

# Introducing the new super fruit

The citrus fruit 'yuzu' has been found by researchers to help improve bone health



Citrus fruits are beneficial too humans for a number of reasons. Alongside providing micronutrients such as vitamin C and folic acid, certain citrus fruit has been found to have connotations with bone health. For a few years now, researchers have started looking into the relationship between the citrus fruits oranges and grapefruits, and their relationship with bone health.

Studies have demonstrated that when rats have been supplemented with orange juice, their biomechanical properties have improved (including their overall strength and moderately restored femoral density). In accordance to this, it has also been shown that when rats were fed orange pulp, their vertebral bone and cortical thickness of long bones were improved.<sup>1</sup>

Assistant professor at the Josai University in Japan, Dr Yoshifumi Kimira, research focuses around the effects of collagen-derived peptides contained in citrus fruits on bone metabolism. One of Kimira's main interest involves the East Asian citrus fruit 'yuzu' (also commonly known as 'Citrus junos').

## What are your key research interests?

I am interested in studying the functionality of foods that have similar effects to drugs. The functional ingredients of food can be taken from food while having the effect similar to the medicine. I believe that by maximising this benefit we can propose a meal to lead to disease prevention. The research field is food science, and my key research interest is to elucidate the mechanism of collagen-derived peptides or citrus limonoids on bone metabolism.

## Can you explain your project 'Citrus limonoid nomilin inhibits osteoclastogenesis *in vitro* by suppression NFAT<sub>c1</sub> and MAPK signalling pathway'?

Limonoid has been reported to have antioxidant activity, anti-tumorigenic activity, hypoglycemic effect and improving hyper-lipedema. Although it has already been clarified that fruit intake brings about a good effect on bones, there have been some reports showing the possibility of having limonoids contained in fruit peel and seeds on bone. As a result, we also investigated whether citrus limonoid 'nomilin' have effects on bone metabolism. 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. 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. The cell viability of RAW 264.7 cells and BMMs was measured with the Cell Counting Kit. TRAP-positive multinucleated cells were counted as osteoclast cell numbers. The number and area of resorption pits were measured as bone-resorbing activity. Osteoclast-specific genes expression was evaluated by quantitative real-time PCR, and proteins expression was evaluated by western blot.

It was also discovered that nomilin significantly decreased TRAP-positive multinucleated cell numbers compared with the control and exhibited no cytotoxicity. Nomilin decreased bone resorption activity. Nomilin downregulated osteoclast-specific genes, NFATc1 and TRAP mRNA levels. Furthermore, nomilin suppressed MAPK signalling pathways. This study demonstrates clearly that nomilin has inhibitory effects on osteoclastic differentiation *in vitro*. These findings indicate that nomilin-containing herbal preparations have potential utility for the prevention of bone metabolic diseases.

### Why yuzu and nomilin? What is the importance of this research?

We focused on yuzu because it is mainly associated with being squeezed for its fruit juice. In addition to this, when you scrub a small amount of its peel (it is highly fragranced), the yuzu discards about half the weight of the production. As this fee for disposal is seriously damaging for yuzu farmers, we created a paste named 'Tanemadeyuzuran' containing yuzu's fruit, peel and seeds in consideration of effective use of yuzu. This type of research is particularly important because it is thought that the range of yuzu utilisation as food will widen.

### How is this project going so far? Would you say you are getting closer to halting the rise in the number of people suffering from osteoporosis both in Japan and internationally?

It has been confirmed that nomilin has an action on osteoclast differentiation and activity. However, the results of the animal tests have not been seen



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yet but considering that 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.

Our study considers the problems related to local foods to be solved by food processing approaches and food functional approaches. In this project I believe that it was a project that got the help of yuzu farmers and a very good outcome that we can achieve results that can be expected to be effective for people's health.

### What is next? Do you currently have plans for your next research project?

I think that it is in the first step of basic research. I know that there are many steps to be recognised as functional food, but I would like to make research that can contribute to people's health through the functionality of yuzu. As an approach

to the problem of agriculture, I think that we have made considerable progress.

The amount of wasted yuzu will be reduced, and yuzu farmers will be able to produce lots of fragrant yuzu energetically. Also, I would like to present research results to those who enjoy the citrus fruits as food, without necessarily knowing the health benefit.



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