Journal of Aging Research & Clinical Practice©
Volume 1, Number 2, 2012

EFFECT OF AGAVE FRUCTANS ON SELECTED PARAMETERS OF CALCIUM METABOLISM AND BONE CONDITION IN RATS

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Abstract: *Objective:* A prevalence of chronic diseases such as osteoporosis is observed because of the increasing life expectancy coming with a backlog of elderly (especially women). In this regard, functional foods containing fructans might play an outstanding role. The aim of the study was to assess the effects of agave fructans on selected parameters of calcium metabolism and bone condition in rats. *Design:* 28 days, 6 rats for each feeding group. *Setting:* University of Agriculture in Krakow, Poland. *Subjects:* 36 male Wistar rats, weighing about 120 g. *Intervention:* The current work studies the development of Wistar rats (experiment duration: 28 days) by Ca-modulated diet (Ca 100 – recommended doses, Ca 50 – 50% deficiency of doses, Ca 25 – 75% deficiency of doses) with/without agave fructan. The commercial product "Metlin" (Nekutli) - organic fructan from agave was used. After an adaptation period, the rats were distributed into six groups and kept in steel cages under controlled conditions. Finally, the rats were anaesthetized; blood and femora were sampled. *Measurements:* The levels of Ca2+, inorganic phosphorous, and the activity of alkaline phosphatase in rat blood serum were performed. BMC as well as BMD of femora were determined. Right femora were also scanned with peripheral quantitative computed tomography. The following parameters were determined: cortical thickness, periosteal and endoosteal circumferences as well as polar strength strain index. Statistical analysis was carried out. The Tukey's-test was used to determine differences between the experimental groups. *Results:* The level of calcium- and phosphate ions and the biochemical parameters (ICTP, ALP) in the blood are relatively constant with positive trend to higher availability of Ca2+ with agave fructan. The content of calcium in the femur increases clearly in the groups with agave fructan. Additionally the results of femora analysis show a higher stability and density of rat bone with agave fructan, particularly in the Ca 50 group. *Conclusion:* The results support the assumption that intake of agave fructan could provide the prevention of bone mass loss and bone weakening, what in turn could improve quality of life, by helping prevent osteoporosis.

Key words: Agave, fructans, rat femur, serum parameters.

Introduction

A prevalence of chronic diseases is observed in industrialized countries because of the increasing life expectancy coming with a backlog of elderly (especially women). In this respect osteoporosis - meaning increased bone fragility - has become a particular problem worldwide. Calcium is critical to achieve optimal peak bone mass and modulate the rate of bone loss associated with ageing (1, 2). Unfortunately, food consumption patterns clearly show that calcium intakes are inadequate in many countries (2). Strategies to improve the absorption of calcium are needed. In this regard, functional foods might play an outstanding role (3). Among them, fructans have attracted special attention (3-10). Studies carried out in animals and humans have demonstrated that fructans affect bone mineralization (11, 12). The positive effects in intestinal absorption, mineral retention, and biomechanical properties of the bones were observed (13-16).

Fructans are natural constituents of many common foods. Among plants being industrially important due to their fructans content, chicory and Jerusalem artichoke are the most studied. Recent data reported that Agaves, abundant in Mexico, contain significant amounts of fructans. Particularly the pine of Agave tequilana Weber var. azul, called blue agave, is rich in fructans, holding interesting nutritional properties (17). This paper will show the effect of agave fructans on selected parameters of calcium metabolism and bone condition in rats.

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Received January 2, 2012
Accepted for publication January 10, 2012

Material and methods

Agave fructan characteristics (Metlin, Nekutli)

The commercial product "Metlin" (Nekutli) is organic fructan from blue agave. The agave plants are cultivated under controlled conditions in the highlands of Jalisco state (Mexico) and are harvested before flowering (after 5 to 7 years of growth) for processing. This fructan is composed of β(2-1) and β(2-6) linked fructopyranosid units, forming a branched molecule with a degree of polymerization up to 60.

The chemical composition of Metlin (Product Data Sheet from Nekutli) is: moisture 0.0-5.5%, total carbohydrates 98.0-99.5%, fructan 90-99.5%, fructose-glucose-sucrose 0.0-5.0%, ash <0.2%, fat 0.0%, cholesterol 0.0% of dry matter.

Animals and experimental conditions

The feeding experiment was performed on 36 male Wistar rats, weighing about 120 g. After an adaptation period (7 days), the rats were distributed into six groups and kept one per wire-bottomed steel cage under controlled conditions (20-23°C environmental temperature and a 12h /12h light/dark cycle). Animals had free access to deionised water and were fed with AIN'93 G according to Reeves (18) or the same diet containing 75 g of agave fructans. The feeding groups had various amounts of calcium in mineral mix, where Ca deficiency in mix was supplemented with starch (Table 1). Agave fructans were introduced into diets instead of starch. The groups were: "Ca 100" – 100% daily dose of calcium; "Ca 100+F" – 100% Ca, and fructans addition; "Ca 50" – 50% Ca; "Ca 50+F group" – 50% Ca, and fructans addition; "Ca 25 group" – 25% Ca, "Ca 25+F group" – 25% Ca, with fructans in rat diet.

Body weight of rats was monitored weekly. Body mass gain was calculated as the difference between the final and initial weights. Finally, the rats were anaesthetized; blood and femora were isolated, cleaned of soft tissues and frozen for further analysis.

The procedures of animal experiment in this study were permitted by Local Ethics Commission in Krakow, Poland.

Determination of blood serum parameters in rats

The levels of calcium, inorganic phosphorous, and the activity of alkaline phosphatase (AlP), were performed using Roche Cobas Integra 400 chemistry analyzer (Roche Diagnostics).

The calcium content in serum was measured colorimetrically according to the method of Schwarzenbach (19), and total protein on the basis of the method of Weichselbaum (20), at wavelenght 552 nm. The calculation of free Ca^{2+} ions was as follows: [(protein (every g above 72 g/l) x 0.036) + total Ca in serum]/2.

Table 1
The composition of experimental diets (g/kg diet)

Ingredient	Groups	
	Control	+ fructans
Cornstarch	532.486	453.126
Casein	200	200
Sucrose	100	100
Soybean oil	70	70
Fiber	50	50
Mineral mix*	35	35
Vitamin mix	10	10
Choline	2.5	2.5
Tert-butylhydroquinone	0.014	0.014
Fructans	0	75.00

* The content of mineral mix (g/kg mix): calcium carbonate – 375.00, (groups Ca100 and Ca 100+F), 187.50 (groups Ca 50 and Ca 50+F), 93.75 (groups Ca 25 and Ca 25+F); starch – 0.00 (groups Ca 100 and Ca 100+F), 187.50 (groups Ca 50 and Ca 50+F), 281.25 (groups Ca 25 and Ca 25+F); potassium phosphate–196.00, potassium citrate–70.78; sodium chloride–74.00; potassium sulfate–46.60; magnesium oxide–24.00; ferric citrate– 6.06; zinc carbonate–1.65; manganese carbonate–0.63; cupric carbonate–0.30; potassium iodate–0.01; sodium selenate–0.01; ammonium molybdate–0.008; powdered sucrose– 204.952.

Inorganic phosphorus level in blood was determined using a method with ammonium molybdate, at wavelength 340 nm (21).

The activity of alkaline phosphatase was measured using IFCC method, with 1,2-amino-2-methyl-1-propanol buffer, at wavelength 409 nm (22).

The cross-linked carboxyterminal telopeptide of type I collagen (ICTP) (23) was measured using commercially available ICTP-RIA according to manufacturer's (Orion Diagnostica) instructions.

Ca content in femur (AAS)

Standard solution of calcium (10000 mgL-1) was obtained from Fluka. Nitric acid (65% w/v) Suprapur reagent was purchased from Merck KGaA.

0.5 g of sample was digested with 10 ml of nitric acid (65% w/v) in a microwave digestion system (MARS Xpress, CEM). Next, samples were analyzed by the use of Varian AA240FS spectrometer at wavelength 422.7 nm.

Densitometric analysis (DEXA)

Bone mineral content (BMC) as well as bone mineral density (BMD) of isolated right femora were determined using a Norland Excell Plus Densitometer (Fort Atkinson WI, USA) equipped with Small Subject Scan software. The machine was calibrated daily with the use of the

quality assurance phantom (QA-Phantom) provided by the manufacturer.

Peripheral quantitative computed tomography (pQCT)

Right femora were scanned with peripheral quantitative computed tomography XCT Research SA Plus system with software version 5.5 D (Stratec Medizintechnik GmbH, Pforzheim, Germany). The femoral metaphysis was scanned 5 mm from distal end. The scan line was adjusted using scout view of the pQCT system. During measurement the bones were fixed in a test tube filled with 70% ethanol. Upon completion of scanning, the following parameters were determined: cortical thickness (CRT THK), periosteal (PERI C) and endoosteal (ENDO C) circumferences as well as polar strength strain index (polar SSI).

Analyses of cortical bone were performed with the threshold of 0.900cm-1 and cortical mode 2. The initial scan was performed with the speed 20 mm/s, and CT-scan 4 mm/s. Daily calibration of the system using hydroxyapatite-containing quality assurance phantom precedes the measurements.

Statistical analyses

All values were expressed as mean ± SD. Statistical analysis was carried out considering a 5% significance level and a 95% confidence interval. Analysis of variance (Statistica software) was used as statistical model. The Tukey's-test was used to determine statistically significant differences in variables between the experimental groups.

Results

The mean rat weight, taking into account all groups, increased from 129 ± 13.1 g at the beginning to 262 ± 14.5 g at the end of the experiment (Table 2).

The concentrations of calcium ions in the animals' blood serum did not differ significantly in dependence on the level of this element in diet (Table 2). However, there was a tendency to increase this value after the consumption of agave fructan. Despite the lack of significance of differences between feeding group, a tendency to decrease the inorganic phosphorus concentration was also observed (Table 2). The standard deviation of the results for alkaline phosphatase is in an extremely broad range allowing no differentiation of the main value. There were statistically significant changes in ICTP level in rat serum (Table 2). The values of this marker in the animals fed with diet containing 25% of recommended calcium dose were significantly lower (2.32 mmol/l) than in the other groups. Moreover, there was a tendency to increase it after adding fructans, at all three studied calcium levels.

In the groups Ca 100, Ca 50 and Ca 25 the calcium content in femur was decreased from 130 to 122mg/g; in the groups Ca100+F, Ca50 + F and Ca25+F from 135 to 130 mg/g (Table 3).

Table 2

Body mass gain and selected biochemical blood parameters in controls (100, 50 and 25% recommended Ca dose) and agave fructans-fed rats[1]

Parameter	Ca 100	Ca 100+F	Ca 50	Ca 50+F	Ca 25	Ca 25+F
Body mass gain (g)	133.0± 23.8 a	129.0± 12.5 a	136.0± 8.2 a	130.7± 12.2 a	134.2± 19.7 a	136.3± 14.8 a
ALP (U/l)	257.9 ± 51.9 a	249.9 ± 22.6 a	268.9 ± 114.1 a	214.6 ± 51.1 a	239.9 ± 63.8 a	257.8 ± 53.2 a
ICTP (ug/l)	3.40 ± 0.23 a	3.45 ± 0.65 a	2.60 ± 0.12 ab	2.92 ± 0.71 ab	2.32 ± 0.22 b	2.65 ± 0.40 ab
Ca^{2+} (mmol/l)	1.49 ± 0.05 a	1.52 ± 0.09 a	1.53 ± 0.05 a	1.51 ± 0.04 a	1.47 ± 0.04 a	1.55 ± 0.04 a
P (mmol/l)	3.61 ± 0.16 a	3.45 ± 0.40 a	3.60 ± 0.05 a	3.54 ± 0.15 a	3.63 ± 0.34 a	3.50 ± 0.16 a

1. Results are expressed as mean ± SD; results shown correspond to the end of the experiment, different letters mean statistical differences ($p < 0.05$)

Table 3

Selected parameters connected with bone in controls (100, 50 and 25% recommended Ca dose) and agave fructans-fed rats[1]

Parameter	Ca 100	Ca 100+F	Ca 50	Ca 50+F	Ca 25	Ca 25+F
Ca in femur (mg/g)	130.5 ± 4.7ab	134.7 ± 4.9a	127.1 ± 6. b	130.4 ± 7. ab	122.2 ± 7.3ab	130.1 ± 4.8ab
ENDO C (mm)	6.96 ± 0.29a	7.02 ± 0.48a	6.86 ± 0.46a	6.70 ± 0.39a	7.15 ± 0.46a	6.99 ± 0.63a
PERI C (mm)	10.38 ± 0.31a	10.29 ± 0.29a	10.08 ± 0.28a	10.13 ± 0.26a	10.23 ± 0.44a	10.17 ± 0.40a
CRT THK (mm)	0.54 ± 0.03a	0.52 ± 0.04a	0.51 ± 0.04a	0.55 ± 0.05a	0.49 ± 0.03a	0.51 ± 0.05a
polar SSI (mm3)	5.14 ± 0.40a	4.87 ± 0.32ab	4.62 ± 0.28b	4.84 ± 0.45ab	4.52 ± 0.52bc	4.44 ± 0.29c

1. Results are expressed as mean ± SD; results shown correspond to the end of the experiment, different letters mean statistical differences ($p < 0.05$)

Bone mineral content and bone mineral density, decreased continuously in the groups from Ca 100 to Ca 25 without fructans (Figure 1, Figure 2). There were statistically important changes after adding these compounds to rat diet, but only at the level of 50% recommended Ca dose.

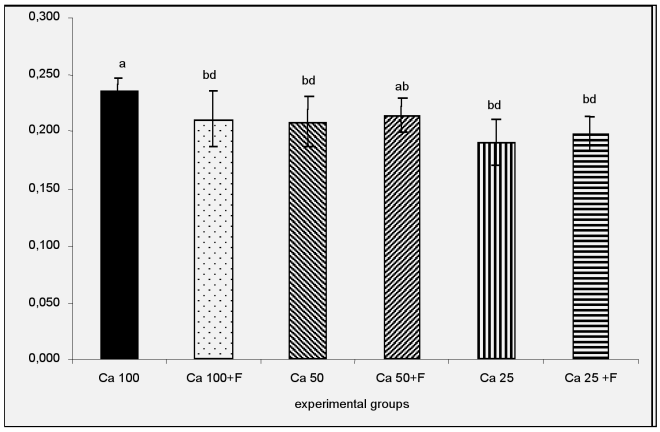

Figure 1. Bone mineral content (BMC)[1] in rat groups over the study. 1. Bars (treatments) with different letters are significantly different ($p<0.05$)

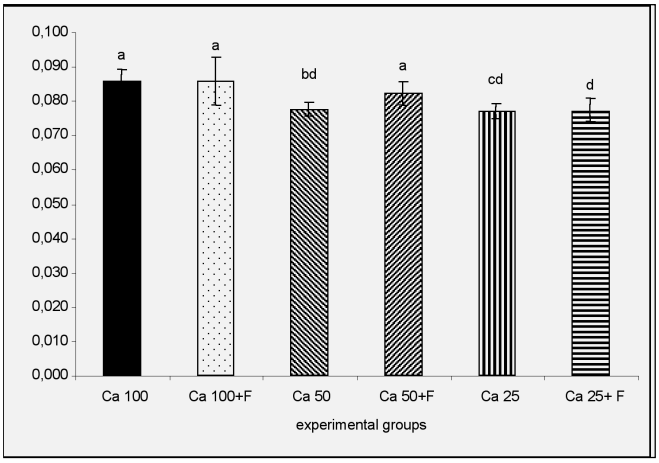

Figure 2. Bone mineral density (BMD)[1] in rat groups over the study. 1. Bars (treatments) with different letters are significantly different ($p<0.05$)

The results obtained for the PERI C and ENDO C, as well as cortical thickness of rat femur did not show statistical changes (Table 3). However, it was a tendency to increase CRT THK after adding fructans, but only in the condition of calcium dietary deficiency.

The analysis of polar strength strain index of animal femur showed significant reduction in the value of this parameter with lowering of dietary calcium level (Table 3) - it was 5.14 mm^3 in Ca 100 group, and 4.52 mm^3 in Ca 25 group. Animals from Ca 50 group which consumed diet with fructans had higher polar SSI results (4.84 mm^3) than these from control group (4.62 mm^3).

Discussion

Body mass and blood biochemical parameters

There were no significant differences among groups for body mass gain. Similarly, other authors did not find effects of body mass gain in rats depending on Ca level (24) or after adding chicory inulin or FOS (25) into animal diet.

Calcium is one of the essential ions for the organism. Younes et al. (26) observed that the ingestion of different carbohydrates, was without effect on plasma calcium concentration in rats. In our experiment similar results were observed for Ca2+ in the serum, independent from dietary calcium levels. A reason for this could be the homeostasis for calcium content in the blood; only at strong Ca deficiency in the diet, fructan supplement supported the stabilization of Ca level.

Biochemical markers for bone turnover are substances that reflect bone formation or resorption (27). The values of ICTP, marker of bone resorption, showed decrease in Ca-deficient groups compared to control animals and no effects of fructan enriched diet.

Femora assays

An increase of Ca content in the bone was observed with fructan, 5% in the Ca 100 group, 3% in the Ca 50 group and 8% in the Ca25 group (Table 3). That could possibly mean that fructan intake stabilized the Ca level in the femur. The obtained results coincide with those of Scholz-Ahrens et al. (16) who showed that FOS increased mineral content of the femur and Kruger et al. (2003) (11) who showed that high polymer inulin (DP > 23) augmented the calcium intake. Moreover, Lobo et al. (28) reported higher mineral absorption and bioavailability (Ca, Zn) after feeding rats with a combination of inulin and fish oil as part of diet.

The mechanical efficiency of the bone structure is governed by the directional modulation of bone modeling and remodeling (29). Experimental data do already support the hypothesis that the beneficial effects of fructan target not only the mineral absorption phase but also other aspects of bone health. However, it requires information concerning an improved Ca absorption regarding bone mineralization, density and structure (6). Diverse studies of animal models have shown the beneficial effect of fructans on the colonic epithelium as well as on calcium absorption and bone mass (11, 15-16, 30-31).

Fructan in the groups seemed to stabilize bone mineral content and bone mineral density, only in the group Ca50 and Ca50 + F a significant augmentation was observed (Figure 1, Figure 2). This assumption will be supported by Roberfroid et al. (32), who reported a significant

increase of BMC and BMD dependent from supplements of inulin in a diet with different Ca doses.

Different intensity of formation and resorption processes in femur – dependent from different calcium level in the diet - possibly causes a change of geometric parameters. PERI C value in every groups with/without fructan was similar, however a slight decrease from Ca100 to Ca50 as well as from Ca100 + F to Ca50 + F was notable. A similar effect was found in the ENDO C analysis (Table 3).

Cortical thickness, defined as the distance between outer and inner edge of the cortical shell, showed relatively constant values at different Ca levels with and without fructan. Only the group Ca 50 + F compared to Ca50 show slight increase of this parameter (Table 3). This fact allows the assumption that in this case possibly more bone tissue was produced. This was divergent to the report of Scholz-Ahrens et al. (16), who observed the highest cortical thickness in the presence of FOS, and at raised calcium level.

Regarding the mechanical properties, polar strength strain index (SSI) had the same tendency as the forgoing parameters (Table 3). It was higher in Ca 50+F group than in Ca 50 group, so the bones tended to be harder in rats consuming fructans with 50% of recommended calcium dose, and their predicted resistance to the rotation (the most often force in nature) was increased compared to the other groups with dietary calcium level 100 and 25 with and without fructan. Similarly, Lobo et al. (15) observed a strong trend toward higher values of studied biomechanical parameters in the Ca 50 + F group compared to the control.

Generally the better availability of calcium in blood and bone of rats will be supported by continuous intake of fructan with the diet. The background of this statement is the fact that fructan, in particular Agave fructan with high DP and branched structure serves as fermentative substrate for bacterial growth in the colon (cit from 33). Chain length of fructans is an important factor in determining physiological effect, because differences in mineral absorption, fermentability, and transit time have been influenced by this parameter (33-35). The rate and proportions of SCFAs produced is also influenced by chain length (33). This opens new alternatives for agave fructans as food health-promoting ingredients (36), especially in period with high demand or insufficient calcium intake with diet (25).

Conclusion

The present study provides insight into the effect of agave fructans on calcium level and availability in blood and bone of rats. The level of calcium- and phosphate ions and the biochemical parameters in the blood were relatively constant with positive trend to higher availability of Ca^{2+} in the groups with agave fructans. The content of calcium in the femur was found to be clearly higher in all groups with fructans containing diet compared to the groups with different Ca level without fructans; similar trend showed the phosphorous content. Femora analysis delivered a higher stability and density of rat bone in case of agave fructans supplemented diet, significantly in the Ca 50 group. These results could possibly indicate an important role of agave fructans in the maintenance of bone health and avoidance of osteoporosis. For clearer response of the fructan effects on the Ca levels and their parameters in blood and bone more detailed studies and long term investigations will be of advantage.

The studies were sponsored by Nekutli SA de CV company.

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