FINAL REPORT

RANDOMIZED CLINICAL TRIAL TO ANALYZE THE EFFICACY OF A FOOD SUPPLEMENT EXTRACTED FROM EGGSHELL MEMBRANE IN DOGS WITH OSTEOARTHRITIS

Date: June 16, 2022

Version 2

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1. Title

Randomized clinical trial to analyze the efficacy of a food supplement extracted from eggshell membrane in dogs with osteoarthritis.

2. Clinical researchers

The veterinary researchers responsible for carrying out the study were:

Miren Jaione Zurbano

Pablo Messia.

3. Research Center

Lur Gorri-Barañain Veterinary Clinic
Address: Plaza de Lur Gorri 4 bajo, 31010 Barañain (Navarre)
Spain Lur Gorri-Orkoien Veterinary Clinic
Address: Plaza Mendikur, 3, 31160 Orkoien (Navarre) Spain

4. Background

Osteoarthritis (OA) is a chronic, painful, degenerative and inflammatory disease that affects the synovial joints. It is highly prevalent in dogs and can significantly compromise the welfare of the animals that have it (1). The disease is accompanied by chronic pain, limping, and stiffness, especially following prolonged activity. These clinical manifestations reduce the quality of life of the animals and ultimately cause them to lose joint function and mobility (2).

There is currently no cure for OA and drug treatment is limited to alleviating the clinical symptoms. For that reason, the therapeutic management of OA in dogs most often uses non-steroidal anti-inflammatory drugs (NSAIDs), whose purpose is to treat the symptoms of the OA and reduce pain and inflammation (3). However, treatment with NSAIDs causes adverse intestinal affects like discomfort, pain, and diarrhea (4).

Therefore, in recent years research has been done into the development of food supplements that can slow the onset of OA through prevention, alleviate its symptoms, and improve the quality of life of pets without causing the side effects associated with NSAID treatment.

Membrapet is a food supplement made with eggshell membrane that contains biologically active compounds like hyaluronic acid, collagen, glucosamine, chondroitin sulphate, lysozyme, and elastin.

Eggshell membrane has been shown to be effective in alleviating joint pain and stiffness (5-10) in clinical studies on humans. However, its efficacy for treating OA in animals is still unknown.

A randomized, double-blind, controlled clinical study was performed to evaluate the efficacy of eggshell membrane, administrated in the form of the Membrapet food supplement, in treating OA in dogs that have owners.

5. Objectives

5.1. Primary objective

To determine the efficacy of a food supplement made with eggshell membrane on the functionality of patients diagnosed with osteoarthritis, after a treatment period of 10 weeks.

5.2. Secondary objectives

- To evaluate the effect of ingesting eggshell membrane for 10 weeks on the assessment of the knees, hips, musculature, pain, flexibility, limping, quality of life, functional limitation, and joint mobility.
- To assess the changes that occur in cartilage degeneration during 10 weeks of ingesting the food supplement made with eggshell membrane.
- To study the evolution of the overall condition of the patients with osteoarthritis during the study.
- To evaluate the safety of the food supplement after ingesting it for 10 weeks.

6. Study design

It is a randomized, double-blind, controlled clinical study with two groups being studied in parallel (Eggshell membrane and Placebo).

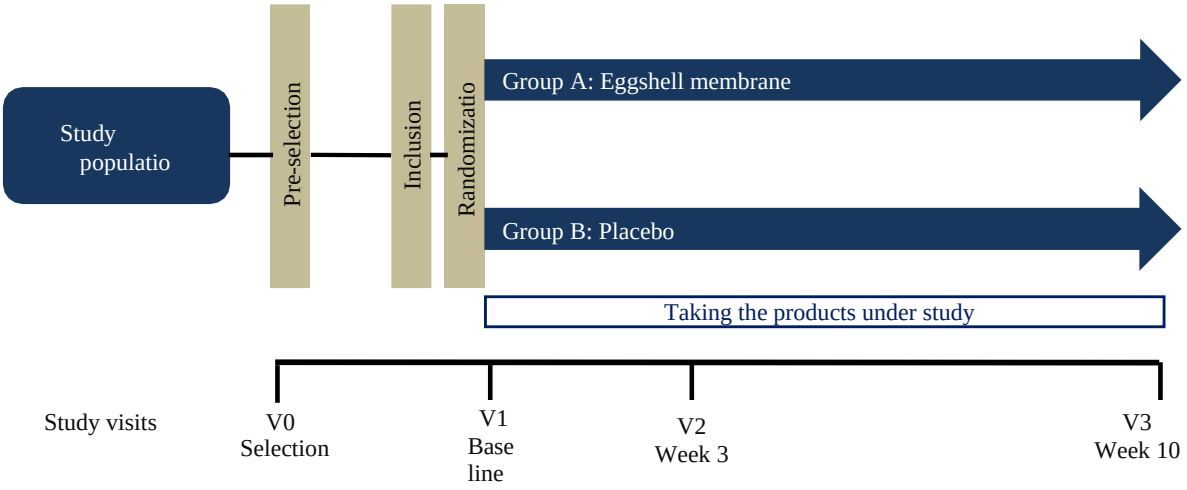

Figure 1. Outline of the study

7. Study population

The subjects included in this study met the following inclusion criteria:

Inclusion criteria

- Diagnosed with osteoarthritis
- Presence of persistent pain associated with osteoarthritis
- Presence of symptoms associated with musculoskeletal problems like limping, loss of activity, etc.

Additionally, to be included in the study, the subjects could not meet any of the following exclusion criteria.

Exclusion criteria

- Subjects in treatment with chondroprotectors, with supplements made from chondroitin sulphate, glucosamine, hyaluronic acid, and/or collagen
- Subjects with egg allergies
- Lack of willingness or inability to comply with the study procedures
- Subjects whose condition makes them ineligible for the study at the researcher's discretion.

Recruitment

The individuals were selected by reviewing their medical records. When an individual was selected as a possible candidate to participate in the study, the researcher contacted the owner by telephone and offered them the possibility of taking part in the study.

Otherwise, the participants were selected at a visit to the veterinary clinic.

7.1. Randomization and masking

Randomization method

A computerized random-number generator was used to assign each participating subject a code that put them in one of the two study groups (Membrapet and placebo). The randomization was simple with a 1:1 proportion, so each subject had the same probability of being assigned to either study group.

Technique used for masking and blinding

As a control, a placebo with identical organoleptic characteristics and appearance as the product under study was selected, and both the product under study and the placebo were manufactured by the sponsor. The packages for the products were also identical. Neither the researchers nor the owners of the participants ever knew to which group the individuals included in the study belonged.

7.2. Criteria for withdrawal or leaving

Criteria for withdrawal and their analysis

Participation in the study was always voluntary. The owners of the participating subjects could leave the study without needing to specify the reasons for doing so and without suffering any disadvantage in terms of the care received by the veterinarian. Likewise, the researcher could withdraw a subject from the study if they considered that the subject could no longer fully meet all the requirements or if any of the procedures were considered harmful for the subject.

8. Product under study

The characteristics of the product under study are as follows:

Membrapet

Pharmaceutical form: Tablets for both the product and the placebo.

Content: The capsules of the product under study contain 200 mg of eggshell membrane (ESM®). ESM® is an ingredient rich in collagen, hyaluronic acid, chondroitin sulphate, dermatan sulphate, and other amino acids and carbohydrates.

Route of administration: Oral

Dosage: 1 tablet a day for small and medium individuals (weighing 0-25kg) and 2 tablets a day for large individuals (weighing over 25kg).

Duration of the treatment: 10 weeks

Prior and concomitant treatments: The participating individuals were being treated with anti-inflammatories, and were prohibited from withdrawing that treatment during their participation in the study. Any treatment, pharmacological or otherwise, taken during the monitoring period had to be recorded in the data collection logbooks (DCL). The lead researcher of the study decided whether or not to allow the participants to continue in the study.

Criteria for treatment modifications: The treatment regimen was adapted to the body weight of the participating individuals as follows: individuals with body weight between 0 and 25 kg took a daily dose of one tablet, and individuals with body weight of over 25 kg took a daily dose of two tablets.

Procedures for monitoring the subject's therapeutic compliance: Following the randomization plan, the corresponding treatment was provided to the participants under the supervision of the lead researcher, who noted the assigned treatment in the indicated section of the DCL. Given that the treatment was administrated by the owner at home, compliance monitoring was done through the returning and counting of all the packages, both full and empty.

The lead researcher maintained a file with the dates, quantities, and batch codes of the product under study/placebo that was given out and returned.

9. Study variables

9.1. Efficacy variables

To evaluate the efficacy of eggshell membrane in OA treatment in dogs, a series of measurements called efficacy variables were taken.

9.1.1. Functional assessment

The functional assessment was carried out using the Bioarth scale (Annex I).

The Bioarth scale is a tool for evaluating different functional problems that affect joints, with an organized and systematized scoring system. It is highly useful for doing an initial patient assessment, as well as for monitoring their evolution over time.

The scale establishes a scoring system (from 0 to 3 or from 0 to 2 depending on the parameter being evaluated) for each of the 12 parameters examined. This assessment lets us

determine the status of the three basic functional parameters: functional limitation, joint mobility, and muscular atrophy.

The first block evaluates the functional limitation of the affected joint using a scoring system that quantifies posture changes, limping, and resistance to walking and playing (items 5 and 6 can be answered directly by the owner). The total score for this block is 23.

The second block assesses joint mobility limitations to see if there is any loss in the degree of flexion and extension of the examined joint and if this examination causes any pain. The range of motion is measured using the standard parameters[3]. The total score for this block is 7.

The third block assesses the degree of muscular atrophy, which is classified as: no atrophy (0 points), slight atrophy (1 point), or severe atrophy (2 points).

The sum of the 3 functional parameters (functional mobility, joint mobility and muscular atrophy) determines the degree of arthrosis (11).

Finally, combining the different questions of the 3 main blocks of the Bioarth scale makes it possible to assess limping, the condition of the knee and hip, pain, and flexibility.

The Bioarth Evaluation Scale was presented at the 4th International Symposium on Rehabilitation and Physical Therapy in Veterinary Medicine in Arnhem, The Netherlands.

The Bioarth scale was completed at the baseline visit, after 3 weeks of treatment (visit 1), and after 10 weeks of treatment (final visit).

9.1.2. Collagen degradation. CTX-II

In OA, the destruction of joint cartilage translates into the loss of its two principal components, proteoglycans and type II collagen, which makes them markers in assessing cartilage metabolism. Among the effects of type II collagen degradation, the C-terminal telopeptide of type II collagen (CTX-II) is one of the most studied markers, appearing in high levels in patients with osteoarthritis compared to healthy individuals.

This parameter was measured using the ELISA Kit, which detects canine C-terminal telopeptides of type II collagen.

CTX-II was measured at the baseline visit and after 10 weeks of treatment.

9.1.3. Anthropometric variables

Body weight was measured at the baseline visit and after 10 weeks of treatment (at the final visit). The weight results can be used to determine whether the individual's appetite had varied and to draw conclusions about tolerances to the treatment under study.

9.2. Safety variables

Any adverse events that had taken place were recorded and evaluated at every visit.

Any type of adverse event was recorded when the participant's owner reported it, spontaneously or at the researcher's instructions, and was described in the corresponding log for documenting tolerance to the product under study.

These logs contain information about the nature, severity, start time, and duration of the adverse events, the actions taken, and the probability of them being related to the products under study.

9.3. Statistical methodology

Descriptive study of the variables.

A descriptive analysis of the characteristics of the sample was done at the baseline visit, and at visits 1 and 2, describing every variable in every treatment group. The categorical variables are described with proportions and the continuous variables with means and standard deviations.

Comparative study between groups in baseline status.

We analyzed each target variable separately, also separating visit 1 from visit 2, with a linear regression model, where the dependent variable is the difference in the target variable between a baseline visit and a follow-up visit and the explanatory variables are the treatment group and the target variable at the baseline visit when it was measured. This allows us to measure the regression toward the mean. We thus calculated the "Average change" expected for each group between the two visits, adjusted for the baseline value. The effect of the treatment is the difference in this average estimated change between the two groups.

Additionally, where the data are not parametric, the non-parametric Kruskal-Wallis test or a robust test for truncated means were used. In both cases, the target dependent variable was the difference between the respective visit (1 or 2) and the baseline visit, and the independent variable was the intervention group. Any significant and appropriate results of these tests have been included in their respective sections.

The statistical analysis was performed using the R statistical program.

10. Results

The study included 51 dogs that were randomized into one of the two treatment groups (27 individuals in the Membrapet group and 24 in the placebo group). Fifty-one dogs completed the study and all of them were included in the analysis.

10.1. Demographic characteristics.

Table 1 shows the breeds of the dogs that participated in the study

Table 1. Breeds by treatment group.

Breed	Membrapet (N=27) n (%)	Placebo (N=24) n (%)
Bichon Maltese	0 (0)	2 (8.3)
Border Collie	3 (11.1)	1 (4.2)
Boxer	1 (3.7)	0 (0)
Bernese Mountain Dog	0 (0)	1 (4.2)
Brittany	0 (0)	1 (4.2)
Bulldog	1 (3.7)	0 (0)
Bully Kutta	1 (3.7)	0 (0)
Poodle	0 (0)	1 (4.2)
Yorkie Poo	0 (0)	1 (4.2)
Chihuahua	1 (3.7)	1 (4.2)
Cocker Spaniel	3 (11.1)	0 (0)
Labrador/Shar Pei Mix	1 (3.7)	0 (0)
Husky	1 (3.7)	0 (0)
Labrador	0 (0)	6 (25)
Labrador/Shar Pei	0 (0)	1 (4.2)
Pyrenean Mastiff	1 (3.7)	0 (0)
Mixed breed	5 (18.5)	2 (8.3)
German Shepherd	2 (7.4)	0 (0)
Basque Shepherd	2 (7.4)	0 (0)
Spanish Water Dog	0 (0)	2 (8.3)
Warren Hound	0 (0)	1 (4.2)
Ratter	1 (3.7)	1 (4.2)
Yorkshire	4 (14.8)	3 (12.5)

n(%)= Number of individuals (percentage of the treatment group)

The age and sex are shown in table 2. We can observe that there were no significant differences in the characteristics of the population between the two treatment groups.

Table 2. Sex, fertility and age

Demographic characteristics	Membrapet (N=27)	Placebo (N=24)
Male/female	14 / 12	8 /16
Fertile/Castrated	11 / 6	8 / 5
Age (years)	10.3 (3.12)	10.7 (3.41)

The sex and fertility status variables are expressed as the number of individuals and the age variable is expressed as a mean (standard deviation).

10.2. Anthropometric characteristics

The body weight was measured at the beginning, after 3 weeks, and at the end of the study (10 weeks), and no significant differences were found between the groups, nor were any significant weight changes observed over the course of the study for each group separately (table 3).

We can therefore conclude that the products under study were well tolerated and did not cause any appetite changes in the participating individuals.

Table 3. Body weight in Kg measured at the baseline visit and at 3 and 10 weeks for each treatment group

Body weight (Kg)	Membrapet (N=27)	Placebo (N=24)
Baseline visit	18.4 (13.7)	15.2 (11.6)
3 weeks	20.7 (15.8)	13.4 (10.6)
10 weeks	19.4 (14.3)	13.5 (9.98)

Conclusion: The Membrapet treatment was well tolerated and did not affect the appetite of the participants.

10.3. Functional assessment

The functional assessment was measured using the Bioarth scale. The sum total of the scores obtained on each of the 12 questions was calculated. The total maximum score that can be obtained on the scale is 32 points and the minimum is 0.

Various authors have linked the degree of arthrosis with the total score obtained on the Bioarth scale (12). Thus, a score of 0 to 2 indicates no evidence of arthrosis, 3 to 8 indicates slight arthrosis, 9 to 18 indicates moderate arthrosis, and more than 18 indicates severe arthrosis.

The individuals participating in this study presented slight-moderate arthrosis, so the average Bioarth scale score at the beginning of the study was 8.02 ± 5.81.

Figure 2 shows the total score results obtained at the beginning, and at 3 and 10 weeks of treatment, for the Membrapet group and for the placebo group.

As shown in table 4, we can observe a significant reduction in the total score after 3 weeks in both groups (p=0.004 Membrapet and p=0.004 placebo). However, after 10 weeks of treatment, only the group treated with Membrapet shows a statistically significant reduction in the total score (p=0.002).

Moreover, when we compare the evolution of the total Bioarth scale score of the two groups in the study, there was a nearly statistically significant (0.084) reduction in this variable for the Membrapet group compared to the placebo after 10 weeks of treatment (Table 4).

This result indicates that the individuals treated with Membrapet improve their functionality at the end of the study compared to the individuals treated with placebo.

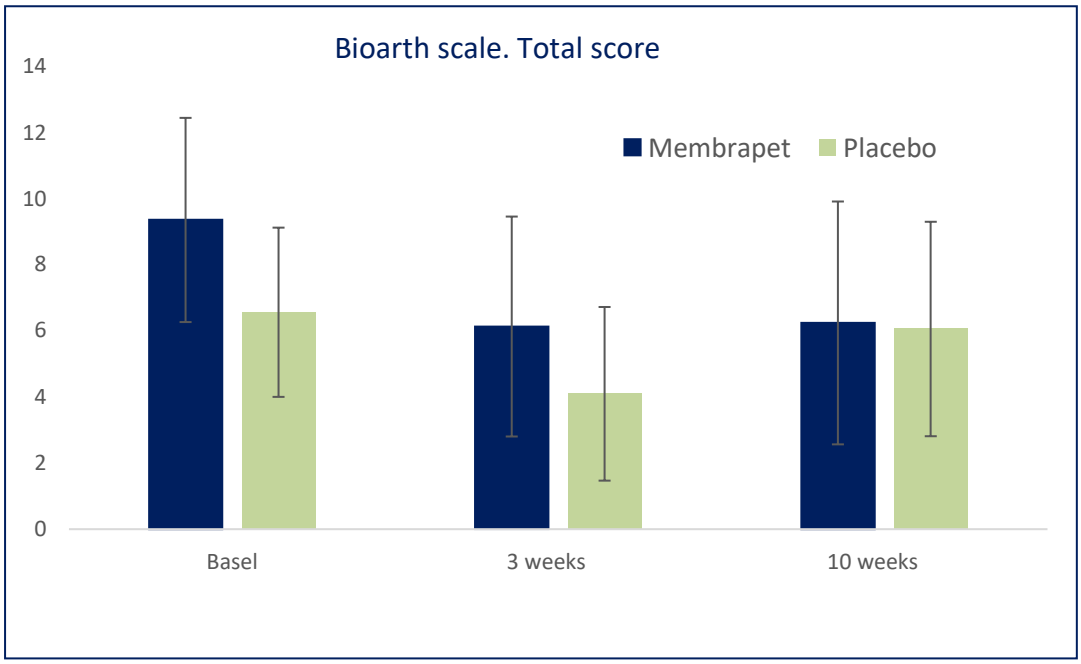

Figure 2. Total Bioarth scale score for each group at each measurement

The paragraph below explains the content of the tables shown in this report, taking table 4 as a model.

Table 4 shows the differences between the total score at 3 and 10 weeks and the score at the beginning of the study, and the difference between those differences with their respective P values.

Thus, -2.785 is the difference between the total Bioarth scale score at 3 weeks and the total score at the beginning of the study for the Membrapet group, and -2.997 is the difference between the total Bioarth scale score at 10 weeks and the total score at the beginning of the study for the Membrapet group. Next to each of those values is p= 0.004 and p= 0.002, respectively indicating that this difference between the two measurements is statistically significant (p<0.05).

In the fifth column, the value -0.164 is the difference between -2.950 and -2.785 (difference between the differences) and it is used to compare the evolution of the two groups in the study (taking time and treatment into account). Thus, comparing how the Membrapet group evolved compared to the placebo group we get a value of p= 0.905, which indicates that the total score reduction in the Membrapet group at 3 weeks is not significant compared to the placebo. However, at 10 weeks it gets much closer to statistical significance (p= 0.084)

Table 4. Differences between the total results of the Bioarth scale for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	**Initial** Mean	S.D.	**3 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	9.33	6.16	6.11	6.64	-2.785	0.004	-0.164	0.905
Placebo (N=24)	6.54	5.11	4.08	5.24	-2.950	0.004		

Product	**Initial** Mean	S.D.	**10 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	9.33	6.16	6.22	7.33	-2.997	0.002	-0.087	0.05
Placebo (N=24)	6.54	5.11	6.04	6.47	-0.628	0.518		

Conclusion: The Membrapet treatment for 10 weeks produces functional improvement in the individuals of the study

10.4. Functional limitation

The evolution of functional limitation during the study is shown in figure 3.

We can see that both the group treated with Membrapet and the placebo group improve functional limitation at 3 weeks and at 10 weeks of treatment. This improvement is significant for both groups at 3 weeks of treatment, but after 10 weeks only the group treated with Membrapet shows a significant improvement (p=0.000), while the functional limitation of the placebo group worsens compared to the values obtained at 3 weeks.

Comparing the improvement in functional limitation between the two groups at 10 weeks, we find that the group treated with Membrapet shows a significant reduction in functional limitation (p=0.029) compared to the placebo group (Table 5).

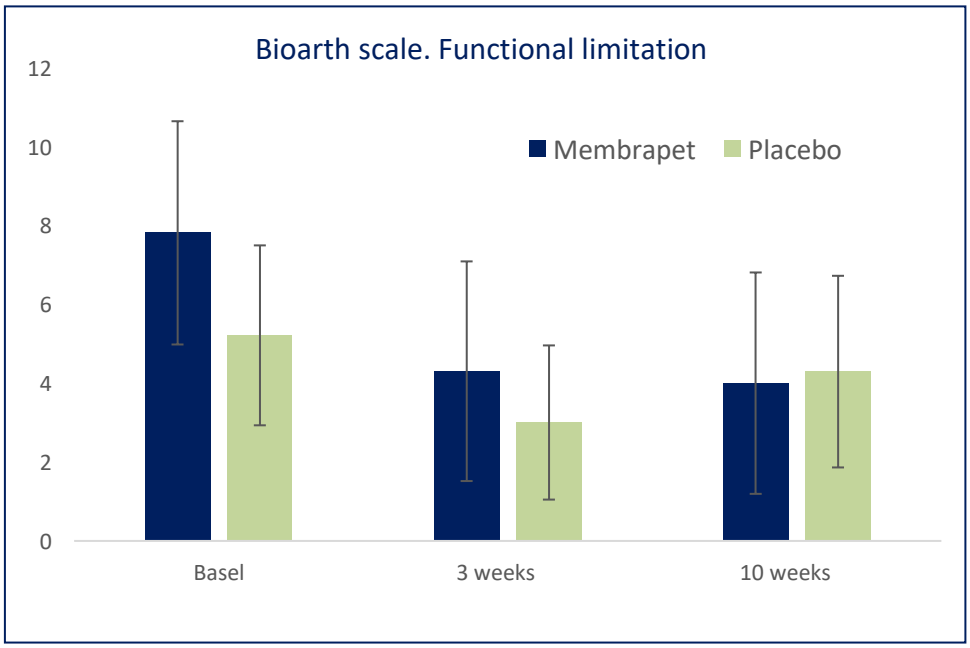

Figure 3. Functional limitation for each group at each measurement

Table 5. Differences between the functional limitation results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	Initial Mean	Initial S.D.	3 Weeks Mean	3 Weeks S.D.	Difference between visits	P	Difference between the differences	P (prod x time)
Membrapet (N=27)	7.81	5.66	4.30	5.57	-3.037	0.000	0.288	0.792
Placebo (N=24)	5.21	4.56	3.00	3.91	-2.750	0.001		

Product	Initial Mean	Initial S.D.	10 Weeks Mean	10 Weeks S.D.	Difference between visits	P	Difference between the differences	P (prod x time)
Membrapet (N=27)	7.81	5.66	4.00	5.61	-3.560	0.000	-2.357	0.029
Placebo (N=24)	5.21	4.56	4.29	4.86	-1.203	0.090		

Conclusion: The Membrapet treatment for 10 weeks produces a reduction in functional limitation for the individuals in the study

10.5. Joint mobility

The behavior of the individuals in the study in terms of joint mobility was very similar to what occurred when looking at functional limitation (Figure 4). In both treatment groups, this parameter improved significantly over time (p= 0.014 and p=0.007 in the Membrapet group at 3 and 10 weeks, respectively; p= 0.000 and p= 0.005 in the placebo group at 3 and 10 weeks, respectively).

However, when we compare the evolution of the two treatment groups, taking time and treatment into account, the differences between the groups are not significant (p=0.192 and p=0.861 at 3 and 10 weeks, respectively) (Table 6)

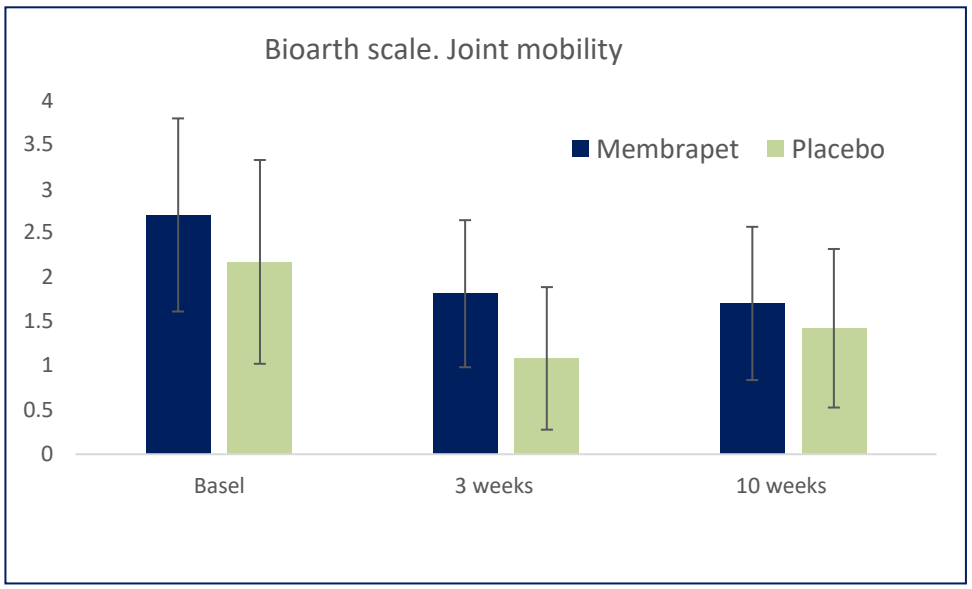

Figure 4. Joint mobility for each treatment group at each measurement

Table 6. Differences between the joint mobility results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	Initial		3 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	2.70	2.18	1.81	1.66	-0.722	0.014	-0.549	0.192
Placebo (N=24)	2.17	2.30	1.08	1.61	-1.271	0.000		

Product	Initial		10 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	2.70	2.18	1.70	1.73	-1.444	0.007	-0.952	0.861
Placebo (N=24)	2.17	2.30	1.42	1.79	-1.558	0.005		

Conclusion: It cannot be concluded that the Membrapet treatment for 10 weeks improves joint mobility for the individuals in the study. However, there is an upward trend.

10.6. Muscular atrophy

The results of the muscle assessment did not show significant differences in either of the treatment groups, both when looking at evolution over time and when comparing the evolutions of the two treatment groups.

However, it was shown that the fertility status of the individual (fertile or castrated) did have a significant influence on this variable. Thus, as shown in figure 5, fertile animals showed significantly less muscular atrophy than castrated animals (p=0.01). However, even though the statistical tests included fertility status as an explanatory variable, no significant differences were found between the treatment groups.

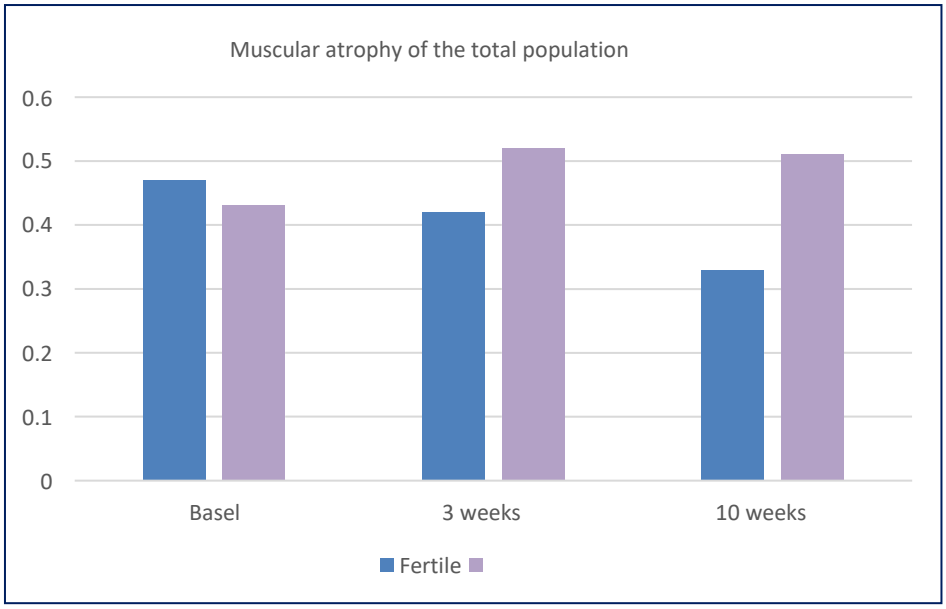

Figure 5. Muscular atrophy of the total population under study

Table 7. Differences between the muscular atrophy results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	**Initial** Mean	S.D.	**3 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	0.423	0.643	0.479	0.580	0.096	0.368	-0.119	0.445
Placebo (N=24)	0.476	0.512	0.425	0.591	-0.024	0.836		

Product	Initial Mean	S.D.	10 Weeks Mean	S.D.	Difference between visits	P	Difference between the differences	P (prod x time)
Membrapet (N=27)	0.423	0.643	0.480	0.653	0.054	0.635	-0.181	0.288
Placebo (N=24)	0.476	0.512	0.333	0.577	-0.127	0.312		

These results take on great importance in terms of designing new studies aimed at evaluating the efficacy of products intended to improve muscular atrophy.

10.7. Limping.

Limping was assessed through a combination of two questions from the Bioarth scale: Limping in cold and Limping while walking.

It was observed that after 3 weeks of treatment there was a significant reduction in the values of this variable for subjects treated with Membrapet and subjects treated with placebo (p= 0.008 and P= 0.000, respectively). After taking the treatment for 10 weeks, the placebo group did not improve limping and even showed increased values for this variable compared to at 3 weeks. However, the group treated with Membrapet significantly reduced limping (p= 0.009) after 10 weeks (table 8).

Comparing the evolution of limping between the two treatment groups over the entire study, we not find significant differences.

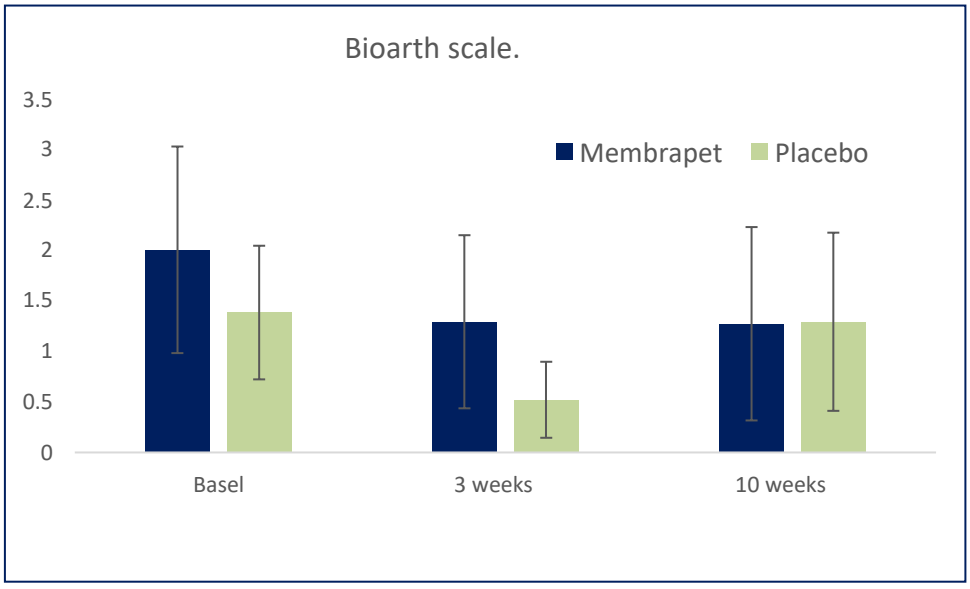

Figure 6. Limping for each treatment group at each measurement

Table 8. Differences between the limping results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	Initial Mean	Initial S.D.	3 Weeks Mean	3 Weeks S.D.	Difference between visits	P	Difference between the differences	P (prod x time)
Membrapet (N=27)	2.00	2.04	1.29	1.71	-0.545	0.008	-0.455	0.120
Placebo (N=24)	1.38	1.32	0.524	0.750	-1.000	0.000		

Product	Initial Mean	Initial S.D.	10 Weeks Mean	10 Weeks S.D.	Difference between visits	P	Difference between the differences	P (prod x time)
Membrapet (N=27)	2.00	2.04	1.27	1.91	-1.167	0.009	0.501	0.184
Placebo (N=24)	1.38	1.32	1.29	1.76	-0.723	0.539		

Figures 7 and 8 show the percentage of individuals that showed different levels of limping at the beginning of the study and after 10 weeks of treatment: not limping, slight limp, severe limp, and very severe limp.

We can observe that in the group treated with Membrapet for 10 weeks, the percentage of individuals that stopped limping doubled from the beginning of the study, going from 20.8% to 50%. In the placebo group, however, there was only a 9.1% increase in individuals that had stopped limping by the end of the study.

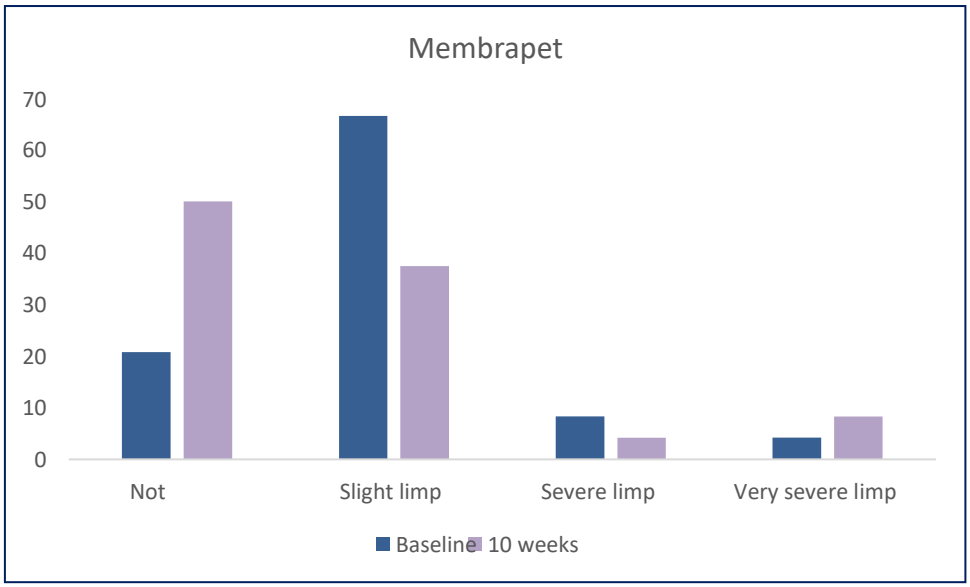

Figure 7. Percentage of individuals with a different level of limping at the beginning and after 10 weeks of Membrapet treatment

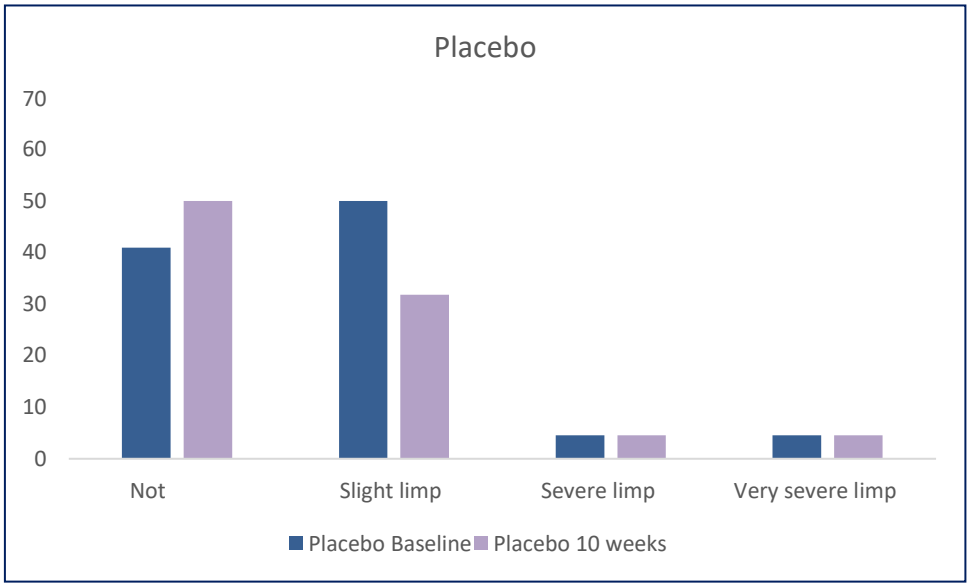

Figure 8. Percentage of individuals with a different level of limping at the beginning and after 10 weeks of Placebo treatment

Conclusion: It cannot be concluded that the Membrapet treatment for 10 weeks improves limping for the individuals in the study. However, there is an upward trend that is not observed in the placebo group.

10.8. Pain

Pain was assessed by combining two of the responses obtained for questions 1 and 2 of the Bioarth scale: Shifting when standing still and Awkward posture when standing up.

In both groups in the study, we observe a significant reduction in pain over time (table 9). However, the individuals treated with placebo suffer increased pain between week 3 and week 10 of treatment.

Comparing the evolution of pain between the two treatment groups, there are no significant differences at 3 or at 10 weeks (p=0.195 and p=0.739, respectively)

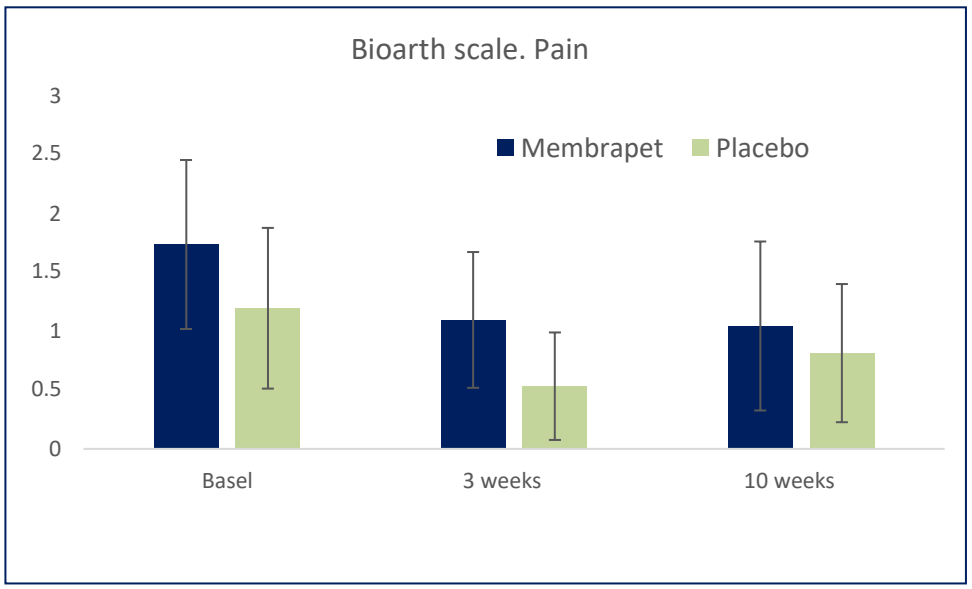

Figure 9. Pain for each treatment group at each measurement

Table 9. Differences between the pain results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	**Initial** Mean	S.D.	**3 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	1.73	1.43	1.09	1.15	-0.505	0.009	-0.364	0.195
Placebo (N=24)	1.19	1.36	0.526	0.905	-0.870	0.000		

Product	**Initial** Mean	S.D.	**10 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	1.73	1.43	1.04	1.43	-0.599	0.005	0.101	0.739
Placebo (N=24)	1.19	1.36	0.810	1.17	-0.497	0.031		

Conclusion: It cannot be concluded that the Membrapet treatment for 10 weeks reduces pain for the individuals in the study. However, there is a trend toward pain reduction.

10.9. Quality of life

The quality of life of the study participants was evaluated using a combination of questions 1 and 2 of the Bioarth scale: Shifting when standing still and Awkward posture when standing up.

The quality of life of the individuals treated with Membrapet improved significantly at 3 and 10 weeks of treatment (p=0.001 and p=0.000). The individuals treated with placebo showed some improvement in their quality of life at 3 weeks, but without statistical significance (p= 0.168), and at 10 weeks no improvement was observed (Table 10).

Comparing the evolution of quality of life between the two treatment groups, it was found that at 10 weeks of treatment, the group treated with Membrapet showed a statistically significant increase in improved quality of life compared to the placebo group (p= 0.045) (Table 9). It can therefore be concluded that treatment with Membrapet improved the quality of life of the dogs that participated in the study.

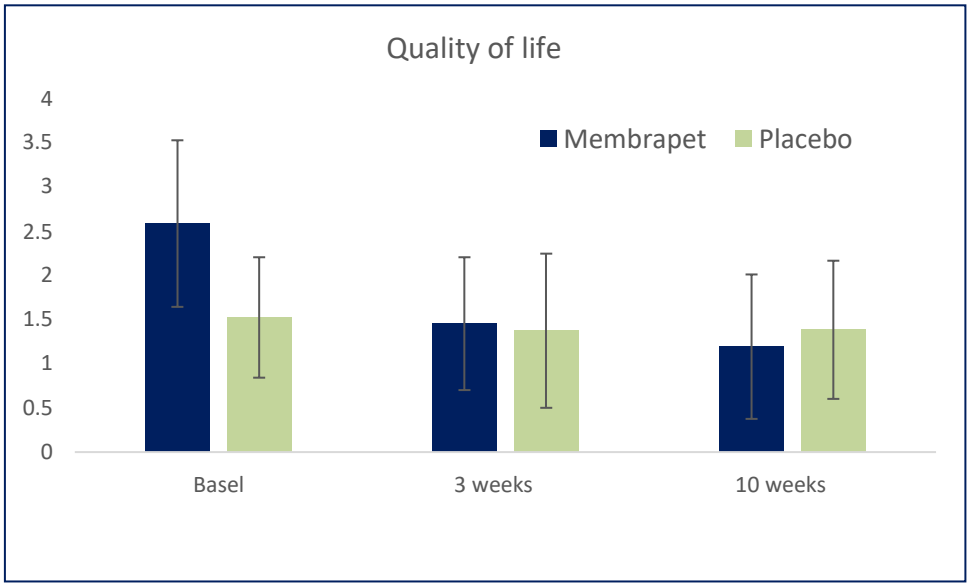

Figure 10. Quality of life for each treatment group at each measurement

Table 10. Differences between the quality of life results for the two treatment groups as a function of time and differences, between the groups taking time and treatment into account

Product	Initial		3 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	2.58	1.88	1.45	1.50	-0.932	0.001	0.530	0.190
Placebo (N=24)	1.52	1.36	1.37	1.74	-0.402	0.168		

Product	Initial		10 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	2.58	1.88	1.19	1.63	-1.191	0.000	0.809	0.045
Placebo (N=24)	1.52	1.36	1.38	1.56	-0.382	0.188		

Conclusion: The Membrapet treatment for 10 weeks improved the quality of life for the individuals participating in the study.

10.10. Overall condition

The overall assessment by the veterinarian was done after 10 weeks of treatment, taking into account the overall condition result from the baseline visit. Following the assessment, there are three possible results: Better, same, or worse

Of the 27 individuals treated with Membrapet, 20 (74.1%) improved by the end of the study, 4 (14.8%) stayed the same, and one (3.7%) got worse. In the group treated with placebo, with 24 individuals, 13 (54.2%) of them improved, one individual stayed the same and 6 individuals (25%) got worse. The differences between the Membrapet group and the Placebo group were statistically significant (p= 0.04), so it can be concluded that the Membrapet treatment improves the overall condition of individuals with osteoarthritis.

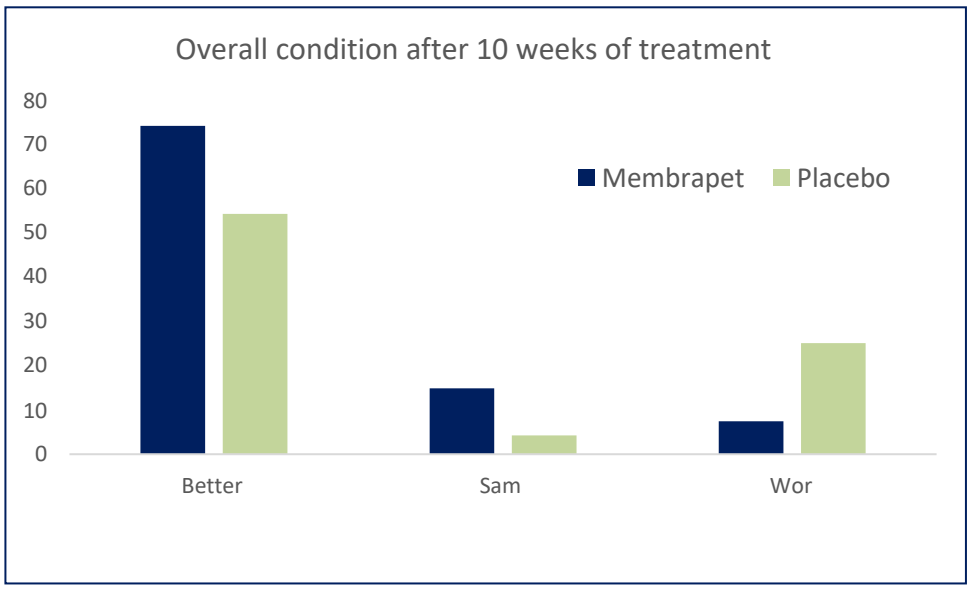

Figure 11. Percentage of individuals at every level of overall condition in each treatment group at the end of the study.

Conclusion: The Membrapet treatment for 10 weeks improved the overall condition of the individuals participating in the study

10.11. Collagen degradation. CTX-II

The CTX-II parameter was measured at the beginning of the study and after 10 weeks of treatment.

In both treatment groups, a significant reduction (p=0.000) in collagen degradation was observed over time. Comparing the change in this parameter between the two groups did not result in significant differences (p=0.244). However, it can be said that collagen degradation was the same whether Membrapet or placebo were ingested.

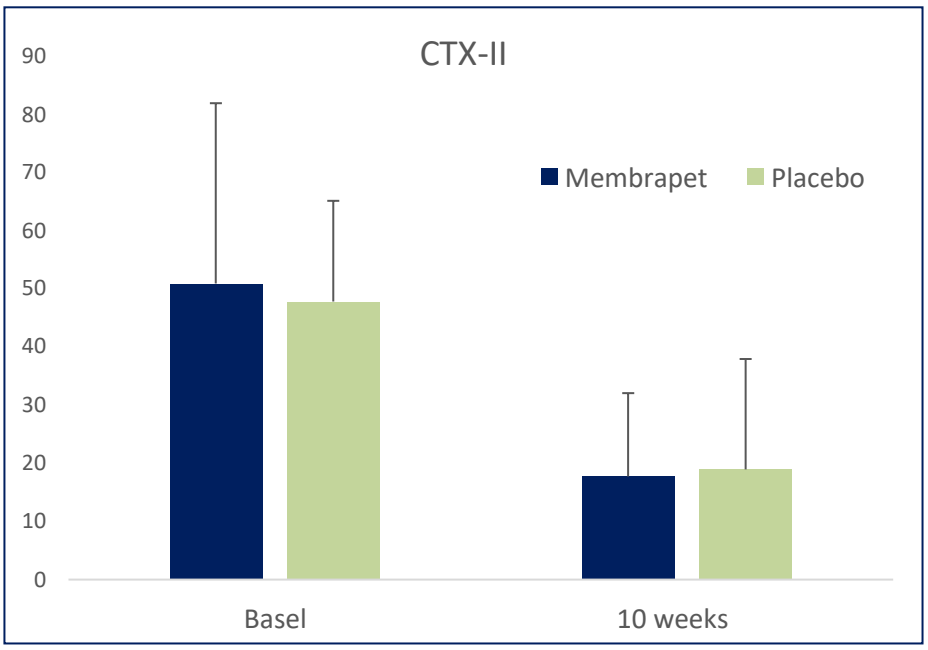

Figure 12. CTX-II for each treatment group at each measurement

The measured results of the CTX-II parameter showed great variability among individuals, as demonstrated by the standard deviation bars in figure 12. Therefore, the individual changes in the CTX-II parameter between the measurement taken at 10 weeks and the baseline measurement were analyzed in individuals where reduced collagen was observed. These measurements are shown in figures 13 and 14. They show that 42.8% of the individuals treated with Membrapet and 31.57% of the individuals treated with placebo have a CTX-II reduction of 50 ng/ml or more.

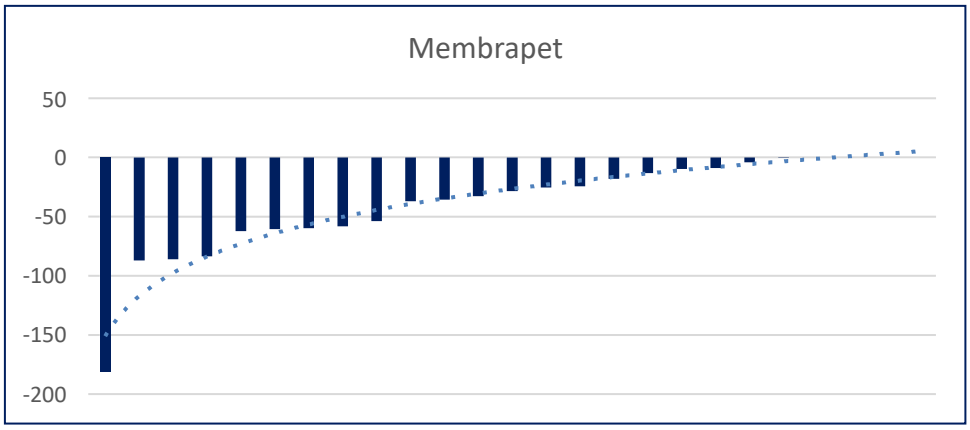

Figure 13. Individual changes in CTX-II measured at 10 weeks in the group treated with Membrapet

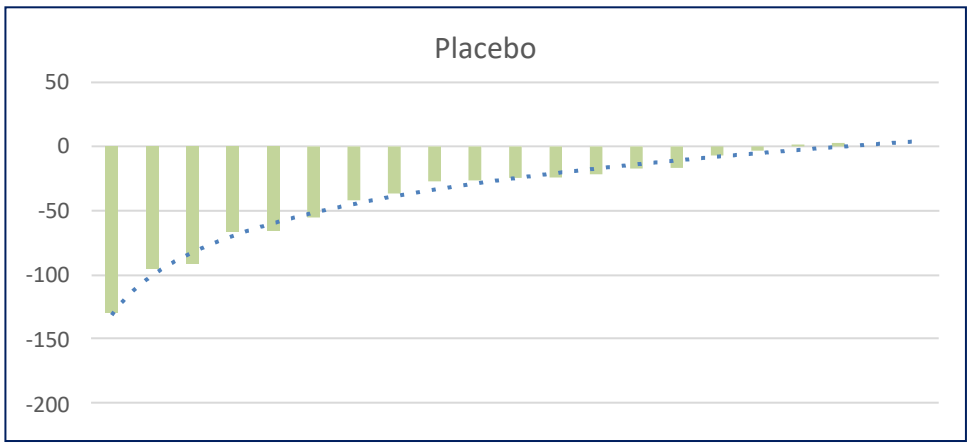

Figure 14. Individual changes in CTX-II measured at 10 weeks in the group treated with Placebo

Table 11. Differences between the CTX-II analysis results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	Initial		10 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	50.80	6.19	17.70	28.60	-31.892	0.000	2.127	0.824
Placebo (N=24)	47.70	34.60	18.90	37.90	-29.765	0.000		

10.12. Knee examination

The knee examination was done by combining questions 10 and 11 pf the Bioarth survey: Knee flexion and Knee extension.

At 3 weeks of treatment, a significant improvement was observed in the knees of the individuals in the placebo group (p= 0.056) but not in the group treated with Membrapet (p=0.141). However, at 10 weeks, no significant improvement was found in either of the groups in the study.

Moreover, comparing the evolution of this parameter in the Membrapet group and the placebo group, there was also no significant difference found (p= 0.664 at 3 weeks and p= 0.901 at 10 weeks).

Table 12. Differences between the knee examination results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	Initial		3 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	1.50	1.36	1.15	1.16	-0.308	0.141	-0.133	0.664
Placebo (N=24)	1.33	1.49	0.975	1.11	-0.441	0.056		

Product	Initial		10 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	1.50	1.36	1.08	1.12	-0.361	0.098	-0.040	0.901
Placebo (N=24)	1.33	1.49	1.00	1.14	-0.400	0.093		

10.13. Hip examination

The hip was examined using question 9 of the Bioarth survey: Manual passive joint mobility in the hip.

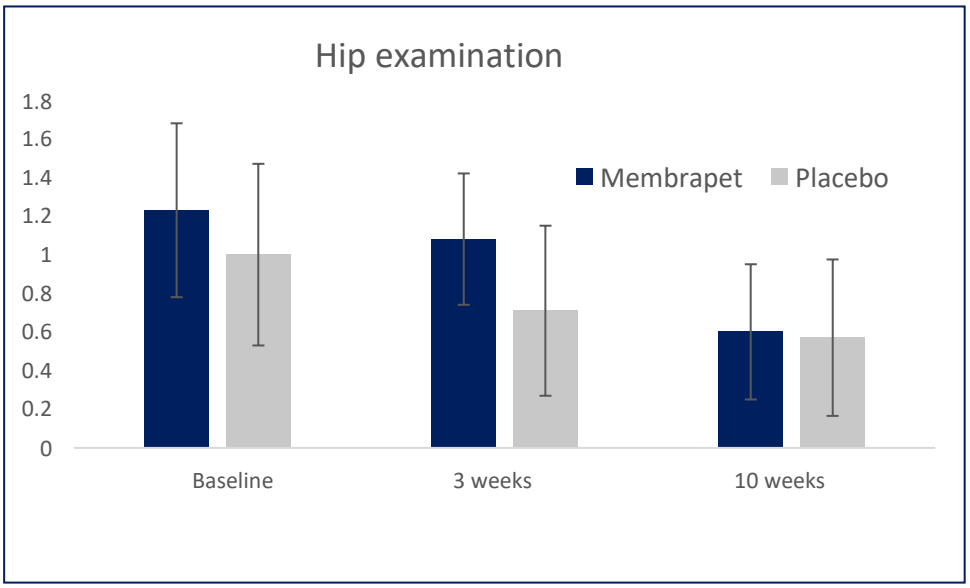

Figure 15. Hip examination for each treatment group at each measurement

Both at 3 weeks and at 10 weeks there was an improvement in the hip conditions of the two treatment groups that was significant for the placebo group at 3 weeks (p= 0.027) and for both treatment groups at 3 and 10 weeks (p= 0.000 Membrapet and p= 0.001 placebo).

Comparing the evolution of the two treatment groups, no significant differences were found at 3 weeks or at 10 weeks (table 13).

Table 13. Differences between the hip examination results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	Initial		3 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	1.23	0.908	1.08	0.687	-0.077	0.615	-0.303	0.185
Placebo (N=24)	1.00	0.949	0.714	0.888	-0.380	0.027		

Product	Initial		10 Weeks		Difference between visits	P	Difference between the differences	P (prod x time)
	Mean	S.D.	Mean	S.D.				
Membrapet (N=27)	1.23	0.908	0.600	0.707	-0.570	0.000	0.061	0.764
Placebo (N=24)	1.00	0.949	0.571	0.811	-0.509	0.001		

10.14. Flexibility

Flexibility was assessed by combining the scores for questions 7 and 8 of the Bioarth scale: Going up stairs and Limitations in small jumps.

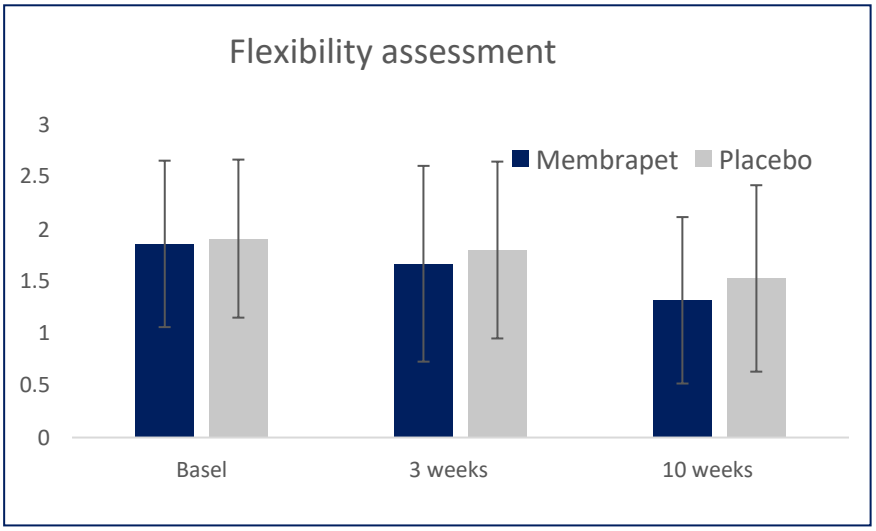

Figure 16. Flexibility assessment for each treatment group at each measurement

Taking into account the difference between the results obtained for each measurement (3 and 10 weeks) compared to the baseline situation, there was only a significant difference found in the group of individuals treated with Membrapet for 10 weeks (p=0.024) (Table 12).

Comparing the evolution of the two groups in the study did not result in significant differences.

Table 14. Differences between the flexibility results for the two treatment groups as a function of time, and differences between the groups taking time and treatment into account

Product	**Initial** Mean	S.D.	**3 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	1.85	1.59	1.66	1.87	0.012	0.960	0.216	0.520
Placebo (N=24)	1.90	1.51	1.79	1.69	-0.204	0.404		

Product	**Initial** Mean	S.D.	**10 Weeks** Mean	S.D.	Difference between visits	P	Difference between the differences	**P** (prod x time)
Membrapet (N=27)	1.85	1.59	1.31	1.59	-0.544	0.024	0.171	0.625
Placebo (N=24)	1.90	1.51	1.52	1.78	-0.374	0.156		

10.15. Safety.

Over the course of the study there were no adverse events reported related to ingesting the products under study. Ingesting Membrapet is therefore considered to be safe.

11. Conclusions

1. The Membrapet treatment for 10 weeks was well tolerated by the participants of the study and did not alter their appetite or their weight.
2. The Membrapet treatment for 10 weeks produced a functional improvement for the individuals in the study.
3. The Membrapet treatment for 10 weeks reduced functional limitation for the individuals in the study.
4. The Membrapet treatment for 10 weeks tended to improve joint mobility for the individuals in the study.
5. The individuals treated with Membrapet for 10 weeks showed a tendency to improve limping.
6. The individuals treated with Membrapet for 10 weeks showed a tendency to reduce pain.
7. The Membrapet treatment for 10 weeks improved the quality of life for the individuals in the study.
8. The Membrapet treatment for 10 weeks improved the overall condition of individuals in the study.
9. The Membrapet treatment for 10 weeks was shown to be safe for the individuals in the study.

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