Study Design and Protocol

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

Background:
The process of bone remodeling exhibits pronounced diurnal pattern that is important for bone health. A balanced rate of bone resorption is required to maintain bone health, a balance that can be disturbed during the life-cycle to effect net rate of formation (as occurs during growth and development to adulthood) or net resorption (as occurs, for example, during the menopause). Bone turnover is a nutritionally modulated process. Research recently completed by this research group (2017_06_03_EHS) suggests that a milk-based protein supplement (MBPS) can modulate beneficially the rate of bone resorption over the time period when bone remodeling is most active i.e. late evening/overnight. In this 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.

Study design:
A block randomised, controlled study among healthy, post-menopausal women with osteopenia receiving a milk-based protein supplement (MBPS) during late night or not (CONTROL) for a period of 24 weeks.
Procedures:
Subjects: Post-menopausal women aged 50 to 70 y
Subject screening: Osteopenia as determined by site-specific BMD (DXA), clinical history, blood screen, previous 3m of intake of calcium and Vit D by Food Frequency Questionnaire (FFQ) will be conducted to qualify inclusion/exclusion criteria

Inclusion Criteria: Post-menopausal women aged 50-70y assessed by site-specific BMD to be osteopenic (BMD T-score between -1.0 to -2.5), assessed by clinical screen to be otherwise healthy and free from other illness, women with normal blood sugar (assessed by fasting blood sugar 3.9 to 5.8 mmol/L), thyroid function (serum TSH 0.50 - 4.4 mU/L), vitamin D (above 50 nmol/L), kidney function (serum creatinine 50 - 98 umol/L), not on medications likely to influence the study outcome (steroid or anti-convulsion drugs)
Exclusion criteria: Women with osteoporosis (BMD T-score less than -2.5) or/and any other illness. Women with blood sugar, TSH levels, vitamin D and/or creatinine measurements outside of the values mentioned above and/or on medications.

Experimental protocol: subjects qualifying and willing to participate will undertake the following tasks;

Week 1: Test Day 1
1. Arrive between 08:00 to 09:00h after an overnight fast from 22:00h the previous evening; 2.Empty bladder and collect and retain urine for a period of 24h.
3. Provide a blood sample from a superficial arm vein; total blood draw is 5ml
4. Subjects will be instructed as to the completion of a 7-day weighed food intake record and asked to complete a trial 24h record prior to returning the following day.

Week 1: Test Day 2
1. Return the following day and provide a first-pass urine sample, provide a blood sample from a superficial arm vein; total blood draw is 5ml
2. The dietitian will then review the entries on the 24h dietary record sheet with the subject and resolve any potential conflicts. The subject will then be asked to complete the full 7 day weighed food intake record.

Week 2: Test Day 3

1. Once the food intake record is completed, the dietitian will review the entries and resolve any potential conflicts with the participant.

2. MBPS group: The subject will be asked to take the supplement the MBPS as prescribed i.e. prior to retirement each night.

Control group: The subject will be free living. No change in dietary behaviour will be required.

Composition of MBPM - 25g of milk-based proteins + 1000mg dairy-based calcium fortified with 40ug Vit D flavoured and textured. All formulations to be supplied food grade and product tested by Dairygold Co-operative Society, Mitchelstown, Ireland.

Week 12: Test Day 4

1. Subjects will return for a DXA scan between 07:30 and 09:30h after an overnight fast from 22:00h the previous evening.

Week 24: Test Day 5

1. Subjects will return and follow the same protocol as on Test Day 1 and Test Day 2.
2. Subjects will also receive a DXA scan on the first return visit.

Throughout the study period:
The MBPS group will be contacted at regular intervals to check compliance and will return to PESS to collect additional supplements.

Analytical methods:
The International Osteoporosis Foundation I.O.F. recommended the biomarker CTX for bone resorption and P1NP for bone formation in serum and pyridinoline (PYD), deoxypyridinoline (DPD) and NTX excretion normalised for urinary creatinine as the respective biomarkers in urine.

i. In total, 240 (60 subjects * 2 samples * 2 d) morning blood samples will be collected. Separated aliquots will be frozen @ -80C and batch analysed for CTX (biomarker of resorption), P1NP (biomarker of formation).
ii. In total, 240 (60 subjects * 2 samples * 2 d) 24h urine samples will be collected. Aliquots will be frozen @ -80C and batch analysed for uPYD, uDPD, and uNTX (biomarkers of resorption) normalised to urinary creatinine.

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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.

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 24 week trial of taking a dairy supplement or NONE as prescribed i.e. prior to retirement each night for a period of 24 weeks.

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

Week 1: Test Day 1

1. Arrive between 08:00 to 09:00h after an overnight fast from 22:00h the previous evening;
2. Empty bladder and collect and retain urine for a period of 24h.
3. Provide a blood sample from a superficial arm vein; total blood draw is 5ml
4. You will be instructed as to the completion of a 7-day weighed food intake record and asked to complete a trial 24h record prior to returning the following day.

Week 1: Test Day 2

1. Return the following day and provide a first-pass urine sample, provide a blood sample from a superficial arm vein; total blood draw is 5ml
2. Your 24h dietary record sheet will be reviewed and any potential conflicts resolved. You will then be asked to complete the full 7 day weighed food intake record.

Week 2: Test Day 3

1. Once the food intake record is completed, the dietitian will review the entries and resolve any potential conflicts with you.
2. You may be asked to take a drink provided by the research team on each night before bed for 24 weeks depending on your group allocation.

Week 12: Test Day 4

1. You will return for a DXA scan between 07:30 and 09:30h after an overnight fast from 22:00h.

Week 24: Test Day 5

1. You will return and follow the same protocol as on Test Day 1 and Test Day 2.
2. You will also receive a DXA scan on the first return visit.

Throughout the study period:

The group consuming the drink each night will be contacted at regular intervals to check compliance and will return to PESS to collect additional supplements in monthly intervals. The second group will be free living. No change in dietary behaviour will be required.

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?

No, there is no remuneration for participation in this study.

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

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Email: Manjula.Hettiarachchi@ul.ie

Ms. Rachel Cooke
Email: Rachel.Cooke@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