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Virgin Coconut Oil Improved Discriminative Learning and Working Memory in Aging Cycling and Non-Cycling Female Sprague-Dawley Rats Supporting Its Beneficial Effect in Retarding Age-Related Cognitive Decline

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Abstract

Aim: Beneficial effects of virgin coconut oil (VCO) consumption to improve cognition in menopausal females remain inconclusive. This study examined the effect of VCO supplementation in aging cycling and non-cycling rodents to assess its impact on cognition. **Methods:** Sprague-Dawley rats (10 - 18 months) were randomly assigned to a supplemented VCO group (SVCO) that received oral doses of 1.42 mL/kg/day VCO (n = 10) and a non-supplemented (NVCO) group (n = 10). Their performance in a biased Y-maze discriminative learning paradigm was assessed over a 16-week period. Rats were initially allowed 3 minutes to explore the maze (*habituated*) and subsequently *pre-trained* in the non-preferred, white chamber to associate the presentation of a tone with a treat (reward). *Training* involved 4 daily trials initially for 3 weeks during which rats were rewarded if they entered the white arm within 15 sec after tone presentation. Time (days) to attain at least 75% correct responses (CR) determined acquisition latency (AL). Memory retention (MR1) of the learned task was assessed following a 1-week break from training and absence of supplementation (session T1). Following an additional 2-week break, supplementation of SVCO animals resumed and continued to week 16. In week 14, all animals received *re-training* for 1 week (session T2) followed by another 1-week break and subsequent assessment of memory (MR2). Vaginal smear cytology determinations were performed throughout the study to identify cycling and non-cycling rats. Student's t-test and ANOVA with Brown-Forsythe and Tukey's post-hoc tests were used to compare means. **Results:** C-SVCO rats attained lower AL, and higher CR and MR scores vs their NVCO counter-

parts ($p < 0.05$). At session T2, NC-SVCO rats out-performed other groups ($p = 0.048$, $F = 2.64$), attaining highest CR scores between sessions ($p = 0.026$). **Conclusion:** VCO supplementation attenuated cognitive decline with a more positive impact on non-cycling rodents suggesting a beneficial effect on brain health in females in menopausal transition.

Keywords

Dementia, Menopause, Estrous Cycle, Cognitive Impairment

1. Introduction

Aging menopausal females are at increased risk of cognitive decline due in part to the sudden decrease in bioavailable estrogen that causes diminished neuroprotective effects on learning and memory [1] [2]. Women in the fifth decade of life with reproductive organs intact, will begin the transition through perimenopause to menopause at which stage ovarian secretion of estrogen and progesterone will decline [3]. The reproductive estrous cycle of female rats starts at 50 to 60 days of age, and is a measure of the integrity of the hypothalamic-pituitary-ovarian axis [4] [5]. Estrogen regulates the cycle, peaking by the end of proestrus and returning to baseline at estrus [6]. Aged female rats enter a non-cycling stage between 15 and 20 months of age, corresponding to the onset of menopause in women [7].

Thus far, intervention strategies during the postmenopausal stage to correct progressive memory impairment have not resulted in significant success for those women experiencing cognitive decline. During the perimenopausal transition phase, humans and rodents share common features that include decline in follicles, irregular cycling and fertility, steroid hormone fluctuations, and estrogen insensitivity [3]. Hormonal dysregulation may cause rodents to exhibit a prolonged acyclic phase prior to frank ovarian failure. During this period, there may also be persistent estrus [8] [9] [10] [11] in which FSH levels are elevated and 17β-estradiol levels are normal, a hormonal milieu similar to human perimenopause [8] [12] [13].

The use of ovariectomized animals to model a menopausal-like state has provided substantial information on the physiological effects of postmenopausal hormonal changes but provides limited information on the transition phase of perimenopause, as well as the hormonal variations associated with the hypothalamic-pituitary-ovarian axis. Chemical induction, using 4-vinylcyclohexene diepoxide or VCD for example, to induce a perimenopausal-like state in young rodents, can induce acute toxicity effects with high mortality rates, lymphopenia and hepatotoxicity [8]. Thus, studies of female rats that are transitioning normally between the cycling and non-cycling stages of their reproductive cycle may provide useful information for intervention strategies. Timely intervention may be key to retarding the cognitive impairment associated with normal aging, par-

ticularly in women.

Virgin coconut oil (VCO) is an important cognitive nutritional supplement. It has been found to enhance baseline metabolic activity, while providing ongoing neuronal protection for the aging brain [14] [15]. Deranged brain glucose metabolism leads to diminished executive function, and has been linked to the cognitive decline of dementia. Ketones provide an important alternative to glucose, and have been shown to be valuable to renewed activity in aging brain. VCO has a high content of medium-chain fatty acids (MCTs) that can provide this alternative energy source for the brain [14], supporting its role as a dietary supplement to improve cognitive function [15]. Lauric acid is reported to provide most of the nutritional content and healing property of VCO and is a major component of the MCTs [16] [17]. MCTs are also easily metabolized by the liver to provide energy and are converted to ketones in astrocytes. Astrocytes provide the initial cellular barrier encountered by nutrients and other substances and there is evidence to suggest that they have a greater preference for fatty acids as their main energy source. VCO therefore potentially provides both cellular nutrition and neuroprotection, in a form which is easily accessible via the gastrointestinal tract [16] [17]. The MCTs may therefore be important for brain energy metabolism, producing ketone bodies which can be taken up by neurons to provide oxidative fuels during hypoglycemic and hypoxic states [18].

Like estrogen, VCO has properties against oxidative damage that are neuroprotective and may provide significant benefits to learning and memory. The aim of this study was therefore to investigate the effect of VCO supplementation on learning and memory in aging Sprague-Dawley rats that are transitioning normally between the cycling and non-cycling stages of their estrous cycle, to assess whether it has any beneficial impact on age-related cognitive decline.

2. Materials and Methods

Subjects

Twenty female *Sprague-Dawley* rats, 14 to 22 months old (starting ages: 10 - 18 months), completed the study. Rats were obtained from the animal house facility following institutional ethical approval of the protocol by The University of the West Indies (UWI), Mona Campus Ethics Research Committee (MREC). Rats were housed three per cage in a behavioral laboratory and provided with food and water *ad libitum*. Habituation, handling and other procedures were followed in accordance with the National Institutes for Health guidelines on the use and care of laboratory animals.

Each rat was weighed and coded at the start of the study, and its estrous cycle status determined twice weekly in the first 4 weeks and then daily from week 10. Recordings of their performance in the behavioral paradigm were collated with their estrous cycle status at the end of the study. During the VCO supplementation phases of the study, animals were allowed water *ad libitum* but rat chow was restricted over-night and provided in the morning. During the break periods in

which training ceased, all animals were provided with food and water *ad libitum.* Data collection was carried out blindly as treatment groups were unknown to the experimenter who recorded the behavioral data. Figure 1 outlines the organizational flow chart of the procedures followed.

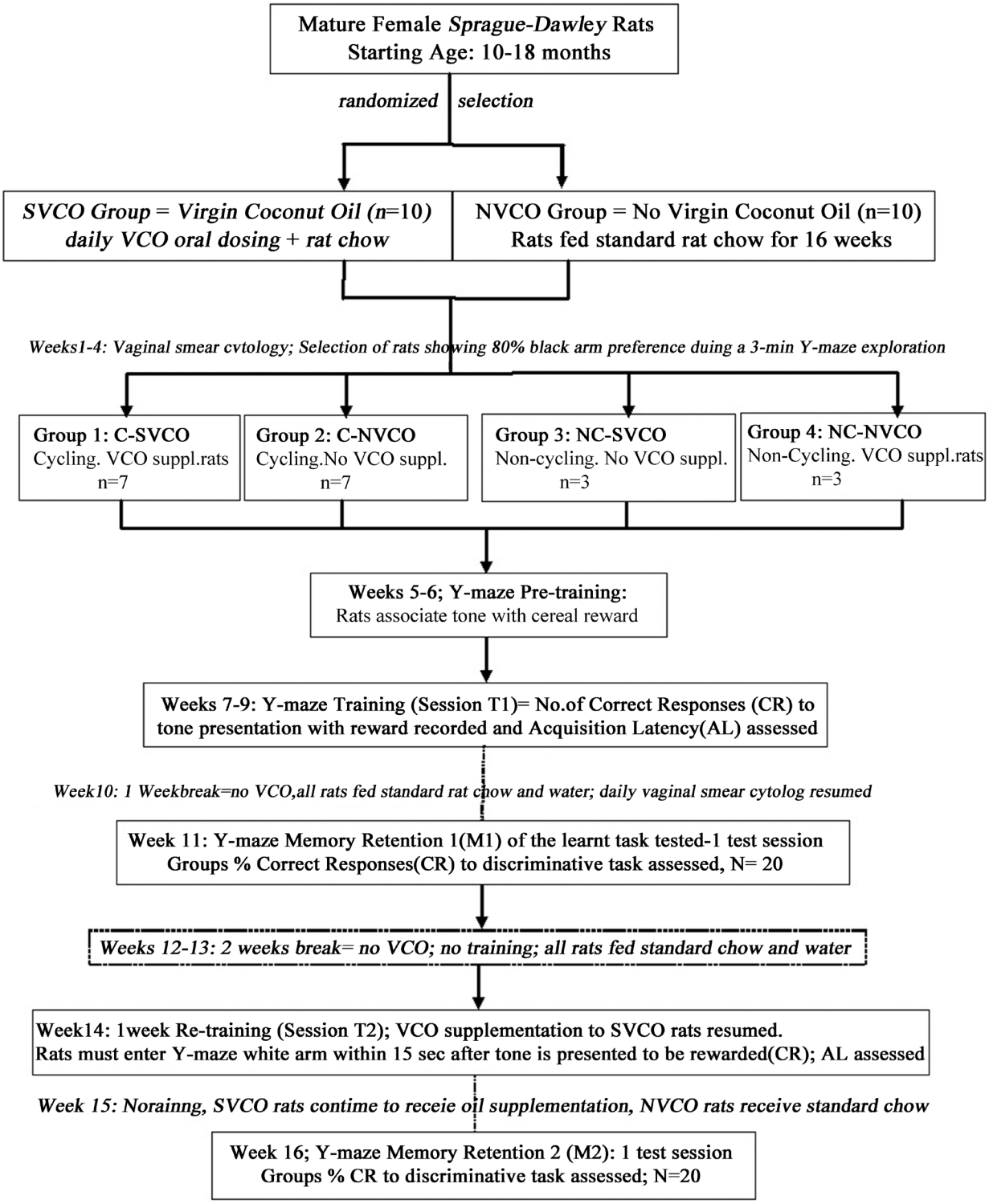


Figure 1. Organizational flow chart of the methodological procedures.

Virgin coconut oil administration

A locally produced and commercially available (Manufacturer: Michael Black Farms Ltd., Jamaica) 100% cold pressed virgin coconut oil (VCO) was used in this study. Animals were randomly divided into: 1) a VCO-treated (SVCO) group and 2) a non-VCO (NVCO) group representing the untreated controls. SVCO animals received (between 10:00 am and 12:00 noon) a daily oral dose of 1.42 mL/kg of the cold pressed VCO, which was calculated as an average of the dose range of 0.5 - 2.5 mL/kg recommended in humans [14] [15] [19]. NVCO untreated rats only had access to standard rat chow and water throughout the 16 weeks of the study.

Estrous cycle staging

Vaginal smear cytology according to the method of [20] was conducted on all animals by 12 noon on alternate days in weeks 1 to 4 and subsequently daily, starting from week 10. Vaginal smears were morphologically characterized based on all four phases of the 4-day cycle, namely, i) successively occurring proestrus, estrus, metestus and diestrus (*i.e.* regular cyclers); ii) there was a prolonged phase lasting 2 to 3 days (*i.e.* irregular cyclers); or iii) non-cycling, if there was a persistent estrus or diestrus or no successive change in any phase over 10 days, indicating an acyclic estrous cycle that was locked in phase (*i.e.* non-cyclers). There was no presumption of estrous status—that is, whether cycling or non-cycling, based on the chronological age of the rat.

Vaginal secretions were obtained by carefully restraining the rat and gently placing the tip of a plastic pipette, filled with 0.9% saline solution, into the vaginal opening. The vagina was then gently flushed three consecutive times with 10 μL of the same saline solution. Fluid collected from the final flush was placed on a glass slide and observed under a light microscope using the 10× magnified objective lens. Results from the final vaginal smear cytology were collated and used to classify the animals into their final respective cycling/non-cycling groups.

The Apparatus

A simple, wooden Y-maze was used to conduct the cue-directed, discrimination learning task according to a modified method of [21]. The Y-maze consisted of 3 symmetrical arms separated by 120˚ (dimensions: 38 cm long × 13 cm wide × 33 cm high) and differentiated by color, that is, each arm was painted black, white or grey. The grey arm represented the neutral or starting arm of the Y-maze.

The Discriminative Behavioral Paradigm

Habituation phase

Y-maze arm preference for each animal was determined at the beginning of the study in week 1. Each animal was placed individually in the grey arm and allowed 3 minutes to freely explore the maze. Arm preference was determined based on the frequency of entries into, and length of time spent in each arm. Rats that displayed a minimum 80% preference for the black arm based on these

indices, were selected for the study.

Pre-training

In weeks 5 and 6, each rat was confined to the non-preferred, white arm and trained to associate the presentation of a brief sound (tone) with a cereal treat (reward). Each rat was allowed to eat the treat within 15 sec after presentation of the tone or was removed from the arm unrewarded. Each animal received 4 trials daily between 2:00 pm and 5:00 pm over a period of 10 days.

Training

A 15-day training period followed pre-training in weeks 7 to 9. Each rat was individually placed in the grey arm and allowed to freely explore the maze for 1 min. In the subsequent 2 min, the tone was introduced and the rat was rewarded with a cereal treat only when all 4 paws entered the white arm within 15 sec of presentation of the tone. Each animal received 4 trials daily (between 2:00 pm and 5:00 pm). A correct response (CR) was recorded when the rat placed all 4 paws in the white arm within 15 sec after presentation of the tone, and was rewarded. An incorrect response meant that the animal entered the arm before the tone was presented or did not enter the arm, and was not rewarded. The number of days taken to learn to associate the tone with entry to the white arm to be rewarded was recorded. The 'learned' task was measured in terms of acquisition latency (AL), that is, the number of days taken to attain and maintain over a 5-day period, a minimum performance criterion of 75% CR.

*End of Session T*1: *Assessment of memory retention of the learned task with discontinuation of VCO supplementation*

Memory retention (MR1) of the learned task, defined as the number of correct responses performed by SVCO and NVCO animals, was assessed blindly in a single session on the first day of week 11. This was done following a 7-day break in week 10, during which animals received no further training and were provided only with standard rat chow and water *ad libitum.* After assessment of MR1 in week 11, animals received an additional 2-week break from training and were maintained on standard rat chow and water *ad libitum.*

*End of Session T*2: *Assessment of memory retention following resumption of VCO supplementation*

In week 14, daily VCO supplementation by oral gavage was reinstated for the SVCO rats and continued throughout weeks 15 and 16. NVCO control rats received only standard rat chow during this period. Both SVCO and NVCO animals also received an additional 1-week of re-training in the discriminative learning paradigm. The number of correct responses for each rat was recorded and AL determined. Following an additional 1-week break from training while maintaining SVCO animals on the daily oil supplement, memory retention (MR2) was again assessed in both SVCO and NVCO groups in week 16.

Data Analysis

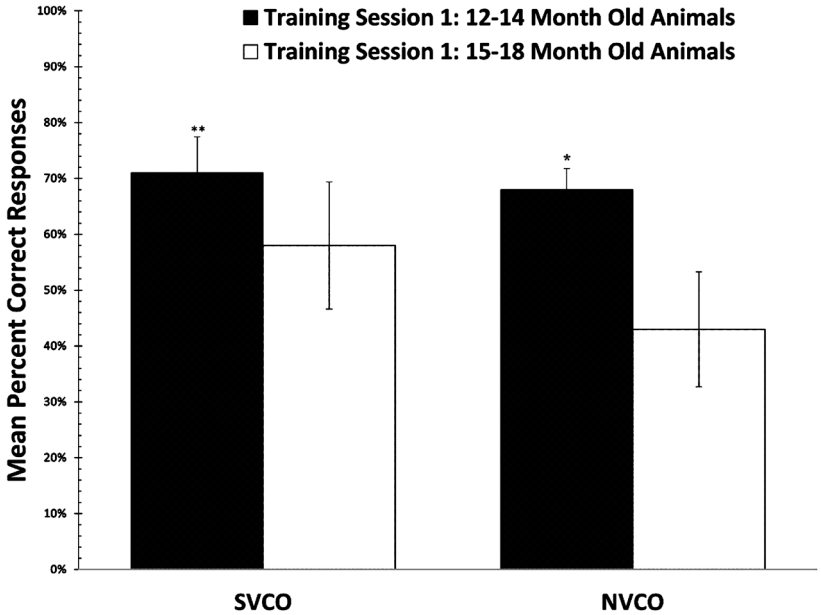
Data were analyzed using one-way Analysis of Variance (ANOVA) and Brown-Forsythe tests followed by the Tukey HSD post-hoc test (IBM SPSS for

Statistics Version 23). Student's t-test was also used to conduct pairwise comparisons of group means of the effect of VCO supplementation on the mean percent correct responses (CR), rate of acquisition of learning (AL), and memory retention (MR) between VCO supplemented cycling and non-cycling rats as well as their non-supplemented cycling and non-cycling counterparts. Results are presented as means ± SEM (standard error of the mean) with significance taken at $p \leq 0.05$

3. Results

*Effect of VCO Supplementation on Performance of Aging Rats at Training Session T*1

Chronologically, there were two aged cohorts of rats identified at the completion of training session T1, namely, rats that were 12 - 14 months old (≤14-mo-old) and 15-18 months old (≥15-mo-old). Both ≤ 14-mo-old SVCO and NVCO rats attained more correct responses (CR) on the discriminative task than did older rats. However, SVCO ≥ 15-mo-old rats had higher CR scores than their non-supplemented (NVCO) counterparts ($p < 0.05$) (Figure 2). Pairwise comparisons revealed significant increases in CR by SVCO ≤ 14-mo-old rats compared to NVCO ≥ 15-mo-old rats ($p = 0.04$). Also, NVCO ≤ 14-mo-old rats made significantly more CR than NVCO ≥ 15-mo-old rats ($p = 0.04$). In general, VCO supplementation improved the performance of older rats in the learning paradigm (Figure 2).



Values are expressed as mean percent correct responses (CR) ± S.E.M. *p = 0.04 compared to NVCO 15 - 18 months old rats.

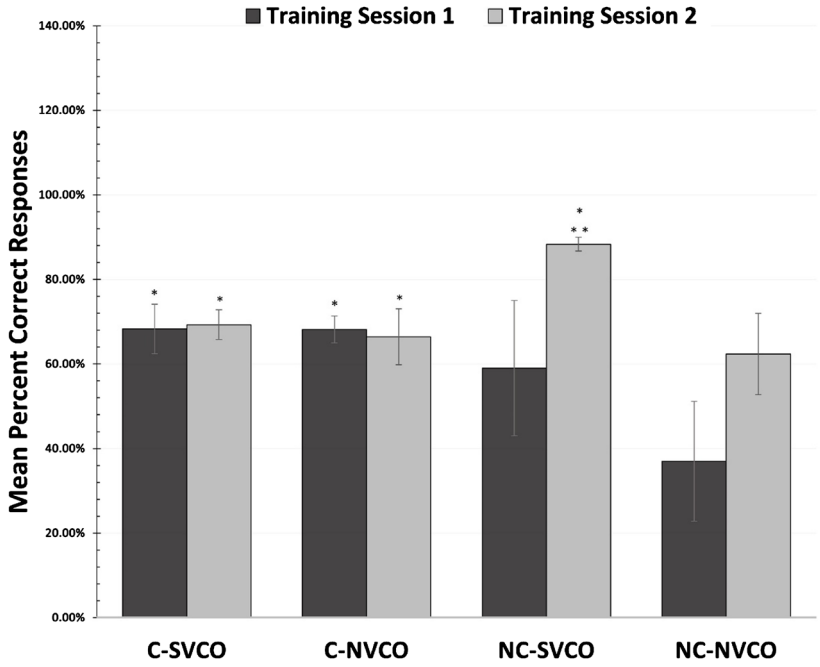
Figure 2. A comparison of the performances of virgin coconut oil supplemented (SVCO, n = 10) and non-supplemented (NVCO, n = 10), 12 - 14 and 15 - 18 months old female *Sprague-Dawley* rats following 3 weeks of training (sessions T1) in the discriminative learning paradigm (N = 20).

Reproductive Status versus Chronological Age of the Rats

At the end of week 4, results of the vaginal smear cytology revealed that 50% of the ≤14 mo-old (younger) rats were identified as regular cyclers and 50% as irregular cyclers. In contrast, 78% of the ≥15 mo (older) rats were identified as acyclic, while 22% were identified as irregular cyclers. By week 10 of the study, approximately 70% of rats irrespective of age, were identified as irregular cyclers and 30% as non-cyclers. Two rats in the ≤14-mo-old age cohort became acyclic and 4 rats in the ≥15-mo-old age cohort continued to show signs of irregular cycling. Thus, 14 rats classified as "cycling rats" and 6 rats as "non-cycling rats" completed the study. The unevenness in group numbers was as a result of the higher mortality rate among the non-cycling, older aged rats and the transition of two rats to the non-cycling group.

*Effect of VCO Supplementation on Performance during Training sessions T*1 *and T*2: *Cycling vs Non-Cycling Rats*

There was no difference in CR rates at sessions T1 and T2 between cycling, supplemented (C-SVCO) and non-supplemented (C-NVCO) aging rats (Figure 3). However, supplemented groups achieved significantly higher mean percent CR scores ($p < 0.05$) than did their non-cycling, non-supplemented (NC-NVCO) counterparts during the first training session T1. NC-NVCO rats at session T1 displayed the poorest performance, attaining the lowest mean percent CR (37%) score among the groups (Figure 3). During training session T2, non-cycling,



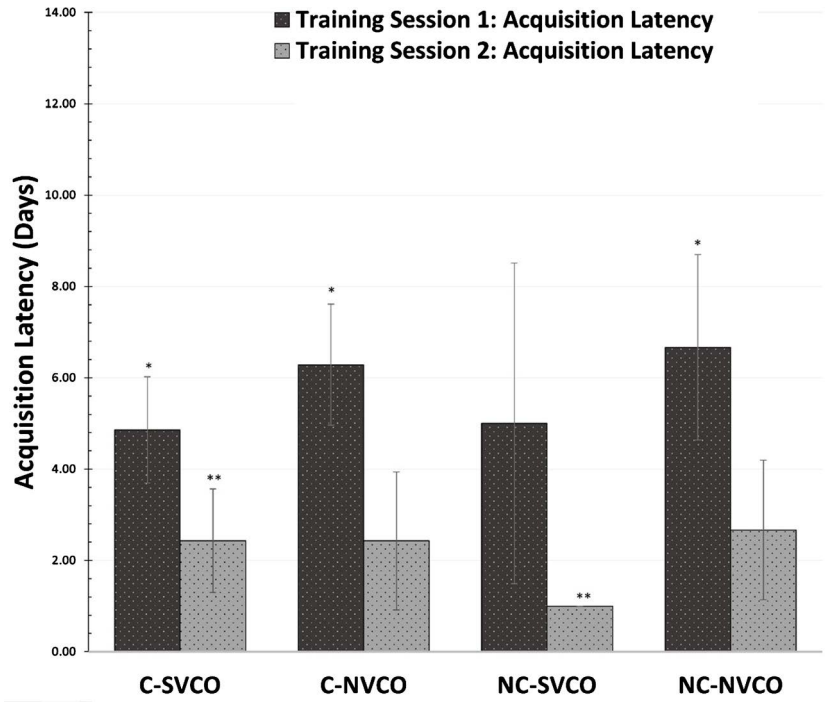
Values are expressed as mean percent CR ± S.E.M. F = 2.64. *p < 0.05 compared to NC-NVCO at T1. **p = 0.026 compared to NC-SVCO at T1.

Figure 3. A comparison of the correct responses (CR) at training sessions T1 and T2 among VCO supplemented cycling (C-SVCO) and non-cycling rats (NC-SVCO) and their non-supplemented cycling (C-NVCO) and non-cycling (NC-NVCO) counterparts. N = 20.

supplemented (NC-SVCO) rats achieved a mean 88.33% CR score, out-performing all other groups at $p < 0.048$ ($F = 2.64$). Additionally, pairwise comparison between training sessions T1 and T2 revealed that the NC-SVCO group achieved a significantly higher mean percent CR score ($p = 0.026$; $F = 2.64$) following re-training at session T2 (Figure 3).

Effect of VCO Supplementation on Acquisition Latencies during Training Sessions T1 and T2: Cycling vs Non-Cycling Rats

All groups attained lower ALs during re-training at session T2 than at training session T1 (Figure 4). During training session T1, VCO supplemented groups (C-SVCO and NC-SVCO) attained relatively lower AL scores than their non-supplemented counterparts. Non-supplemented rats regardless of estrous status (C-NVCO and NC-NVCO) performed similarly on the discriminative task at both sessions T1 and T2 (Figure 4). Pairwise comparisons revealed a significantly lower AL times for both supplemented cycling (C-SVCO) and non-cycling (NC-SVCO) groups than NC-NVCO at session T1 ($p < 0.05$). Of note, NC-SVCO rats attained the shortest AL time (*i.e.* 1 day) to maintain 75% CR following re-training during session T2, which was significantly lower than the AL time scores achieved by C-SVCO rats ($p = 0.014$), and both C-NVCO and NC-NVCO groups ($p < 0.021$) at session T1 (Figure 4). NC-NVCO rats also took significantly longer times to maintain a 75% CR during session T1 compared to



Values are expressed as mean AL ± S.E.M. $F = 2.38$. *$p < 0.05$ compared to NC-SVCO in T2. **$p < 0.05$ compared to NC-NVCO in T1.

Figure 4. A comparison of the acquisition latencies (days) for training sessions T1 and T2 among VCO supplemented cycling (C-SVCO) and non-cycling rats (NC-SVCO) and their non-supplemented cycling (C-NVCO) and non-cycling (NC-NVCO) counterparts. $n = 20$.

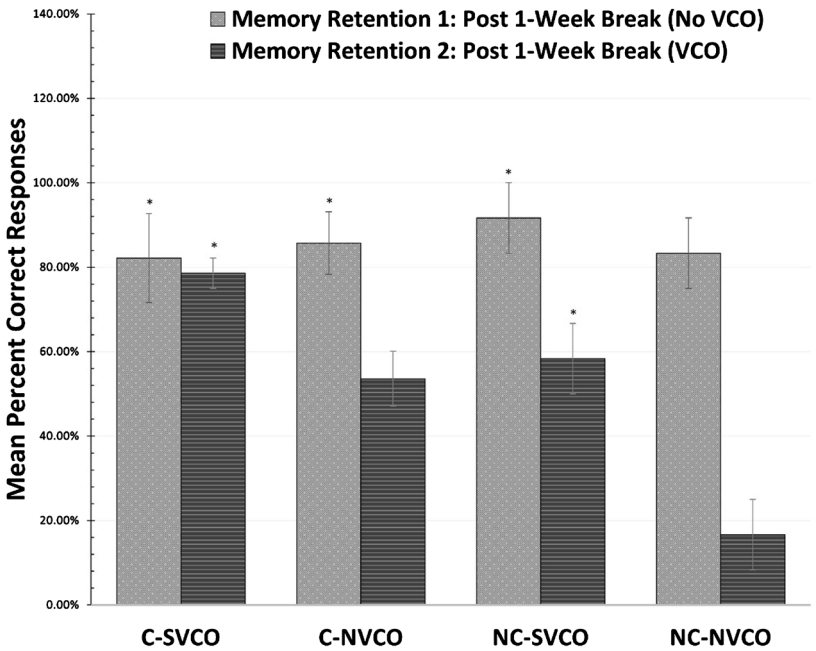
C-SVCO and NC-SVCO rats during re-training at session T2 ($p = 0.037$; $F = 2.38$).

On the final day of each training session, there was a fairly uniform performance of the learned task by all groups. However, C-SVCO rats relatively out-performed the other groups, attaining a 96.43% CR score; while the lowest performer was the NC-NVCO group that attained only the minimum 75% CR in the discriminative paradigm. ANOVA however revealed no significant difference in mean percent CR scores between groups on the final day of training sessions T1 and T2.

Effect of VCO Supplementation on Memory Retention of the Learned Task at Weeks 11 *and* 16: *Cycling vs Non-Cycling Rats*

Memory retention of the learned task was fairly consistent across all groups tested following the first 1 week break from training (MR1) with no significant difference between groups. However, assessment of memory of the learned task at MR2 revealed significantly higher CR scores achieved by C-SVCO, NC-SVCO as well as C-NVCO groups than obtained for the NC-NVCO group ($p < 0.05$; $F = 6.672$). Notably, NC-NVCO animals had the poorest recall and attained the lowest CR scores at MR2 ($p < 0.05$) following re-training (Figure 5).

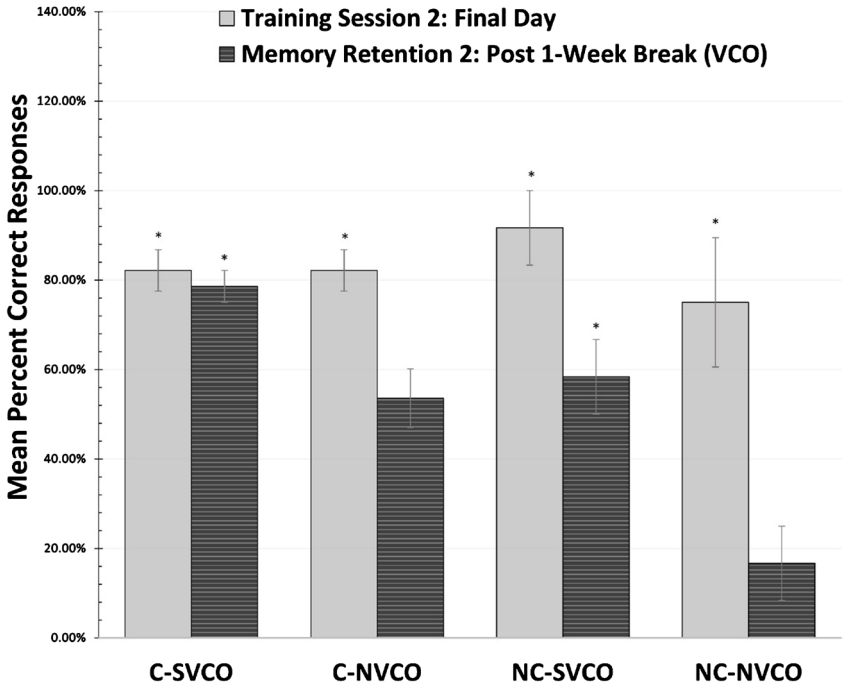
A Comparison of Performance on the Final Day of Re-training Sessions T2 and Memory Retention (MR2) of the Learned Task: Effect of Supplementation on Cycling vs Non-Cycling Rats



Values are presented as the mean percent CR ± S.E.M. $F = 6.672$. *$p < 0.05$: compared to NC-NVCO at M2.

Figure 5. A comparison of memory of the learned task at weeks 11 (Memory Retention 1) and 16 (Memory Retention 2) following a 1-week cessation of training. Virgin coconut oil supplementation was terminated at week 10 prior to testing at MR1 and was reinstated at week 14, prior to testing of MR2 (n = 20).

Performance in the discriminative task on the final day of re-training session T2 was approximately similar across all groups, with NC-SVCO rats demonstrating the highest CR scores. Assessment of memory retention (MR2) revealed that C-SVCO rats maintained a consistently high CR score on the discriminative task, while other groups demonstrated some extinction in memory of the learned task (Figure 6). Notably, NC-SVCO rats performed equally well on the memory retention test as C-NVCO rats, but attained a significantly higher CR score than NC-NVCO rats ($p < 0.05$). That is, non-cycling, non-supplemented rats attained the lowest mean percent CR score and exhibited the highest degree of memory extinction of the learned task after re-training as compared with all other groups ($p < 0.05$; $F = 10.304$).



Values are presented as mean percent CR ± S.E.M. $F = 10.304$. *$p < 0.05$ compared to M2 for NC-NVCO.

Figure 6. A comparison of the mean percent correct responses of groups on the final day of training session T2 and memory retention of the learned task (M2) following 3 weeks of VCO reinstated supplementation (N = 20).

4. Discussion

This study aimed to determine the effect of virgin coconut oil (VCO) dietary supplementation on discriminative learning and working memory in aging female rats that were transitioning normally between the cycling and non-cycling phases of the estrous cycle. The results show a positive effect of VCO treatment on the acquisition latency (AL) and memory retention (MR) of the discriminative-learned test, especially among the older, non-cycling (NC-SVCO) rats that were among the 15 months and older cohort.

Chronological age alone however, is not an accurate measure to determine the

integrity of the hypothalamic-pituitary axis and reproductive status in rodents. Humans are known to enter menopause at varying ages, before or after the average age of 51 years [22] [23]. This is equivalent to between 15 and 20 months in female rats entering reproductive senescence, a state similar to menopause in women [7]. Circulating levels of estrogen, the hormone that is neuroprotective to learning and memory function [24] [25] [26] [27] [28] are normally low in both humans experiencing menopause and rodents in reproductive senescence. In this study, aging rats were therefore grouped according to whether they were transitioning between regular and irregular "cycling", and those that had become acyclic or "non-cycling". During the first training phase for acquisition of the new task, the "cycling" animals performed at a higher correct response rate, which validates the methodological baseline of our laboratory. Data also revealed that VCO supplementation starting prior to, and maintained during the non-cycling period, significantly improved learning outcome and memory retention in these acyclic rats. This was further highlighted by the significantly poorer performance in acquisition latency of the discriminative task by untreated, non-cycling, (NC-NVCO) rats following retraining in week 14 and memory retention of the task in week 16 (**Figure 4** & **Figure 6**).

The ovarian cycle confers vulnerability on learning and memory functions of the female brain after the loss of circulating estrogen. This was clearly demonstrated in this study, as it was accompanied by the observation that non-cycling animals that had received VCO demonstrated similar performance on the discrimination-learning test as their cycling counterparts (**Figure 3** & **Figure 4**). Additionally, VCO supplementation significantly improved retention of the memory of the previously learned task among these non-cycling rats (**Figure 5** & **Figure 6**). In contrast, non-cycling (NC-NVCO) rats that were only fed standard rat chow were the poorest performers on the discrimination-learning test. They took longer to learn the task and exhibited a faster rate of extinction of memory of the learned task than all other groups (**Figure 6**). Our results also show that notwithstanding their ovarian status, and even after the weeks without supplementation with VCO, the NC-SVCO group performed significantly better than their cycling counterparts that had not received the oil supplement (C-NVCO). The NC-SVCO group also out-performed the NC-NVCO group on all parameters. Additionally, whereas the data shown in AL1 provided evidence that VCO-treated groups acquired 75% correct responses more quickly, information revealed by AL2 showed the significant role of VCO-treatment for both cycling and non-cycling animals upon retraining after a period of non-training and removal from the paradigm (**Figure 4**), and the expected extinction of memory of the discriminative learned task that would naturally ensue. At session T2, the fastest learners were surprisingly the NC-SVCO group, a result that was associated strongly with the administration of VCO. The NC-NVCO group were indeed the slowest learners in both training sessions and exhibited the highest rate of extinction of the memory of the learned task even after re-training.

In this study, we have presented evidence that VCO enhanced learning and memory retention in aging, cycling and non-cycling animals. This presents a new opportunity for the protection of the female from age-related decline in executive function. Commencement of VCO therapy prior to the onset of menopause and maintained during the menopausal transition may therefore improve brain health. Studies conducted in humans and rodents with varying levels of cognitive impairment have linked increased levels of ketones in the plasma with improved memory performance [17] [29] [30]. VCO can provide the ketones that can avert decreases in brain glucose metabolism, correct hypometabolism, and thus maintain brain health during the aging process. VCO also contains phenolic compounds which have been shown to improve cholinergic activity and enhance memory retention [31] which support the conclusions of this study. VCO and estrogen both have antioxidant properties which play essential roles in neuroprotection [17] [28] [32]. Estrogen contains a phenolic hydroxyl group on the A-ring of its chemical structure which is thought to provide its antioxidant effects [28]. The removal of this group, and hence the reduction or absence of estrogen, is thought to eliminate the antioxidant effects which leads to the oxidative damage in brain tissues. Similarly, VCO contains compounds such as squalene, polyphenols, sterols and tocopherols which are potent anti-oxidative agents [28] [32]. This is akin to the mechanism of action of *Ginko biloba* extract, which similarly exhibited potent mitigation of age-related cognitive decline and impairment via reduction of the quantity of reactive oxygen species [33]. Oxidative stress is thought to be a part of the normal aging process, thus making the combined effects of estrogen and VCO supplementation significant factors to achieve cognitive enhancement.

5. Conclusion

This work presents evidence which may impact not only women experiencing age-related cognitive decline, but who have undergone oophorectomy, as it opens up memory-enhancing therapy relevant to the younger female. The data revealed a beneficial effect of VCO supplementation on discriminative learning and working memory function in aging, cycling and non-cycling rodents. This finding supports the moderate dietary use of virgin coconut oil to improve brain health in women during the menopausal transition.

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Author Disclosure Statement

The authors declare that no competing financial interest exists.

References

[1] Pike, C.J. (2017) Sex and the Development of Alzheimer's Disease. *Journal of Neuroscience Research*, **95**, 671-680. https://doi.org/10.1002/jnr.23827

[2] Merlo, S., Spampinato, S.F. and Sortino, M.A. (2017) Estrogen and Alzheimer's Disease: Still an Attractive Topic Despite Disappointment from Early Clinical Results. *European Journal of Pharmacology*, **817**, 51-58. https://doi.org/10.1016/j.ejphar.2017.05.059

[3] Yin, F., Yao, J., Sanchetti, H., Feng, T., Melangi, R.C., Morgan, T.E., Finch, C.E., Pike, C.J., Mack, W.J., Cadenas, E. and Brinton, R.D. (2015) The Perimenopausal Aging Transition in the Female Rat Brain: Decline in Bioenergetic Systems and Synaptic Plasticity. *Neurobiology of Aging*, **36**, 2282-2295. https://doi.org/10.1016/j.neurobiolaging.2015.03.013

[4] Westwood, F.R. (2008) The Female Rat Reproductive Cycle: A Practical Histological Guide to Staging. *Toxicologic Pathology*, **36**, 375-384. https://doi.org/10.1177/0192623308315665

[5] Paccola, C., Resende, C., Stumpp, T., Miraglia, S. and Cipriano, I. (2013) The Rat Estrous Cycle Revisited: A Quantitative and Qualitative Analysis. *Animal Reproduction*, **10**, 677-683.

[6] Marcondes, F., Bianchi, F. and Tanno, A. (2002) Determination of the Estrous Cycle Phases of Rats: Some Helpful Considerations. *Brazilian Journal of Biology*, **62**, 609-614. https://doi.org/10.1590/S1519-69842002000400008

[7] Sengupta, P. (2013) The Laboratory Rat: Relating Its Age with Human's. *International Journal of Preventive Medicine*, **4**, 624-630.

[8] Frye, J.B. Lukefahr, A.L., Wright, L.E., Marion, S.L., Hoyer, P.B. and Funk, J.L. (2012) Modeling Perimenopause in Sprague-Dawley Rats by Chemical Manipulation of the Transition to Ovarian Failure. *Comparative Medicine*, **62**, 193-202.

[9] Huang, H. and Meites, J. (1975) Reproductive Capacity of Aging Female Rats. *Neuroendocrinology*, **17**, 289-295. https://doi.org/10.1159/000122367

[10] Peluso, J., Steger, R., Huang, H. and Meites, J. (1979) Pattern of Follicular Growth and Steroidogenesis in the Ovary of Aging Cycling Rats. *Experimental Aging Research*, **5**, 319-333. https://doi.org/10.1080/03610737908257208

[11] Vom Saal, F.S., Finch, C.E. and Nelson, J.F. (1994) Natural History and Mechanisms of Reproductive Aging in Humans, Laboratory Rodents, and Other Selected Vertebrates. *The Physiology of Reproduction*, **2**, 1213-1314.

[12] Burger, H. (2008) The Menopausal Transition—Endocrinology. *The Journal of Sexual Medicine*, **5**, 2266-2273. https://doi.org/10.1111/j.1743-6109.2008.00921.x

[13] Prior, J.C. and Hitchcock, C.L. (2011) The Endocrinology of Perimenopause: Need for a Paradigm Shift. *Frontiers in Bioscience* (*Scholar Edition*), **3**, 474-486. https://doi.org/10.2741/s166

[14] Fernando, W.M., Martins, I.J., Goozee, K., Brennan, C.S., Jayasena, V. and Martins, R.N. (2015) The Role of Dietary Coconut for the Prevention and Treatment of Alzheimer's Disease: Potential Mechanisms of Action. *British Journal of Nutrition*, **114**, 1-14. https://doi.org/10.1017/S0007114515001452

[15] De la Rubia Ortí, J.E., AM, B.C., Rochina, M. and Yang, H. (2017) How Does Co-

conut Oil Affect Cognitive Performance in Alzheimer Patients? *Nutricion Hospitalaria*, **34**, 352-356. https://doi.org/10.20960/nh.780

[16] Krishna, A.G., Gaurav, R., Singh, B.A., Kumar, P.P. and Preeti, C. (2010) Coconut Oil: Chemistry, Production and Its Applications—A Review. *Indian Coconut Journal*, **53**, 15-27.

[17] Rahim, N.S., Lim, S.M., Mani, V., Abdul Majeed, A.B. and Ramasamy, K. (2017) Enhanced Memory in Wistar Rats by Virgin Coconut Oil Is Associated with Increased Antioxidative, Cholinergic Activities and Reduced Oxidative Stress. *Pharmaceutical Biology*, **55**, 825-832. https://doi.org/10.1080/13880209.2017.1280688

[18] Nonaka, Y., Takagi, T., Inai, M., Nishmura, S., Urashima, S., Honda, K., Aoyama, T. and Terada, S. (2016) Lauric Acid Stimulates Ketone Body Production in the KT-5 Astrocyte Cell Line. *Journal of Oleo Science*, **65**, 693-699. https://doi.org/10.5650/jos.ess16069

[19] Nurul-Iman, B.S., Kamisah, Y., Jaarin, K. and Qodriyah, H.M.S. (2013) Virgin Coconut Oil Prevents Blood Pressure Elevation and Improves Endothelial Functions in Rats Fed with Repeatedly Heated Palm Oil. *Evidence-Based Complementary and Alternative Medicine*, **2013**, Article ID: 629329. https://doi.org/10.1155/2013/629329

[20] Caligioni, C.S. (2009) Assessing Reproductive Status/Stages in Mice. *Current Protocols in Neuroscience*, **48**, A.4I.1-A.4I.8. https://doi.org/10.1002/0471142301.nsa04is48

[21] Lamptey, M.S. and Walker, B.L. (1976) A Possible Essential role for Dietary Linolenic Acid in the Development of the Young Rat. *The Journal of Nutrition*, **106**, 86-93. https://doi.org/10.1093/jn/106.1.86

[22] Gold, E.B. (2011) The Timing of the Age at which Natural Menopause Occurs. *Obstetrics and Gynecology Clinics*, **38**, 425-440. https://doi.org/10.1016/j.ogc.2011.05.002

[23] Delavar, M.A. and Hajiahmadi, M. (2011) Factors Affecting the Age in Normal Menopause and Frequency of Menopausal Symptoms in Northern Iran. *Iranian Red Crescent Medical Journal*, **13**, 192-198.

[24] Warren, S.G., Humphreys, A.G., Juraska, J.M. and Greenough, W.T. (1995) LTP Varies Across the Estrous Cycle: Enhanced Synaptic Plasticity in Proestrus Rats. *Brain Research*, **703**, 26-30. https://doi.org/10.1016/0006-8993(95)01059-9

[25] Foy, M., Xu, J., Xie, X., Brinton, R., Thompson, R. and Berger, T. (1999) 17β-Estradiol Enhances NMDA Receptor-Mediated EPSPs and Long-Term Potentiation. *Journal of Neurophysiology*, **81**, 925-929. https://doi.org/10.1152/jn.1999.81.2.925

[26] Romeo, R.D., McCarthy, J.B., Wang, A., Milner, T.A. and McEwen, B.S. (2005) Sex Differences in Hippocampal Estradiol-Induced N-Methyl-D-Aspartic Acid Binding and Ultrastructural Localization of Estrogen Receptor-Alpha. *Neuroendocrinology*, **81**, 391-399. https://doi.org/10.1159/000089557

[27] Kramár, E.A. Chen, L.y., Brandon, N.J., Rex, C.S., Liu, F., Gall, C.M. and Lynch, G. (2009) Cytoskeletal Changes Underlie Estrogen's Acute Effects on Synaptic Transmission and Plasticity. *Journal of Neuroscience*, **29**, 12982-12993. https://doi.org/10.1523/JNEUROSCI.3059-09.2009

[28] Pourganji, M., Hosseini, M., Soukhtanloo, M., Zabihi, H. and Hadjzadeh, M.A.-R. (2014) Protective Role of Endogenous Ovarian Hormones against Learning and Memory Impairments and Brain Tissues Oxidative Damage Induced by Lipopolysaccharide. *Iranian Red Crescent Medical Journal*, **16**, e13954. https://doi.org/10.5812/ircmj.13954

[29] Reger, M.A., Henderson, S.T., Hale, C., Cholerton, B., Baker, L.D., Watson, G.S., Hyde, K., Chapman, D. and Craft, S. (2004) Effects of β-Hydroxybutyrate on Cognition in Memory-Impaired Adults. *Neurobiology of Aging*, **25**, 311-314. https://doi.org/10.1016/S0197-4580(03)00087-3

[30] Krikorian, R., Shidler, M.D., Dangelo, K., Couch, S.C., Benoit, S.C. and Clegg, D.J. (2012) Dietary Ketosis Enhances Memory in Mild Cognitive Impairment. *Neurobiology of Aging*, **33**, 425.e19-425.e27. https://doi.org/10.1016/j.neurobiolaging.2010.10.006

[31] Marina, A., Che Man, Y., Nazimah, S. and Amin, I. (2009) Antioxidant Capacity and Phenolic Acids of Virgin Coconut Oil. *International Journal of Food Sciences and Nutrition*, **60**, 114-123. https://doi.org/10.1080/09637480802549127

[32] Abujazia, M.A., Muhammad, N., Shuid, A.N. and Soelaiman, I.N. (2012) The Effects of Virgin Coconut Oil on Bone Oxidative Status in Ovariectomised Rat. *Evidence-Based Complementary and Alternative Medicine*, **2012**, Article ID: 525079. https://doi.org/10.1155/2012/525079

[33] Gruenwald, J., Eckert, A. and Kressig, R.W. (2020) The Effects of Standardized Ginkgo Biloba Extracts (GBE) on Subjective Cognitive Decline (SCD) in Middle-Aged Adults: A Review. *Advances in Aging Research*, **9**, 45-65. https://doi.org/10.4236/aar.2020.93005