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Original Article

Hylonome

Some vegetables (commonly consumed by humans) efficiently modulate bone metabolism

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Abstract

We have hypothesized that some vegetables which are part of the regular human diet may contain modulators of bone metabolism. To mimic a typical Western diet with large proportions of refined components, rats were pair-fed a semi-purified diet to which, in the treated animals, the dried material under investigation was added. Effects are expressed as % of untreated control. Bone parameters in rats were assessed in the proximal tibia by pQCT. Bone resorption (BR) was assessed by the urinary excretion of [^{3}H]-tetracycline from prelabeled rats. Daily administration of 1 g of onion during 4 weeks increased total bone mineral content by 17.4% ($p<0.05$), trabecular bone mineral density by 13.6% ($p<0.05$). One g of onion/day administered to male rats blunted BR by 23±5% ($p<0.05$). Daily administration of onion to ovariectomized rats inhibited BR in a dose-dependent manner. At the highest dose (1.5 g of onion) BR was inhibited by 26±4% ($p<0.01$) as compared to 24±3% ($p<0.001$) for estradiol (27µg/kg/day). An additional 13 vegetables displayed significant effects on BR at the dose of 1g/day. Interestingly, 1g/day of soy did not inhibit BR in this model. Also, skimmed milk, meat and egg (all 1 g/day) were ineffective. Thus, common vegetables consumed by humans potently modulate bone metabolism in the rat. This opens the possibility to develop the basis for a low-cost, safe and effective nutritional approach to osteoporosis.

Keywords: Bone Metabolism, Bone Resorption, Osteoporosis, Ovariectomy, Rats, pQCT, Onion

Introduction

Osteoporotic fractures, besides causing suffering to the patient, are a major burden to health care as the direct expenditure for osteoporosis and associated fractures is around US$ 14 billion/year in the USA and exceeds US$ 10 billion/year in Europe[1]; novel strategies to prevent osteoporosis are therefore required.

To date no nutritional approach has been shown to be able to prevent osteoporosis; even the effect of milk consumption on the relative risk of hip fractures seems to be restricted to the 10% of the female population with the lowest intake of calcium[2,3].

Manipulation of bone resorption by pharmacological doses of calcium plus vitamin D_3 may, however, decrease the fracture rate by some 30%[3]. Nutritional strategies aimed at preventing osteoporosis based on plant components with oestrogenic effect[4-6] as alternatives to oestrogen treatment have not yet been shown to be effective in humans and are hampered by the low potency of phytoestrogens, necessitating a fundamental modification of nutritional habits such as the daily consumption of large amounts of soy.

Clearly, a successful nutritional approach to prevent osteoporosis should be applicable to the whole population and should be characterised by effects beyond that of treatment with calcium or hormones. In this paper we describe a novel nutritional approach which could lead to such a strategy.

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Materials and Methods

Processing of foodstuffs

If not otherwise stated, the foodstuffs were purchased locally, carefully washed, minced, air-dried at about 50 °C and ground to a fine powder. Dried onion flakes and Italian parsley were purchased from Landolt & Hauser Inc., Näfels, Switzerland, the moisture removed by adsorption over silica gel before grinding. Garlic powder was purchased from McCormick Inc. Regensdorf, Switzerland. Heat inactivated white hylum soy-beans were purchased from Klingen-

talmühle, Kaiseraugst, Switzerland.

Seven vegetables were cooked (as customary for human nutrition) before drying. Thus, potatoes were baked for 45 minutes in an oven set to 225 °C and were peeled before drying. Carefully washed cut broccoli, Brussels sprouts and cauliflower were steamed until tender (about 10 minutes), frozen and freeze-dried. Chinese, white and red cabbage were cut, carefully washed, brought to the boil with a minimum amount of water and simmered until tender (about 15 minutes), frozen with the accumulated liquid and freeze-dried. Fresh eggs were whipped slightly, frozen and freeze-dried. Finally, skimmed milk (Rapilait) was purchased from a local retailer (Migros).

Diet

To mimic a typical Western diet with large proportions of refined components and to avoid any interference from natural components of a normal rat diet, rats were pair-fed a "semi-purified" diet with a similar composition to AIN 76 from the first day of treatment[7]. To avoid possible effects of the calcium and phosphate content of the vegetable additions to the rat diet, we used a modified diet with a high calcium (1.1%) and a high phosphate (1.2%) content, purchased from Klingentalmühle, Kaiseraugst, Switzerland (SoDi 2160). The diet was given as wet food - deionised water was added to the food powder to reach a wet but not soupy consistency. Demineralised water was given *ad libitum.*

In vivo treatments

If not otherwise stated, one g of powder of the foodstuff under investigation was added to the daily ration of wet food. The rats received the same total amount of food (24 g) including the additions. This way, the additions produced at the most a minor lowering of total calcium content, a procedure which does not affect bone[8].

Synthetic salmon calcitonin (Miacalcic®, Novartis Inc. Basel, Switzerland) was injected subcutaneously during the 10 days of the study. It was injected daily at doses of 1.25 IU/kg and 2.5 IU/kg body wt in a volume of 1 ml/kg body wt, three hours before food was given, a time which has been established earlier to be the optimal time of administration[9]. The calcitonin was diluted with a solvent consisting of 0.1 M sodium acetate containing 167 µl/ml heat-inactivated rat serum. According to the product information, the activity of calcitonin administered as nasal spray is about half that of the injected form[10]. Thus, the active dose of 200 IU/day of nasal calcitonin given to a postmenopausal osteoporotic woman corresponds to about 1.7 IU/kg body wt of the injected form, assuming a body weight of 60 kg[11].

Assessment of bone "mass"

The bone parameters were measured in the proximal metaphysis of the tibia by quantitative computed tomography (pQCT), as previously described[8,12].

In vivo monitoring of bone resorption

Rats were injected from birth for 6 weeks with [^{3}H]-Tetracycline ([^{3}H]-Tc). [^{3}H]-Tc is deposited into bone and is released when bone is resorbed. After discontinuation of labelling, the rats were housed in metabolic cages and bone resorption was monitored by measuring the daily urinary [^{3}H]-excretion[9,13-15]. After 10 days of baseline monitoring of bone resorption the dietary intervention was started.

Results

In rats which consumed onion (1 g/rat/day for 4 weeks), the total bone mineral content was increased by 17.7% ± 6.4% ($p<0.05$; n=6 each), mean cortical thickness by 14.8% ± 7.6% and trabecular bone mineral density by 13.5% ± 3.1% ($p<0.05$) relative to control. Consumption of onion therefore appears to increase bone mass.

Onion inhibited bone resorption assessed by the urinary excretion of [^{3}H]-Tetracycline from prelabelled rats, a model sensitive to all inhibitors of bone resorption used clinically[9,13-16].This model was therefore chosen to investigate the presence of inhibitory activity in foodstuffs.

Figure 1 shows that 14 vegetables consumed by humans as salads, herbs or cooked vegetables significantly inhibit bone resorption in the rat. Many of the active vegetables stem from the species of allium ("onion"; lanes 1-7), petroselinum ("parsley"; lanes 8+9) and brassica ("cabbage"; lanes 19, 27-29 and 31-33). The degree to which vegetables inhibit bone resorption is independent of their calcium content: vegetables significantly inhibiting bone resorption contain 0.65% ± 0.14% of calcium/g of dry matter (n=10/14), while those with no significant activity contain 0.43% ± 0.11% calcium (n= 8/9; ns)[17]. None of the foodstuffs from animal origin (lanes 20, 21, 25) inhibited bone resorption to any significant extent. The effect of milk powder was not significant, despite its 1.29% calcium content. Nine different lots of onion were used as positive controls over a period of eighteen months. Eight out of 9 lots of onion significantly inhibited bone resorption, the mean effect being about 20% inhibition. Thus, the effect of 1g of onion/day is reproducible - the magnitude of effect being slightly larger than that of calcitonin at doses comparable (per kg body weight) to those effective to treat postmenopausal osteoporosis[11].

The experiments described above were performed in male rats. As the incidence of osteoporosis in humans is much more frequent in women as bone resorption increases after the menopause, an animal model for this condition, the ovariectomized (OVX) rat, has been used in further studies[18]. OVX was performed 1-2 days prior to the dietary intervention. Onion powder was administered at doses of 30, 100, 300, 1000 and 1500 mg/rat/day for 10 days. OVX increased cumulative bone resorption compared with sham operated animals by 32% ± 3% $p<0.001$, whereas 27 µg/kg/day of 17β-estradiol decreased bone resorption by 24% ± 3% $p<0.001$. Onion inhibited bone resorption in a dose-dependent manner; at the highest dose resorption

decreased by 25% ± 4% $p<0.01$. Thus, onion inhibits resorption not only in intact male rats but also in females in which bone resorption is stimulated by oestrogen withdrawal.

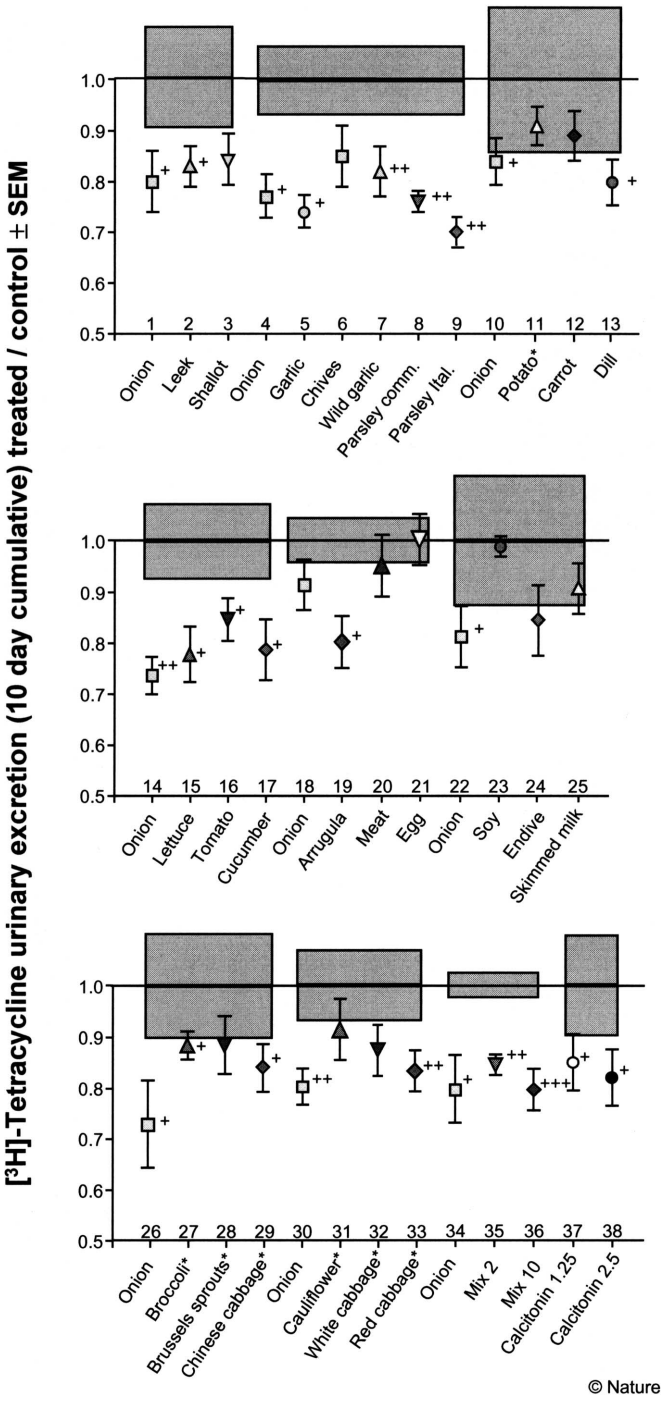

Figure 1. Effect of foodstuffs and calcitonin on bone resorption as assessed with the urinary excretion of previously administered radio-labelled tetracycline[9,13,15]. Data are plotted as the ratio treated / untreated control (n = 5 per group). The 95% confidence interval of the untreated control groups (n= 5-6, 10 experiments) is shown as the shadowed area. Onion was used as positive control for all foodstuffs. All rats received the same total daily amount of food including 1 g of the dried test foodstuff. * cooked before drying ; $^{+}$ $p< 0.05$, $^{++}$ $p< 0.01$ and $^{+++}$ $p< 0.001$.

This study clearly shows that many foodstuffs of vegetable origin consumed by humans inhibit bone resorption in the rat. As shown by the primary vegetable onion, the treatment increases bone mass by inhibiting bone resorption. Thus, bone resorption in humans may be reduced by the consumption of various vegetable foodstuffs. This implies, however, that the different active vegetables are not antagonising each other.

We have therefore mixed various vegetables and tested them at the standard one g dose. Both, a mixture of equal parts of onion and Italian parsley (Fig. 1; lane 35) as well as a mixture of 10 active vegetables each contributing with 100 mg to the daily dose, significantly inhibited bone resorption (Fig. 1; lane 36). Thus, the effects of lettuce, tomato, cucumber, arrugula, onion, garlic, wild (bear's) garlic, common parsley, Italian parsley and dill, all eaten by humans as salads with/or herbs appear to be additive.

Discussion

The above data clearly indicate that a variety of vegetables and herbs, but not soy (at the dose of 1 g/day), all common components of the human diet, can inhibit bone resorption in the rat[19]. The effect which is independent of their calcium content occurs in animals even when they are being fed a high calcium diet.

Should this effect also occur in man, inclusion of an appropriate amount of these vegetables and herbs in the human daily diet could be an effective and inexpensive way to decrease the incidence of osteoporosis.

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