers, a living room with fully equipped kitchen and laboratory to handle human blood samples.
The subjects' programmed protocol is as follows;

1. Arrive @ 17:00h with overnight bag;
2. Empty bladder and then provide and retain urine samples for the duration of the stay (assisted collection by researchers);
3. Consume a standardised evening meal (pre-prepared by the research dietitian) and then relax reading/watching films etc in the living room;
4. At 20:00h a research nurse will insert a cannula into a superficial arm vein and a blood draw (5ml) will be taken and processed for later analysis;
5. Further blood draws (5ml) will be taken at 22:00h, 2300h, 2400h,0100h and 0200h and the cannula withdrawn;
6. At 22:00h consume ***either*** maltodetran control (**PLACEBO**) (day 1) OR supplement (**MBPM**)(day 2) - or *vice versa;*
7. Retire to bedroom;
8. Consume a standardised breakfast and lunch (pre-prepared by the research dietitian) whilst living in and around the University grounds
(i.e. in close proximity to ensure 24h urine collection is complete);
9. Repeat from 2 above to end of 2nd day.

Total blood draw over 48h is 14 x 5ml = 70ml

All formulations to be supplied food grade and product tested by Dairygold Co-opertaive Society, Mitchelstown, Ireland.

4. Analytical methods:

i. In total 224 (16 subjects * 7 samples * 2 d) blood samples will be collected. Separated aliquots will be frozen @ -80C and batch analysed for CTX (biomarker of resorption), P1NP (biomarker of formation). In total 32 (16 subjects * 2 d) 24h urine samples will be collected. Aliquots will be frozen @ -80C and batch analysed for uPYD, uDPD (biomarkers of resorption) normalised to urinary creatinine.

ii. The I.O.F. recommended serum marker for bone resorption is CTX and formation P1NP. The magnitude and time course of the acute response (0-4h) change in bone turnover following ingestion of the MBPM *vs*. PLACEBO will be analysed by repeated measures ANOVA of the serum biomarkers of resorption and formation.

iii. The overall influence on the diurnal change in bone turnover is best measured by a global biomarker of bone resorption specific to the type I collagen in bone. This is provided by the measurement of 24h urinary pyridinoline (PYD) and deoxypyridinoline (DPD) excretion normalised for urinary creatinine. Thus, the magnitude of effect of 24h change in bone resorption following ingestion of the MBPM *vs*. PLACEBO will be analysed by ANOVA(R) of the urinary markers of bone resorption (PYD/creatinine and DPD/creatinine).

Schematic Flow Chart of theTrial

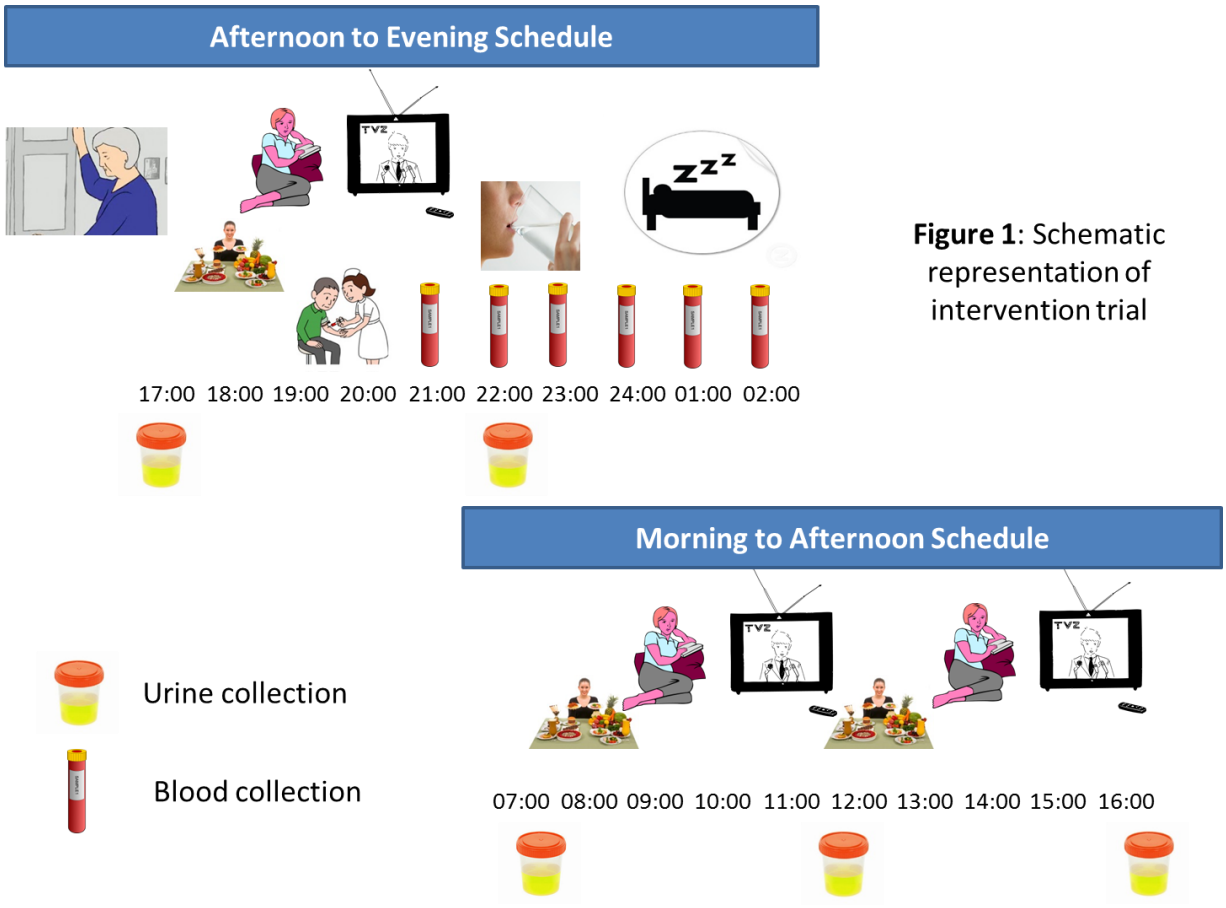

Figure 1: Schematic representation of intervention trial

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Volunteer Information Sheet

An Evaluation of a Milk-Based Nutritional Supplement to Effect a Positive Change in Bone Health in Post-Menopausal Women at Risk of Osteoporosis.

Thank you for considering this research study.

What is the project about?

It is an attempt to use a milk-based, nutrient supplement to make a positive modification to bone health in post-menopausal women at risk of osteoporosis, i.e. diagnosed as osteopenic.

FIVE key facts related to bone health

1. Over the lifespan the tendency to poor bone health is influenced by your genetic make-up and environmental factors such as diet and physical activity.

2. Poor bone health is characterised by a diminution to bone quality (i.e. the protein content of bone) and low bone mass (i.e. the mineral content of the bone).

3. We now know that bone regulates both quality and mineral content through a coupled process of bone resorption (the dissolution of old bone) and bone formation (the formation of new bone), a process termed bone turnover.

4. Bone turnover has an underlying 24 clock, greater rates of bone resorption occur at night than in the day, and

5. Bone turnover is modulated by calcium, Vit D and protein intake. Calcium and vitamin D intake, as recommended by the International Osteoporosis Foundation (I.O.F.), can modify bone turnover to reduce potential development of poor bone health. Equally, appropriate protein intake can increase the to the protein content of bone in support bone health.

In a novel application of the 5 key facts listed above, this study seeks to modify the rate of bone turnover by feeding a milk-based nutritional supplement at night, ***i.e. when the rate of bone turnover is highest***. We propose a milk-based supplement because milk contains a natural source of calcium and protein. ***So***, if you are post-menopausal, diagnosed as at risk of osteoporosis but otherwise in good health you may wish to consider taking part in this study.

What will you have to do?

Firstly, we will make every effort to explain fully all aspects of the study and allow you time to question and reflect on your participation. If you are happy to proceed, you will be asked to sign a written, statement of consent.

You will be consenting to the following;

1. Screening:

Following an overnight fast (from 10pm the previous evening) to attend the University of Limerick at a scheduled date between 7:30 and 9:00am to undergo a clinical examination, provide a blood sample, have a bone scan (DXA) and complete a dietary intake analysis. This will take approximately 2h to complete. You will be provided with a snack, tea/coffee before leaving.

It will take a few days to process these screening data. Should the results indicate that you are a suitable candidate for this study you will be invited to proceed to participate in the intervention trial.

2. Intervention:

This is a 2-day, 2-night trial requiring you to be resident at the University for 48 hours.

At a designated date we require you to undertake the following;

Attend a University Residence located in Kilmurray Village. This facility is equipped to conduct residential human trials and has on-site individual bedroom accommodation and living room with fully equipped kitchen. There are 6 rooms, so you will be 'sharing' the house with 5 other participants for the 2 days.

The programmed protocol is as follows;

1. Arrive @ 17:00h with overnight bag;
2. Settle into your residence before emptying your bladder. From this tie point and for the 48 hours that follow you will be required to provide urine samples for the duration of the stay (assisted collection by researchers);
3. Consume a standardised evening meal (pre-prepared by the research dietitian) and then relax reading/watching films etc. in the living room;
4. At 20:00h a research nurse will insert a cannula into a superficial arm vein and a blood draw (5ml) will be taken and processed for later analysis;
5. At 22:00h consume ***either*** a carbohydrate-based drink (**CON**) (day 1) OR milk-based protein supplement (day 2) - or *vice versa* and a further blood sample will be drawn;
6. Retire to bedroom;
7. Further blood draws (5ml) will be taken at 2300h, 2400h, 0100h and 0200h after which the cannula is withdrawn. This will require the researcher to enter your room during the night. Though blood can be drawn without waking, there may be occasions when the disturbance will wake you.
8. In the morning, consume a standardised breakfast, then a lunch (both breakfast and lunch are pre-prepared by the research dietitian) whilst living in and around the University grounds
   (i.e. in close proximity to ensure 24h urine collection is complete);
9. Repeat from 2 above to end of 2nd day.

AND THAT's IT!

What are the potential benefits to you?

The benefits for partaking in this study are:

- You will receive a comprehensive report of current bone mineral density, body composition and dietary intake of essential nutrients related to bone health.
- You will be contributing to the evaluation of a novel, diet-based intervention to enable bone health in those whom, like you, are at risk of osteoporosis.

What are the potential risks?

You may be assured that the procedures to be employed have been used extensively by the researchers conducting this study and are generally well tolerated by participants.

- There is minimal risk involved in the study. The bone scan is attained by exposing you to a mild X-ray when lying on a bed, termed a DXA scan. This exposure is equivalent to approximately 1/30th of a normal X-ray which is 'trivial'.

- You may experience some discomfort during the blood draw. Blood will be drawn by a qualified research nurse or clinician.

- The food supplement is based on milk, a naturally source dietary food. The study would NOT be suitable for a person who has a known allergy to dairy produce, e.g. lactose intolerant.

What if I do not want to take part?

- You can discontinue your participation in the research study at any time and this will be dealt with in an unhesitating and confidential manner.

What happens to the information?

- The information retrieved will be dealt with and handled in complete confidence. After the completion of the study, information will be kept electronically on the principal investigator's password-protected computer

Who else is taking part?

Other post-menopausal women at risk of poor bone health.

What if something goes wrong?

In the unlikely event that anything untoward occur the testing procedure will immediately cease and the PESS department emergency procedures will be followed.

What happens at the end of the study?

At the end of the study the information garnered will be used anonymously to present the results as a conference or academic journal communication and may result in the supplement being further evaluated over a longer period of time.

Your personal details/information and data from the study will be held by the principal investigator for up to 7 years in a password-protected computer at UL.

Upon completion, a report containing the overall study outcome will be available to participants on written request to the Principal Investigator.

What if I have more questions or do not understand something.

If you do not understand any aspect of the study we would urge you to come forward to any of the researchers (see contact details below) and discuss any questions that you might have.

It is important that participants feel completely at ease throughout the experiment.

Will I receive payment for participating?

Subjects receive an overnight allowance for the time committed to the study.
The allowance is €108 per night.

Project Investigator Contact Details:

Principal Investigator: Professor Phil Jakeman
P1027, University of Limerick,
Office Telephone: 061 202800
Email: phil.jakeman@ul.ie

Other investigators: Dr. Catherine Norton
Email: catherine.norton@ul.ie

If you have any concerns about this study and wish to contact someone independent, you may contact The EHS Research Ethics Contact Point of the Education and Health Sciences Research Ethics Committee, Room E1003, University of Limerick, Limerick.
Tel: (061) 234101 / Email: ehsresearchethics@ul.ie

Informed Consent

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Should you agree to participate in this study please read the statements below and if you agree to them, please sign the consent form.

- I have read and understood the participant information sheet.
- I understand what the project is about, and what the results will be used for.
- I understand that what the researchers find out in this study may be shared with others but that my name will not be given to anyone in any written material developed.
- I am fully aware of all of the procedures involving myself, and of any risks and benefits associated with the study.
- I know that my participation is voluntary and that I can withdraw from the project at any stage without giving any reason.
- I consent to the data obtained from the conduct of this project to be used, anonymously, for presentation and publication.

I consent (or agree) to my involvement in this research project after agreeing to all the above statements.

Name: (please print): _____

Signature: _____ Date: _____

Investigator's Signature _____ Date: _____

This study has been approved by the ethics committee of the Faculty of Education and Health Sciences. If you have any concerns about this study and wish to contact someone independent, you may contact The EHS Research Ethics Contact Point of the Education and Health Sciences Research Ethics Committee, University of Limerick, Limerick. Tel: (061) 234101 / Email: ehsresearchethics@ul.ie