



Canine Research

Effects of chronic supplementation of homotaurine on cognitive processes and spatial cognition in aged dogs: Preliminary results



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ABSTRACT

Aged dogs may develop age-related neuropathological and behavioral changes including the reduction in their memory and learning abilities. The aim of the study was to assess the effects of dietary supplementation with tramiprosate (homotaurine) on spatial cognition of aging dogs. Twenty-four aged dogs, sheltered in the same kennel, were randomly assigned into two groups—one group receiving homotaurine supplementation (500 mg once daily) and the other group, living in the same environment, without any supplementation. Cognitive testing was conducted using a T-maze. The experimental procedure consisted of four stages: a one-time direction-determination trial and a learning task, memory task, and reversal learning task that were done at T zero (immediately before supplementation start) and repeated after 4, 8, and 12 months. Data were collected from video-recordings by a single observer. The homotaurine-supplemented dogs were able to maintain a better performance in learning and memory trials with the largest differences observed in the reversal learning test, with a higher number of dogs able to pass the test ($P < 0.05$). The results suggest that dietary supplementation with homotaurine together with the additional stimulation and exercise of a learning activity seems to slowdown the cognitive aging in dog.

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Introduction

As a result of progress in veterinary medicine and nutrition, dogs have experienced an increase in their average life expectancy, and consequently, as in human beings, the pathologies of aged dogs, including those related to the cognitive sphere, are being recognized. Cognitive dysfunction syndrome in pet dogs is characterized by behavioral and cognitive changes that could be manifested by alterations in sleep-wake cycle (the dog starts to sleep more during daytime and less at night), social interaction (there is decreasing interaction with other pets and family members), house training, orientation, and/or impairing in learning and memory (Heath, 2002; Ruehl and Hart, 1998; Ruehl et al., 1995). Cognitive dysfunction impairs the dog's overall welfare and directly affects their day-to-day

relationship with their owners. In challenging cases, the deterioration of the pet-owner relationship may underlie owners' requests for euthanasia. Therefore, age-related cognitive dysfunction is becoming more relevant as the dog population ages (Nagasawa et al., 2012).

Dogs may naturally suffer from age-related cognitive deficits that share several key aspects in common with those of the early-onset human Alzheimer's disease (AD) (Gonzalez-Martinez et al., 2011). Beta-amyloid ($A\beta$) deposition, along with other structural and functional changes in brain (for example, oxidative damage, mitochondrial dysfunction, glutamate-mediated neuronal damage, alteration of glucose metabolism, and microglial and astrocyte dysfunction) and alteration of gray-white matter ratio have been shown to also occur in dogs (Borras et al., 1999). Other studies have shown neurodegeneration and oxidative damage in aged dogs (Bernedo et al., 2009; Head and Torp, 2002; Head et al., 2002; Insua et al., 2010; Pugliese et al., 2006; Rofina et al., 2006; Tapp et al., 2004). The similarities between human and canine brain senescence, especially in the context of human aging and AD, have stimulated interest in assessing the effects of age on canine cognitive function and its decline, along with the neuropathological changes—associated decline (Adams et al., 2000; Cotman and Head,

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2008; Landsberg, 2005). Several studies have already shown that age-related changes negatively affect the learning ability in old dogs (Milgram et al., 2002; Salvin et al., 2011). Reversal learning, which is the ability to reverse a previously learned stimulus-rewarded contingency, has been shown both in a laboratory canine model of pet dogs and humans to be even closer related to aging than other simpler learning tasks (Mongillo et al., 2013; Studzinski et al., 2006). Some studies have highlighted that cognitive aging could be slowed, and possibly reversed, by appropriately increasing levels of antioxidants and decreasing production of free radicals, and these studies have found beneficial effects from counteracting oxidative stress by dietary intervention with antioxidants (Bickford et al., 2000; Socci et al., 1995).

Studies in humans have shown the effect of homotaurine in mild-to-moderate AD (Aisen et al., 2011) and the benefits on cognitive function, especially on memory (Caltagirone et al., 2012). Tramiprosate (homotaurine) is a small, orally administered compound that binds to soluble A β and reduces amyloid aggregation and subsequent deposition (Gervais et al., 2007) and may have a protective role against oxidative stress on DNA caused by free radicals produced by the oxidation of catecholamines. *In vitro*, tramiprosate provided neuroprotection against A β -induced neurotoxicity in neuronal and mouse organotypic hippocampal cultures and had reversed A β -induced long-term potentiation inhibition in the rat hippocampus (Krzywkowski et al., 2007), in part, through activation of β -aminobutyric acid A (GABA-A) receptors (Azzi et al., 2007). *In vivo*, tramiprosate produced dose-dependent reductions of A β in the brain of transgenic mice (hAPP-TgCRND8) (Krzywkowski et al., 2007). Clinical studies in humans (Aisen et al., 2006) showed that tramiprosate was well tolerated, with a high-safety margin. In patients with mild-to-moderate AD, tramiprosate also reduced A β_{42} levels in cerebro-spinal fluid (Aisen et al., 2006), as well as being beneficial for maintenance of the cognitive state in the observed patients (Aisen et al., 2011). In dogs, homotaurine has been investigated once before with positive effects reported after an 8-month daily supplementation regime when orally administered (Benedetti et al., 2017). The aim of this study was to assess if chronic dietary supplementation with homotaurine over a twelve-month period was able to decrease the expected deterioration of cognitive processes and spatial cognition in aged dogs.

Materials and methods

Subjects

Twenty-four aged dogs (age 10 to 16 years), sheltered in the same kennel, were randomly assigned into two groups. Dogs had been present in the shelter for eight years; they were fed once daily and were allowed to interact with volunteers for twice-daily walks. The homotaurine-supplemented dogs (HT group, 7 males and 5 females; 1 female withdrawn after three months due to chronic renal failure) received daily 500 mg homotaurine supplementation as in the scheme in Figure 1; the control dogs (CTRL group) (8 males and 4 females) did not receive homotaurine supplementation. Inclusion criteria consisted of an age range between 10 and 16 years and the absence of behavioral or clinical conditions that could negatively influence the study. All dogs were screened by routine physical and neurological examination, and each dog's behavior was assessed by a veterinary behaviorist. The animals were treated according to the European legislations on animal handling (European Parliament Directive 2010/63/EU), local laws governing this type of study were followed, and the Ethical Committee of the University of Camerino approved the study.

Blood samples were taken from all dogs at enrollment for complete blood count and serum biochemistry analysis and

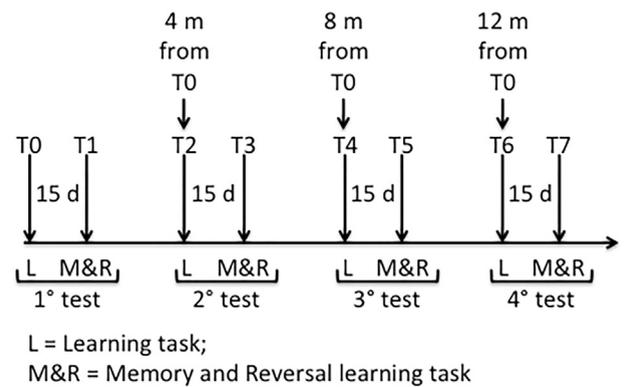


Figure 1. Time scheme of the study procedure.

repeated after 12 months (except for the dog withdrawn from the study) to check for any possible effects on blood parameters.

All blood sampling procedures were conducted in accordance with the normal clinical screening standards for shelter dogs. Serum biochemistry parameters tested were as follows: potassium (Ise K), sodium (Ise Na), chlorine (Ise Cl), gamma-glutamyl transferase, alkaline phosphatase, aspartate amino transferase, alanine amino transferase, unconjugated bilirubin, conjugated bilirubin, total bilirubin, cholesterol, triglyceride, creatinine, azotemia (BUN), phosphorus, calcium, correct calcium, calcium/phosphorus ratio, glucose, albumin, globulin, total protein, albumin/globulin ratio.

Experimental procedure

The apparatus used for the cognitive testing was a maze (Figure 2), housed in the same shelter. The maze was adapted from other versions previously employed for testing spatial learning and

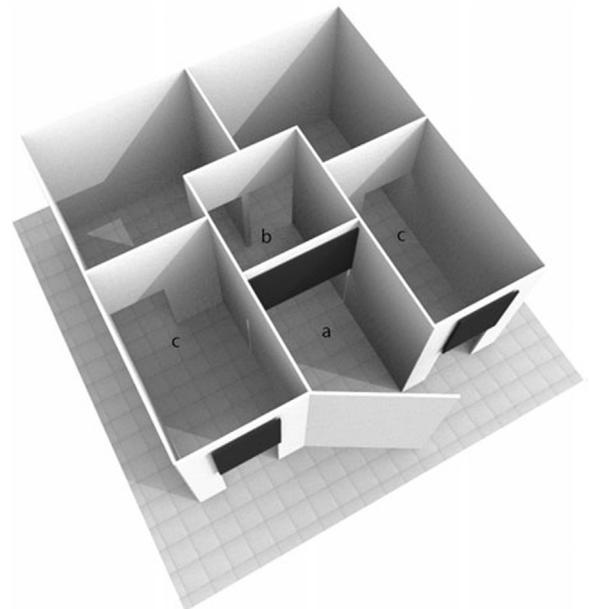


Figure 2. Representation of the T-maze used in the study (picture from Mongillo et al., 2013). A door at the center of the front wall leads into the start compartment (1 × 1 m; A). A sliding panel divides the start and the central compartment (1 × 1 m; B), from where two identical and symmetrical openings lead into the lateral arms of the maze (3 × 1 m at their narrowest point; C). Each of these arms terminates with an aperture (0.8 m in height), equipped with a remotely operated sliding panel. Sliding panels are represented in black in the picture.

memory in a different species (Regolin and Rose 1999; Regolin et al. 1995) and adapted for dogs by Mongillo et al. (2013). Externally, the maze measured 3 meters in both width and length and 1.5 meters in height. The entrance to the apparatus consisted of a 1-meter-wide door, leading into the start compartment, (1 × 1 meter; compartment “a” in Figure 2). The apparatus contained three sliding panels that were remotely controlled; one panel permitted access to the central compartment (1 × 1 meter; compartment “b” in Figure 2) from the start compartment, and the remaining two served as exit at the end of each lateral arm (3 × 1 meters at their narrowest point; see compartments “c” in Figure 2). The dogs' responses within the apparatus were monitored in real time using video cameras and recorded for subsequent analysis.

The procedure used was the same performed by Mongillo et al. (2013) and consisted of four stages. The first stage was a single direction-determination trial, which was used to determine the correct exit path for each subject in subsequent learning and memory retention stages. The second stage of the procedure was the learning task, in which dogs were required to learn which of the two arm choices resulted in the correct exit from the maze. Retention of this information was assessed two weeks later in the third long-term retention test stage. In the last stage, reversal learning task, dogs were required to learn to exit from the maze via the opposite arm to the one reinforced in the learning task.

In the present study, the procedure was repeated for four times: just before the beginning of homotaurine supplementation (time zero) and after four, eight, and twelve months from time zero.

Direction-determination trial

This procedure was used to rule out any possible side preference of the individual dogs and to ensure the dog explored the entire maze at least once.

The trial began when the dog was in the start compartment and with all doors and panels closed. The operator immediately thereafter opened the sliding panel leading into the central compartment, which remained lifted until the end of the trial. The dog could freely enter either of the lateral arms. The arm first entered by the dog became the incorrect entry arm for that dog in the subsequent learning task and memory retention test. The sliding panel at the end of the lateral arm initially entered by the dog remained closed throughout the trial, as well as all the trials of the subsequent learning task. Therefore, the dog had to navigate back to the central compartment and then to the opposite arm to exit from the apparatus. When the dog reached the half-way point of the correct arm, the sliding panel at the end of this was raised and the trial ended when the dog stepped out of the apparatus.

Learning task

Immediately after the direction-determination trial, the dogs underwent one session of continuous consecutive trials during which the exit panel of the correct lateral arm remained lifted. To reduce the risk to develop a negative association between stepping out of the apparatus and subsequent reintroduction, the operator praised, petted, or played with the dog for about 45 seconds during the intertrial intervals. On each trial, a correct response was recorded when the dog entered the correct side of the compartment first and an incorrect response was recorded when the dog entered the incorrect side first. Each dog was tested until it achieved the learning criterion of three consecutive correct trials within the maximum 15 trials. If the learning criterion was not achieved within 15 trials, the dog failed the task and was not included in the subsequent stages.

Retention test

This test took place 2 weeks after the learning phase with the aim of determining if dogs retained the information acquired in the learning phase over a long-term delay. The memory test consisted of four trials in which the exit panels for both lateral arms were open, thereby allowing the dog to exit the apparatus from either lateral arm or preventing new learning from confounding the results of the retention test. A dog successfully passed the retention test if it initially entered the correct lateral arm, as acquired during the learning task, at least three of the four trials.

Reversal learning task

Only dogs that passed the retention test were included in this phase, which took place immediately after the retention test. The reversal learning task was intended to evaluate the dogs' ability to contrast and modify previously acquired behavioral responses (i.e., navigating the previously reinforced direction). Thus, the protocol and learning criterion were identical to that of the learning task, but the arm choice resulting in the exit from the apparatus was reversed. Because the dogs were expected to respond incorrectly on the first trial, this was not included in the 15 trial maximum, nor was it considered in the statistical analysis.

Time scheme of the procedure

The experiment started in July and subsequent tests conducted in November, March, and July of the following year. See Figure 1 for a complete time scheme of the experiment.

Data collection and statistical analysis

Trials were performed by a single observer, and data were collected by reviewing the video-recording, and the number of dogs able to pass each test (Learning, Memory, and Reversal Learning) and the number of attempts necessary to pass for the Learning and Reversal Learning tests were counted, for the total number of dogs enrolled in the study. Contingency tables (Pearson Chi-square test and Fisher exact test when needed) were used to verify the hypothesis that the proportion of dogs able to pass the test was the same in the two groups. One-way analysis of variance was performed to compare the means of the attempts to pass the test within the groups, and an independent sample *t*-test was used to compare the mean number of attempts to pass the test between the two groups at the same time session. All the statistical analyses were performed by SPSS 12.0 statistical software package (SPSS, 2003).

Results

Complete blood count and serum biochemistry parameters evaluated before the administration of homotaurine and after 12 months of administration were within normal range for all dogs.

The results are presented as the percentage of dogs able to pass each test (Figure 3 for Learning, 5 for Retention and 6 for Reversal Learning), and for Learning and Reversal Learning tests, the mean number of incorrect attempts (when the dog chooses the incorrect arm after entering the maze) is also presented in Figures 4 and 7.

At the first test (T0 and T1), no differences were detected between performance of both groups in the 3 tasks, for both the percentage of dogs able to pass the tests (Figures 3, 5 and 6) and the mean number of failed attempts (Figures 4 and 7).

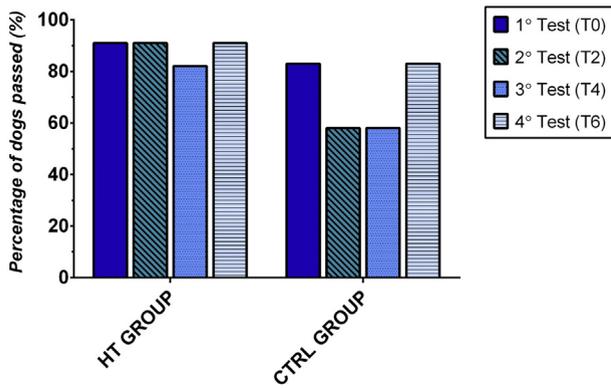


Figure 3. Percentage of aged dogs that passed the learning task at each of the tests. Percentages were calculated on the total number of dogs enrolled in each group of the study. CTRL group, control dogs; HT group, homotaurine-supplemented dogs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Learning task

The percentage of HT dogs that passed the learning test remained very stable throughout the 12-month period (Figure 3), whereas the mean number of failed attempts decreased slightly compared with T0 (Figure 4). In the CTRL group, we see a slight decrease in the pass percentage at T2 and T4, but at T6, it returns to the initial level at T0 (Figure 3). The mean number of failed attempts increased at T2 and T4 but then decreased at T6 (Figure 4), when compared with T0. Also in the CTRL group, the mean number of incorrect attempts was different between T4 and T6 ($P < 0.05$). A difference was also seen between groups at T4 ($P < 0.05$), in favor of the HT group.

Retention test

The HT dogs appeared to be able to maintain their memory throughout the study period, whereas a decrease was observed in CTRL dogs in the second and third tests (T3 and T5) when compared with first test at T0 (Figure 5, $P < 0.01$). The difference at these timepoints (T3 and T5) between the groups tends to be significant ($P = 0.10$). In the fourth test (T7), the HT dogs' pass rates improve over the initial level, whereas in the CTRL, this improvement does not reach the initial level. Overall, it appears the HT dogs are able to maintain their memory ability better than the CTRL group.

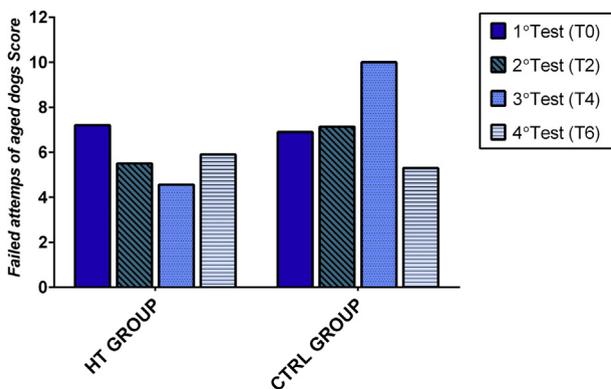


Figure 4. Average numbers of incorrect attempts of the aged dogs at each test. CTRL group, control dogs; HT group, homotaurine-supplemented dogs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

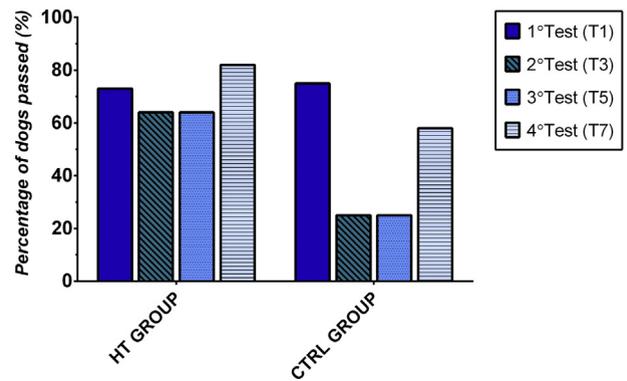


Figure 5. Percentage of aged dogs that passed the Retention test at each test. Percentages were calculated on the total number of dogs enrolled in each group of the study. CTRL group, control dogs; HT group, homotaurine-supplemented dogs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Reversal learning task

The percentage of CTRL dogs passing the test results significantly higher at the first test with respect to the subsequent ones ($P < 0.01$ vs. T3 and $P < 0.05$ vs. T5 and T5), showing there is decline in the CTRL dogs' ability to reverse learning (Figure 6). At the fourth test (T7), there are significant differences between the pass rates of the two groups ($P < 0.05$).

The data about the mean number of wrong tries do not appear to change in the HT group (Figure 7).

Discussion and conclusion

These preliminary results on chronic supplementation of homotaurine in aged dogs suggest homotaurine dietary supplementation might limit the age-related deterioration of learning, memory, and reversal learning processes in aged dogs.

Despite the relatively low number of dogs assessed and the study not being powered for significance, better cognitive results can be seen consistently in favor of the dogs receiving the homotaurine-supplemented diet.

The performance of the HT dogs in the cognitive tests remains relatively unchanged throughout the 12-month period, with the exception of a drop in the reversal learning task performance at 4 months (second test).

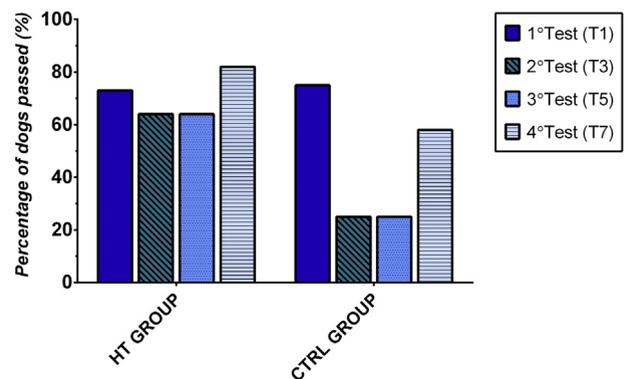


Figure 6. Percentage of aged dogs that passed the reversal learning task at each test. Percentages were calculated on the total number of dogs enrolled in each group of the study. CTRL group, control dogs; HT group, homotaurine-supplemented dogs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

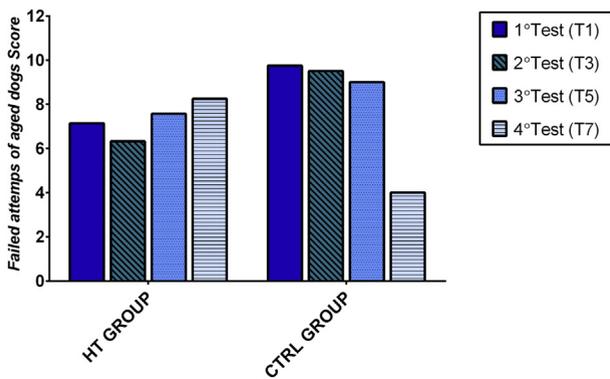


Figure 7. Average numbers of incorrect attempts of the aged dogs at each test. The means of CTRL group are from a too small sample to be considered. CTRL group, control dogs; HT group, homotaurine-supplemented dogs. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

In contrast, a decline in performance of the CTRL group was observed throughout the study period in all tasks, which is what could be expected due to the advancing aging.

In the learning and retention tasks, differences between the two groups were also observed, particularly at the second and third test (at 4 and 8 months). Interestingly, at 12 months, we note a distinct improvement in performance in the CTRL group for these 2 tests, back to T0 levels for the learning task and not quite so for the retention task, but still clear improvement over second and third tests. This was not an unexpected finding, as these dogs were subjected to increased levels of exercise and mental stimulation during the study, which has been found to lead to improved cognitive performance in both humans and dogs (Christie et al., 2009). Furthermore, it has been shown that dogs with previous experience of cognitive tests will perform better, so an improvement was expected in all dogs as the study progressed and repeated the tasks (Milgram, 2003).

Reversal learning can be considered the most challenging cognitive test in this study. The age-dependent reversal learning deficits has been largely reported in canine cognitive studies and has been shown to be more age sensitive than other basic cognitive processes, such as simple associative learning (Adams et al., 2000; Mongillo et al., 2013). From the preliminary results presented herein, homotaurine-supplemented dogs showed significantly better performance, indicating they were able to maintain their ability to reverse-learn better than nonsupplemented animals, which showed the age-related decline expect for reverse learning.

As in AD in humans, there is still much to learn about age-related cognitive decline and cognitive dysfunction syndrome in dogs. Also, similarly to AD, A β deposition is believed to play a role in disease progression. Homotaurine's anti-amyloid activity is well demonstrated, and its GABAergic activity could also be beneficial as a mood enhancer. In this study, the data indicate it to be well tolerated by dogs and to have no effect on blood parameters after one-year supplementation. These preliminary findings indicate that homotaurine supplementation slows down the age-related cognitive decline and in particular in preserving the ability to reverse-learn, which is more closely related to age than simpler learning tasks. As demonstrated by other studies, physical and mental stimulation can also contribute for the improvement in cognitive ability although the improvements will take longer to take effect: in this study, these were noted only after 12 months and only in learning and retention exercises.

With an aging dog population, cognitive decline and dysfunction will become more relevant to pet owners, their dogs, and the veterinarians caring for them. This study indicates homotaurine

supplementation could potentially play a role in helping dogs maintain their cognitive ability for as long as possible.

Further studies involving a larger number of subjects are encouraged to further understand the effect of chronically orally administered homotaurine in cognitive function of aged dogs.

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Authors' contribution: The idea for the paper was conceived by Riccardo Benedetti, Alessandro Malfatti, Andrea Spaterna, and Andrea Marchegiani. The experiments were designed by Riccardo Benedetti, Alessandro Malfatti, Adolfo Maria Tambella, and Andrea Spaterna. The experiments were performed by Riccardo Benedetti, Andrea Marchegiani, Alessandro Fruganti, and Evelina Serri. The data were analyzed by Alessandro Malfatti, Evelina Serri, and Alessandro Fruganti. The paper was written by Riccardo Benedetti, Andrea Marchegiani, Adolfo Maria Tambella, Alessandro Fruganti, Evelina Serri, Alessandro Malfatti, and Andrea Spaterna. This work was supported and financed by FB Maria, Ascoli Piceno, Italy.

Conflict of interest

The authors declare no conflict of interest.

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