

## Impact Objectives

- Gain a greater understanding of the potential benefits of the yuzu fruit, particularly in relation to bone health
- Delve deeper into the physiological effects of nomilin, so as to understand the effects of the citrus limonoid on other areas of the body
- Ultimately halt the rise in the number of people suffering from osteoporosis both in Japan, where numbers are on the increase, and internationally

# Freshly squeezed research

*Dr Yoshifumi Kimira is exploring how the humble citrus fruit, with a focus on yuzu, can help improve bone health*



**Could you introduce yourself and your key research interests?**

I graduated from the Department of Physical Education, Tokai University, Japan, in 2007 and entered the Department of Food and Nutrition, Graduate School of Agriculture, Tokyo University of Agriculture, Japan. I finished my doctoral course in 2012 and took up the position of research assistant at Josai University, Japan. Currently, I am Assistant Professor and am doing research and education in food science. My research subject is the effect of collagen-derived peptides or food functional factors contained in citrus fruits on bone metabolism.

**What do you find most interesting about your field?**

I found the idea of studying the functionality of foods with an approach that is closer to medicine and adopted it. The functional ingredients of foods have effects close to those of medicines and can be ingested from foods, so there is a safety aspect as well. I believe that by maximising this benefit we can propose a meal that will lead to disease prevention.

**Can you introduce the basis of your project 'Citrus limonoid nomilin inhibits osteoclastogenesis *in vitro* by suppression of NFATc1 and MAPK signalling pathways'?**

Limonoids show various physiological effects, but the effect of limonoids on bone metabolism remains unknown. We studied the major citrus limonoid, nomilin, on osteoclast differentiation and activity signal transduction.

**What are the known benefits of nomilin and what led you to question the potential in relation to bone health?**

Limonoid has been reported to have antioxidant activity, anti-tumourigenic activity and hypoglycaemic effect, and improve hyper-lipoedema. Although it has already been clarified that fruit has a positive effect on bones, there have been some reports showing the possibility that the limonoids contained in fruit peel and seeds can also affect bone. We also investigated whether nomilin has an effect on bone metabolism.

**Are you able to confirm any benefits to nomilin, particularly in relation to osteoporosis?**

It has been confirmed that nomilin has

an action on osteoclast differentiation and activity. The results of the animal test have not been seen yet, but considering there are many targeted osteoclasts in the mechanism of action of the therapeutic agent for osteoporosis, nomilin is considered to be beneficial for the prevention of the development of osteoporosis.

**At what stage of your research are you at and what is the immediate next step?**

We are still in the first phase of basic research and have a way to go. I would like to conduct research that can contribute to people's health through the functionality of yuzu (a citrus fruit). As an approach to the problem of agriculture, I think that we have made considerable progress.

**How do you see your research progressing in the next five to 10 years?**

The amount of waste yuzu will be reduced, and yuzu farmers will be able to produce lots of fragrant yuzu abundantly. Also, I would like to present research results that show there are many people who enjoy the citrus fruits as food, but at the same time are also enjoying the health benefits. ●



# Introducing yuzu

*A team of researchers based at Josai University, Japan, wants to help improve population bone health by introducing a special citrus fruit*

You have surely heard of oranges, lemons and limes, but what about yuzu? This is a citrus fruit originally stemming from central China and Tibet, and associated with Japanese cuisine. Although it is rarely eaten as a fruit, its scented juice and zest can be added to sweet and savoury dishes to add unique flavour. A team of Japanese researchers is exploring the potential of the fruit to be used medicinally, with a focus on bone health.

Dr Yoshifumi Kimira is part of a research team that is investigating how citrus fruits could potentially alleviate various types of bone disease. He is based in the Department of Clinical Dietetics and Human Nutrition in the Faculty of Pharmacy and Pharmaceutical Sciences at Josai University, Japan. He is working alongside fellow Josai University researchers, as well as collaborators from Liberty House, a food processing company, and from Moroyama town, Saitama. These collaborations were necessary, as Kimira explains: 'I'm not a specialist in food processing, so collaborating with companies specialising in food processing was very important.'

## NATURE'S MEDICINE

The team's project is entitled 'Citrus limonoid nomilin inhibits osteoclastogenesis *in vitro* by suppression of NFATc1 and MAPK signalling pathways'. Limonoids are

phytochemicals found in citrus fruit, 53 of which have been identified, including the limonoids nomilin and nomilinic acid. These have been studied in anti-cancer research and now Kimira and his team are studying the potential effects of nomilin on osteoclastogenesis, which is the development of osteoclasts – a type of bone cell that breaks down bone tissue – from blood cells. Despite nomilin having been reported to have many biological activities in animal systems, its mechanism on bone metabolism is unclear.

Kimira and his team are trying to fill the gaps in the research on nomilin and how it affects osteoclasts. 'Although the effect of nomilin on the gene expression of osteoclasts and the effect on intracellular signalling can be seen, we have not been able to examine how nomilin exerts influence on cells from outside the cell,' Kimira explains. 'Since nomilin has been reported to be a ligand for TGR5, which is a bile acid receptor expressed in the intestinal tract, if it can be confirmed that TGR5 is expressed in osteoclasts, I believe the effect of nomilin can be extracellularly considered.' There are recommendations in place for the prevention of osteoporosis in Japan. For example, Japan's 2015 Guidelines for Prevention and Treatment of Osteoporosis state that in order to prevent the onset of the disease, it is recommended that people

under the age of 18 begin exercising, in addition to consuming the right nutrition, while those who are middle aged or elderly are advised to maintain a 'healthy' weight, follow nutritional guidelines, avoid smoking and perform daily exercise. However, despite these recommendations, osteoporosis is on the increase in Japan, as Kimira explains: 'The number of osteoporosis patients in Japan is 12.8 million (three million men and 9.8 million women). From an international perspective, incidence rates have begun to decline, such as in Europe, Canada and the US. However, in Japan, the number of osteoporosis patients is rising and it is considered very important to prevent disease onset.'

## MYSTERIOUS MECHANISMS

In their current project, the researchers are building on existing animal studies that have shown a positive correlation between intake of citrus fruits and bone health. Specifically, their aim is to reveal the mechanism of nomilin on osteoclastic differentiation of mouse primary bone marrow-derived macrophages (BMMs) and the mouse RAW 264.7 macrophage cell line into osteoclasts. This could shed light on the prevention of bone diseases. 'The most common cause of osteoporosis is the balance collapse of bone resorption and bone formation caused by the reduction of oestrogen by menopause. ►



## *Nomilin can demonstrate efficacy against the onset mechanism of osteoporosis by controlling the differentiation and activity of osteoclasts*

If osteoclast differentiation and activity can be kept normal, bone density is preserved,' explains Kimira. 'We believe that nomilin can demonstrate efficacy against the onset mechanism of osteoporosis by controlling the differentiation and activity of osteoclasts.'

To do this, the team is using a cell counting kit to measure the cell viability of RAW 264.7 and BMMs, with TRAP-positive multinucleated cells counted as osteoclast cell numbers. The number and area of resorption pits were measured as bone-resorbing activity. Osteoclast-specific gene expression was evaluated by quantitative real-time polymerase chain reaction (PCR); and protein expression was evaluated by western blot.

The team found that nomilin significantly decreased TRAP-positive multinucleated cell numbers compared with the control, and exhibited no cytotoxicity. In addition, nomilin decreased bone resorption activity, as well as down regulating osteoclast-specific genes, NFATc1 and TRAP mRNA levels. Furthermore, nomilin suppressed MAPK signalling pathways. This led the researchers to conclude that nomilin has inhibitory effects on osteoclastic differentiation *in vitro*. They believe the findings suggest that nomilin-containing herbal preparations could potentially be

utilised in the prevention of bone metabolic diseases.

### MAGIC PASTE

Kimira believes this can be done in the form of a special paste from yuzu, as he explains: 'I believe that by using yuzu paste containing high dose nomilin developed by us, we can introduce nomilin into our diets and reap the benefits.' Currently, large amounts of yuzu go to waste, as the rest of the fruit tends to be discarded once the juice has been obtained. Kimira and the team want to reduce this waste, using the fruit's peel and seeds. 'Because the use of yuzu is mostly squeezing fruit juice and grating a small amount of peel for the scent, about half the weight of the production is discarded. The fee for disposal is seriously damaging for yuzu farmers. We have created a paste named Tanemadeyuzuran that contains yuzu's fruit, peel and seed, using the fruit effectively.'

In the future, the researchers hope that the use of yuzu in food will broaden. In addition, they believe it would be beneficial to collaborate with a group studying the physiological effects of nomilin in areas other than bone metabolism to further develop their research. Ultimately, they are en route to helping improve population health and, in doing so, reducing the waste of a valuable fruit. ●

## Project Insights

### FUNDING

This work was supported by the Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Number JP16K16286.

### COLLABORATORS

Professor Hiroshi Mano, Professor Akiyo Matsumoto and Associate Professor Hyounju Kim – Josai University, Japan • Kenji Nishida – Liberty House

### CONTACT

**Yoshifumi Kimira**  
Project Coordinator

**T:** +81 492717208  
**E:** kimira@josai.ac.jp  
**W:** [www.josai.ac.jp](http://www.josai.ac.jp)

### PROJECT COORDINATOR BIO

**Yoshifumi Kimira** is an Assistant Professor at the Department of Clinical Dietetics and Human Nutrition in the Faculty of Pharmacy and Pharmaceutical Sciences at Josai University, Japan. His research focuses on how to prevent skeletal diseases by functional food factors contained in citrus fruits and to solve the problems of disposal in food production to reduce the burden of the farmers.

