The Effects of Ginkgo Biloba and Docosahexaenoic Acid on the Memory of Mice

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Abstract

This study examined the effects of two memory enhancers, Ginkgo biloba and docosahexaenoic acid (DHA) on mice. Ten mice received Ginkgo biloba, another ten received DHA, while a third group of ten mice served as the control. Each mouse was placed in a maze in order to observe his/her performance. Performance was measured as the time to run the maze and the number of deadends encountered. It was hypothesized that all groups would improve in maze performance, but anticipated that the control group would learn at a slower rate than mice receiving Ginkgo biloba or DHA. No significant difference was found between treatments; however, it appeared that DHA may be more effective in improving mouse memory than Ginkgo biloba. Therefore, an additional study was performed to determine if higher DHA concentrations had varying effects on memory performance.

In the second study, ten mice received 1/8 the human dosage of DHA, another ten received 1/10, a third group of ten received 1/20, and a fourth set of ten received 1/40 the human dosage of DHA. Another group of ten mice served as the control. The groups consisted of both old and young mice in order to determine the effects of different DHA concentrations on age. Each mouse completed two trials on each of three different mazes, in order to test both the ability to learn a new maze and the ability to learn a previously seen maze. It was anticipated that the group receiving the highest DHA concentrations would show a slight improvement in time and the number of wrong turns, but the change would not be as significant and drastic as that of the mice

receiving the lower concentrations. It was expected that the control group would also learn the maze and improve with each trial; however, it was anticipated that they would learn at a slower rate than the mice receiving DHA supplements. It was also hypothesized that the young mice would show greater signs of memory improvement as a result of the DHA. Treatment had a significant effect on the learning of new mazes (trials 1.1, 2.1, and 3.1). Age and treatment also had a significant interaction in several trials. Both old mice and young rnice were positively affected by the DHA supplements; however, the young mice seemed to show signs of greater memory improvement as a result of receiving DHA.

Introduction

The first part of this study examined the effects of two types of memory enhancers, Ginkgo biloba and docosahexaenoic acid, on mice. Docosahexaenoic acid (DHA), is one of the chemicals found in fish oils. DHA is an Omega 3 long chain fatty acid that is commonly found in red meats, animal organs, and eggs, as well as the human brain. DHA is a nutritionally important polyunsaturated fatty acid that has many positive benefits (Wander 1998). DHA is one of the primary building blocks of the brain and is important in brain development in infants. "Brain phospholipids are very rich in DHA, with ~20% of phospholipid fatty acids being DHA," (Ellis, et. al. 1990). DHA has been linked to memory and visual functions, and previous studies have found it to be a key factor in maintaining optimal memory performance. Studies have shown that "high levels of DHA in the brain are associated with optimal brain function--from mental sharpness to memory to mood regulation..." (Epstein 2001). Ginkgo biloba is one of the most widely used herbal drugs on the market. It is believed that more than 10 million Americans are now taking Ginkgo biloba and that the total sales of the herb exceeds $100 million (Glisson, et al. 1999). While Ginkgo biloba has been shown to improve concentration and short-term memory in people with impaired blood flow to the brain, there are still differing opinions as to whether or not it is as effective in people who do not have an impaired blood flow (Tufts University Health & Nutrition Letter 1997).

It is a common belief that eating fish and taking Ginkgo biloba supplements can increase one's memory. Due to the conflicting reports, however, it was of particular interest to perform this study with the hopes of understanding the effects of Ginkgo

biloba on memory in mice. Understanding the effects of DHA on memory is also important because studies have shown that this chemical in fish oil is beneficial to improving memory, therefore, leading many people to believe that by eating fish, they can increase and enhance their memory capabilities. DHA tends to be related to and incorporated in the development of memory functions, whereas Ginkgo biloba is generally used for impaired memory; it is used to strengthen one's memory. Therefore, after researching the possible effects of the chemicals and analyzing past studies, it is hypothesized that mice under the influence of Ginkgo biloba and/or DHA will show signs of an increased memory and learning ability. I expect that the time that it takes the mice receiving the Ginkgo biloba and the DHA treatments to complete the maze will decrease after subsequent trials. I also hypothesize that the treated mice will make fewer wrong turns before reaching the end of the maze. I anticipate that the control group will show a slight improvement in time and the number of wrong turns, but the change will not be as significant and drastic as that of the treated mice. In other words, it is hypothesized that the control group will learn at a slower rate than the groups receiving Ginkgo biloba and DHA. A similar study performed by Gajewski and Hensch in which Ginkgo biloba was given to mice, found equivalent results: "The mice showed an improved memory for the maze as evidenced by a decrease in the number of errors in reaching the goal box when they received ginkgo biloba as a dietary supplement" (1999). Although DHA was not used in this study, the results indicate that memory enhancers such as Ginkgo biloba may be effective in improving a mouse's memory capabilities. Due to the results of similar tests, it is expected that ingesting

Ginkgo biloba and DHA will improve the mice's memory and will therefore, increase their performance in a maze. I also hypothesize that both males and females will be affected equally by both Ginkgo biloba and DHA.

This study also examined the effects of different doses of DHA on memory performance. DHA has been found to have antithrombotic, antivasorestrictive, antihypertensive, and antiarrhythmic influences, leading to a decrease in cardiovascular disease risk (Wander 1998). Fatty acids like DHA also have anti-flammatory properties, improve skin-lesions (psoriasis), have beneficial effects on ulcerative colitis, and appear to decrease the number and size of tumors and impede their growth in cancer studies with animal models (1998). The effects of DHA on the brain have been examined for many years. DHA is essential for the normal functional development of the brain, and along with arachidonic acid, it is one of the predominant structural fatty acids in the gray matter of the brain (1998). It is of interest to test the effects of DHA on mice of various ages, because studies have shown that DHA is crucial to the developing brains of infants. "Human brain growth takes place from the 25th week of gestation until two years after birth. During this period, DHA and arachidonic acid are supplied by the placenta *in utero* and in the diet after birth," (1998). Studies on DHA have shown that it may have an impact on human intelligent. "Infants fed (n-3) fatty acids later demonstrated better cognitive development than those not fed these fatty acids. One study measured the IQ of eight-year old children who had been given, as premature infants, (n-3)-poor formula or human milk. Those who received breast milk [breast milk contains DHA] had scores 8 point higher," (1998). Until recently, the FDA, despite its

use in more than 60 countries, did not approve the addition of DHA to infant formula. However, in May 2001, the FDA approved the addition of the nutritional oils to infant formulas in the United States (Chea 2001).

Visual functions are also positively affected by DHA. Past studies on low birth weight or preterm infants suggest that DHA is essential for neural and visual functions; better visual acuity and mental development were displayed in preterm infants supplemented with DHA (Wasantwisut 1997). Therefore, in this study, various dosages of DHA were administered to mice of varying age and sex in order to determine if different compound concentrations have varying affects on mice memory capabilities. It is hypothesized that the DHA treatments will be more effective in improving memory functions of younger mice. The DHA concentrations were 1/8, 1/10, 1/20, and 1/40 of the human dosage. Previous studies with lab mice have revealed that the intake of DHA improves maze-learning ability, however, it is believed that it may take time after the incorporation of DHA into the brain for any actual improvement in memory to appear (Lim and Suzuki 2001). Therefore, I hypothesize that if given a daily treatment, mice under the influence of DHA will show signs of an increased memory and learning ability. I anticipate that the lower the concentration (within the set limits), the more effective it will be in increasing memory and learning skills. This is anticipated because even though all the concentrations are extremely large doses for mice (in comparison to humans), I expect that the mice will be able to more effectively utilize the smaller concentrations of DHA. The higher the dose, the more toxic it may be to the mouse. However, a dosage of 1/8 the human dose should not have averse effects,

because studies have shown that the "Administration of DHA at 300 mg/kg/day over 10 weeks caused a significant increase in the value of DHA in the cerebral cortex and the hippocampus, indicating that high-dose administration of DHA extending over a long period may induce an increase in brain DHA content in weaned rats," (Gamoh, et. al. 1999). Therefore, it may be possible for mice under all treatments to utilize the DHA; however, I anticipate that the mice receiving the smaller concentrations of DHA will show greater signs of increased memory performance. It is expected that the time that it takes the mice to complete the maze will decrease after subsequent trials. It is also hypothesized that the mice will make fewer wrong turns before reaching the end of the maze. It is anticipated that the group receiving the highest of the DHA concentrations will show a slight improvement in time and the number of wrong turns, but the change will not be as significant as that of the mice receiving the lower concentrations. It is expected that the control group will also learn the maze and improve with each trial; however, it is anticipated that they will learn at a slower rate than the mice receiving DHA supplements.

Methods

Experiment #1

Thirty white laboratory mice were used for this experiment. The mice were divided into three groups of ten. Each group consisted of five females and five males. The first group did not receive any special treatment and was therefore, deemed the control. The second group received treatments of Ginkgo biloba and the third group received treatments of DHA. The initial plan was to orally inject the mice with the medications twice a week by using a diluted mixture of the medicine in water. The mixture was fed to the mice via a syringe. Unfortunately, this method was unsuccessful. The mice refused to ingest any of the medication through this technique. Therefore, an alternative method was adopted. The mice received the DHA or Ginkgo biloba mixed with a small amount of peanut butter.

The control group received a biweekly dosage of 0.1 grams of a peanut butter mixture, consisting of 75 percent peanut butter and 25 percent flour. The DHA group received a biweekly dosage of a 0.1 grams peanut butter mixture; however, their mixture contained the chemical DHA (10 mg), along with the 3:1 ratio of peanut butter and flour. The Ginkgo biloba group also received a biweekly dosage of 0.1 grams peanut butter mixture; however, their mixture contained 6 mg of Ginkgo biloba along with the 3:1 ratio of peanut butter and flour. The dosage of the Ginkgo biloba and DHA given to the mice was the equivalent of 1/10 of the human dosage. The Freshlife Ginkgo biloba extract contained 24% Ginkgoflavonglycosides and 6% Terpene

Lactones. The Solaray DHA Neuromines dietary supplement was in the form of 100 mg softgel capsules. The 100 mg of DHA came from Microalgae Vegetable Oil. The supplements were bought at a local vitamin supplier, Freshlife, located in Williamsport, Pennsylvania. Both supplements were used as received. The mice were treated twice a week, with a 48-hour period between treatments.

Approximately 24 hours after the second treatment, the mice were placed in a maze to evaluate their performance. The maze was a standard maze for hamsters, gerbils, and mice, and was bought at a local pet store. Before placing the mice in the maze, a small amount of peanut butter was placed at the end of the maze. The peanut butter provided the mice with the motivation to reach the end of the maze. The mice were evaluated based on the number of dead ends they encountered and the total time it took them to complete the maze (up to five minutes). If the mouse reached the opposite end of the maze (the goal) within five minutes, it completed the maze and was rewarded with a small amount of peanut butter. A standard stopwatch was used to record the time for each trial. There were five trials over the course of five weeks. Each trial lasted one week. The mice were treated twice a week and then placed in the maze 24 hours after the second treatment. Therefore, all 30 mice ran the maze a total of five times. At the end of the experiment all of the data was tallied and statistically analyzed in order to determine if the groups receiving Ginkgo biloba and DHA had better memory performance than the control group. SPSS was used for the statistical analysis and Multivariate and One-Way ANOVA tests were performed.

Experiment #2

Fifty laboratory mice were used in this part of the study. The mice were divided into five groups of ten. Each group of ten rnice received a different treatment of DHA. Each group consisted of five older mice and five young. Old mice were classified as mice over the age of six months. Mice between the ages of four weeks and six months were classified as young mice. The gender of the mouse was not taken into consideration and therefore, the mice were a random rnixture of both males and females. The mice were all kept in individual cages, which were cleaned and supplied with fresh water and food on a regular basis.

The control group received a dosage of 0.1 grams of a peanut butter mixture, consisting of 66.6 percent peanut butter and 33.3 percent flour. The 1/8 DHA group received a dose of 0.1 grams of the peanut butter mixture along with 12.5 mg of DHA (1/8 the human dosage). The 1/10 DHA group also received a dosage of 0.1 grams peanut butter mixture; however, their mixture contained 10 mg of DHA. The 1/20 DHA group received 0.1 grams of the peanut butter mixture along with 5 mg of DHA. Finally, the 1/40 DHA group received 0.1 grams of the peanut butter mixture along with 2.5 mg of DHA.

Treatment was given daily for approximately three months. The researchers gave each mouse 0.1 grams of the corresponding mixture every day at about the same time. The peanut butter ball was placed in the cage on a clean watch glass. In addition to the treatment, the mice were given one nugget of mouse/rat chow. Only one nugget of dry

food was given so that the mice would be encouraged to eat the peanut butter treatment. All mice appeared very eager to eat the peanut butter treatments and usually ate the mixture within five minutes. At the end of the first two weeks of treatment, each mouse was placed in a maze to evaluate his or her performance. A small quantity of peanut butter was placed at the end of the maze. The peanut butter was used to provide the mouse with the motivation to reach the end of the maze. The time that it took the mouse to go from start to finish was recorded, and the number of wrong turns into dead ends was counted. After running the maze, the mice were placed back in their individual cages and given that day's treatment.

There were three different mazes used for this experiment. Each mouse ran each maze twice. Different mazes were used to test for the ability to learn a new stimulus (maze), and the mice ran two successive trials on each maze to test the learning of a previously seen maze. Mice were given two weeks of continuous treatment before any mazes were run so that the DHA treatments could begin to accumulate in their systems. Therefore, after the first two weeks of treatment, the mice were placed in maze #1 (trial 1) and all data was recorded. Seventy-two hours later, the mice were again placed in maze #1 (trial 2), and all data was recorded. One week later, the mice were tested in maze #2 (trial 1); 72 hours later, they ran maze #2 for a second time (trial 2). Approximately one week later, the mice were placed in maze # 3 for the first trial. Seventy-two hours later, the mice were again tested in maze # 3 (trial 2). The trials were labeled as trials 1.1, 1.2, 2.1, 2.2, 3.1, and 3.2, where the first number

signifies the maze number and the second digit identifies the trial number. Therefore, all 50 mice completed a total of six trials, two trials in each of three different mazes. For each trial, the number of deadends and the total time it took the mouse to complete the maze were documented. At the end of the experiment all data was tallied and run through statistical analysis from which conclusions were made. SPSS was used for the statistical analysis and both Multivariate and One-Way ANOVA tests were performed.

Results

Experiment #1

A multivariate statistical analysis (Table 1) shows the effects the various treatments (DHA or Ginkgo biloba), mouse gender, and the trial number had on the number of deadends, time, and whether or not the mouse completed the maze. The fixed factors were treatments, sex, and trial, and the dependent factors were deadends, time, and completion. This test only analyzed the data from trials 1 and 5 to see if there was an overall difference (α=0.05) from the first trial to the last. Probability values for treatment and sex were not significant (P=0.096). However, significance levels for trial are less than 0.05 for all three dependent variables.

Table 2 displays the results of another multivariate statistical analysis, however, it examined data from all five trials. Again, the significance levels of treatment and sex were not significant while the number of deadends (P=0.001) and time to complete the maze (P=0.001) were significantly influenced by trial.

Figure 1 depicts the mean number of deadends for each treatment in each trial. Notice that while not statistically significant, the DHA group had the lowest mean number of deadends in Trials 1, 3, 4, and 5, and that the Ginkgo biloba group had the highest mean out of those trials.

Figure 2 is a bar chart that shows the mean times for each treatment in each trial. Again, although not significant, notice that in the majority of the trials, trials 3, 4, and 5, the DHA treatment group had the fastest (lowest) mean time, whereas the Ginkgo

biloba group had the highest mean time. Note that in trials 1 and 2, the control group had the fastest mean time.

A One-Way ANOVA analysis (Table 3) shows the means and standard errors for the number of deadends and run times dependent on the type of treatment in each trial.

Although the results were not significant, they offered a research area for the second part of this experiment. DHA appeared to be the most effective type of treatment due the treatment group's low number of deadends and fastest mean time in the majority of the trials.

Experiment #2

A multivariate statistical analysis (Table 4) shows the effects of different variables (treatment, age, and trial) on the number of deadends and the time it took the mice to complete the maze. The fixed factors were treatment, age, and trial, and the dependent factors were the number of deadends and time. When a factor was not significant, it was not included in any of the following graphics. The data in Table 4 is a compilation of the multivariate analysis results from four tests on various trial combinations: trials 1.1, 2.1, and 3.1; trials 1.1 and 1.2; trials 2.1 and 2.2; and trials 3.1 and 3.2. Asterisks indicate significant findings (α=0.05). Neither age; age & treatment; treatment & trial; nor age, treatment, & trial, were significant in the learning of new mazes.

I found that among trials 1.1, 2.1, and 3.1, the type of treatment was significant for both the number of deadends and time (P=0.009 and P=0.018, respectively). In

comparing these three trials, trial was also significant for time (P=0.001). Note that age and trial interacted to have a significant effect on both the number of deadends and time (P=0.008 and P=0.018, respectively).

In comparing trials 1.1 and 1.2, trial was again significant for both dependent factors (P=0.001). Age and treatment interacted to have a significant effect on both the number of deadends (P=0.007) and time (P=0.017).

In the analysis of trials 2.1 and 2.2, the only variable having a significant effect was the interaction of treatment and trial; P=0.026 for the number of deadends and P=0.001 for time.

Only one fixed factor was significant in the comparison of trials 3.1 and 3.2; age was just barely significant for the number of deadends (P=0.049). An interaction between age and trial was not significant but it was the next important value in the analysis (P=0.091).

Table 5 is a table of means, and it provides mean values for each of the variables in the six trials. These values were used in the construction of the bar graphs.

Figure 3 is a bar graph of the mean times for each treatment group in trials 1.1, 2.1, and 3.1. Treatment was significant for time, P=0.018. Individually, trials 1.1 and 3.1 were not significant (P=0.283 and P=0.728, respectively). In trial 2.1, however, treatment was significant in determining the time it took the mice to run the maze (P=0.010). To investigate significant differences among the five treatment levels, I analyzed data in pair-wise groups from individual significant trials with One-Way

ANOVA analyses. In this graph, one can see the relationships in trial 2.1 among the control group and 1/10 (P=0.049), 1/8 and 1/10 (P=0.044), 1/10 and 1/20 (P=0.001). and 1/20 and 1/40 (P=0.004). The relationship between 1/8 and 1/40 was not significant, however, it was close (P=0.068). These specific combinations were all significant and contributed to the significant effect of treatment on time for these trials. Note that the 1/8 and 1/10 groups progressively improve over trials.

Figure 4 is a bar graph for the mean number of deadends for each treatment in trials 1.1, 2.1, and 3.1. Treatment was significant for the number of deadends at P=0.009. Although not significant, trial 2.1 was the closest trial to have a significant treatment effect (P=0.068). Therefore, to see if there were any significant differences between the groups, I analyzed data in pair-wise groups from trial 2.1 with several One-Way ANOVA analyses. The relationships in this graph occur among groups 1/8 and 1/10 (P=0.039), 1/10 and 1/20 (P=0.010), and 1/20 and 1/40 (P=0.022). All treatments tend to either improve or remain the same from trial 1.1 to 3.1.

Figures 5 and 6 depict the relationship between mouse age and the mean time and mean number of deadends for trials 1.1, 2.1, and 3.1. There was a significant interaction between age and trial on both the mean time (P=0.018) and mean number of dead ends (P=0.008). All treatments were combined in this graph, in order to only examine the effects of age and trial. Both graphs follow a similar trend. The young mice progressively improved in time and decreased in the mean number of deadends, ie. they improved with each trial. The older mice, however, improved from trial 1.1 to

2.1 but then got worse (had an increase in time and the number of deadends) between trials 2.1 and 3.1.

Figures 7 and 8 show the relationship, when separated by treatment level, between treatment and age and how they affect the mean time for trials 1.1 and 1.2. In Figure 7, the old mice in the control group generally had lower mean times when compared to the groups receiving varying dosages of DHA. In Figure 8, the young mice in the control group appear to have the worse mean times when compared to all other treatment groups. The interaction between age and treatment was significant for mean time (P=0.017).

Figures 9 and 10 show the relationship, when separated by treatment level, between treatment and age and how they affect the mean number of deadends for trials 1.1 and 1.2. The old mice in the control group have the lowest mean number of deadends (Figure 9) and the young mice in the control group have the highest number of mean deadends when compared to mice in other treatment groups (Figure 10). The interaction between age and treatment was significant for the mean number of deadends (P=0.007).

Figures 11 and 12 depict the relationship between treatment and trial and how the two factors together affect the time and number of deadends in trials 2.1 and 2.2. Both age groups (young and old) were combined in order to only examine the effects of treatment and trial. In both figures, the control, 1/8, and 1/20 groups all improve, decreasing their number of deadends and mean times. The 1/10 and 1/40 groups,

however, both increase in mean time and number of deadends from trial 2.1 to 2.2. Treatment and trial interacted to produce a significant difference in both the mean time and mean number of deadends (P=0.001 and P=0.026, respectively).

Figure 13 shows how age affected the number of deadends in trials 3.1 and 3.2 (P=0.049). Notice that the old mice improved, going from 12.69 to 8.84. The young mice, however, stayed relatively the same, going from a value of 8.08 to 8.48. Even though the young mice have a slightly lower number of deadends in trial 3.2, notice that the old mice improved a lot more from trial 3.1 to trial 3.2.

Table 1

Multivariate Analysis: The Effects of Treatment, Sex, and Trial on Number of Deadends, Time, and Maze Completion For Trials 1 and 5

(* denotes a significant effect)

Source	Dependent Variable	Sig.
Treatment	Deadends	0.378
	Time	0.694
	Complete	0.096
Trial	Deadends	* 0.001
	Time	* 0.000
	Complete	* 0.023
Sex	Deadends	0.972
	Time	0.944
	Complete	1.000
Treatment*Trial	Deadends	0.746
	Time	0.871
	Complete	0.169
Treatment*Sex	Deadends	0.575
	Time	0.999
	Complete	1.000
Trial*Sex	Deadends	0.165
	Time	0.420
	Complete	0.437
Treatment*Trial*Sex	Deadends	0.743
	Time	0.877
	Complete	0.545

Table 2

Multivariate Analysis: The Effects of Treatment, Sex, and Trial On Number of Deadends, Time, and Maze Completion for All Trials

(* denotes a significant effect)

Source	Dependent Variable	Sig.
Treatment	Deadends	0.142
	Time	0.382
	Complete	0.166
Trial	Deadends	* 0.001
	Time	* 0.000
	Complete	0.113
Sex	Deadends	0.819
	Time	0.956
	Complete	1.000
Treatment*Trial	Deadends	0.818
	Time	0.512
	Complete	0.211
Treatment*Sex	Deadends	0.313
	Time	0.783
	Complete	0.838
Trial*Sex	Deadends	0.135
	Time	0.346
	Complete	0.113
Treatment*Trial*Sex	Deadends	0.992
	Time	0.996
	Complete	0.956

Figure 1

Mean Number of Deadends For Each Treatment Group - All Trials

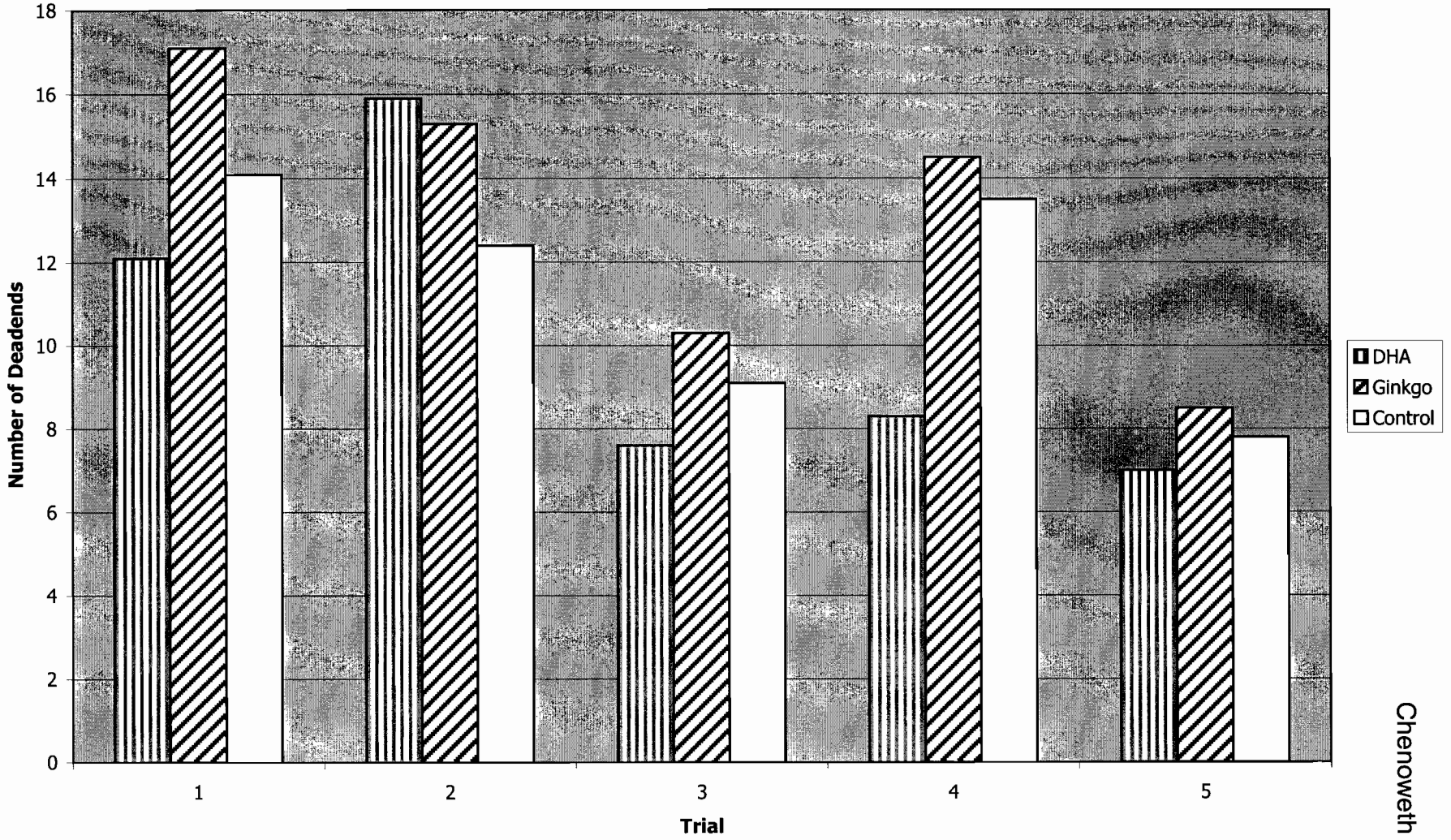


Figure 2

Mean Time For Each Treatment Group - All Trials

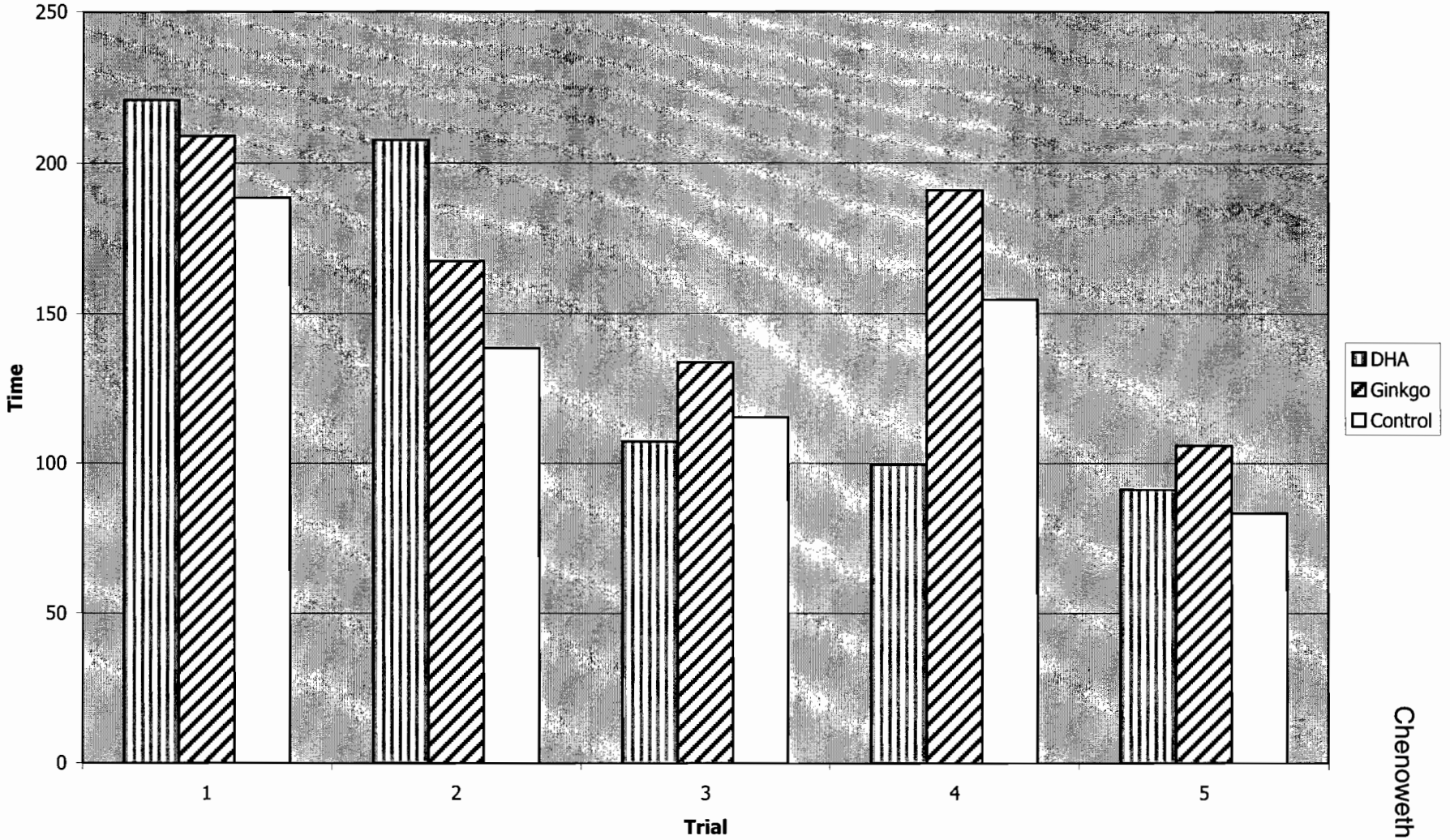


Table 3

One-Way ANOVA Analysis for All Trials - The Means and Standard Errors for Number of Deadends and Run Times for Each Treatment

Trial Number	Variable	Treatment	Mean	Std. Error
1	Deadends	DHA	12.1	1.923
		Ginkgo	17.1	2.976
		Control	14.1	1.278
	Time	DHA	220.8	32.604
		Ginkgo	209.0	31.937
		Control	188.5	16.261
2	Deadends	DHA	15.9	2.100
		Ginkgo	15.3	2.241
		Control	12.4	2.202
	Time	DHA	207.7	28.986
		Ginkgo	167.5	29.651
		Control	138.5	29.988
3	Deadends	DHA	7.6	2.202
		Ginkgo	10.3	2.329
		Control	9.1	2.369
	Time	DHA	107.3	33.870
		Ginkgo	133.8	23.637
		Control	115.4	28.211
4	Deadends	DHA	8.3	1.944
		Ginkgo	14.5	3.181
		Control	13.5	2.535
	Time	DHA	99.6	21.332
		Ginkgo	191.0	32.236
		Control	154.6	31.880
5	Deadends	DHA	7.0	1.758
		Ginkgo	8.5	2.738
		Control	7.8	2.453
	Time	DHA	91.2	20.004
		Ginkgo	105.9	29.521
		Control	83.4	25.318

Table 4

Multivariate Analysis: The Effects of Age, Treatment, and Trial on the Number of Deadends and Time for Various Trials

(* denotes a significant effect)

Trials	Source	Dependent Variable	Sig.
1.1, 2.1, 3.1	Age	Deadends	0.767
		Time	0.886
	Treatment	Deadends	* 0.009
		Time	* 0.018
	Trial	Deadends	0.082
		Time	* 0.000
	Age*Treatment	Deadends	0.361
		Time	0.210
	Age*Trial	Deadends	* 0.008
		Time	* 0.018
	Treatment*Trial	Deadends	0.595
		Time	0.197
	Age*Treatment*Trial	Deadends	0.191
		Time	0.248
1.1, 1.2	Age	Deadends	0.754
		Time	0.389
	Treatment	Deadends	0.633
		Time	0.623
	Trial	Deadends	* 0.000
		Time	* 0.000
	Age*Treatment	Deadends	* 0.007
		Time	* 0.017
	Age*Trial	Deadends	0.671
		Time	0.134
	Treatment*Trial	Deadends	0.123
		Time	0.406
	Age*Treatment*Trial	Deadends	0.843
		Time	0.792

Table 4 – continued

Trials	Source	Dependant Variable	Sig.
2.1, 2.2	Age	Deadends	0.121
		Time	0.828
	Treatment	Deadends	0.850
		Time	0.518
	Trial	Deadends	0.840
		Time	0.645
	Age*Treatment	Deadends	0.544
		Time	0.389
	Age*Trial	Deadends	0.250
		Time	0.690
	Treatment*Trial	Deadends	* 0.026
		Time	* 0.001
	Age*Treatment*Trial	Deadends	0.920
		Time	0.868
3.1, 3.2	Age	Deadends	* 0.049
		Time	0.104
	Treatment	Deadends	0.835
		Time	0.738
	Trial	Deadends	0.169
		Time	0.689
	Age*Treatment	Deadends	0.139
		Time	0.763
	Age*Trial	Deadends	0.091
		Time	0.198
	Treatment*Trial	Deadends	0.496
		Time	0.716
	Age*Treatment*Trial	Deadends	0.864
		Time	0.542

Table 5

Means and Standard Deviations for the Number of Deadends and Time for All Treatment Groups in All Trials

Mean Number of Deadends and Std. Deviations

Age	Treatment	Trial 1.1		Trial 1.2		Trial 2.1		Trial 2.2		Trial 3.1		Trial 3.2	
		Means	Std. D.	Means	Std. D.	Means	Std. D.	Means	Std. D.	Means	Std. D.	Means	Std. D.
Old Mice	Control	10.40	6.58	4.20	4.49	12.00	13.58	7.20	7.85	17.00	7.94	9.80	6.06
	1/8 DHA	20.60	9.40	7.80	4.97	11.40	5.22	10.60	7.80	12.60	7.83	6.40	5.64
	1/10 DHA	9.60	5.73	6.00	3.74	3.40	1.52	12.00	7.91	6.50	2.86	9.20	2.77
	1/20 DHA	10.20	6.34	8.00	3.08	10.60	5.90	8.40	4.34	15.60	11.72	10.80	6.61
	1/40 DHA	13.00	3.94	8.20	7.05	2.80	0.45	8.60	6.35	12.00	4.12	8.00	5.15
Young Mice	Control	17.20	6.76	10.00	6.12	14.40	11.06	9.80	5.12	7.20	5.07	7.60	3.29
	1/8 DHA	12.80	3.77	4.40	2.80	10.60	6.58	8.60	8.50	10.40	9.45	10.40	8.38
	1/10 DHA	13.20	4.09	7.80	5.90	8.60	4.83	11.80	6.61	7.80	4.60	10.40	6.54
	1/20 DHA	11.80	5.89	9.00	5.43	15.20	6.30	6.00	3.24	6.60	4.39	6.80	4.71
	1/40 DHA	8.20	3.27	7.00	3.61	10.20	5.07	13.40	7.16	8.40	4.34	7.20	3.96

Mean Times (seconds) and Std. Deviations

Age	Treatment	Trial 1.1		Trial 1.2		Trial 2.1		Trial 2.2		Trial 3.1		Trial 3.2	
		Means	Std. D.	Means	Std. D.	Means	Std. D.	Means	Std. D.	Means	Std. D.	Means	Std. D.
Old Mice	Control	123.20	97.45	52.20	50.36	90.60	68.22	52.80	57.61	164.20	89.05	107.00	79.70
	1/8 DHA	203.60	69.29	106.80	105.26	149.40	102.91	113.20	101.69	114.60	87.51	64.40	74.95
	1/10 DHA	121.20	62.13	66.60	39.78	37.60	15.96	168.20	60.13	63.40	35.28	133.40	96.16
	1/20 DHA	100.80	46.79	117.00	76.88	152.60	93.51	71.80	21.02	132.40	112.36	106.80	70.50
	1/40 DHA	139.20	59.65	97.80	68.77	20.60	10.53	108.40	98.33	101.20	25.01	93.00	75.22
Young Mice	Control	212.20	86.89	106.60	110.98	115.20	92.12	93.00	53.54	69.40	76.84	85.60	91.50
	1/8 DHA	168.60	38.21	47.80	32.40	91.60	104.42	65.00	68.63	74.40	67.30	99.20	115.79
	1/10 DHA	204.00	83.48	106.00	54.46	58.80	31.32	164.00	120.09	89.60	59.68	102.60	77.02
	1/20 DHA	166.20	91.33	76.00	48.40	148.80	80.72	80.20	40.33	55.80	49.42	128.80	135.24
	1/40 DHA	100.20	20.49	59.60	31.37	82.00	50.46	98.80	63.97	53.00	33.97	61.00	28.04

Figure 3

Treatment vs. Time For All Ages in Trial 1.1, 2.1, and 3.1

***Treatment is Significant: P=0.018**

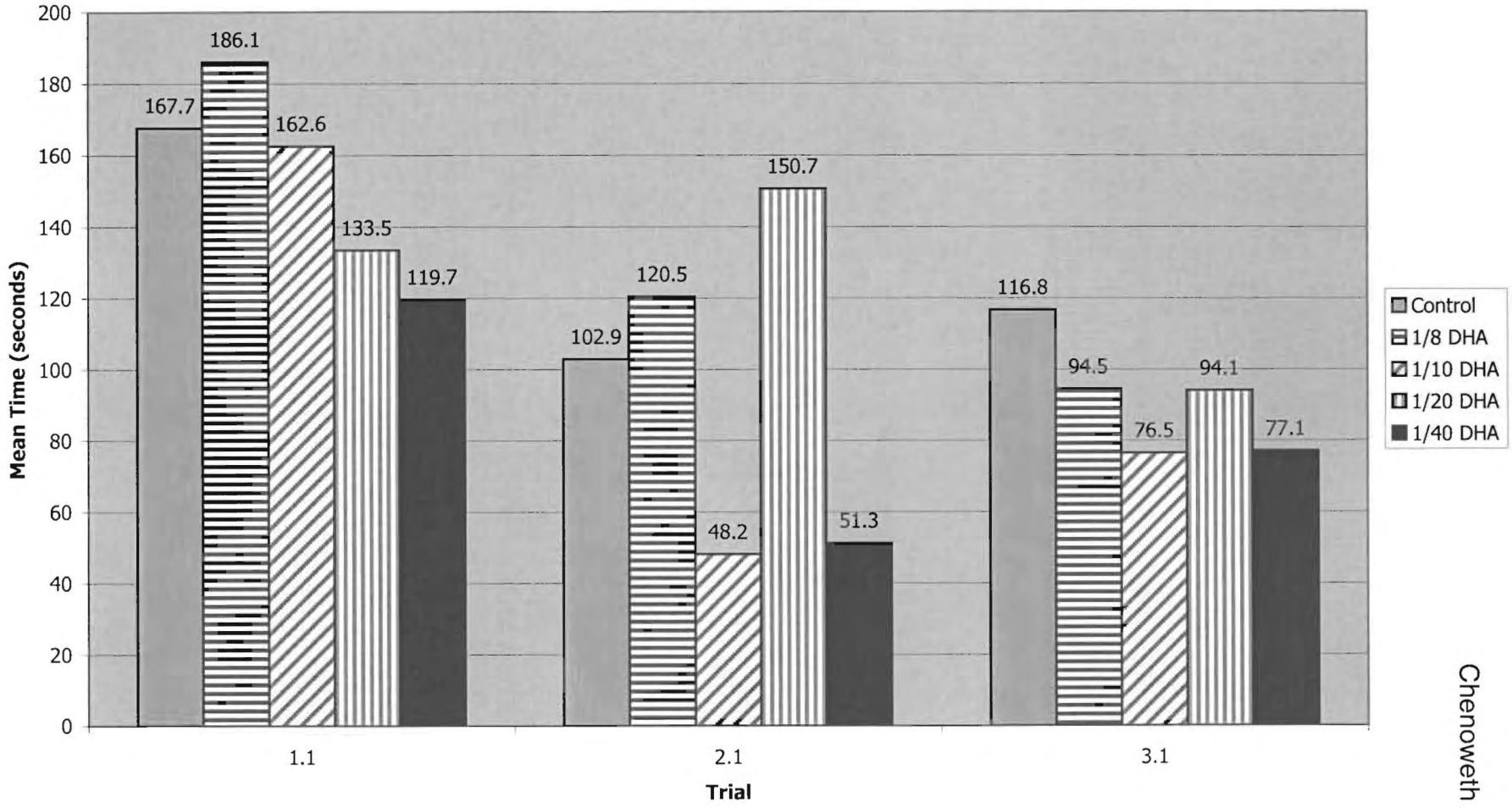


Figure 4

Treatment vs. Deadends For All Ages in Trial 1.1, 2.1, and 3.1
***Treatment is Significant: P=0.009**

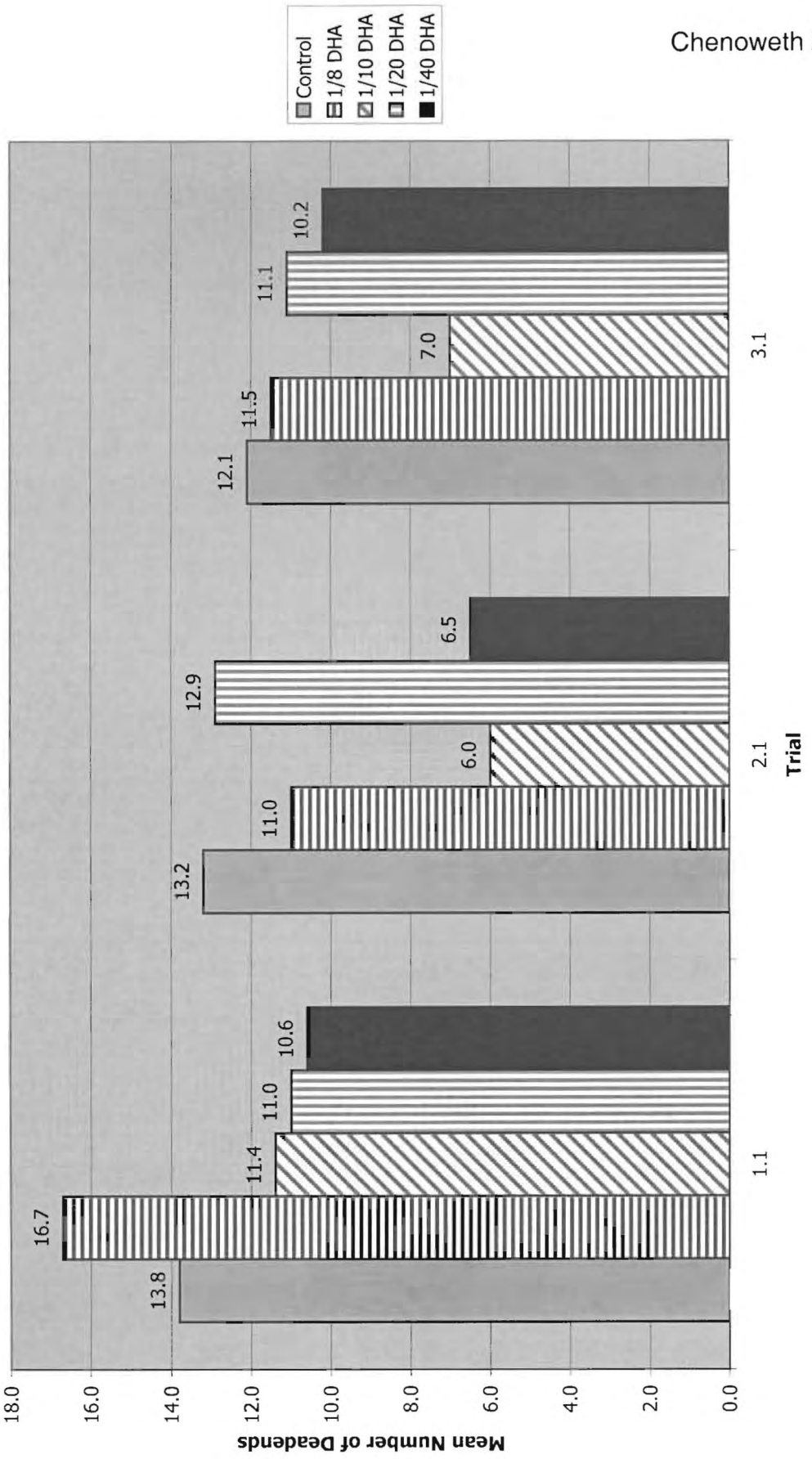


Figure 5

Age vs. Time For Trials 1.1, 2.1, and 3.1

***Age and Trial are Significant: P=0.018**

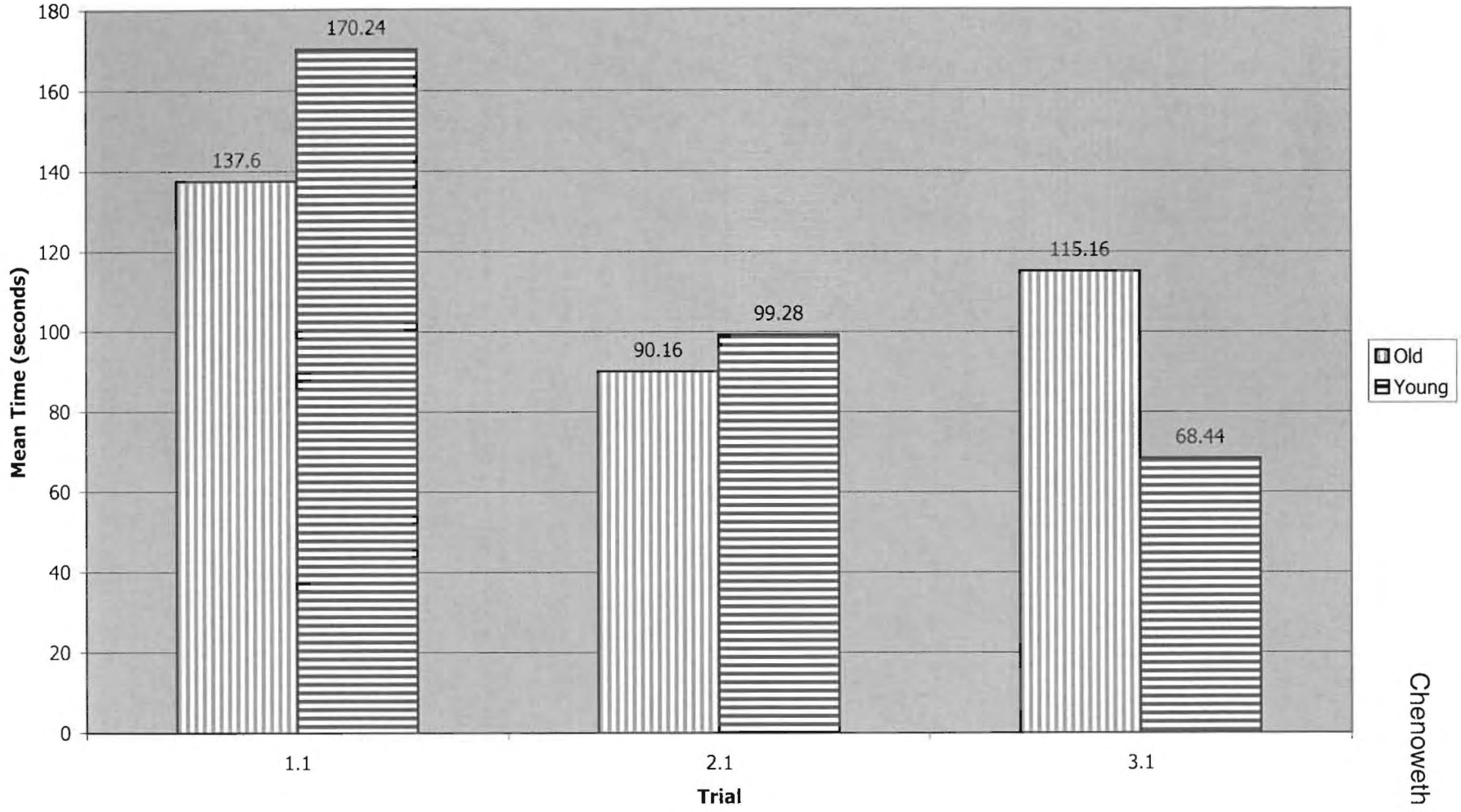


Figure 6

Age vs. Deadends For Trial 1.1, 2.1, and 3.1

***Age and Trial are Significant: P=0.008**

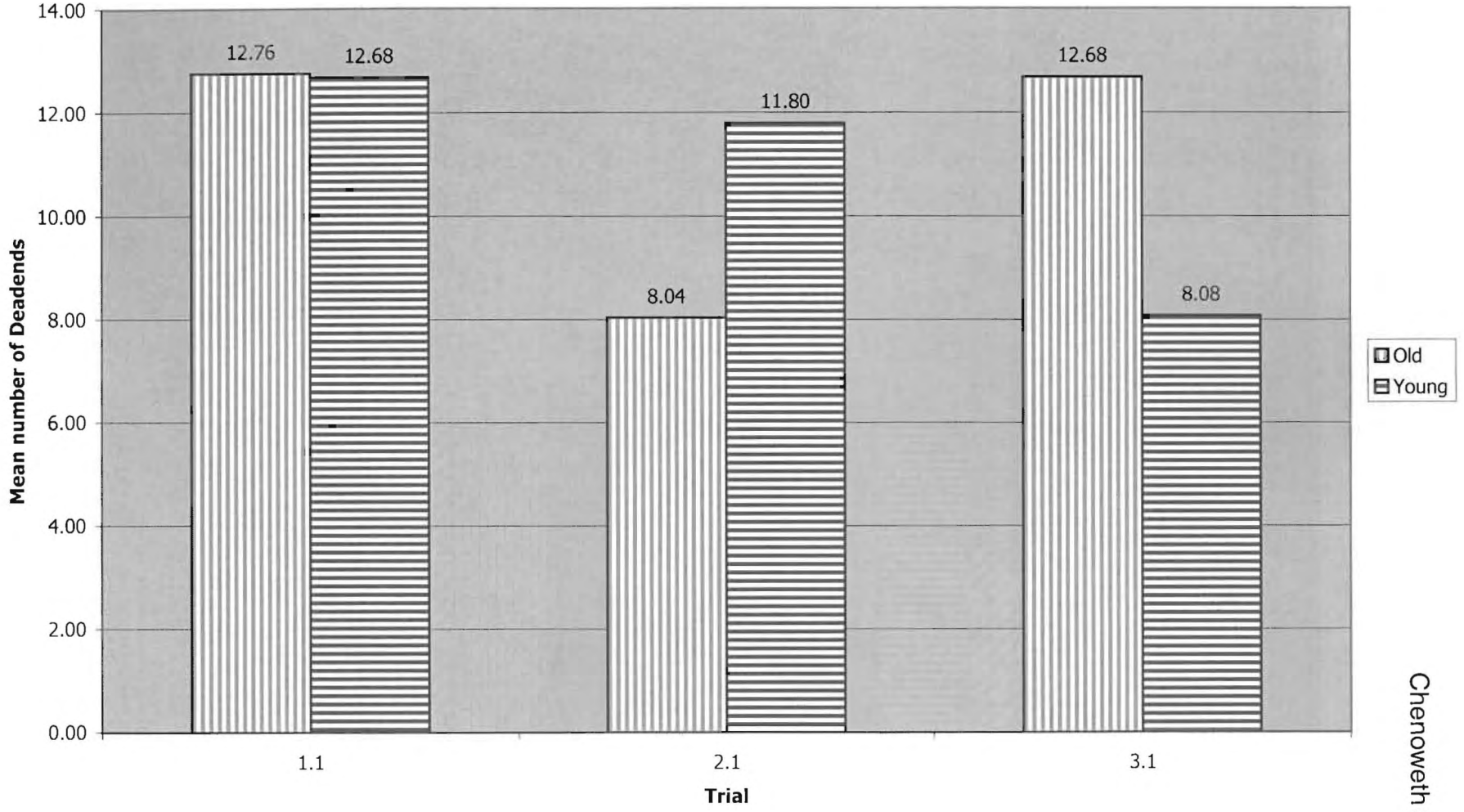


Figure 7

Treatment vs. Time For Trials 1.1 and 1.2 - Old Mice

***Age and Treatment are Significant: P=0.017**

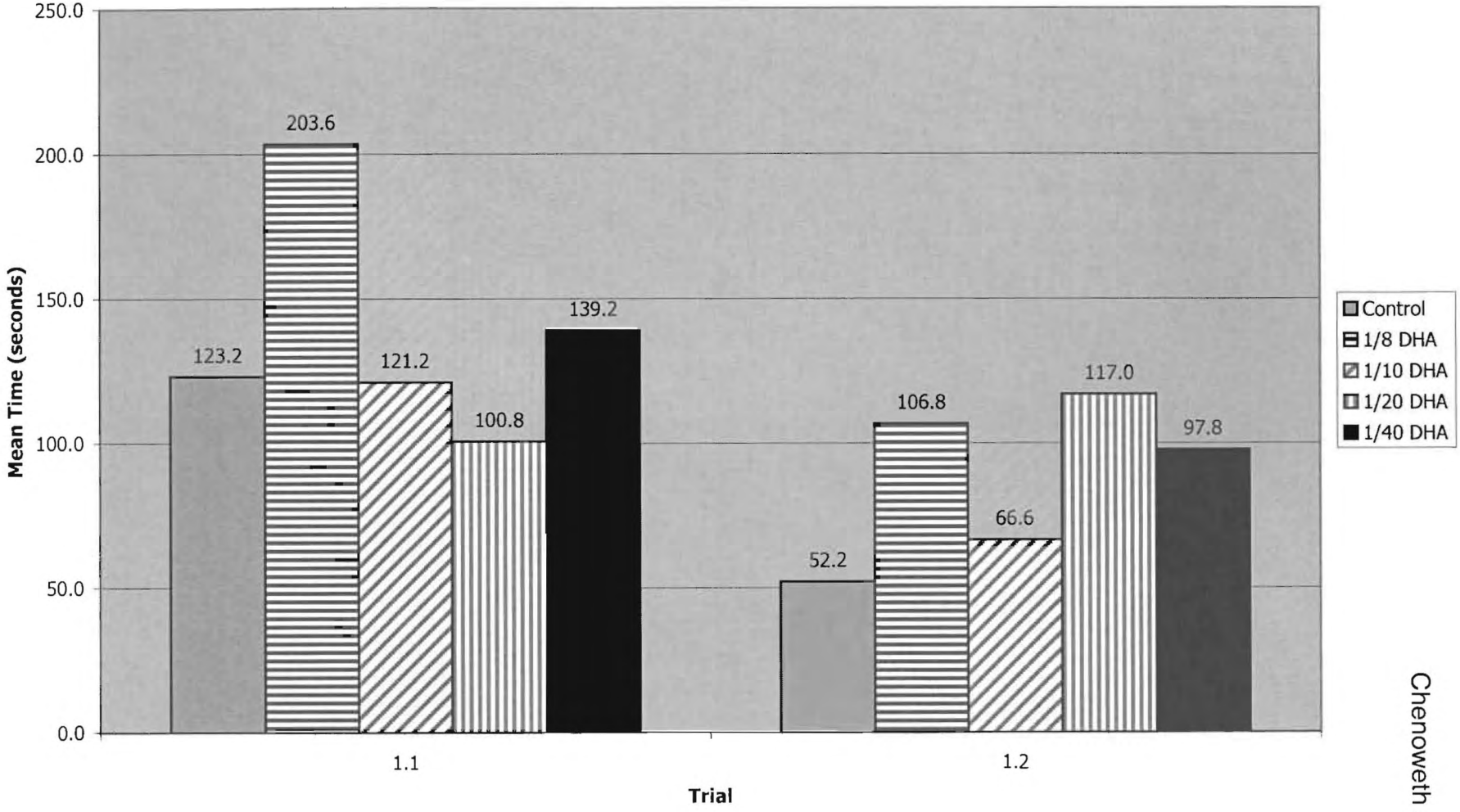


Figure 8

Treatment vs. Time For Trials 1.1 and 1.2 - Young Mice

***Age and Treatment are Significant: P=0.017**

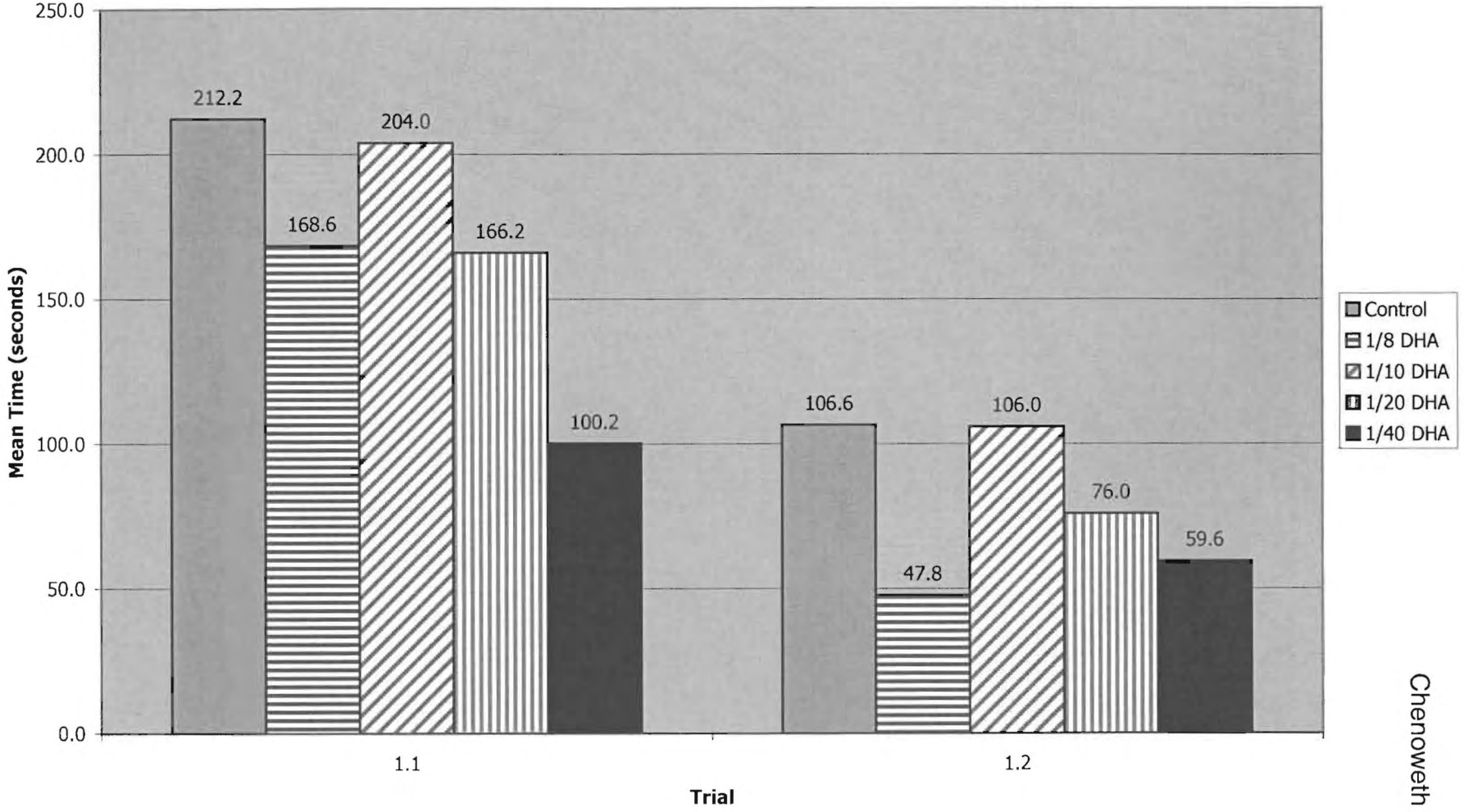


Figure 9

Treatment vs. Deadends For Trials 1.1 and 1.2 - Old Mice

***Age and Treatment are Significant: P=0.007**

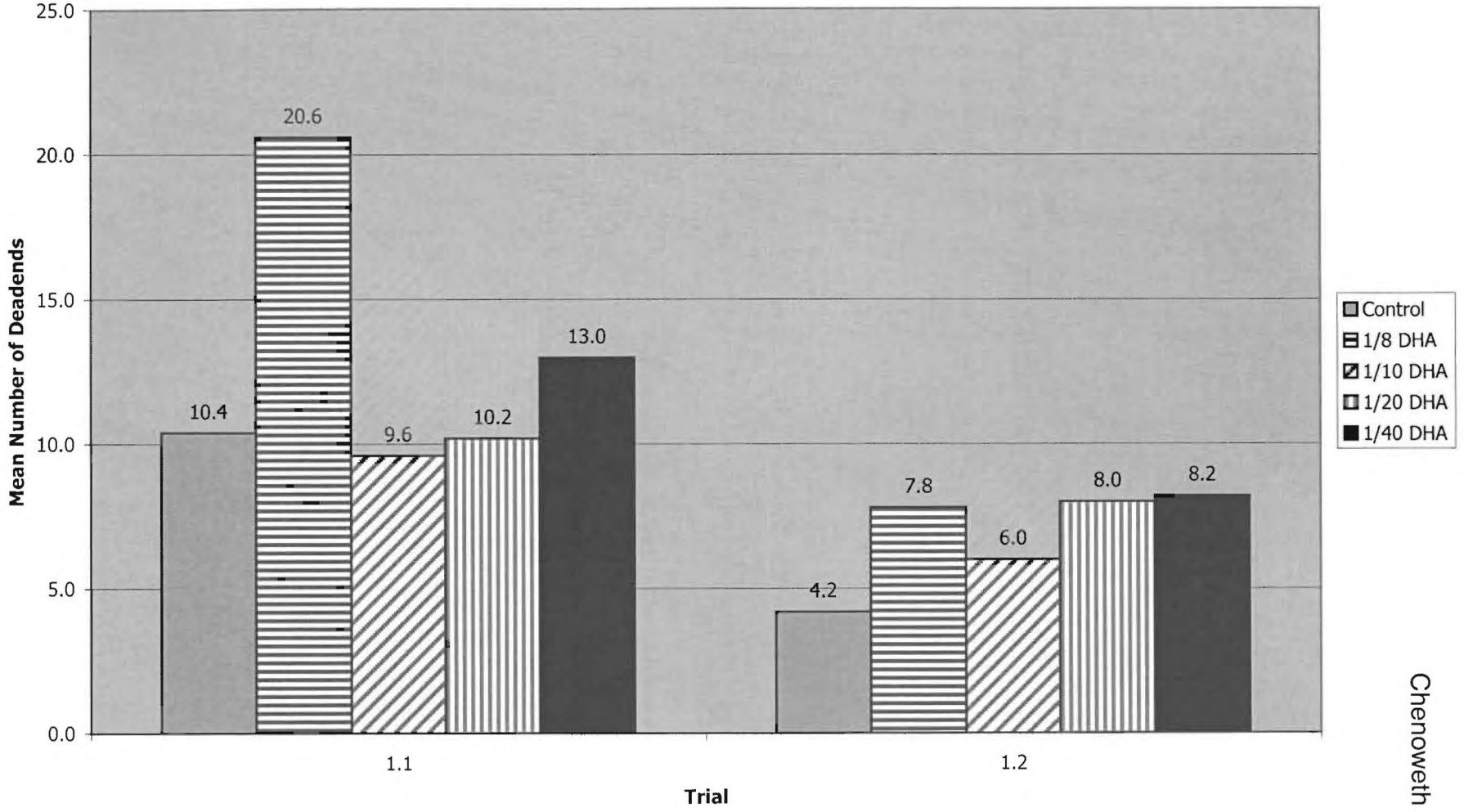


Figure 10

Treatment vs. Deadends For Trials 1.1 and 1.2 - Young Mice

***Age and Treatment are Significant: P=0.007**

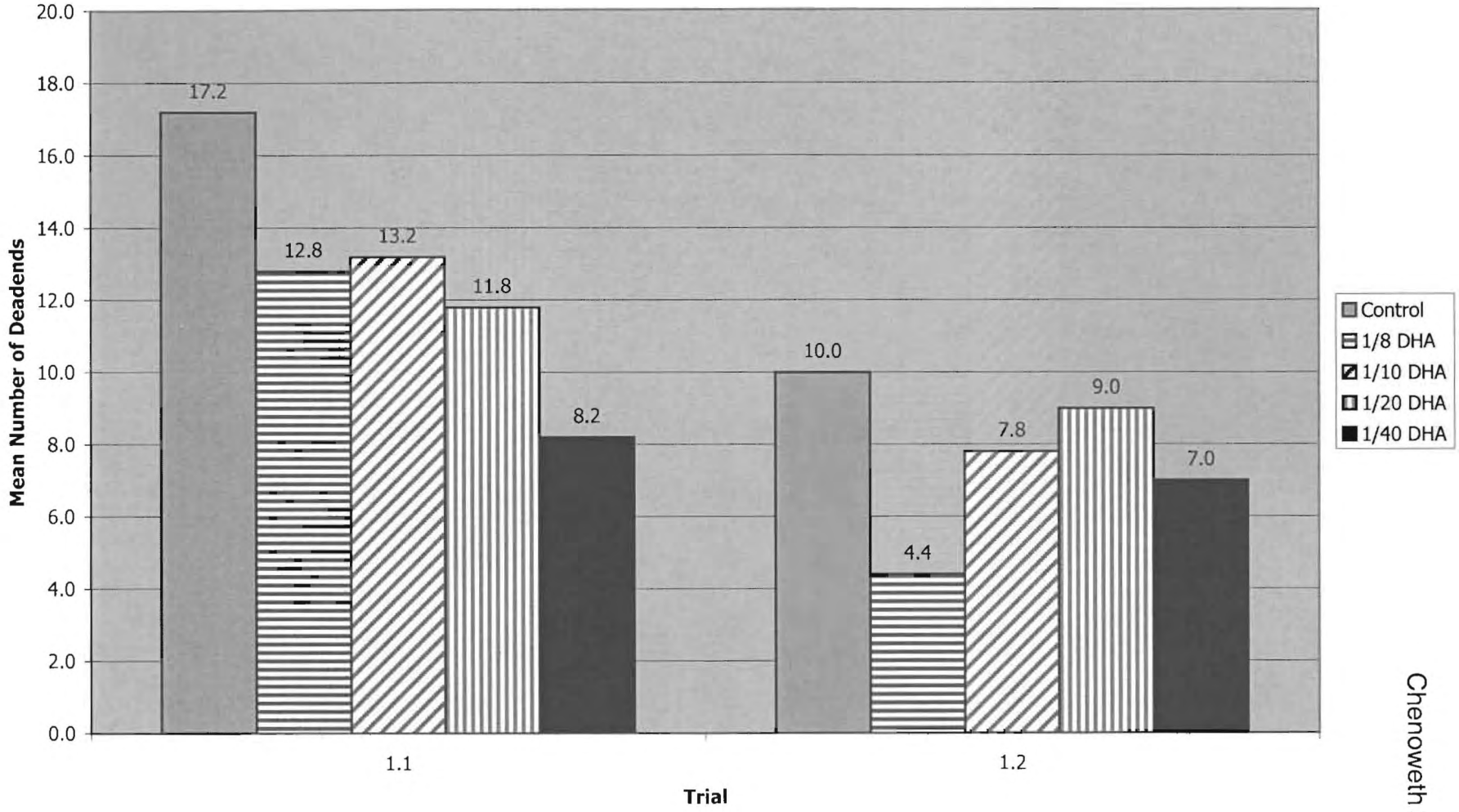


Figure 11

Treatment vs. Time For Trials 2.1 and 2.2

***Treatment and Trial are Significant: P=0.001**

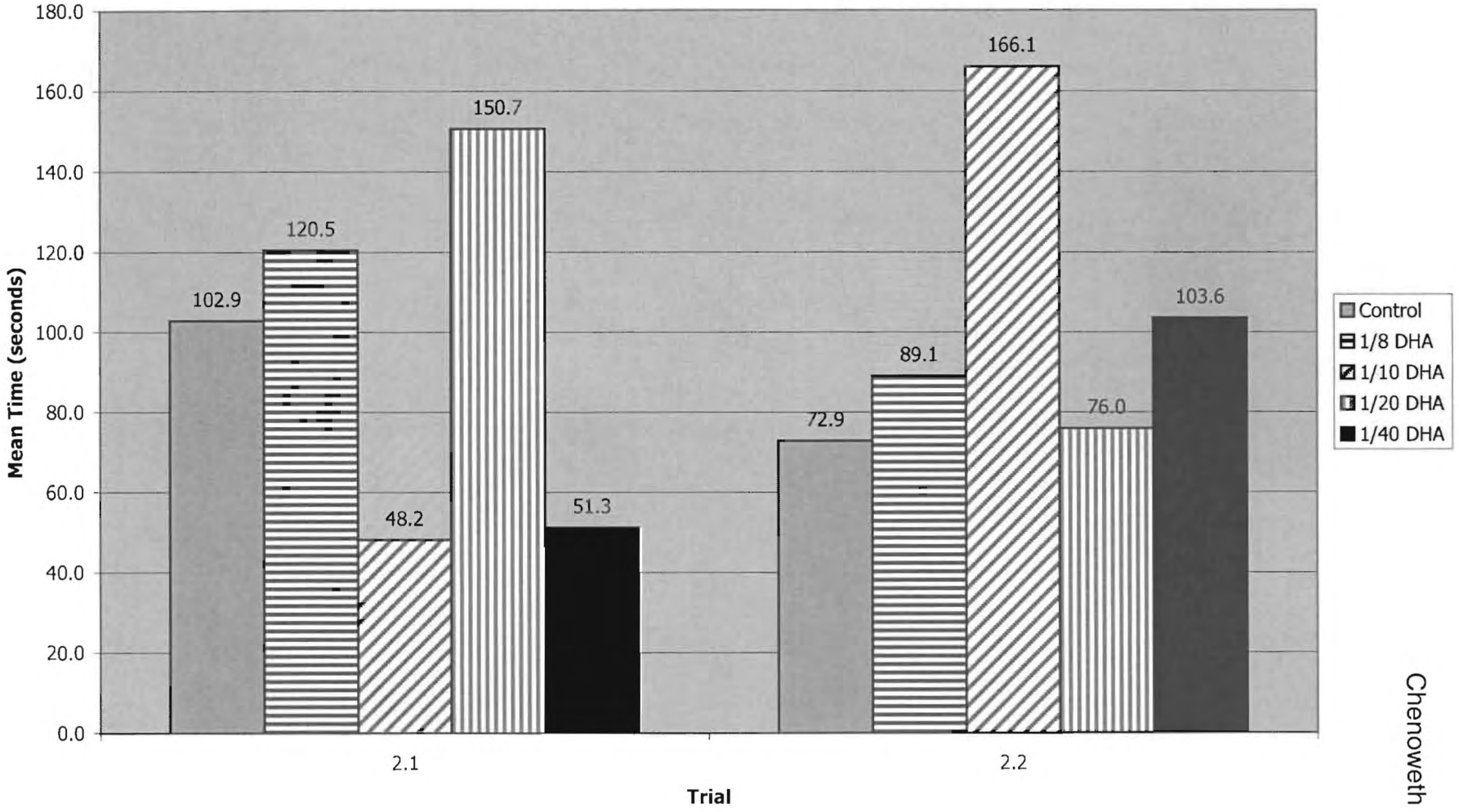


Figure 12

Treatment vs. Deadends For Trials 2.1 and 2.2

***Treatment and Trial are Significant: P=0.026**

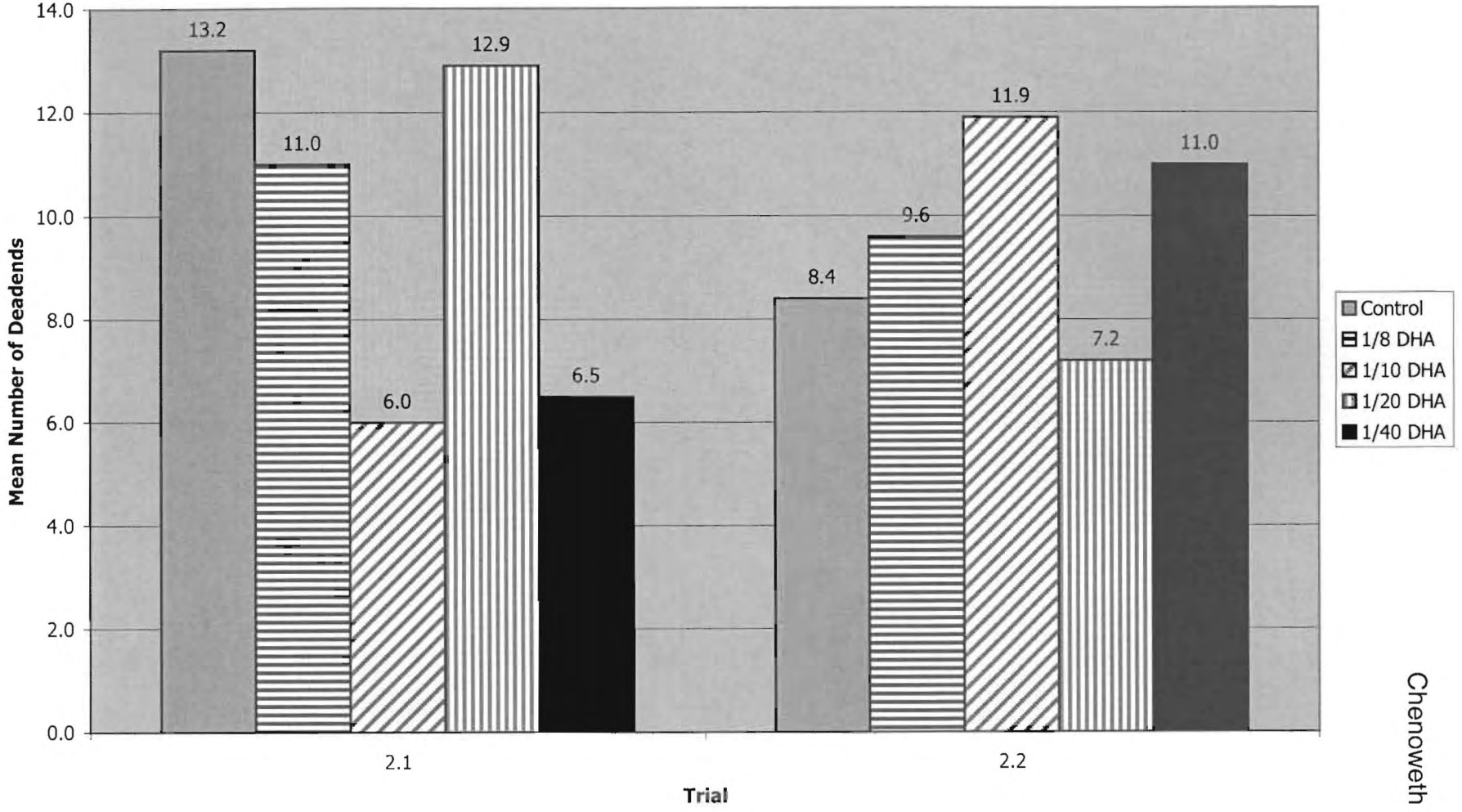
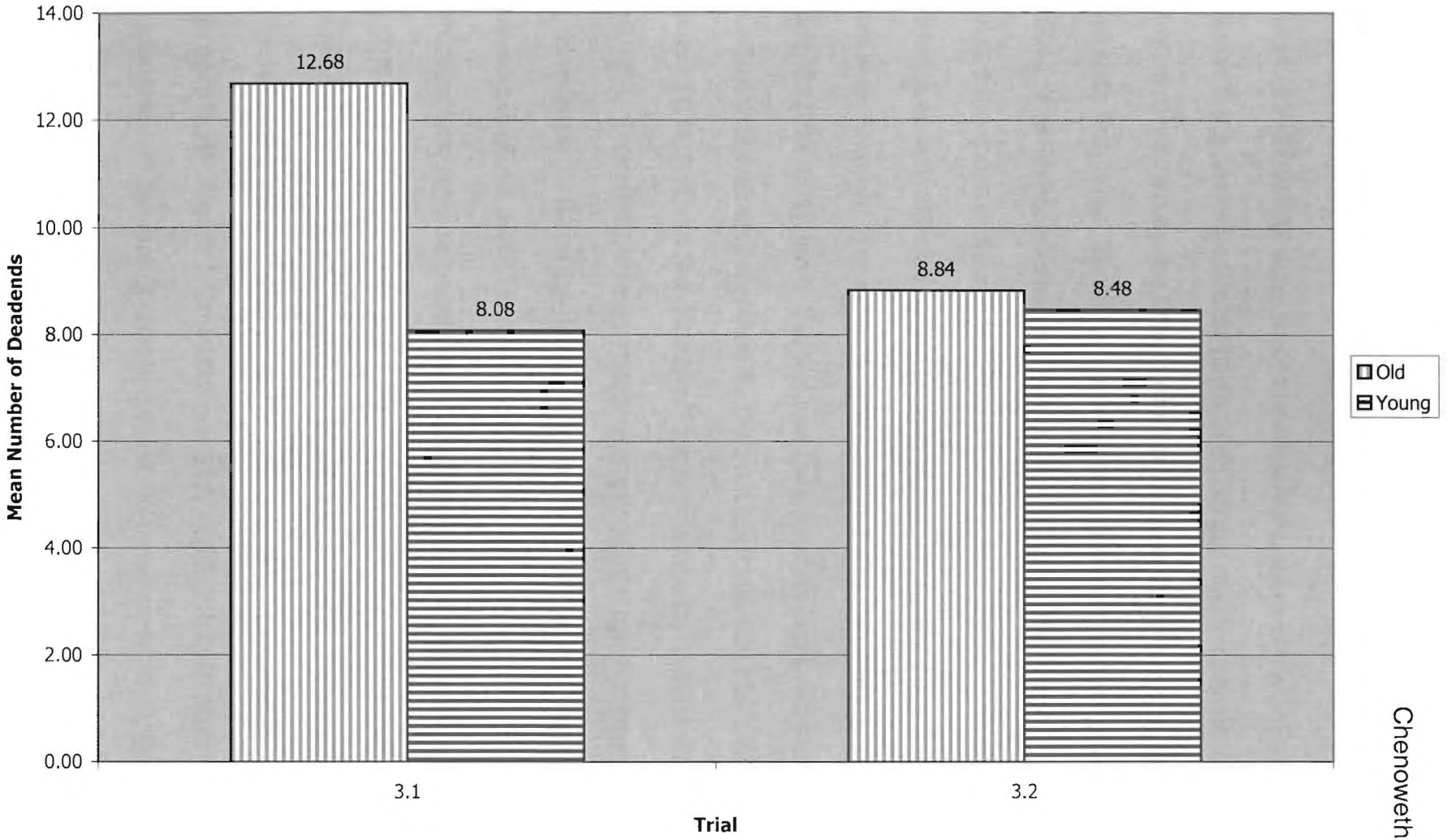


Figure 13

Age vs. Deadends For Trials 3.1 and 3.2

***Age is Significant: P=0.049**



Discussion of Experiment #1

According to the statistical analysis of experiment #1, there is not a significant difference between the types of treatment (DHA and Ginkgo) and the effects they have on mice memory as measured by the time to run a maze and number of deadends. Table 1 displays data from trials 1 and 5 and since the significance values for sex are higher than 0.0944, it can be concluded that sex did not make a difference in how fast the mice completed the maze or how many wrong turn the mice made. Females and males seemed to be equally affected by the Ginkgo biloba, DHA, and control treatments ($P>0.05$). There was, however, a significant difference in the run time ($P<0.001$) and number of deadends ($P=0.001$) due to the trial number. All mice improved from the first trial to the fifth. Run time was also significantly different from trial 1 to trial 5 with a significance value of 0.001. Even the dependent factor of maze completion was significant ($P=0.023$). Therefore, although treatment and sex did not significantly affect maze running, the number of times the mice ran the maze did. This difference is evident in comparing weeks 1 and 5, and all weeks.

Although the relationship between the type of treatment and the number of deadends and time was not significant, there did seem to be some trends that indicate that DHA is more effective than Ginkgo biloba in improving the mice's memory. This finding helped to determine the direction of further research and investigation. Notice the box plot represented by Figure 1. In all but one trial, trial 2, the DHA treatment group had the lowest mean number of deadend turns. In those same trials, Ginkgo biloba had the most deadends, signifying that even the control group preformed better

than the Ginkgo biloba group. The only exception was in trial 2 where the control group seemed to do the best. Therefore, in four out of five trials, the DHA group performed better than both the control and Ginkgo biloba groups. Similarly, the DHA group also had faster times for completion in the majority of the trials. The DHA group had the fastest times in trials 3, 4, and 5. In these trials, the control group again came in second, with the Ginkgo biloba group exhibiting the slowest times. In the first two trials, where DHA did not have the fastest times, the control group proved to be the quickest group. Therefore, out of all five trials, the Ginkgo biloba group never had the lowest mean number of deadends or the fastest time for maze completion. Therefore, it appears that the DHA group performed better than the Ginkgo biloba group. This could be interpreted to mean that the mice receiving DHA had improved memory capabilities. This statement is only a trend, since the multivariate statistical analysis proved that there was no significant difference between the three treatment groups.

Although DHA was not proven to be statistically different, the data indicates that it may have been slightly more effective than the Ginkgo biloba. One of the possible reasons for the lack of a significant difference could be due to the concentrations of DHA and Ginkgo biloba that were used in this study. The mice were given 10 percent of the human dosage. There might have been larger improvements and a difference in the number of deadends and time as a result of the treatments if either a higher or lower quantity was used. Therefore, it would be interesting to analyze DHA in order to determine if varying concentrations have different effects on mice memory performance. A variety of DHA doses were used in subsequent studies, along with altering age

structures, in order to determine how different compound concentrations affected mice memory capabilities. Perhaps the mice were given too much DHA in experiment #1, and therefore, would perform better under lower concentrations. Previous studies with lab mice have revealed that the intake of DHA does improve maze-learning ability, however, it is believed that it may take time after the incorporation of DHA into the brain for any actual improvement in memory to appear (Lim and Suzuki 2001). Therefore, it is hypothesized that mice under the influence of DHA will show signs of an increased memory and learning ability as the levels of DHA accumulate. In other words, I anticipated that the longer the mice were exposed to continuous DHA levels, the more effective it would be in increasing memory and learning skills.

Discussion of Experiment #2

When mice were given daily dosages of DHA, treatment had a significant effect on the time it took the mice to run the maze ($P=0.018$), as well as on the mean number of deadends ($P=0.009$), among trials 1.1, 2.1, and 3.1. In other words, the type of treatment (control, 1/8 DHA, 1/10 DHA, 1/20 DHA, 1/40 DHA) effected how the mice performed when presented with a new stimulus. Although the trials were not all individually significant for type of treatment, treatment was significant when all three were compared. Therefore, treatment did affect the mice's ability to learn a new maze. Figure 3 provides the data for mean times. Each treatment group had faster mean times from trial 1.1 to trial 2.1, however, only the 1/8 DHA and 1/10 DHA groups progressively improved after each trial. It is interesting to note that the control and 1/40

DHA groups decreased in time from trial 1.1 to trial 2.1, however, they increased in time from trial 2.1 to trial 3.1. The 1/20 group increased from trial 1.1 to trial 2.1, but then decreased in time from trial 2.1 to trial 3.1. According to this data, the 1/8 DHA and 1/10 DHA groups seem to have been the most effective treatment groups for mean time. This contradicts the original hypothesis that the groups with smaller DHA concentrations (1/20 and 1/40) would have better rates of performance.

To better understand the relationship between time and treatment in trials 1.1, 2.1, and 3.1, the significant differences among the significantly different groups were examined. Trial 2.1 was the only group to be individually significant for treatment (P=0.010), therefore, several pair-wise One-Way ANOVA tests were completed from the data. The results of the analyses indicated that the control, 1/8, and 1/20 groups were similar while the 1/10 and 1/40 DHA groups were similar. The similarity between the 1/20 DHA group and the control and 1/8 groups was unexpected, because I thought that the groups receiving the lower concentrations would be more effective (similar).

Treatment had a similar effect on the mean number of deadends among trials 1.1, 2.1, and 3.1. The similar trends in the mean time and the number of deadends were expected since they are relative to one another. Again, all the treatment groups either improved or stayed comparatively the same from trial 1.1 to trial 3.1. None of the trials were independently significant for treatment, however, 2.1 was close (P=0.068). In order to check to see if there were any significant differences between the groups in trial 2.1, I again ran several pair-wise One-Way ANOVA tests. The data once more shows the similarities between the control, 1/8, and 1/20 DHA groups and the 1/10 and

1/40 DHA groups. Again, I anticipated that the lesser concentration groups would have been more similar in their results.

There was a significant interaction between the age of the mouse and the trial number for trials 1.1, 2.1, and 3.1 on the mean time and number of deadends ($P=0.018$ and $P=0.008$, respectively). In other words, the age of the mouse in combination with the trial number affected how the mouse performed in the maze. Figures 5 and 6 show how the young mice progressively improved in their performance (their mean time and number of deadends decreased), whereas the old mice decreased from trial 1.1 to trial 2.1, but then had an increase from trial 2.1 to trial 3.1. Therefore, the younger mice seemed to have a better rate of memory performance when introduced to a new stimulus (maze). I expected DHA to increase the rate of learning and have more of an effect on the young mice, when compared with older mice, since DHA is very important to the developing brain (Wander 1998). In a previous study, researchers also found that the chronic administration of DHA improved reference memory related learning in young rats (Gamoh et. al. 1999).

Trial was also significant in the comparison of trials 1.1, 2.1, and 3.1, as well as many of the individual trials and other trial combinations. However, since this was shown in experiment #1 it will not be discussed in any detail. Based on the results of experiment #1, it was expected that all mice would learn over the course of several trials.

This study also examined how various quantities of DHA would affect the learning of a maze. Results indicated that the different treatment groups had a

significant effect on the learning of new mazes; however, I also wanted to determine whether or not higher or lower concentrations would affect the learning of a previously seen maze. Since three different mazes were used, the following sections will look at the effects the variables had on each type of maze. The only variables to have a significant effect on the learning of maze #1 were trial, and an interaction between age and treatment. Neither age nor treatment had a significant individual effect on the mean time or the number of deadends. However, working together, they did influence the mice's maze performance. Again, the results for time and the number of deadends are relevant to one another and have a positive correlation. Figures 7 and 8 show the results for the effects of treatment and age on time (P=0.017). The older mice in the control group had a lower mean time than the groups receiving any type of DHA treatment (Figure 7). The younger mice in the control group, however, had higher mean times than the groups receiving DHA supplements (Figure 8). This same trend can be found for the mean number of deadends (P=0.007). The older mice in the control group seemed to do better than the old mice receiving DHA (Figure 9), and the young mice in the control group seem to have encountered more deadends then the young mice receiving DHA supplements (Figure 10). In other words, the DHA treatments were more effective in improving the memory of younger mice than improving the memory of older mice as determined by the mean time to complete the maze and the mean number of deadends.

Trials 2.1 and 2.2 involved two trials of the same maze, maze #2. The only variables that had a significant effect on maze performance were the interactions of

treatment and trial. This interaction was significant for the mean time to complete the maze and the number of deadends (P=0.001 and P=0.025, respectively). The data displayed in Figures 11 and 12 includes both sets of data for old and young mice. The age groups were combined since age was not a significant factor on neither time nor number of deadends (P=0.828 and P=0.121, respectively). It is interesting to note that the 1/10 and 1/40 DHA groups increased in time and number of deadends from trial 2.1 to trial 2.2, while the other treatment groups (control, 1/8, and 1/20) all decreased. This data is unexpected since the previous experiment (experiment #1) indicated that all treatment groups would improve. However, on this particular maze, the 1/10 and 1/40 groups did not improve in their mean time or number of deadends.

The only significant variable for the trials with maze #3 was age. The age of the mouse did affect the mean number of deadends. In these trials, the old mice had a greater rate of improvement. The younger mice stayed relatively the same in their number of deadends from trial 3.1 to 3.2 (Figure 13). Therefore, even though the younger mice had a lower mean number of deadends when compared to the old mice, the old mice had a greater rate of improvement. Although I did not expect the older mice to perform better than the younger mice, I did anticipate that the older mice would show signs of improvement. One previous experiment examined the effects of chronic administration of docosahexaenoic acid on the memory performance of older rats in a radial arm maze. Gamoh, et al. discovered that the chronic administration of DHA "significantly decreased the number of reference memory errors and working memory errors," (2001). Their study examined the levels of LPO (lipid peroxide), (a chemical

that damages genes, enzyme proteins, and membrane lipids) in the hippocampus of the brain and discovered that the levels tended to decrease with the administration of DHA and had a positive correlation with the number of errors; therefore, DHA decreases the level of LPO, thus improving learning ability (2001). The authors found that in their study, the chronic administration of DHA improved the performance of radial arm maze tasks in older rats.

This experiment supported several aspects of my hypotheses, however, it also presented some interesting, new, and unexpected results. It was found that the level of DHA did affect the mice's ability to run a new maze. Although, the data did not follow the anticipated trend, it was significant for both the mean time and mean number of deadends. There are several reasons for why the 1/20 group may have yielded unexpected results. First, there is always the possibility that there was something wrong with the treatment. Although all of the treatment mixtures were carefully measured, there is always the possibility of error. The treatment mixtures were made several times throughout the experiment in order to eliminate any errors. After the initial results, all data entries were double-checked to ensure that the math and calculations were correct. Another possibility could be that some of the mice were simply smarter than others. The mice were all either obtained from the same supplier or bred from mice that were originally obtained from the supplier, and therefore should have been of a similar genetic species. To eliminate any previous experience or intelligence factors, the mice were randomly assigned to groups. Perhaps a larger sample size would have provided more accurate results.

I anticipated that the younger mice would learn at a faster rate than the older ones, and this was partially supported with data from trials 1.1, 2.1, and 3.1. The younger mice progressively improved in their time and number of deadends. However, in trials 3.1 and 3.2, even though the younger mice still had faster times and lower numbers of deadends, the older mice seemed to improve at a higher rate. This data shows that mice, of all ages, can learn and improve their memory performance. In addition, I hypothesized that DHA treatments would be more effective at improving memory performance in young mice versus old mice. I expected the old mice receiving the DHA treatments to improve, but I expected to see a greater rate and level of improvement among young mice, due to the large part DHA plays in the development of the brain. My results for trials 1.1 and 1.2 support this hypothesis. The younger mice receiving DHA had better memory performance than the young mice in the control group. The old mice in the control group did better than the old mice receiving DHA. Therefore, DHA seemed to be more effective among the younger mice.

Conclusions

My findings correspond with previous studies on DHA and how it affects memory performance. In previous studies, researchers found that the chronic administration of DHA improved reference memory related learning in young rats as well as old rats (Gamoh et. al. 1999, and Gamoh et al. 2001). Therefore, the administration of DHA can improve memory performance in both young and old rats. These findings parallel mine in that both age groups were positively affected by treatments of DHA. My study,

however, found that younger mice's memory might be more greatly affected by the administration of DHA.

Another previous experiment examined whether dietary manipulations that result in a decreased concentration of DHA in the brain would interfere with olfactory-based learning (Greiner, et. al. 2001). This study revealed that the group receiving a deficient source of n-3 fatty acids (DHA) had 81% less brain DHA compared to the n-3 adequate group. In addition, the n-3 deficient group made significantly more errors than the n-3 adequate group in several odor discrimination tasks (2001). The use of olfactory cues and smell is crucial for macrosmatic animals, such as rats and mice, to learn about their environment. "Recent studies have demonstrated that rats rapidly acquire a number of relatively complex tasks when provided with odor cues," (2001). Therefore, it is logical to assume that mice who receive DHA supplements will have improved olfactory/odor performance, and would be able to more precisely detect the peanut butter at the end of the maze. This detection of a food source could serve as a motivator for completion of the maze. It would be interesting to conduct a future study on how DHA affects olfactory-based learning within a maze. Another point to take into consideration is the mouse's level of motivation. Greiner, et al. used partial water deprivation in their study and wondered if the differences in the subjects' responses to the water deprivation could have lead to their differences in motivation. This could relate to my study since I used partial food deprivation. I gave the mice a limited amount of dry food (about one piece per day) so that they would be motivated to reach the end of the maze where a treat of peanut butter was waiting. The more the mouse was motivated, the faster

he/she may have run the maze. Therefore, it would be of interest to complete another study that took the possibility of different reactions to food deprivation into consideration.

This research project supported the hypotheses that mice, under the influence of DHA, would have increased levels of memory performance and would learn new mazes at a faster rate. All mice learned the mazes after subsequent trials, but there were specific treatment groups that seemed to learn at faster rates. Experiment #1 may have been unsuccessful due to the lack of a continuous treatment schedule and the age of the mice. Ginkgo biloba did not appear to be as effective as DHA, but that may have been influenced by the age of the mouse. Only young mice were used in the first experiment, so the interaction between the age of the mouse and the Ginkgo biloba treatment could not be studied. If Ginkgo biloba is more effective for old mice, and both old and young mice were used in the first study, different results might have been obtained. Additional studies should examine the effects of Ginkgo biloba on old mice as well as young; perhaps DHA is more effective for young mice and Ginkgo biloba is more beneficial for old mice. A future study could also involve a larger sample size and use even more precise methods of DHA administration. DHA could be given via injection into the blood stream instead of administered orally. Additional trials and mazes could also be used to acquire more detailed data. A future study could also incorporate pregnant females, in order to see if several generations of offspring exposed to additional dosages of DHA would have better memory performance than generations of offspring that received the normal amount of DHA that is naturally found in the mother's

milk. A preliminary version of this study was attempted, but failed due to the lack of time and successful pregnancies.

References

"A Pill That Helps You Think?" Tufts University Health & Nutrition Letter 15 (1997): 10

Briskin, Donald P. "Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health." Plant Physiology 124.2 (2000): 507-514.

Brody, Jane. "An enriching change for infant formulas." New York Times 150.51817 (2001): F6

Chea, Terence. "Martek Oils Safe, FDA Concludes." Washington Post 18 May 2001: E05.

Ellis, Earl F., Police, Richard J., and Lyn M. Yancey. "Effect of fish oil n-3 fatty acids on cerebral microcirculation." American Journal of Physiology 258 (1990): H1780-5.

Gajewski, Ann and S. A. Hensch. "Ginkgo biloba and memory for a maze." Psychological Reports 84.2 (1999): 481-484.

Gamoh, S., Hashimoto, M., Sugioka, K., Shahdat Hossain, M., Hata, N., Misawa, Y., and S. Masumura. "Chronic administration of docosahexaenoic acid improves reference memory-related learning ability in young rats." Neuroscience 93.1 (1999): 237-241.

Gamoh, Shuji., Hashimoto, Michio., Hossain, Shahdat., and Sumio Masumura. "Chronic administration of docosahexaenoic acid improves the performance of radial arm maze task in aged rats." Clinical and Experimental Pharmacology & Physiology 28.4 (2001): 266-270.

Glisson, James., Crawford, Rebecca., and Shannon Street. "The Clinical Applications of Ginkgo Biloba, St. John's Wort, Saw Palmetto, and Soy." The Nurse Practitioner 24.6 (1999): 28.

Greiner, Rebecca Sheaff., Moriguchi, Toru., Slotnick, Burton M., Hutton, Ana., and Norman Salem, Jr. "Olfactory discrimination deficits in n-3 fatty acid-deficient rats." Physiology & Behavior 72.3 (2001): 379-385.

Lim, Sun-Young and Hiramitsu Suzuki. "Changes in Maze Behavior of Mice Occur after Sufficient Accumulation of Docosahexaenoic Acid in Brain." The Journal of Nutrition 131.2 (2001): 319.

Oken, Barry S. "The Efficacy of Ginkgo biloba on Cognitive Function in Alzheimer Disease." The Journal of the American Medical Association 281.5 (1999): 402.

Sasaki, Keiko., Hatta, Shinichi., Wada, Keiji., Ohshika, Hideyo., and Masanobu Haga. "Bilobalide prevents reduction of gamma-aminobutyric acid levels and glutamic acid decarboxylase activity induced by 4-0-methylpyridoxine in mouse hippocampus." Life Sciences 67.7 (2000): 709-715.

Wainwright, P.E., Xing, H. C., Ward, G. R., Huang, Y. S., Bobik, E., Auestad, N., and M. Montalto. "Water maze performance is unaffected in artificially reared rats fed diets supplemented with arachidonic acid and docosahexaenoic acid." Journal of Nutrition 129.5 (1999): 1079-1089.

Wander, Rosemary. "A Primer on Dietary Fat: The Good, the Bad, and the Unknown." The Linus Pauling Institute Fall/Winter (1998): Internet. 28 January 2002. Available: http://www.orst.edu/dept/lpi/f-w98/primer.html

Wasantwisut, E. "Nutrition and development: Other micronutrients' effect on growth and cognition." The Southeast Asian Journal of Tropical Medicine and Public Health 28.2 (1997): 78-82.