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Short-term Intake of Calcium Fructoborate Improves WOMAC and McGill Scores and Beneficially Modulates Biomarkers Associated with Knee Osteoarthritis: A Pilot Clinical Double-blinded Placebo-controlled Study

Tania Reyes-Izquierdo[1], Boris Nemzer[2*], Ana Elizabeth Gonzalez [3], Qing Zhou[1], Ruby Argumedo[1], Cynthia Shu[1], and Zb Pietrzkowski[1]

[1]Applied BioClinical, Inc., Irvine, CA, USA
[2]VDF FutureCeuticals, Inc, Momence, IL, USA,
[3]NutraClinical Inc., San Diego, CA, USA
***Corresponding Author**
Boris Nemzer
Director of Research and Development
FutureCeuticals, Inc.
Momence, IL
USA
Email: bnemzer@vandrunen.com

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Abstract

Knee osteoarthritis (OA) is a common degenerative joint disease which contributes significantly to the burden of physical disability. Conventional management of OA mainly focuses on relief of symptoms using analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). Some dietary supplements have shown some potency to reduce symptoms associated with OA conditions. Calcium Fructoborate (commercially marketed under the trade name FruiteX-B®), is a naturally occurring borate complex as first described by Patrick Brown [1]. For this study, FruiteX-B® was characterized by NMR and data from solid and liquid-state ^{11}B NMR have been obtained and compared against boric acid and fructose for quality purposes. In this study, FruiteX-B® was tested for fourteen days at a serving of 108 mg twice a day on subjects diagnosed with minor osteoarthritis conditions of the knees by CT scan. FruiteX-B® was well tolerated by all study subjects with no reports of adverse effect and resulted in several positive outcomes. On Day 14, WOMAC and McGill indexes were reduced by an average 29% and 14% respectively over Day 1 pre-ingestion values. Blood level of C-Reactive Protein (CRP) in 7 out of 10 subjects was found reduced up to 37% compared to Day 1 baseline levels. Interestingly, the study also showed that blood level of endogenous 1, 25(OH) vitamin D was increased more than 19% compared to baseline. However, differences in 25(OH) vitamin D were not observed. These results indicate that FruiteX-B® at a serving as low as 108mg twice a day provides significant benefits to people experiencing conditions associated with knee osteoarthritis. On the basis of these results, a larger clinical efficacy study is highly justified.

Keywords: Calcium Fructoborate (FruiteX-B®), knee osteoarthritis, WOMAC Index, McGill Index, C-Reactive Protein, vitamin D.

1. Introduction

Osteoarthritis (OA) is a chronic and degenerative joint disease. It is the leading cause of physical disability in the elderly, with a prevalence of 10-30 percent in persons over age 65 [2]. Currently OA affects nearly 27 million individuals in the United States alone. Knee OA is the most common form of OA with an estimated 12.1% of adults in the United States suffering from associated pain and functional limitations [3]. Current OA treatment regimens primarily utilize analgesics and NSAIDs to relieve the symptoms associated with OA. Over the last several years, however, novel agents and nutritional supplements have been intensively investigated as alternative approaches to OA-related symptoms and conditions [4].

Several lines of evidence suggest that various nutritional supplements can improve OA conditions, including antioxidant vitamins (vitamin C and E), non-antioxidant vitamins (vitamin D and B), glucosamine, chondroitin sulfates, trace elements (boron, selenium, zinc and copper), avocado-soybean unsaponifiables and fish oil [2]. More recently, nutraceuticals have been considered as an alternative to stimulate production of needed components of articular cartilage, by slowing down the damage in people with OA [5].

Calcium Fructoborate, commercially marketed under the trade name FruiteX-B® (FrxB), is a plant-based mineral complex prepared according to US patent #5,962,049, and produced exclusively by VDF FutureCeuticals Inc. (Momence, IL.) FrxB contains approximately 2.7% boron, 92.3% fructose and 5% calcium per weight. Previous pilot studies indicated that FrxB increased bone density in vitamin D-deficient mice (unpublished data) and may increase blood levels of 1,25(OH)dihydroxy-vitamin D in humans (data unpublished).

The present study was performed to verify whether FrxB is capable of increasing 1, 25(OH) dihydroxy-vitamin D after two weeks, taking a 108 mg dose twice a day. In this study, 20 volunteers were selected and divided into two groups, one for placebo and one for FrxB. Blood was drawn before treatment and 7 and 14 days after FrxB supplementation was initiated.

2. Material and Methods

FruiteX-B® Calcium Fructoborate was provided by Futureceuticals, Momence, IL, USA, produced according to Miljkovic (US Patent #5,962,049)

2.1 Chemistry analyses

^{11}B NMR Analyses of FrxB. ^{11}B liquid and solid-state nuclear magnetic resonance (NMR) experiments for FrxB characterization were performed for this study. Liquid-state analyses were performed on a Varian Unity-300 spectrometer (Varian USA, Palo Alto, CA) equipped with a Varian 5 mm broadband probe. ^{11}B experiments were performed at a resonance frequency of 96.23 MHz with a 50 kHz spectral width, an 82 ms acquisition time and a relaxation delay of 0.25s. 1024 pulses were signal averaged for each ^{11}B spectrum. All samples were dissolved in D_2O. Solid-State NMR experiments were performed on a Varian UnityPlus-200 spectrometer (Varian USA, Palo Alto, CA) equipped with a Doty Scientific 7mm Supersonic CP-MAS probe. The ^{11}B NMR was acquired at 64.168 MHz with a 100 kHz spectral width, a 10 ms acquisition time, a selective 1.5 µs pulse width, a 0.5 sec relaxation delay. 320 pulses were accumulated. For all solid-state NMR analyses the magic angle spinning speed was approximately 6 kHz.

2.2 Clinical study

Inclusion and exclusion criteria. The pilot clinical study protocol (ABC-NCI-10-03-FrxB/OA) was approved by the Institutional Review Board at Vita Clinical SA, Avenida

Circunvalacion Norte #135, Guadalajara, JAL, Mexico 44270. Twenty subjects were recruited for this study. Subjects were confirmed as having knee OA by physician using CT scan. All study subjects were not using any type of medication or supplements for a period of 15 days prior to the start of the study. The criteria of selection included their age (>44 and <65 years) with a BMI >23 and <35 kg/m² (overweight and moderately obese), with a mean BMI of 28.7 (SD 3.131). Subjects were free of rhinitis, influenza and other upper respiratory tract infections, were non-diabetic and generally free of allergies to dietary products. Also, subjects using anti-inflammatory, anti-arthritis, and/or pain medications such as NSAIDS within 15 days prior to the study, statin or anti-diabetic drugs were excluded as were subjects taking vitamin D, testosterone or any steroids within 30 days prior to the study. Subjects were fasted for 12h prior to the initial blood draw. After participants gave written consent, groups of 10 subjects (each containing four males and six females per group) were randomly selected from the pool to receive: a.) one encapsulated dose of placebo (fructose in amount equivalent to 108mg of Calcium-fructo-borate, FrxB) (Group 1); or, b.) 108mg of encapsulated FrxB (group 2). Body temperature and blood samples were taken prior to and after treatment. Blood samples were always collected during fasting conditions.

2.3 Blood collection

Before FrxB or placebo was orally ingested, blood was collected at "time zero" (T0). For each participant, two 9 mL blood samples were drawn from an antecubital vein in anticoagulant-free (dry tubes). Following time zero (T0), blood was drawn at Day 7 (D7) and Day 14 (D14) of the treatment and always in fasting conditions. Throughout the protocol time course, volunteers were advised to adhere to their regular routine and habits. Immediately after collection, blood samples were allowed to clot and serum was collected. Serum samples were collected upon clot formation after centrifugation. Serum was aliquoted, snap frozen and kept at -70°C until use.

2.4 Evaluation of symptoms of OA before and after FrxB

The WOMAC and McGill questionnaires were used to describe and rate the symptoms of OA by NCI, Inc. at Days 1, 7 and 14. The WOMAC (Western Ontario and McMaster Universities) index is used to assess patients with osteoarthritis of the hip or knee using 24 parameters. WOMAC can be used to monitor the course of the disease or to determine the effectiveness of anti-rheumatic medications. The score ranges from "none" (Value=0) to "extreme" (Value=4) and includes questions such as: "walking", "sitting" and "standing". From these 24 parameters, five are for pain and nineteen are for stiffness. The scores add up to 96 points and the final score is calculated based on the addition of the numbers divided by the amount of answers [6].

The McGill Pain Questionnaire can be used to evaluate a person experiencing significant pain. McGill can be used to monitor the pain over time and to determine the effectiveness of any intervention. It was developed at by Dr. Melzack at McGill University in Montreal Canada and has been translated into several languages [7].

2.5 Blood chemistry

Blood chemistry tests were performed on serum samples collected from each subject at Day 1, Day 7 and Day 14 to monitor changes during the trial. The tests included serum glucose, blood urea nitrogen, creatine, asparate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGTP), total bilirubin, alkaline phosphatase, total proteins, albumin, globulin, uric acid, calcium, phosphorus, iron, sodium, potassium, chlorine, CO_2, triglyceride, total cholesterol, HDL and LDL.

2.6 C-reactive protein (CRP)

Serum CRP was performed by NCI Inc. using the ultrasensitive latex turbidimetry method (Spinreact®) (Sant Esteve De Bas, Spain) following the instructions from the manufacturer.

2.7 Enzyme immunoassay (EIA) of 25-Hydroxy vitamin D and 1, 25-Dihydroxy vitamin D

The human sera were stored at -20°C and were thawed on ice when the assay was ready to be performed. 25-Hydroxy vitamin D and 1, 25-Dihydroxy vitamin D in human serum were measured using EIA kits for the detection of human 25-Hydroxy vitamin D (both D_2 and D_3) and 1, 25-Dihydroxy vitamin D from Immunodiagnostic Systems (Immunodiagnostic Systems, Fountain Hills, AZ, USA.. The assays were completed according to the manufacturer's instructions.

Detection sensitivity for 25-Hydroxy vitamin D is 5nmol/L (2ng/ml) from a 25µl serum sample size. The working range of the EIA kits used for these assays is 6-360nmol/L (2.4-144ng/ml). Detection sensitivity for 1, 25-Dihydroxy vitamin D is 6pmol/L (2.5pg/ml) and the assay range is about 4-500pmol/L. Percentage changes of post ingestion at D7 and D14 versus D1 values were assessed using student paired t-test. In comparison to 1, 25-Dihydroxy vitamin D, one highest and one lowest percentage change numbers from each group (D7 and D14) were considered as outliers and excluded from the analysis.

3. Results and Discussion

3.1 Chemistry study

FruiteX-B® calcium fructoborate is a nature-identical borate complex in the food-form naturally found in fruits, vegetables, nuts, and legumes [8]. The chemistry of FrxB is revealed here in order to show the distinct characteristics of this plant-based mineral complex.

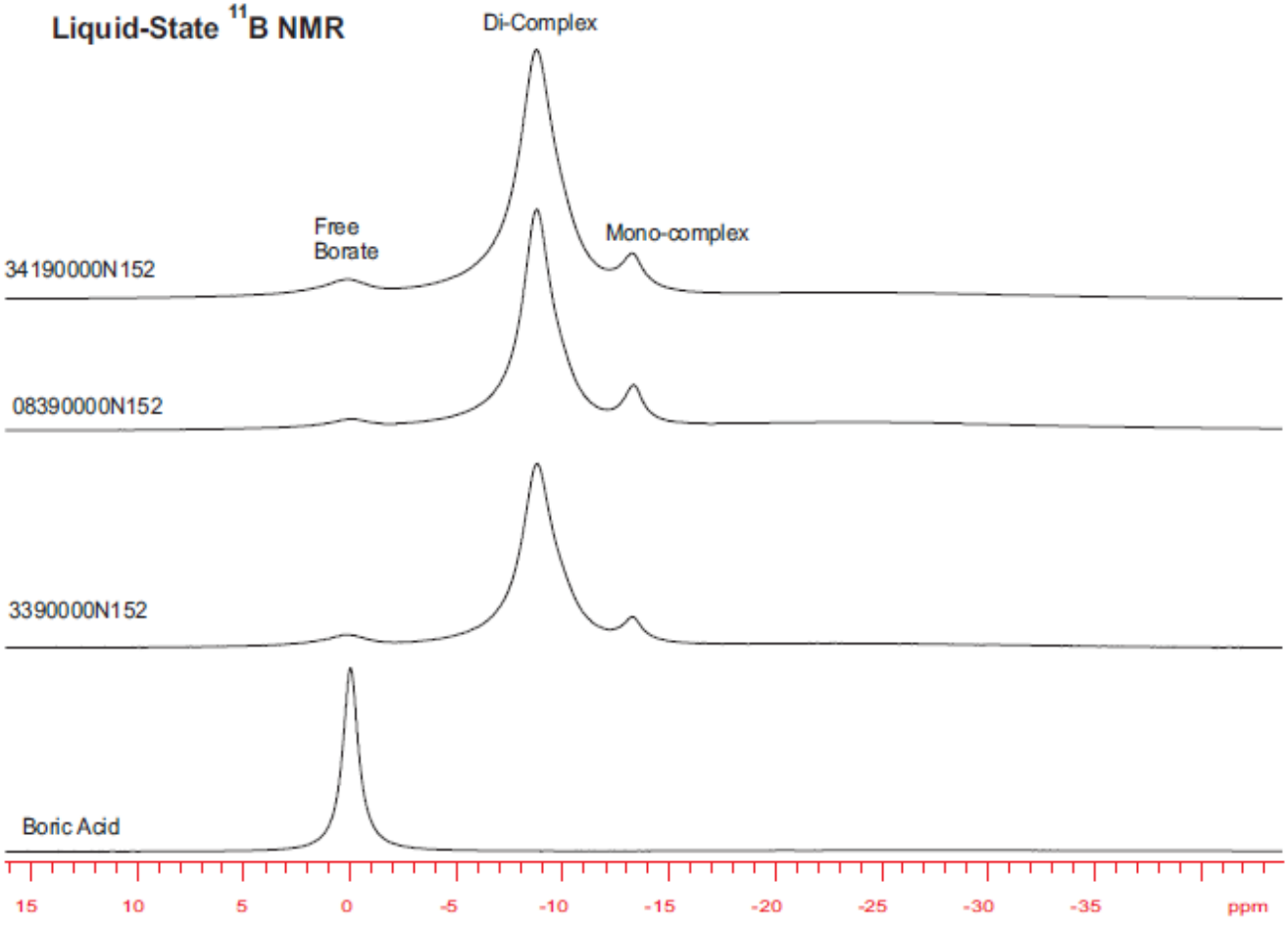

Figure 1. Liquid-state ^{11}B NMR of Boric Acid and three commercially-produced batches of FrxB

Liquid-state ^{11}B NMR data (Fig 1) was obtained for the FrxB samples and for boric acid. In solution, it is assumed that tetrahedral borate is the complexing agent. The symmetrical nature of the borate-complexing ion is shown by the narrow resonances observed in the solid-state NMR. The liquid-state NMR shows three resonances that can be assigned to 1) free borate in solution, at 0 ppm, 2) di-complex (borate complexed to two fructose molecules), at -9 ppm, and 3) mono-complex (borate complexed to one fructose molecule), at -13 ppm.

Figure 2 below shows the spectra of FrxB, the spectra of FrxB spiked with additional amount of fructose, and the spectra of FrxB spiked with additional amount of boric acid. The addition of fructose exerts an effect on the distribution of complex types. Specifically, there appears to be an increase in the number of di-complex boron signals compared to decreasing signals of the mono-complex.

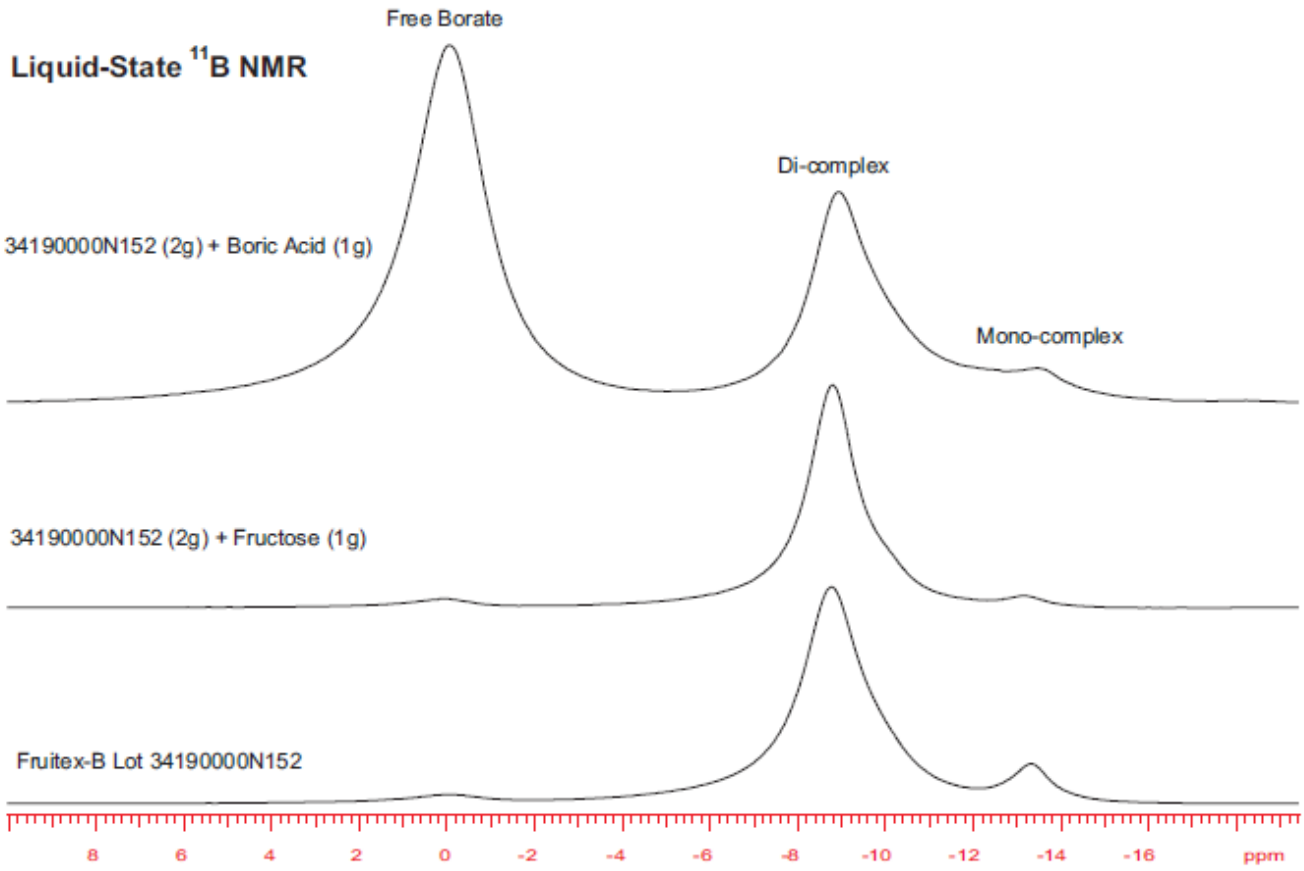

Figure 2. Liquid-state ^{11}B NMR spectra of FrxB and two mixtures of FrxB with fructose and with boric acid, respectively

Free borate clearly decreases due to the formation of a complex with the newly added fructose. The amount of mono-complex also decreases, indicating that some mono-complexes will form the di-complex when additional fructose becomes available in the system. Addition of boric acid to FrxB in solution produces an excess of free borate and a slight increase in the mono-complex over the fructose-supplemented sample.

Solid-State ^{11}B NMR experiments (Figures 3) were also performed on the boron in the sample. Figure 3 shows the ^{11}B spectrum obtained on a sample spinning at 6 kHz at the magic angle. Spinning the solid sample at a special orientation ($54^{o}44'$) with respect to the applied magnetic field from the NMR magnet allows narrowing of the broad solid-state NMR line shapes so that better resolution of chemical species can be obtained.

Figure 4 shows the solid-state ^{11}B with the sample sitting static in the NMR probe. ^{11}B is a quadrupolar nucleus, which is characterized by the manifestation of an electric field gradient across the atomic nucleus due to the fact that the numbers of neutrons and protons are paired.

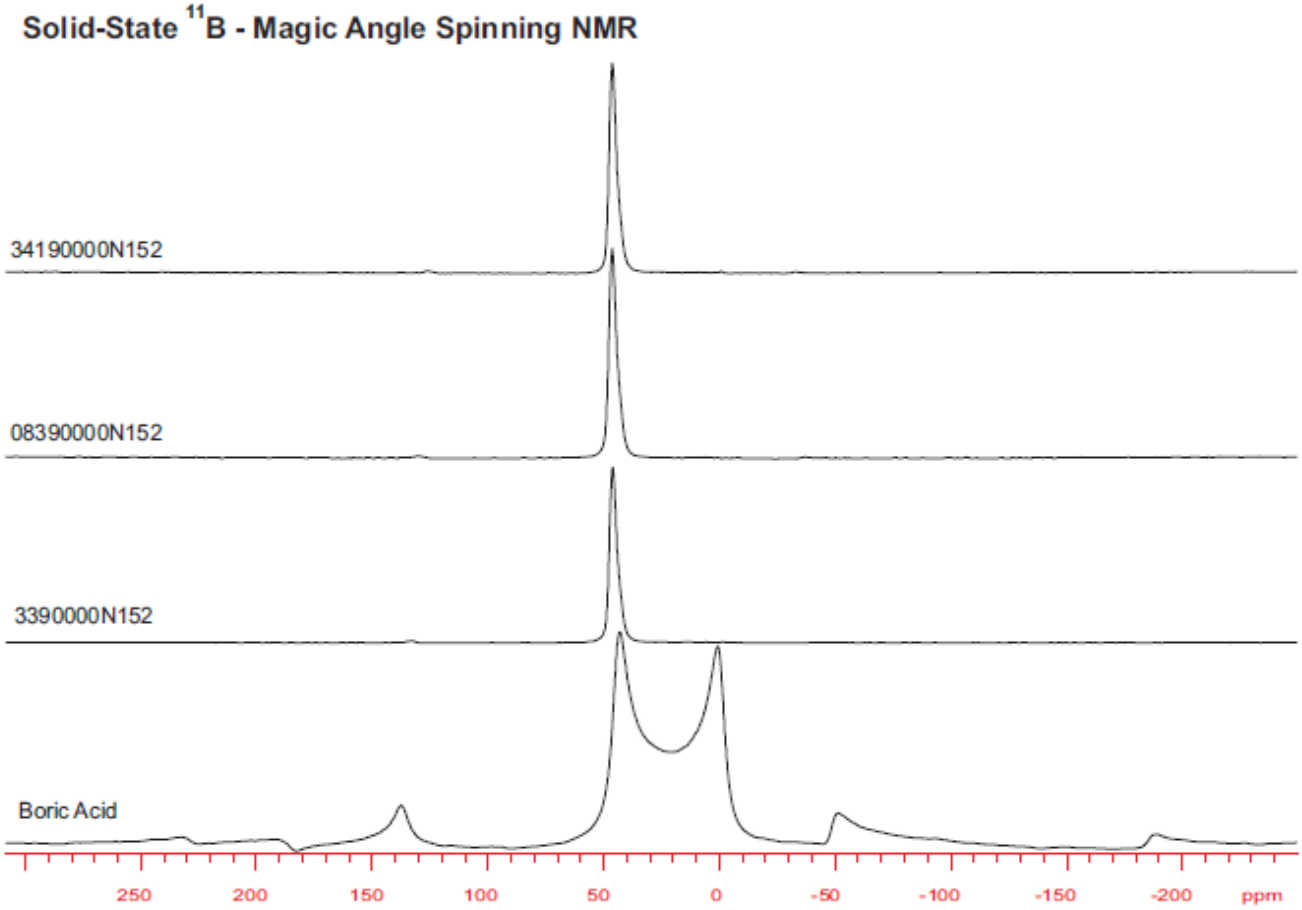

Figure 3. Magic Angle Spinning ^{11}B NMR Line shapes for Boric acid and FrxB

This leads to NMR signals that are not isotropic (symmetric) peaks. The signals are also very broad. The boric acid signal obtained in aqueous solution is used as the chemical shift reference at 0 ppm. The solid boric acid sample yields a broad doublet peak that indicates that the boron is in a rigid, non-symmetric environment (trigonal symmetry is not symmetric). Tetrahedral and octahedral symmetries are considered symmetric and would yield narrow boron signals - which are more along the lines of what is observed in the FrxB sample. The static line shape is much narrower - the presence of a quandrupole coupling (the splitting in the signal) indicates that the symmetry is not perfect but is more like a distorted tetrahedron.

Data presented in Figures1-4 strongly indicate that FrxB is clearly differentiated from boric acid and exists as intact, mostly di-complex Calcium Fructoborate.

3.2 Clinical study

All blood chemistry test parameters in the FrxB group and in the placebo group remained within normal ranges throughout the course of the trial. No undesirable changes were observed (data not shown).

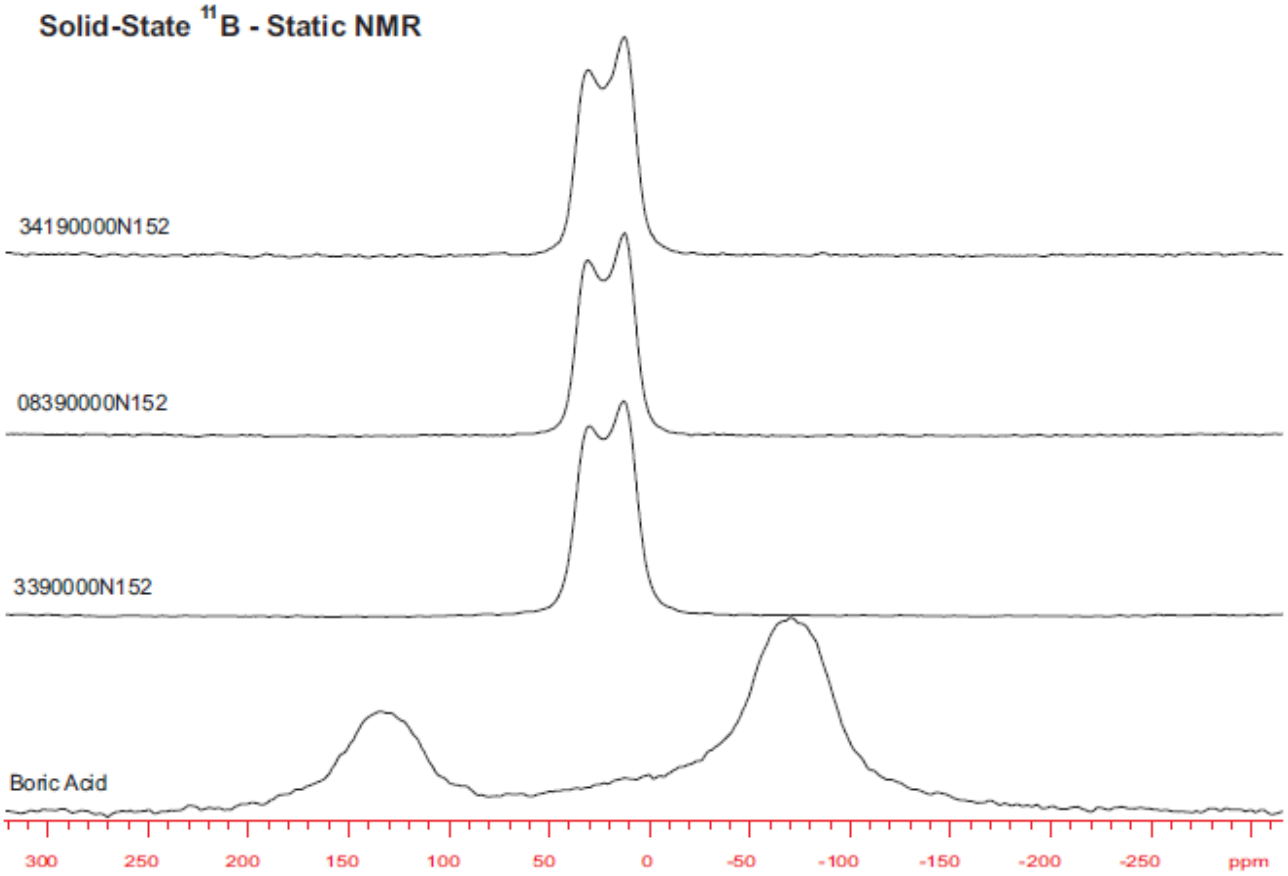

Figure 4. Static ^{11}B NMR Line shapes for Boric Acid and FrxB Products

Table 1. Effect of FrxB on WOMAC Index: Percentage Changes over Baseline Values at Day 1

		Average	N	STDEV	P(T<=t) two-tail
Placebo	D1	100%	10		
	D7	103%	10	24%	0.74
	D14	99%	10	27%	0.95
FrxB	D1	100%	9		
	D7	82%	9	20%	0.026
	D14	71%	9	10%	1.89E-05

3.3 WOMAC index

Placebo-controlled, double blind trial was carried out in age- (placebo group: 55.4±5.3; FrxB group: 56.4±4.3; p=0.65) and BMI-matched (placebo group: 30±2.6; FrxB group: 28.4±3.9; p=0.30) human subjects with minor knee OA conditions as confirmed by CT scan. Ten subjects were recruited for each group. One subject from the FrxB group did not complete the WOMAC questionnaire at Day 1. The WOMAC results from this subject were excluded from the statistical analysis. All other subjects reported full compliance with the study protocol when questioned at each follow-up. None of the subjects reported any adverse effects or new symptoms. WOMAC scores are presented in Table 1. In the FrxB group, WOMAC scores at Day 7 dropped to 82% of the Day 1 value (from 74.0 to 59.9, p<0.05). By Day 14, the WOMAC score reduced to 71% of the baseline (from 74.4 to 52.2, p<0.01).

In contrast, there was no significant reduction in WOMAC scores in the placebo group on either Day 7 or Day 14.

3.4 McGill Pain Questionnaire.

Pain severity was measured by the McGill Pain Questionnaire (MPQ). As compared to averaged individual MPG scores at Day 1, eight of the ten participants in the FrxB group showed a 13% reduction of MPQ scores at Day 7 ($p < 0.05$) and a 14% reduction at Day 14 ($p < 0.01$). Interestingly, subjects treated with the FrxB reported subjective feelings of pain reduction corresponding to McGill data which showed up to 15% improvement compared to Day 1 baseline values.

Table 2. Effect of FrxB on McGill Index: Percentage Changes over Baseline Values at Day 1

		Average	N	STDEV	P(T<=t) two-tail
Placebo	D1	100%	10		
	D7	103%	10	14%	0.583717
	D14	105%	10	13%	0.214467
FrxB	D1	100%	8		
	D7	87%	8	14%	0.034001
	D14	86%	8	10%	0.004505

Table 3. Effect of FrxB on blood level of 25-hydroxy vitamin D: Percentage Changes over Baseline Values at Day 1

		Average	N	STDEV	P(T<=t) two-tail
Placebo	D1	100.00%	10		
	D7	98.39%	10	23.01%	0.83
	D14	101.03%	10	22.63%	0.89
FrxB	D1	100.00%	10		
	D7	104.33%	10	22.83%	0.57
	D14	106.29%	10	16.94%	0.28

3.5 Serum C-reactive protein (CRP)

In the FrxB group, 7 out of 10 subjects showed a consistent reduction of CRP value at Day 7 and 14 compared to Day 1 baseline value. By Day 7, CRP levels of these 7 subjects dropped an average of 27% (73%±16%, p=0.0050); while at Day 14, CRP was reduced 37% (63%±27%, p=0.0102). However, there were three subjects with the opposite response. In two cases, Day 7 results showed no significant change (98% and 100% of Day 1 baseline levels respectively) while on Day 14 CRP was increased 94% and 19%. In the third subject CRP was increased 48% by Day 7 and 47% by Day 14. We could not exclude the possibility that this significant increase was attributable to an infection unrelated to the treatment. CRP in placebo group did not exhibit any significant and consistent changes at Day 7 (159% ±20.4%, p=0.3797) or Day 14 (124%±42%, p=0.1055).

3.6 25-Hydroxy vitamin D and 1, 25-Dihydroxy vitamin D.

As can be observed in table 3, there was no significant difference (P=0.5) between the average baseline (Day 1) serum levels of 25[OH]D of placebo group (19.39 ± 6.10ng/ml) and FrxB group (17.78 ± 4.96ng/ml). Also, no significant changes were found in serum levels of 25-Hydroxy vitamin D (25[OH] D) at Day 7 or Day 14 after FrxB or placebo intake compared to Day 1.

However, FrxB intake significantly increased serum 1, 25[OH]$_2$D levels 16.4% (n = 8, *P* = 0.049) and 19.9% (n = 8, *P* = 0.009) at Day 7 and Day 14 respectively compared to Day 1 (Table 4). In contrast, there was no significant change in serum 1, 25[OH]$_2$D levels at either time points in the placebo group. No apparent association can be identified between the changes of serum 1, 25[OH]$_2$D level and age, gender, BMI, blood chemistry parameters, or baseline serum 1, 25[OH]$_2$D level.

Table 4. Effect of FrxB on blood level of 1, 25-hydroxy vitamin D: Percentage Changes over Baseline Values at Day 1

		Average	N	STDEV	P(T<=t) two-tail
Placebo	D1	100.00%	8		
	D7	99.13%	8	21.65%	0.912661
	D14	131.87%	8	74.76%	0.267148
FrxB	D1	100.00%	8		
	D7	116.44%	8	19.52%	0.048705
	D14	119.93%	8	15.64%	0.008711

The current study confirms the results previously reported by Scorei [9]. Daily oral intake of FrxB for two weeks by people with minor knee OA conditions generated no adverse effects and resulted in significant reductions of indicators of OA and joint discomfort as measured by WOMAC and McGill questionnaires. Also, an apparent decrease in CRP, a general inflammation marker, was also observed. Results also showed an increase in blood level of endogenous 1, 25[OH]$_2$D but not 25-hydroxy Vitamin D in FrxB group.

A great majority of people over the age of 65 have evidence of OA in both industrialized and developing countries. Prevalence increases with age and above 11% of women over the age of 60 have symptoms of knee OA [10]. With a global population increasing in age, the costs for medical treatment and healthcare due to OA conditions are a tremendous burden onto society. Despite its frequency, OA is poorly understood with limited therapeutic options [11]. OA mainly affects hands, knees, hips and spine, which often leads to functional impairment, and is accompanied by signs of inflammation, including discomfort, stiffness and loss of mobility [2]. Drugs commonly used to alleviate symptoms of OA include acetaminophen and NSAIDs such as COX-2 inhibitors. However, due to potential serious side-effects of long term use of NSAIDs and recent safety concerns on COX-2 inhibitors, it is not surprising that OA is the leading condition for which people seek and use alternative therapy [12] [4].

OA is not considered a classical inflammatory arthropathy [11]. However, OA is often associated with signs and symptoms of inflammation, including joint pain, swelling and stiffness [11]. C-reactive protein (CRP), the classical acute phase plasma protein, is a non-specific marker of inflammation and tissue damage. An association between elevated CRP and disease progression in persons with osteoarthritis has been suggested [13]. Recent studies revealed that serum natural log-transformed CRP was powerfully associated with all definitions of radiographic OA, although the association was not independent of BMI. In the present study, serum CRP levels in 7 out of 10 participants exhibited 27-37% reduction after FrxB intake. Because this is a short two week trial and body weights of the subjects were not monitored at the end, it is not clear whether this CRP reduction is directly associated with possible changes in BMI. A recent study by Scorei et al [9] showed that OA subjects treated with 113 mg of FrxB experienced up to a 17% reduction of blood level of CRP. Those results support the CRP data reported in the present study.

The baseline 25[OH]D serum levels determined prior to the treatment of all subjects

participating in these study, suggested that they are either 25[OH]D insufficient or deficient [14]. A significant association between serum 25[OH]D deficiency and knee OA [15] [16]. In a recent study, Bergink et al., reported that low dietary vitamin D intake increased the risk of progression of knee OA [16]. An association between low 25[OH]D levels and prevalent radiographic hip OA has also been reported[17]. In fact, men with vitamin D deficiency are twice as likely to have prevalent radiographic hip OA [14]. On the other hand, sunlight exposure and improved serum 25[OH]D levels may prevent and/or slow cartilage loss, assessed by radiograph or MRI, in knee OA [18]. Furthermore, increased serum 25[OH]D levels are associated with reduced musculoskeletal pain and disease severity [19].

Serum 25[OH]D level is the primary criteria to define vitamin D deficiency since it is considered as "prohormone". 1, 25$[OH]_2$D (calcitriol) is the biologically active hormone and accounts for the majority of vitamin D biological actions. The enzyme CYP27B1 catalyzes 1α-hydroxylation of 25[OH]D and turns it to 1, 25$[OH]_2$D. This conversion occurs primarily in the kidneys of higher animals. Interestingly it is also expressed in human cartilage [20-22]. Moreover, 1, 25$[OH]_2$D, serving as the ligand, activates a transcription factor, vitamin D receptor (VDR). Most, if not all, effects of 1, 25$[OH]_2$D are mediated by VDR to regulate gene expression. Recent evidence revealed that VDR is expressed in human articular chondrocytes of OA cartilage, especially in the superficial zone, indicating direct effects of vitamin D on chondrocytes in OA cartilage [23].

In our study, FrxB did not increase serum 25[OH]D levels after 7 and 14 days of intake. On the other hand, and unexpectedly, serum 1, 25$[OH]_2$D levels in the FrxB group were elevated 16.4% and 19.9% at Days 7 and 14 respectively. The increase is relatively moderate but statistically significant. Although 25[OH]D is the major circulating form of vitamin D, 1, 25$[OH]_2$D can also be detected in blood at pictogram concentrations that are 1000 times less than those of 25[OH]D. Produced mainly in the kidney, 1, 25$[OH]_2$D is transported in blood and acts at a distance principally on intestinal cells to increase calcium absorption [24]. Additionally, it also regulates differentiation and activation of chondrocytes in cartilage and osteoblasts and osteoclasts in bone [25]. We speculate that increased 1, 25$[OH]_2$ D levels may play a positive role in bone and cartilage health in OA subjects. The mechanism of induction by FrxB and whether a moderate increase of 1, 25$[OH]_2$ D induced by FrxB intake over a longer term may be beneficial for OA conditions remain unclear at this point. Based on the results reported here, however, there is clear warrant for further investigation of the biological significance of FrxB.

In summary, FrxB intake over two weeks improved WOMAC and McGill scores, reduced blood levels of the general inflammatory marker CRP, and increased serum levels of 1, 25$[OH]_2$D. A future trial with more subjects experiencing minor OA conditions would help further confirm the efficacy of FrxB.

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