

1-20-2021

Meta-Analysis of Natural vs Pharmaceutical Interventions for Alzheimer's Disease

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Vrublevskaya, Tamara, "Meta-Analysis of Natural vs Pharmaceutical Interventions for Alzheimer's Disease" (2021). *Master's Projects*. 977.

DOI: <https://doi.org/10.31979/etd.b494-dcd5>

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Meta-Analysis of Natural vs Pharmaceutical Interventions for Alzheimer's Disease

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In Partial Fulfilment
of Requirements for the
Degree Master of Science

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November 2020

ABSTRACT

The purpose of this research is to present a way to compare interventions by collecting all available drug clinical trials for a disease of interest. The assessment is done using Meta-Analysis, a method for evaluating data from several studies to arrive at a combined estimate of treatment effect. The evaluation is done in R on a given set of studies to answer the question – which Alzheimer's Disease intervention (pharmaceutical vs. natural) show better outcomes and fewer adverse effects. The research covers many types of interventions across diverse patient populations. On a small subset of papers, natural treatments showed better overall improvements. The evaluation conducted is not medical research, and it lays no claims for determination which intervention is better, but the approach presented can be utilized in medical research provided a more representative dataset is used. The simple and portable way to do Meta-Analysis is presented. As it is not specific to the domain of disease interventions, it could be applied to other domains with minimal adjustments.

Keywords: Meta-Analysis, Alzheimer's disease, Alzheimer's treatments

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Introduction

Alzheimer's Disease

According to the World Health Organization, Alzheimer's Disease (AD) is the 5th leading cause of death around the globe [1]. Worldwide there are 50 million people living with dementia, and staggering 10 million annual cases of dementia are reported. Alzheimer's contributes to approximately a third of those cases [2]. The future predictions are very alarming - between 2017 and 2025, every state in the US is expected to see at least a 14% rise in the prevalence of AD (in some states, the rise is greater than 40%) [2]. In 2018, the mortality rate due to Alzheimer's reached 37.3 deaths per 100,000 people [3]. Globally, the total number of people with dementia is expected to reach 82 million in 2030 and 152 million by 2050. Much of this increase is attributable to the rising numbers of people with dementia living in low- and middle-income countries and an overall population aging [4].

It is important to understand and study all factors that contribute to the significant number of AD cases each year. As an example, one of the most feared long-term consequences of traumatic brain injuries (TBI) is dementia that often leads to more serious conditions [5]. By doing a better job at spreading awareness of the seriousness of head injuries and focusing more on their treatment and prevention, we can improve the Alzheimer's statistics and the quality of life of many seniors, gifting families more time and memories with their loved ones. Head injuries and age are not the only variables that can cause an AD diagnosis. Poor diet, genetic conditions, vascular disease, immune system deficiencies, etc., are all risk factors for Alzheimer's [6][7][8].

Alzheimer's Disease (AD) is an irreversible, progressive brain disorder that slowly destroys brain cells, causing a loss of the connections between neurons, and is the leading cause

of dementia around the globe [9]. AD has a severe negative impact on the quality of life, creates a difficult burden on family members, worsens cognitive functions, memory, and linguistic abilities. The behavioral patterns of severely affected patients closely resemble those of a 3-year-old child that must not be left unattended.

The disease can either be early-onset starting between the age of 30 to 60 or late-onset starting in the mid 60's [10]. The disease has seven distinct phases, the last one ending with death. The starting point of AD, stage one, is undetectable, and the second stage is the usual forgetfulness that is associated with aging, like misplacing a cell phone. The second stage is completely harmless, and family members, as well as the person experiencing the symptoms, will not be able to link this slightly fading of memory to AD. Stage three is called mild cognitive decline and is associated with increased forgetfulness, loss of focus, slight difficulty in communication. This stage may last many years before officially turning into early-stage dementia – stage four. This stage has a more noticeable cognitive decline; a person may have trouble managing a household and finances, traveling alone, or problem-solving. The average duration of stage four is approximately two years. Stage five already requires assistance with everyday tasks. At this stage, patients cannot be allowed to travel alone because they usually do not remember their address or phone number and may lose orientation. Stage five approximately lasts one and a half years. Stage six, severe cognitive decline, is the commonly associated level with Alzheimer's disease- limited memories of past life, trouble recognizing family, difficulty speaking with complete sentences. At stage six, patients required round-the-clock care. This stage lasts approximately two and a half years. Late dementia is the final seventh stage. Patients can become immobile with almost no communication ability and can exist in this stage for approximately two and a half years until death [11].

Alzheimer's neuropathology is mainly characterized by two events - a high level of amyloid-beta ($A\beta$) plaques and neurofibrillary tangles inside neurons (tau protein aggregates) [12]. Amyloid-beta peptide is derived from a transmembrane protein known as an amyloid precursor protein (APP). In the correct biochemical pathway, the ($A\beta$) peptide is cleaved from the APP by the alpha or beta-secretase (protease enzyme which assists in protein/peptide breakdowns) and creates a shorter version peptide - $A\beta$ 40. If the cleavage is done sequentially by beta and then gamma-secretase, it creates a long toxic type of the peptide – $A\beta$ 42 [13]. The second hallmark of AD, neurofibrillary tangles (NFT), are formed by abnormally folded and hyperphosphorylated tau protein. This protein is involved in microtubule formation; however, when hyperphosphorylated, the tubules fall apart, that partitions form insoluble aggregates of tau protein and become the NFT's. Tangle formation is a good indicator of the disease stage - the more advanced the stage of the disease, the more tau tangles in the brain [14]. The $A\beta$ 42 plaques together with NFTs cause neuronal damage and death, leading to the fatal ending of the disease.

Treatments for Alzheimer's can fall into different categories: (1) cholinesterase inhibitors that try to block the breakdown of acetylcholine, which is the main neurotransmitter for both the peripheral nervous system and the central nervous system [15]; (2) antioxidants to minimize the damaging effect of free radicals [12]; (3) monoclonal antibodies (proteins mimicking immune system response) are used to target the amyloid-beta plaques and remove them [16]; (4) BACE1 inhibitors target the APP beta-secretase cleaving enzyme directly to prevent new plaques from forming in the brain [17]. There are many more types of AD interventions in clinical trial stages. Alzheimer's interventions that were evaluated in this paper are presented in the table below. They are split by natural (common vitamins, natural diet, plant extracts) vs. non-natural (human-

derived complexes and other compounds). Clinical trials containing other interventions that were originally collected but not covered by the Meta-Analysis are absent from this table.

Type	Intervention	Treatment Description
natural	Huperzine A	Natural cholinesterase inhibitor derived from the Chinese herb <i>Huperzia serrata</i>
natural	Crocus sativus	Saffron
natural	Extract of <i>Salvia officinalis/lavandulaefolia</i>	Sage. Essential oil composed almost exclusively of monoterpenoids
natural	EGB761	Ginkgo biloba extract
natural	Prolonged-release melatonin (PRM)	Hormone secreted by the pineal gland at night
natural	<i>Melissa officinalis</i>	Herb extract
natural	Folic acid 5 mg, vitamin B12 1 mg, vitamin B6 25 mg	High-dose vitamin supplements
natural	Vitamin E, memantine	Alpha tocopherol; moderate-affinity NMDA antagonist
natural	DHA	Algal docosahexaenoic acid (DHA)
non-natural	Bryostatin	Activator of protein kinase C epsilon
non-natural	Verubecestat	Oral BACE-1 inhibitor
non-natural	Rosiglitazone	Agonist of the peroxisome proliferator-activated receptor-gamma
non-natural	Bapineuzumab	Humanized N-terminal-specific anti-AB monoclonal antibody
non-natural	Donepezil	Cholinesterase inhibitor
non-natural	Tramiprosate	The modified amino acid that binds to soluble amyloid-beta and inhibits the formation of neurotoxic aggregates
non-natural	Edonepic maleate	Low-molecular-weight compound that protects against neurotoxic effects preserves synapses
non-natural	Solanezumab	Humanized immunoglobulin G1 monoclonal antibody
non-natural	RGm	Resveratrol with glucose and malate
non-natural	ABT-126	Novel $\alpha 7$ nicotinic acetylcholine receptor agonist ABT-126
non-natural	Vitamin E, vitamin C, alpha-lipoic acid	Antioxidants
non-natural	Forasyn Connect	Multinutrient combination
non-natural	Semagacestat	Small molecule gamma-secretase inhibitor

Table 1. List of included Alzheimer's treatments with a brief description of the treatment.

It is hard to imagine that such a common disease that has been known for more than 100 years still to this day has no cure and is the only disease in the top 10 causes of death that cannot be prevented or controlled. If many drugs to date have no noticeable positive impact on the

patients, perhaps, science has been moving in the wrong direction? Can natural treatments be the future of AD? If natural treatments have higher success rates, can this relieve the heavy burden of caregiving and cut costs? In 2020 alone, Alzheimer's and other dementia-related healthcare cost the nation \$305 billion, including \$206 billion in Medicare and Medicaid payments. By 2050, Alzheimer's is projected to cost more than \$1.1 trillion unless a remedy will be available [18].

There are two independent goals in this project: to present a simple way to do Meta-Analysis in R and to evaluate if Meta-Analysis is a reliable approach for rating Alzheimer's interventions individually and by type and to assess whether natural treatments for AD perform better than pharmaceutical treatments. Meta-Analysis was chosen as a useful tool that allows working with large amounts of data.

Meta-Analysis

Meta-Analysis is a statistical procedure for combining data from multiple studies first introduced in the 1970s by Gene V Glass [19]. By extracting data from several studies, the goal of a Meta-analysis is to arrive at a combined estimate of treatment effect (effect size) – summary measures that represent the difference in average scores between intervention and control groups [20]. Generally, effect estimates of the results of an applied intervention from different treatment studies are combined using a weighted average, generating a single statistic with a confidence interval that encapsulates how effective the experimental intervention was with respect to control groups. Meta-Analysis is advantageous because it enhances precision by combining information from multiple studies. It could also be used to address a question that is either different from or broader than those targeted by the individual studies. The target question of a Meta-Analysis can address a broader range of interventions and populations. Finally, Meta-Analysis can resolve

conflicts presented by the conclusions of individual studies or fill in the gaps missed by individual studies. However, the disadvantage of Meta-Analysis is the potential to be misleading, as there are numerous design-related decisions that must be made and documented and many sources of bias that must be considered. Specifically, aspects of design such as the selection of studies and data, inclusion/exclusion criteria, and the appropriate comparison of interventions must be carefully considered [21]. Moreover, the data preparation phase of Meta-Analysis is extremely time-consuming because it is a manual and labor-intensive process requiring careful reading and data extraction from each study.

To conduct a comprehensive Meta-Analysis, one must calculate the effect size, make a choice of the correct statistical model to use (fixed-effect vs. random effect or others), understand the concepts of and variables that quantify heterogeneity, then produce a forest plot that shows estimated results along with corresponding statistics, and finally interpret the results [22]. Additional steps include creating a funnel plot and conducting tests on publication bias.

The effect size identifies the magnitude and direction of a treatment effect compared to the placebo. The calculation of the effect size depends on the type of data – continuous or dichotomous. When the experimental and control intervention responses are continuous, studies typically report the mean ($\hat{\mu}_{ek}, \hat{\mu}_{ck}$), standard deviation (s_{ek}, s_{ck}), and sample size (n_{ek}, n_{ck}) for the experimental and control groups for studies $k = 1, \dots, K$. When all outcomes are reported on the same cognitive assessment scale, the mean difference may be used as a measure of the effect. When study outcomes are reported on different scales (e.g., some studies report Alzheimer's Disease Assessment Score - cognitive subscale (ADAS-cog), others Clinical Dementia Rating-Sum of Boxes, etc.), the standardized mean difference, a dimensionless effect measure, is instead used since mean differences cannot be pooled directly. This paper uses continuous data only.

For a study k , the estimated mean difference is given by

$$\hat{\mu}_k = \hat{\mu}_{ek} - \hat{\mu}_{ck}$$

and the variance estimate by

$$\widehat{Var}(\hat{\mu}_k) = \frac{S_{ek}^2}{n_{ek}} + \frac{S_{ck}^2}{n_{ck}}$$

An approximate $(1 - \alpha)$ two-sided confidence interval is then

$$\hat{\mu}_{ek} - \hat{\mu}_{ck} \pm z_{1-\frac{\alpha}{2}} \sqrt{\frac{S_{ek}^2}{n_{ek}} + \frac{S_{ck}^2}{n_{ck}}}$$

where $z_{1-\frac{\alpha}{2}}$ is the $1 - \frac{\alpha}{2}$ quantile of the standard normal distribution.

A common version of the standardized mean difference is the Hedge's g , given by

$$\hat{g}_k = \left(1 - \frac{3}{4n_k - 9}\right) \frac{\hat{\mu}_{ek} - \hat{\mu}_{ck}}{\sqrt{\frac{((n_{ek} - 1)S_{ek}^2 + (n_{ck} - 1)S_{ck}^2)}{(n_k - 2)}}}$$

where $n_k = n_{ek} + n_{ck}$ and a correction for bias in the estimated standard error is achieved via $\left(1 - \frac{3}{4n_k - 9}\right)$. The variance of \hat{g}_k is

$$\widehat{Var}(\hat{g}_k) = \frac{n_k}{n_{ek} \cdot n_{ck}} + \frac{\hat{g}_k^2}{2(n_k - 3.94)}$$

and the two-sided $(1 - \alpha)$ confidence interval is calculated by

$$\hat{g}_k \pm z_{1-\frac{\alpha}{2}} S.E.(\hat{g}_k)$$

where $S.E.(\hat{g}_k) = \sqrt{\widehat{Var}(\hat{g}_k)}$ and $z_{1-\frac{\alpha}{2}}$ is the $1 - \frac{\alpha}{2}$ quantile of the standard normal distribution [23].

There are two commonly used modes: fixed effect model and random effect model.

A fixed effect model is used when all studies $k = 1, \dots, K$ are estimating the same value, share an identical protocol, share the same effect size, and come from one homogenous population. The main model assumption is that every study is evaluating a common treatment effect [12]. Let $\hat{\theta}_k$ be the intervention effect from study k and θ unknown intervention effect in the population, our goal for estimation. Furthermore, let $\hat{\sigma}_k^2$ be the sample estimate of $Var(\hat{\theta}_k)$. The fixed effects model is then

$$\hat{\theta}_k = \theta + \sigma_k \epsilon_k, \quad \epsilon_k \sim N(0,1)$$

where ϵ_k are independent and identically distributed.

The maximum likelihood estimate of θ is given by

$$\hat{\theta}_F = \frac{\sum_{k=1}^K \hat{\theta}_k / \hat{\sigma}_k^2}{\sum_{k=1}^K 1 / \hat{\sigma}_k^2} = \frac{\sum_{k=1}^K w_k \hat{\theta}_k}{\sum_{k=1}^K w_k}$$

This method is called the inverse variance method, since $\hat{\theta}_F$ is a weighted average of $\hat{\theta}_k$, the individual effect estimates and the weights $w_k = 1 / \hat{\sigma}_k^2$. The variance of $\hat{\theta}_F$ is

$$\widehat{Var}(\hat{\theta}_F) = \frac{1}{\sum_{k=1}^K w_k}$$

and a $(1 - \alpha)$ confidence interval for $\hat{\theta}_F$ is

$$\hat{\theta}_F \pm z_{1-\frac{\alpha}{2}} S.E.(\hat{\theta}_F)$$

where $S.E.(\hat{\theta}_F) = \sqrt{\widehat{Var}(\hat{\theta}_F)}$ and $z_{1-\frac{\alpha}{2}}$ is the $1 - \frac{\alpha}{2}$ quantile of the standard normal distribution.

The test statistic $\hat{\theta}_F/S.E.(\hat{\theta}_F)$ may then be used to test for an overall treatment effect [16].

A random effects model, on the other hand, has a completely different sampling frame – assumes a universe of populations, random sampling, and variation in effects. The main model assumption is that the true treatment effects of the individual studies may be different from each other [12]. The random effects model is typically used when a Meta-Analysis combines studies that use different protocols/test groups and are part of a systematic review. If we elect to use a random effects model, we are assuming that the effect estimates, $\hat{\theta}_k$ Vary more than under a fixed effects model.

The random effects model is

$$\hat{\theta}_k = \theta + \mu_k + \sigma_k \epsilon_k$$

where $\epsilon_k \sim N(0,1)$ and $\mu_k \sim N(0, \tau^2)$ are independent, identically distributed, and independent of one another.

The maximum likelihood estimate is given by the DerSimonian-Laird estimator $\hat{\tau}^2$ (describes between-study variability and does not increase with K or sample size).

$$\hat{\tau}^2 = \frac{Q - (K - 1)}{S}$$

$$S = \sum_{k=1}^K w_k - \frac{\sum_{k=1}^K w_k^2}{\sum_{k=1}^K w_k}$$

But if $Q < (K - 1)$, $\hat{\tau}^2 = 0$ and $\hat{\theta}_R = \hat{\theta}_F$.

According to the inverse variance method, the random effects estimate and variance are given by

$$\hat{\theta}_R = \frac{\sum_{k=1}^K w_k^* \hat{\theta}_k}{\sum_{k=1}^K w_k^*}$$

$$\widehat{var}(\hat{\theta}_R) = \frac{1}{\sum_{k=1}^K w_k^*}$$

respectively, with weights $w_k^* = 1/(\widehat{\sigma}_k^2 + \hat{\tau}^2)$.

The $(1 - \alpha)$ confidence interval for $\hat{\theta}_R$ is

$$\hat{\theta}_R \pm z_{1-\frac{\alpha}{2}} S.E.(\hat{\theta}_R)$$

where $S.E.(\hat{\theta}_R) = \sqrt{\widehat{var}(\hat{\theta}_R)}$ and $z_{1-\frac{\alpha}{2}}$ is the $1 - \frac{\alpha}{2}$ quantile of the standard normal distribution.

The test statistic $\hat{\theta}_R/S.E.(\hat{\theta}_R)$ may then be used to test for an overall treatment effect [16].

Heterogeneity is a measure of the variation among effect sizes. For example, if one of the studies were conducted on mice and the other on humans, a high heterogeneity is expected and would suggest that different subgroups (mice vs. humans) are present. In this example, there was known heterogeneity, however, in real life, Meta-Analysis unexplained differences between the studies can be a cause of concern.

There are a few important variables that measure heterogeneity in a Meta-Analysis: τ^2 , I^2 , and Cochran's Q with a p -value [24][25]. The Q -statistic (Cochran's Q) is defined as the weighted sum of squared differences between the observed effects and the weighted average effect [26].

$$Q = \sum_{k=1}^K w_k (\hat{\theta}_k - \hat{\theta}_F)^2$$

where K is the number of studies, $w_k = 1/\hat{\sigma}_k^2$, $\hat{\theta}_k$ the intervention effect and $\hat{\theta}_F$ is a weighted average of $\hat{\theta}_k$.

Under the null hypothesis (no heterogeneity), Q follows a Chi-Square distribution with $df = K - 1$. Greater heterogeneity among studies is indicated by large Q and $\hat{\tau}^2$.

Two additional commonly used statistics are

$$H^2 = \frac{Q}{K - 1}$$

$$I^2 = \begin{cases} \frac{H^2 - 1}{H^2} & \text{if } Q > (K - 1) \\ 0 & \text{otherwise} \end{cases}$$

where large H^2 indicates higher heterogeneity, and $H = 1$ signifies homogeneity. I^2 measures a proportion of observed variance and is independent of K and ranges between 0% and 100%, with larger I^2 indicating greater heterogeneity. By convention, I^2 greater than 30% is considered heterogeneous [23][27].

Lastly, two visual representations are commonly used. Forest plots are the gold standard for the visual representation of Meta-Analysis data. The forest plots show the full comparison of all studies with confidence intervals, the overall effect, and can provide the statistics described above ($\hat{\tau}^2$, I^2 , p -value). One of the most important statistics on the forest plot is the confidence

interval (CI) for each study, which shows a margin of error for the result and p -value, representing the statistical significance of the study. If the study results cross over the middle (no effect) line, they are not statistically significant. The plot also shows the "weight" of each study, the bigger the block, the more weight is given to the study and usually depends solely on the sample size [29][30].

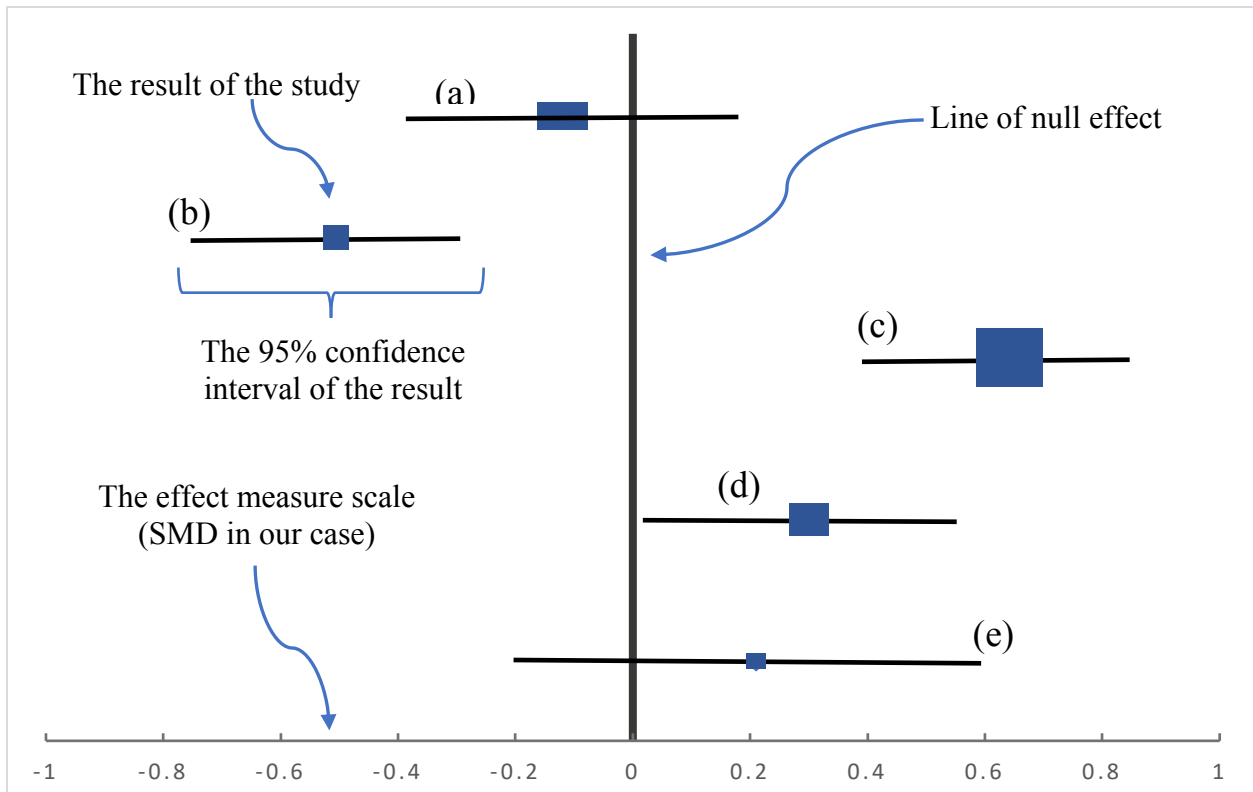


Figure 1. How to read a forest plot.

This is an example forest plot with five results. The bigger "block" of the the result, the more "weight" (influence on the result) was given to the study. For example, (c) has the most weight, and (e) has the lowest weight. The x-axis scale is the scale for the statistic (standard mean difference is used in this paper). If positive results on the scale mean that the treatment is effective, then all the studies to the right show effective treatments. The studies that have no crossing with the line of no effect are considered statistically significant.

Funnel plots are the visual representation of the present publication bias in the study, and if it is present, the plot will look asymmetrical [28]. The y-axis is usually the standard error of

the effect estimate, and the x-axis is the effect estimator. Larger studies with a higher power are placed towards the top. Lower powered studies are placed towards the bottom. The lines of the triangle represent the 95% confidence interval.

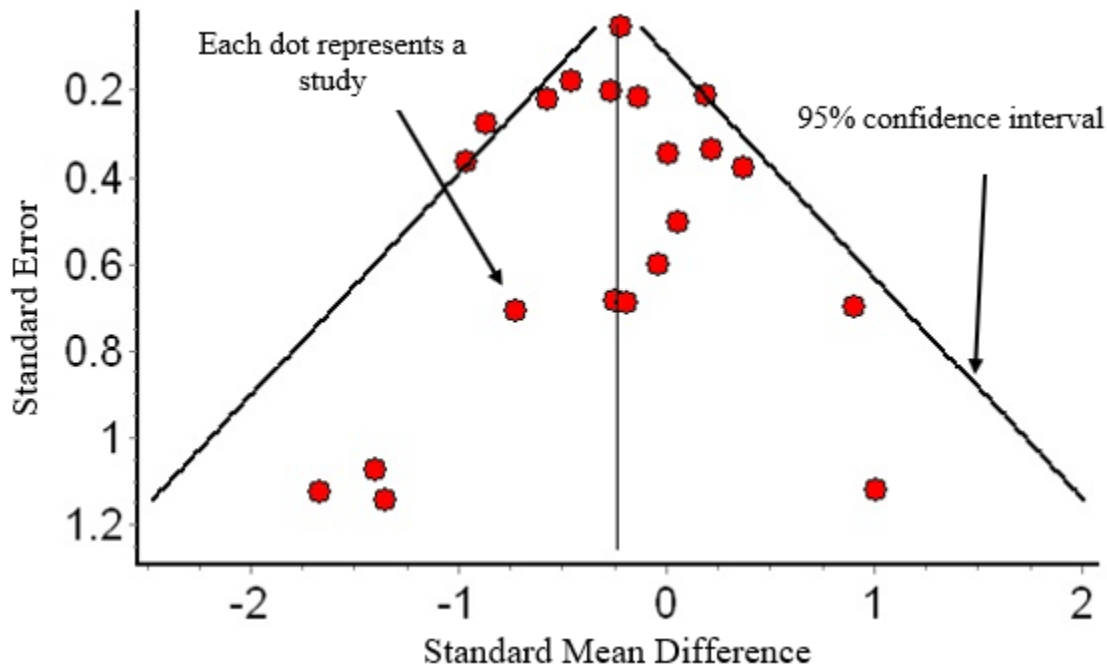


Figure 2. How to read a funnel plot.

The funnel plot can check if publication bias is present (if it is, it will look asymmetrical). The standard error (y-axis) provides a measure of the precision of the effect size as an estimate of the population parameter. More precise studies (smaller standard error) are on top, and others are scattered widely towards the bottom.

Meta-Analysis Approach

When studies provide data for multiple interventions (for example, varying dose of medicine), there are several possible venues for analysis:

- Combine intervention groups into a single group;
- Pair intervention one by one with the control;

- Partition the shared (control) group into more groups such that pairwise comparisons are independent;
- Perform Network Meta-Analysis comparing all interventions simultaneously.

The first option is generally recommended, though the last option makes the most efficient use of the data and will generally produce a similar result to the first option [21]. Both the first and last options are used in this paper.

To combine two interventions into a single intervention group, the following formulas are applied [21].

	Group 1	Group 2	Combined groups
Sample Size	N_1	N_2	$N_1 + N_2$
Mean	M_1	M_2	$\frac{N_1M_1 + N_2M_2}{N_1 + N_2}$
SD	SD_1	SD_2	$\sqrt{\frac{(N_1 - 1)SD_1^2 + (N_2 - 1)SD_2^2 + \frac{N_1N_2}{N_1 + N_2}(M_1^2 + M_2^2 - 2M_1M_2)}{N_1 + N_2 - 1}}$

Table 2. Formulas used for calculating combined groups sample size, mean and standard deviation.

Network Meta-Analysis generalizes Pairwise Meta-Analysis and focuses on addressing the question of how a set of treatments for a particular diagnosis compare to one another by combining direct and indirect evidence. This assumes that (1) studies are independent, and (2) the underlying effects are consistent (so-called transitivity assumption). Network Meta-Analysis allows for the treatments to be ranked based on their summary results in a single analysis [31]. The data collected may be visualized in a network graph. For example, if studies compare three interventions, A, B, C, the network could look like the following graph:

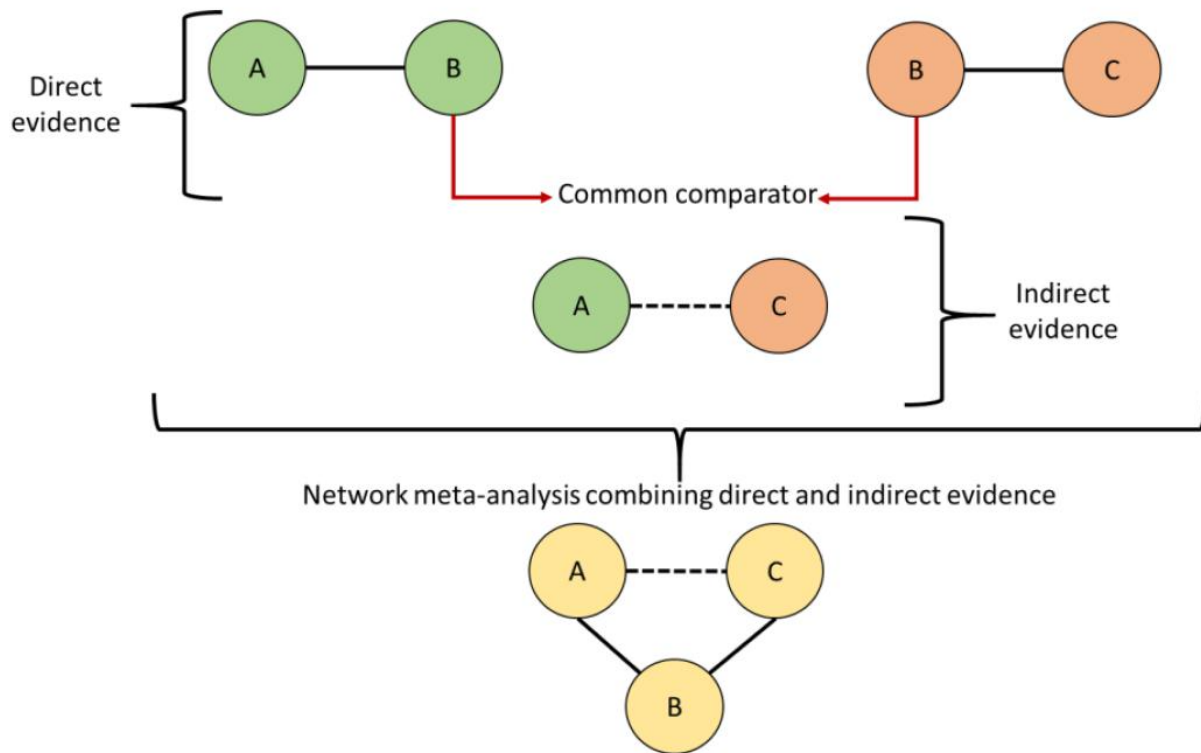


Figure 3. Building a Network Meta-Analysis from direct and indirect evidence [32]. When studies are found directly comparing interventions, the following relationship can be demonstrated: A versus B (green) and B versus C (orange). Each circle represents an intervention, and lines represent direct comparisons. Dashed lines are for indirect comparison. A global effect value is generated for each comparison (direct or indirect). Indirect evidence is generated by using B as a common comparator for the comparison of A versus C.

In Figure 1, the connecting lines between the two treatments mean that there are one or more studies in the data set that compare the treatments. A network is consistent when direct and indirect evidence between two treatments does not differ. The goal of a Network Meta-Analysis for the above network would be to estimate the treatment differences and their standard errors [33][34].

Risk of Bias

The small-study effect may introduce bias into Meta-Analysis, as smaller studies often show larger treatment effects compared to large studies. This often occurs because smaller

studies with larger treatment effects are more likely to be published. Other reasons for the small-study effect include outcome selection bias, clinical heterogeneity, and coincidence. The small-study effect is usually examined via a funnel plot, which shows the estimated treatment effects against their standard errors. If no small-study effect is present, treatment effects will scatter about the average regardless of the size of the study. In the absence of extreme between-study heterogeneity, small studies will scatter more widely due to their larger standard errors, generating the funnel plot in the shape of the symmetric triangle. If the funnel plot is instead asymmetric, this is evidence of the small-study effect. There are additional statistical tests for the small-study effect that test for funnel plot asymmetry, specifically Begg and Mazumdar's non-parametric tests utilizing rank-correlation methods and Egger's tests using regression [35][36].

Adjustments for the small-study effects include the trim-and-fill method, a non-parametric method that provides an estimated number of missing studies, and adjusted treatment effects. This method does not explicitly model selection bias. The Copas method, models publication bias by setting up a model for the treatment effect and a model for the probability of study selection for publication [37]. Another adjustment could be made via regression and shrinkage [23].

Other types of bias include publication bias (positive studies having a higher chance of being published), search bias (how the papers were searched and filtered), and selection bias (inclusion criteria) [38]. Although publication bias is independent of the person performing the Meta-Analysis, both search and selection bias can be easily and unintentionally introduced. Specifically, with an Alzheimer's Disease study, it is given that the patients of the clinical trials are at least over 50 years old, and the sampling frame does not include children and young

adults. This automatically cancels out the assumption of a random sample from the population and introduces bias.

Analysis Goal

One of the goals of this research is to perform a Meta-Analysis on literature to evaluate the efficacy of Alzheimer's Disease treatments. All studies chosen are randomized clinical trials and were largely multi-arm, with two or more treatment groups. In this case, the best options are (1) to combine treatment groups into a single treatment group or split the control group such that a Pairwise Meta-Analysis is possible or (2) to perform a Network Meta-Analysis. Since some of the treatment groups were not simply different dosages but rather different treatments or combinations of treatments, Network Meta-Analysis is a more reasonable approach.

Studies included in the research did not report the same outcomes because they were based on different types of scales, and as a result, effect estimates cannot be pooled. Therefore, the standardized mean difference is used as an effect measure. A further complication is that for some of the scales, higher scores indicate better cognitive function, whereas, for others, higher scores indicate greater cognitive impairment. To resolve this issue, two Meta-Analyses were performed. One Meta-Analysis utilized a single outcome from each study where higher scores signified better cognitive outcomes. The second Meta-Analysis utilized a single outcome from each study, where higher scores signified poorer outcomes. Only a single outcome was selected per study per Meta-Analysis. Network Meta-Analysis sometimes has problems with heterogeneity and inconsistency, so the use of two Network Meta-Analyses provides a more robust approach.

To provide insight into overall trends and incorporate more studies into the analysis, a comparison of study conclusions by treatment type (natural vs. non-natural) was performed.

Adverse effects were evaluated graphically rather than incorporated into the Meta-Analysis. As a supplementary analysis and to examine group-based differences between natural and non-natural treatments, treatment groups were combined into a single treatment such that Pairwise Continuous Outcomes Meta-Analysis could be followed by Subgroup Meta-Analysis.

Methods

Data Extraction

63 studies collected from Pubmed and Clinicaltrials.gov were examined for treatment and placebo differences from baseline. For studies that reported summary statistics, this baseline difference was the most common and consistent result reported. For example, if a study was evaluating ADAS-cog change due to treatment, the mean and standard deviation of the change in ADAS-cog was provided for both the treatment and placebo groups. If standard error or 95% confidence intervals were reported, the standard deviation was derived with two different formulas depending on the sample size. For large sample size (over 60):

$$SD = ((upper\ CI - lower\ CI) *SQRT(sample\ size)) / 3.92$$

and for a small sample size (less than 60) need to use t distribution with degrees of freedom:

$$SD = ((upper\ CI - lower\ CI) *SQRT(sample\ size)) / t\ value$$

the t value in Excel =tinv(1-0.95, N-1), where N is the sample size [39].

When the number of patients in each group was not provided in tabular form or in the body of the paper, the number of patients extracted from the graphs was used in the analysis.

Of the 63 studies, 26 reported treatment and placebo differences from baseline either as mean and standard deviation, standard error, or 95% confidence intervals. Data were manually extracted from these 26 studies and used in the Network Meta-Analysis.

From the remaining studies, six studies reported mean and standard deviation at baseline and after treatment for both treatment and placebo groups. Although the difference in means and standard deviation of the difference in means can be derived, this assumes the two populations (baseline, after treatment) are independent. In the case of all these studies, that assumption is clearly violated, and so these six studies were not incorporated into the analysis.

The remaining 31 studies did not include mean differences and standard deviations. Of these, two were reviews that provided no mean difference data. Three studies presented results in figures with mean difference data not included in tabular form or in the body of the paper. Seven studies were protocols with ongoing trial status and therefore presented no data at all because the clinical trial has not yet been completed, and results reported. Four studies presented hazard ratios rather than mean differences. Six studies did not use a placebo and were excluded because no reasonable comparison to treatment can be made in this situation. Three studies reported adherence or safety/tolerability rather than effectiveness. One study provided results as medians with quartiles rather than mean with standard deviation. Four studies were missing data, either not providing the number of patients in each group, not providing standard deviations, or reporting only for control. One study provided comparisons between groups by time point rather than between time points for each group.

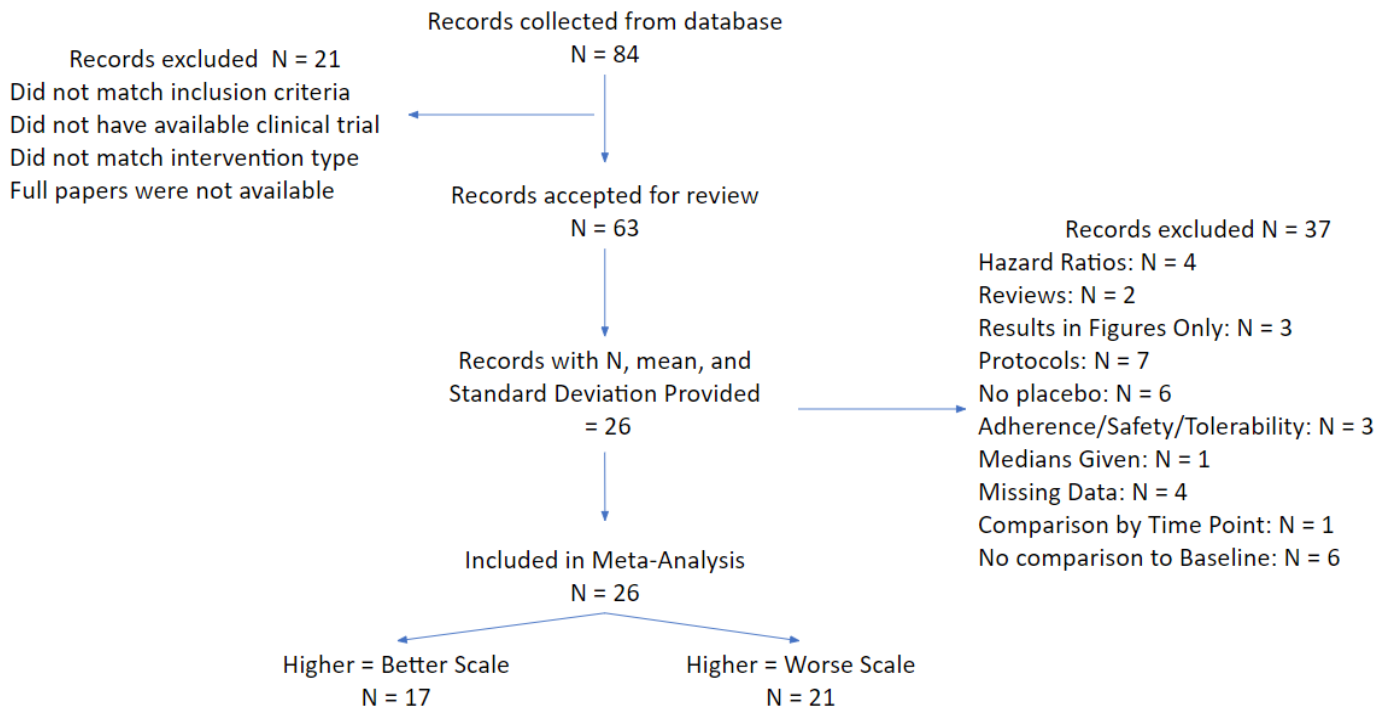


Figure 4. A diagram of study inclusion.

This diagram shows how many studies were originally collected and the stages/reasons for studies being rejected. The final step shows how many studies were included in the Higher-is-Better and Higher-is-Worse scales.

Adverse effects were also manually extracted as the percent of patients in each group with a specific adverse effect (that is the format studies reported). If any study made a conclusion regarding the effectiveness of treatment, this was recorded as a binary variable to be used for descriptive purposes. All treatments were labeled as natural or non-natural.

Excel Spreadsheets

Six Excel Spreadsheets were created during the project to extract and organize data from each study. They are available on GitHub [40].

The *Data Standardized Mean Diff* sheet catalogs basic information about each study, including a unique numerical ID, name, authors, and year. Outcome variables present as a change in score from baseline for placebo, and interventions were recorded. Studies provided

either arithmetic means or least-square means (estimated from a linear model). All applicable outcomes were recorded along with treatment doses. Reference controls/placebo group results were also recorded in a pairwise fashion, even though for many studies, the control/placebo was a reference for two or more arms of the study. Sample size, mean, standard deviation, standard error, or 95% confidence intervals were recorded. Standard deviations were derived from standard errors and 95% confidence intervals when necessary.

The *Standardized Mean Diff* sheet is a cleaned-up version of *Data Standardized Mean Diff*, including only one outcome variable per study per type of scale (either higher better scales or higher worse scales).

The *Combined Groups Analysis* sheet replicates the *Standardized Mean Diff* only it presents the data for the combined groups. Groups were combined using the formulas in the table in the "Combined Groups" section of the introduction, implemented as R functions. Studies in which donepezil was used as placebo were excluded. Only studies that used true placebos were included to minimize bias.

The *Excluded Standardized Mean Diff* and *Need to Derive Mean Diff* sheets catalog studies that were excluded and the reason for exclusion. All studies in the *Need to Derive Mean Diff* sheet were excluded because post-treatment to baseline difference in placebo and intervention groups were not able to be calculated and were not provided in the paper.

The *Data Binary* sheet provides an overview of studies that presented an efficacy conclusion. If the intervention was judged to be efficacious, 1 was placed in the *Treatment Superior* column (otherwise, it was 0).

The *Adverse Effects* sheet catalogs adverse effects per study. Only adverse effects and no severe adverse effects were recorded, though sometimes the distinction between the two was ambiguous in studies. As a result, only the top adverse effects were used for comparison.

Treatment Effects

Outcome scales used are presented in the table below.

Higher-is-Better	Higher-is-Worse
MMSE (Mini-Mental State Examination)	ADAS-Cog (Alzheimer's Disease Assessment Score - cognitive subscale)
ADCS-ADL (Alzheimer's Disease Cooperative Study Activities of Daily Living scale)	CDR-SB, CDR-SOB (Clinical Dementia Rating-Sum of Boxes)
SIB (Severe Impairment Battery)	NPI (Neuropsychiatric Inventory)
Delayed word Recall (number) 4 hr	Simple RT (ms) 4 hr

Table 3. List of scales used to measure the outcome of studies.

This is a collection of the commonly used cognitive measurement scales for the studies included in the project. The effect of treatment was evaluated based on these scales.

Each outcome was cataloged for studies that reported treatment and placebo differences from baseline. These outcomes were then partitioned based on scale type into Higher-is-Better scales (e.g., ADCS-ADL) or Higher-is-Worse scales (e.g., ADAS-cog). For each study, a single outcome was selected for inclusion in the Higher-is-Better group or to the Higher-is-Worse group. Outcomes were prioritized by frequency. ADAS-cog was the most frequent outcome used in the Higher-is-Worse group, and this outcome was selected whenever possible, followed by CDR-SB and NPI. MMSE was the most common outcome used in the Higher-is-Better group, and this outcome was selected whenever possible, followed by ADCS-ADL and SIB. This resulted in two data sets of one outcome per study with mean, standard deviation, and patient number for placebo and treatment groups.

All data manipulation, processing, and analysis were performed in R. The data was structured according to the format presented in [23], such that for each study, each group (treatment, placebo) was accompanied by the number of patients, mean, and standard deviation. From this, the treatment effect and standard error of the treatment effect were calculated for each pairwise grouping. This data processing step was performed twice, for the Higher-is-Better group and then for the Higher-is-Worse group.

A Network Meta-Analysis was performed using the standardized mean difference function of the **netmeta** R package [41], once for the Higher-is-Better group and once for the Higher-is-Worse group. Both fixed and random effects models were fitted, and the results were examined as forest plots with treatments labeled as natural vs. non-natural. Heterogeneity was evaluated using the Q statistic. Publication bias was assessed using a funnel plot, which was tested by a linear regression test of funnel plot asymmetry and method of moments linear regression test of funnel plot asymmetry. In general, the method of moments allows us to solve for parameters by equating sample and theoretic moments. The R documentation states that "the test statistic is based on a weighted linear regression of the treatment effect on its standard error using the method of moments estimator for the additive between-study variance component. The test statistic follows a t distribution with the number of studies – 2 degrees of freedom." [42]

A simple Pairwise Meta-Analysis was performed using the **netmeta** R package [41]. Fixed and random effects models were fitted for both the Higher-is-Better group and the Higher-is-Worse group. Results were summarized using forest plots. Heterogeneity was evaluated using the Q statistic. Publication bias was assessed using funnel plots and rank, linear regression, and method of moments tests. Subgroup analysis was performed to determine whether natural interventions were more efficacious than non-natural interventions.

Network Meta-Analysis and simple Pairwise Meta-Analysis were used because they are the recommended methods when studies present multi-arm results [21]. Network Meta-Analysis makes more efficient use of the data, while simple Pairwise Meta-Analysis presents a more conclusive winner when evaluating natural vs. non-natural interventions.

Treatment Outcomes

To assess the overall effectiveness of natural vs. pharmaceutical methods, conclusions regarding treatment effectiveness (effective vs. not effective or unsure) were compared between natural vs. non-natural studies using bar plots. There was a total of 49 studies used to determine the overall effect. This was done by reading the abstracts and conclusions of each clinical trial/paper to determine if treatment was effective. The total number of effective/non-effective natural and non-natural interventions were summed up and plotted.

Adverse Effects

The top 10 most commonly reported adverse effects were extracted. The percent of patients experiencing adverse effects was summarized using the mean within treatment arms of each study. Boxplots of the percent of patients experiencing adverse effects by type of treatment (natural vs. non-natural) and adverse effects were generated.

Software Code

To conduct the Meta-Analysis, five custom R scripts have been developed.

1. `GroupCombinationFormulas.R`

It contains helper functions to assist in filling Excel file `AlzheimersData.xlsx` with values calculated from studies' data.

2. `Summarize_Data.R`

It produces two plots. The Adverse Effects by Natural and Non-Natural Treatments is plotted based on the Adverse Effects sheet of the `AlzheimersData.xlsx` file. The Overall Effectiveness is plotted based on the Data Binary sheet of the `AlzheimersData.xlsx` file. In addition to the **readxl** package [43] (to import data from Excel files), it uses the **dplyr** package [44]. This script creates figures 11 and 12.

3. `Run_NetworkAnalysis.R`

This script performs the Network Meta-Analysis based on the Standardized Mean Diff sheet of the `AlzheimersData.xlsx` file. It does it twice, first for the Higher-is-Better model, then for the Higher-is-Worse model. Both are done in the same manner by invoking *make_network_ma_data(...)* and *do_network_ma(...)* custom functions defined in the `Custom_Functions.R` script. The only package it uses is the **readxl** package [43] (to import data from Excel files). This script produces figures (5-10) and tables (4-7).

4. `Run_CombinedGroups.R`

It performs Continuous Effects Meta-Analysis for paired outcomes based on the Combined Groups Analysis sheet of the `AlzheimersData.xlsx` file. It does it twice, first for the Higher-is-Better model, then for the Higher-is-Worse model. Both are done in the same manner by invoking *do_continuous_ma(...)* custom function defined in the `Custom_Functions.R` script. The only package it uses is the **readxl** package [43] (to import data from Excel files). This script produces figures (13-18).

5. `Custom_Functions.R`

This script consists of functions to assist `Run_NetworkAnalysis.R` and `Run_CombinedGroups.R` scripts in properly organizing data and conducting Meta-Analysis, and producing data for tables and plots for figures presented in this project. It uses the **dplyr** [44], **grid** [45] and **netmeta** [41] packages. The actual Network Meta-Analysis is performed by the

netmeta function [46] of the **netmeta** package [41]. Tests for bias are performed by the *metabias* [47] function on the *metagen* function [48] results. Forest and funnel plots are produced for the *forest* [49] and *funnel.netmeta* [50] functions respectfully. The actual Continuous Outcome Meta-Analysis is performed by the *metacont* function [51].

The data in the `AlzheimersData.xlsx` file, the processing in the `Summarize_Data.R` and `Custom_Functions.R` scripts are specific to the project's subject. Although, the processing flow in the `Summarize_Data.R` and `Custom_Functions.R` scripts is generic. Moreover, the domain-specific string constants are assigned to variables, thus enabling easy adaptation to conduct the Meta-Analysis in a different data domain.

All R code, as well as the `AlzheimersData.xlsx` file, is placed on the GitHub public repository [40].

Results

Treatment Effects – Network Meta-Analysis

Higher-is-Better Scales - 18 studies with 28 treatments were used in a fixed and random effects Network Meta-Analysis. The multiarm tolerance was relaxed to 0.1. From the R documentation, multiarm tolerance is "A numeric for the tolerance for consistency of treatment estimates and corresponding variances in multi-arm studies which are consistent by design". The default is 0.001. Consistency is when the direct evidence for a treatment effect between two treatments does not differ from the indirect evidence. Therefore, by increasing tolerance, we are allowing it to differ a little [16]. The fitted fixed effects model is provided in Table 4, and the accompanying forest plot is in Figure 5. Heterogeneity was present ($\tau^2 = 0.027$, $\tau = 0.163$, $I^2 = 73.1\%$ (95% CI: 24.2%, 90.4%)). Total Q was 11.14 ($df = 3$, $p = 0.0110$), within designs $Q = 11.21$ ($df = 3$, $p = 0.0106$), and between designs $Q = 0$. The random effects model is provided in

Table 5, and the accompanying forest plot is in Figure 6. In Figures 5 and 6, P-scores are used to order treatments by effectiveness. In both fixed and random effects models, natural treatments like (huperzine A, PRM, and SL essential oil) as well as non-natural treatments like Bryostatin generated the highest difference (improvement) from placebo when assessing Alzheimer's Disease using scales like the MMSE, SIB, and ADCS-ADL. Figure 7 shows a funnel plot to assess publication bias. There is no evidence of publication bias in the plot, and a linear regression test of funnel plot asymmetry did not demonstrate evidence of funnel asymmetry ($t = 1.7, df = 44, p = 0.10$).

Treatment	SMD	95%-CI
0.5 mg/kg Bapineuzumab	0.0823	[-0.0592; 0.2238]
1.0 mg/kg Bapineuzumab	0.0429	[-0.0996; 0.1854]
10 mg/day donepezil	0.2469	[-0.0835; 0.5774]
100 mg semagacestat	-0.0905	[-0.2366; 0.0555]
12 mg/day verubecestat	-0.0248	[-0.1308; 0.0812]
140 mg semagacestat	-0.163	[-0.3126; -0.0135]
2 mg PRM nightly	0.5012	[-0.0096; 1.0120]
200 ug BID <u>huperzineA</u>	0.3343	[-0.0030; 0.6716]
23 mg daily donepezil	0.2863	[-0.0626; 0.6353]
2g/d DGA	0.0854	[-0.1408; 0.3116]
40 mg/day verubecestat	-0.0392	[-0.1460; 0.0677]
400 mg every 4 weeks solanezumab	0.1047	[0.0127; 0.1966]
400 mg of coenzyme Q 3 times/d	-0.0383	[-0.5928; 0.5161]
400 ug BID huperzine A	0.4883	[0.1543; 0.8223]
50 uL of SL essential oil plus olive oil	0.2949	[-0.1698; 0.7596]
5g dextrose, 5 g malate, 5 mg resveratrol with 8 oz glass <u>unsweetened grape juice</u> twice a day	0.3713	[-0.3678; 1.1104]
800 IU/d vitamin E, 500 mg/d vitamin C, 900 mg/d alpha-lipoic acid	-0.6895	[-1.2457; -0.1333]
ABT-126 25 mg/day	-0.1161	[-0.4377; 0.2056]
ABT-126 50 mg/day	0.0974	[-0.1943; 0.3890]
ABT-126 75 mg/day	0.0605	[-0.2638; 0.3849]
Bryostatatin intravenous infusion	0.5685	[0.0957; 1.0413]
edonerpic maleate 224 mg	0.0093	[-0.2116; 0.2302]
edonerpic maleate 448 mg	-0.0113	[-0.2340; 0.2113]
folic acid 5 mg, vitamin B12 1 mg, vitamin B6 25 mg, once daily	0.0949	[-0.1236; 0.3135]
Memantine (10 mg twice a day)	0.0285	[-0.2071; 0.2641]
placebo	.	.
Vitamin E (DL-alpha-tocopheryl acetate 1000IU twice a day)	0.049	[-0.1883; 0.2864]
Vitamin E + Memantine	0.093	[-0.1444; 0.3303]

Table 4. Fixed Effects Model results for the Higher-is-Better outcomes. This tables provides unranked SMD and CI for each study without rounding.

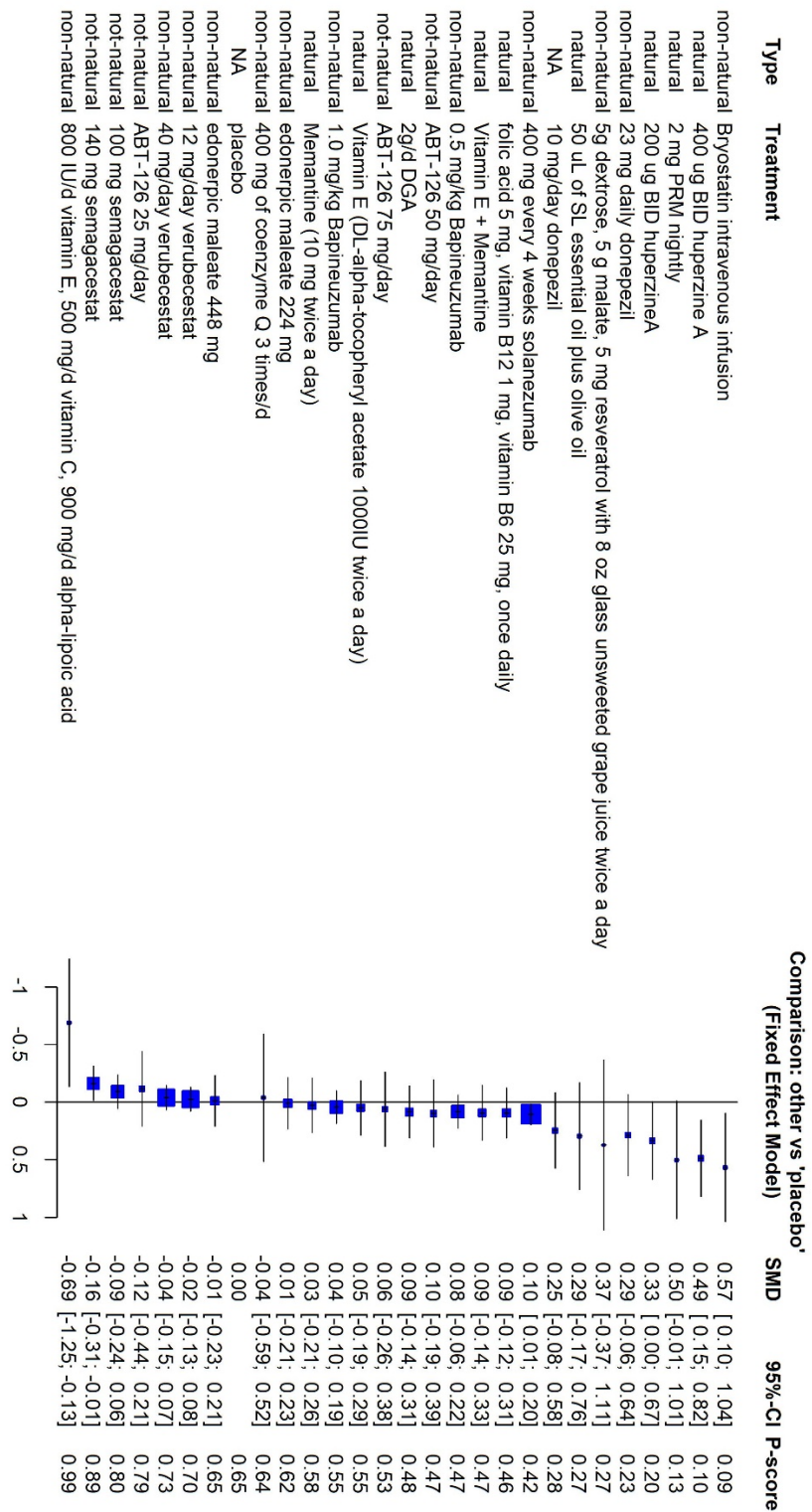


Figure 5. Higher-is-Better Fixed Effects Model Forest plot. Note donepezil is non-natural but listed as NA because some studies used this as a control. Interventions on the top are the highest performing treatments for the Fixed Effect Model.

Treatment	SMD	95%-CI
0.5 mg/kg Bapineuzumab	0.0821	[-0.2674; 0.4316]
1.0 mg/kg Bapineuzumab	0.0431	[-0.3069; 0.3930]
10 mg/day donepezil	0.2473	[-0.2124; 0.7070]
100 mg semagacestat	-0.0906	[-0.4420; 0.2608]
12 mg/day verubecestat	-0.0516	[-0.3031; 0.1999]
140 mg semagacestat	-0.163	[-0.5159; 0.1898]
2 mg PRM nightly	0.5012	[-0.1013; 1.1037]
200 ug BID huperzine A	0.3344	[-0.1303; 0.7990]
23 mg daily donepezil	0.2867	[-0.2843; 0.8577]
2g/d DGA	0.0854	[-0.3061; 0.4770]
40 mg/day verubecestat	-0.079	[-0.3310; 0.1731]
400 mg every 4 weeks solanezumab	0.1047	[-0.2279; 0.4372]
400 mg of coenzyme Q 3 times/d	-0.0383	[-0.6783; 0.6017]
400 ug BID huperzine A	0.4883	[0.0260; 0.9506]
50 uL of SL essential oil plus olive oil	0.2949	[-0.2691; 0.8589]
5g dextrose, 5 g malate, 5 mg resveratrol with 8 oz glass unsweeted grape juice twice a day	0.3713	[-0.4340; 1.1765]
800 IU/d vitamin E, 500 mg/d vitamin C, 900 mg/d alpha-lipoic acid	-0.6895	[-1.3310; -0.0480]
ABT-126 25 mg/day	-0.1161	[-0.5695; 0.3374]
ABT-126 50 mg/day	0.0977	[-0.3350; 0.5303]
ABT-126 75 mg/day	0.0607	[-0.3947; 0.5161]
Bryostatin intravenous infusion	0.6078	[0.0476; 1.1680]
edonergic maleate 224 mg	0.0093	[-0.3792; 0.3978]
edonergic maleate 448 mg	-0.0113	[-0.4008; 0.3782]
folic acid 5 mg, vitamin B12 1 mg, vitamin B6 25 mg, once daily	0.0949	[-0.2923; 0.4821]
Memantine (10 mg twice a day)	0.0285	[-0.3686; 0.4255]
placebo	.	.
Vitamin E (DL-alpha-tocopheryl acetate 1000IU twice a day)	0.049	[-0.3491; 0.4471]
Vitamin E + Memantine	0.093	[-0.3051; 0.4911]

Table 5. Random Effects Model results for the Higher-is-Better outcomes. This table provides unranked SMD and CI for each study without rounding.

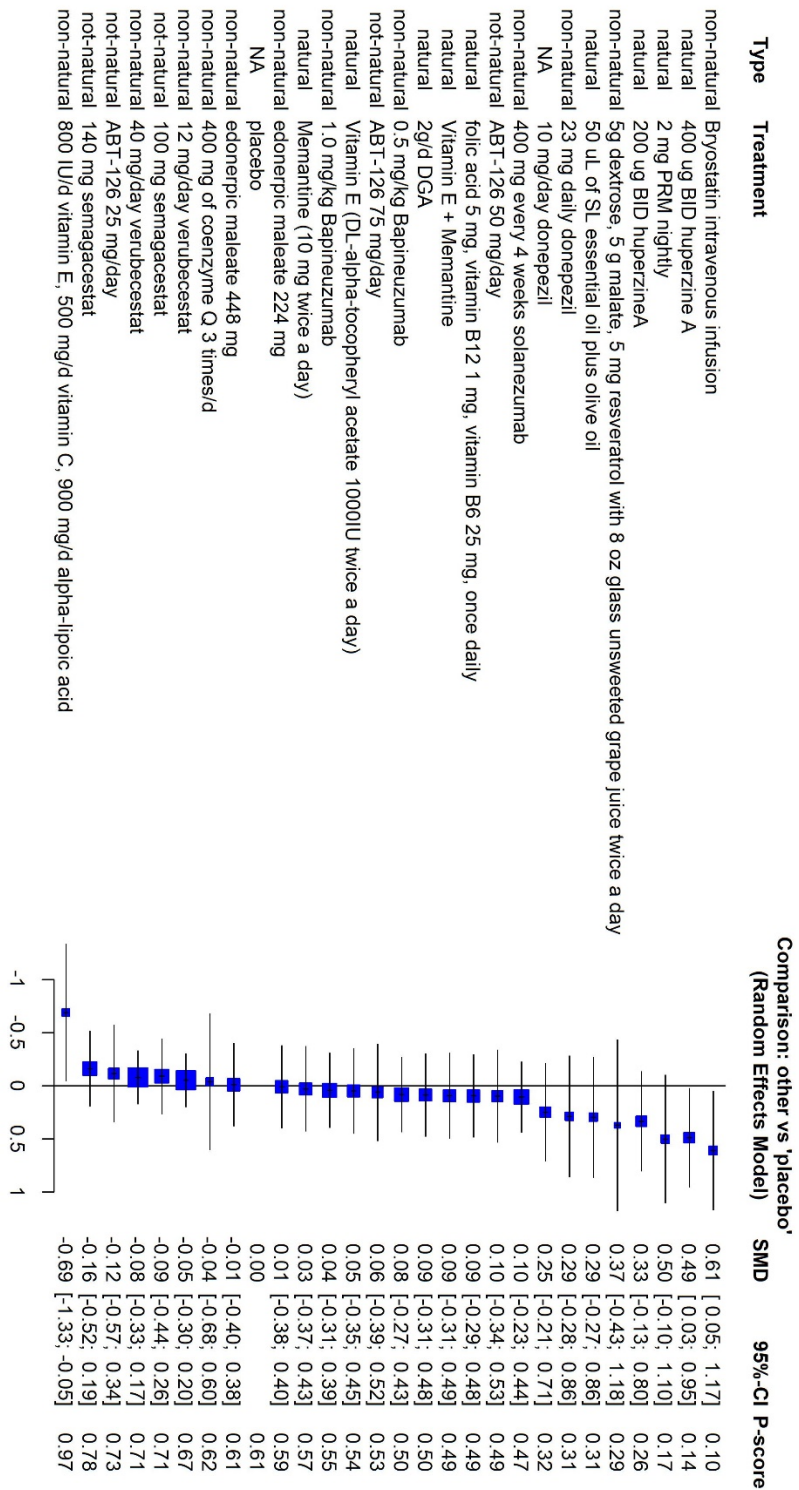


Figure 6. Higher-is-Better Random Effects forest plot. Note donepezil is non-natural but listed as NA because some studies used this as a control. Interventions on the top are the highest performing treatments for the Random Effects model.

Higher-is-Worse Scales - 22 studies with 32 treatments were used in a fixed and random effects network Meta-Analysis. The multiarm tolerance was relaxed to 0.1. The fitted fixed effects model is provided in Table 6, and the accompanying forest plot is in Figure 8. Heterogeneity was present ($\tau^2 = 0.0011$, $\tau = 0.0336$, $I^2 = 10.5\%$ (95% CI: 0.0%, 73.9%)). Total Q was 6.70 ($df = 6$, $p = 0.3494$), within designs $Q = 5.86$ ($df = 5$, $p = 0.3198$), and between designs $Q = 0.59$ ($df = 1$, $p = 0.4430$). The random effects model is provided in Table 7, and the accompanying forest plot is in Figure 9. In Figures 8 and 9, P-scores are used to order treatments by effectiveness. In both fixed and random effects models, largely natural treatments (*Melissa officinalis*, EGb, *Crocus sativus*, huperzine A, vitamin E, SL essential oil, and memantine) generated the highest difference (improvement) from placebo when assessing Alzheimer's disease using scales like the ADAS-cog, CDR-SB, and NPI. Figure 10 shows a funnel plot to assess publication bias. There is no evidence of publication bias in the plot, and a linear regression test of funnel plot asymmetry did not demonstrate evidence of funnel asymmetry ($t = 1.11$, $df = 55$, $p = 0.27$).

Treatment	SMD	95%-CI
0.5 mg/kg Bapineuzumab	-0.0465	[-0.1690; 0.0760]
1.0 mg/kg Bapineuzumab	0.0012	[-0.1223; 0.1248]
10 mg/day donepezil	-0.3151	[-0.5435; -0.0866]
100 mg BID tramiprosate	-0.0878	[-0.2593; 0.0837]
100 mg semagacestat	0.096	[-0.0301; 0.2220]
12 mg/day verubecestat	0.0317	[-0.0739; 0.1373]
125 mL once a day multinutrient drink	-0.3624	[-0.6233; -0.1016]
140 mg semagacestat	0.121	[-0.0042; 0.2463]
150 mg BID tramiprosate	-0.0011	[-0.1736; 0.1714]
2 mg PRM nightly	0.0454	[-0.4839; 0.5748]
2 mg/day RSG XR	-0.1537	[-0.3878; 0.0804]
200 ug BID <u>huperzineA</u>	-0.0539	[-0.3888; 0.2811]
240 mg of EGb 761	-0.4427	[-0.5826; -0.3029]
2g/d DGA	-0.0305	[-0.2597; 0.1986]
30 mg/day Crocus sativus	-0.3663	[-0.9821; 0.2496]
40 mg/day verubecestat	0.1068	[-0.0001; 0.2138]
400 mg every 4 weeks solanezumab	-0.0727	[-0.1646; 0.0192]
400 ug BID huperzine A	-0.2471	[-0.5781; 0.0840]
50 uL of SL essential oil plus olive oil	-0.2086	[-0.6719; 0.2547]
5g dextrose, 5 g malate, 5 mg resveratrol with 8 oz glass <u>unsweetened</u> grape juice twice a day	-0.2163	[-0.9505; 0.5179]
8 mg/day RSG XR	-0.1535	[-0.3902; 0.0832]
ABT-126 25 mg/day	-0.054	[-0.3629; 0.2550]
ABT-126 50 mg/day	-0.1594	[-0.4400; 0.1213]
ABT-126 75 mg/day	-0.1903	[-0.5030; 0.1224]
edonerpic maleate 224 mg	-0.0497	[-0.2706; 0.1711]
edonerpic maleate 448 mg	-0.0892	[-0.3121; 0.1336]
extract 60 drops/day <i>Melissa officinalis</i>	-7.7543	[-9.5316; -5.9770]
folic acid 5 mg, vitamin B12 1 mg, vitamin B6 25 mg, once daily	0.0919	[-0.1266; 0.3104]
Memantine (10 mg twice a day)	-0.17	[-0.4059; 0.0660]
placebo	.	.
Vitamin E (DL-alpha-tocopheryl acetate 1000IU twice a day)	-0.2202	[-0.4585; 0.0182]
Vitamin E + Memantine	-0.2002	[-0.4381; 0.0378]

Table 6. Fixed Effects Model results for the Higher-is-Worse outcomes. This table provides unranked SMD and CI for each study without rounding.

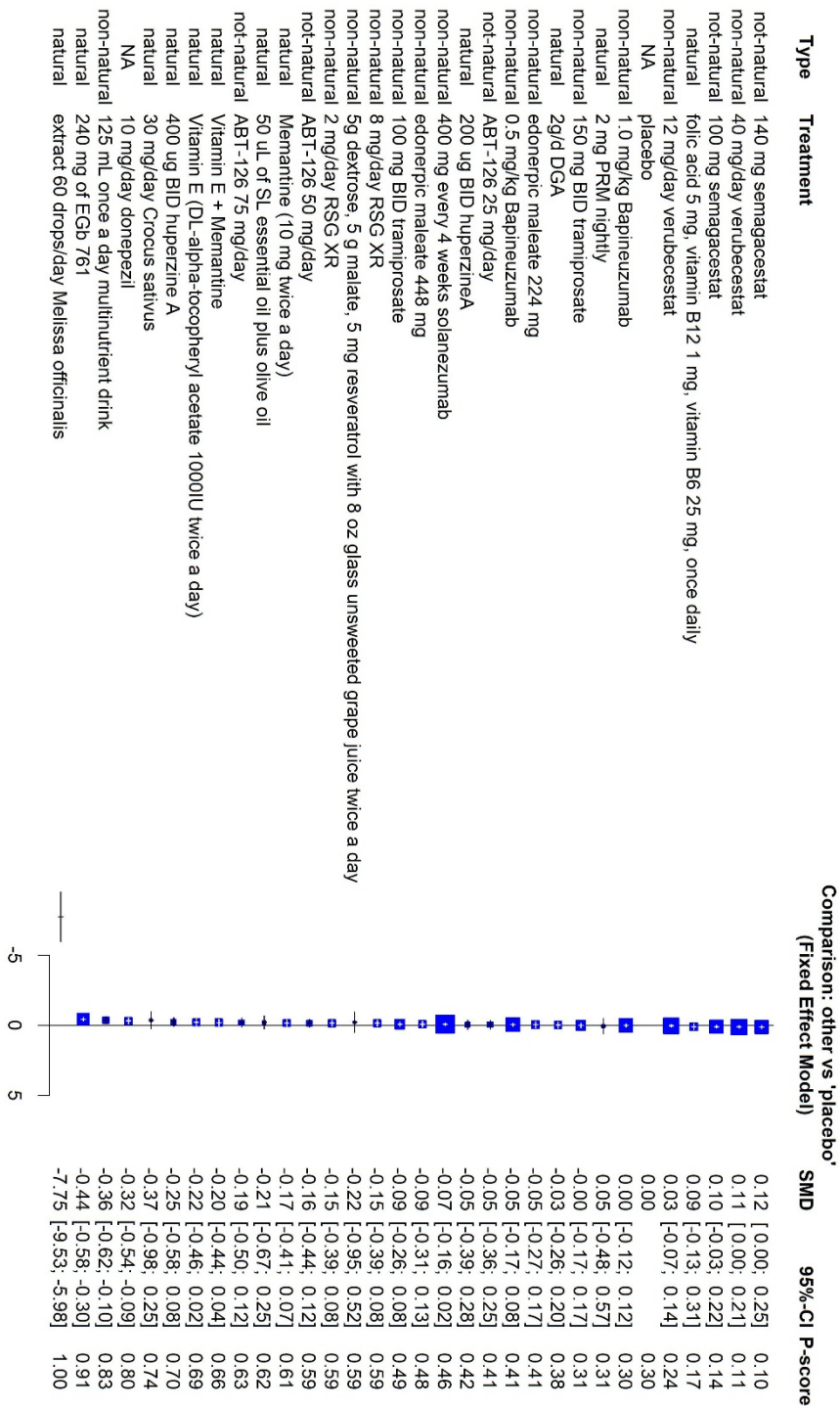


Figure 8. Higher-is-Worse fixed effects model forest plot.

Note: donepezil is non-natural but listed as NA because some studies used this as a control. Interventions on the bottom are the highest performing treatments for the Fixed effect model.

Treatment	SMD	95%-CI
0.5 mg/kg Bapineuzumab	-0.0488	[-0.1818; 0.0841]
1.0 mg/kg Bapineuzumab	-0.0024	[-0.1363; 0.1316]
10 mg/day donepezil	-0.3153	[-0.5484; -0.0821]
100 mg BID tramiprosate	-0.0878	[-0.2715; 0.0959]
100 mg semagacestat	0.096	[-0.0462; 0.2382]
12 mg/day verubecestat	0.032	[-0.0842; 0.1482]
125 mL once a day multinutrient drink	-0.3624	[-0.6315; -0.0934]
140 mg semagacestat	0.121	[-0.0205; 0.2625]
150 mg BID tramiprosate	-0.0011	[-0.1857; 0.1835]
2 mg PRM nightly	0.0454	[-0.4880; 0.5789]
2 mg/day RSG XR	-0.1545	[-0.3967; 0.0877]
200 ug BID <u>huperzine A</u>	-0.0538	[-0.3952; 0.2876]
240 mg of EGb 761	-0.4428	[-0.5902; -0.2954]
2g/d DGA	-0.0305	[-0.2689; 0.2079]
30 mg/day Crocus sativus	-0.3665	[-0.9876; 0.2546]
40 mg/day verubecestat	0.1114	[-0.0061; 0.2289]
400 mg every 4 weeks solanezumab	-0.0727	[-0.1858; 0.0404]
400 ug BID huperzine A	-0.247	[-0.5845; 0.0905]
50 uL of SL essential oil plus olive oil	-0.2086	[-0.6766; 0.2594]
5g dextrose, 5 g malate, 5 mg resveratrol with 8 oz glass <u>unsweetened</u> grape juice twice a day	-0.2163	[-0.9534; 0.5209]
8 mg/day RSG XR	-0.1543	[-0.3991; 0.0905]
ABT-126 25 mg/day	-0.0537	[-0.3687; 0.2614]
ABT-126 50 mg/day	-0.1591	[-0.4464; 0.1283]
ABT-126 75 mg/day	-0.19	[-0.5087; 0.1287]
edonerpic maleate 224 mg	-0.0497	[-0.2802; 0.1808]
edonerpic maleate 448 mg	-0.0892	[-0.3216; 0.1432]
extract 60 drops/day Melissa officinalis	-7.7543	[-9.5328; -5.9758]
folic acid 5 mg, vitamin B12 1 mg, vitamin B6 25 mg, once daily	0.0919	[-0.1363; 0.3201]
Memantine (10 mg twice a day)	-0.17	[-0.4150; 0.0750]
placebo	.	.
Vitamin E (DL-alpha-tocopheryl acetate 1000IU twice a day)	-0.2202	[-0.4674; 0.0271]
Vitamin E + Memantine	-0.2002	[-0.4471; 0.0467]

Table 7. Random Effects Model results for the Higher-is-Worse outcomes. This table provides unranked SMD and CI for each study without rounding.

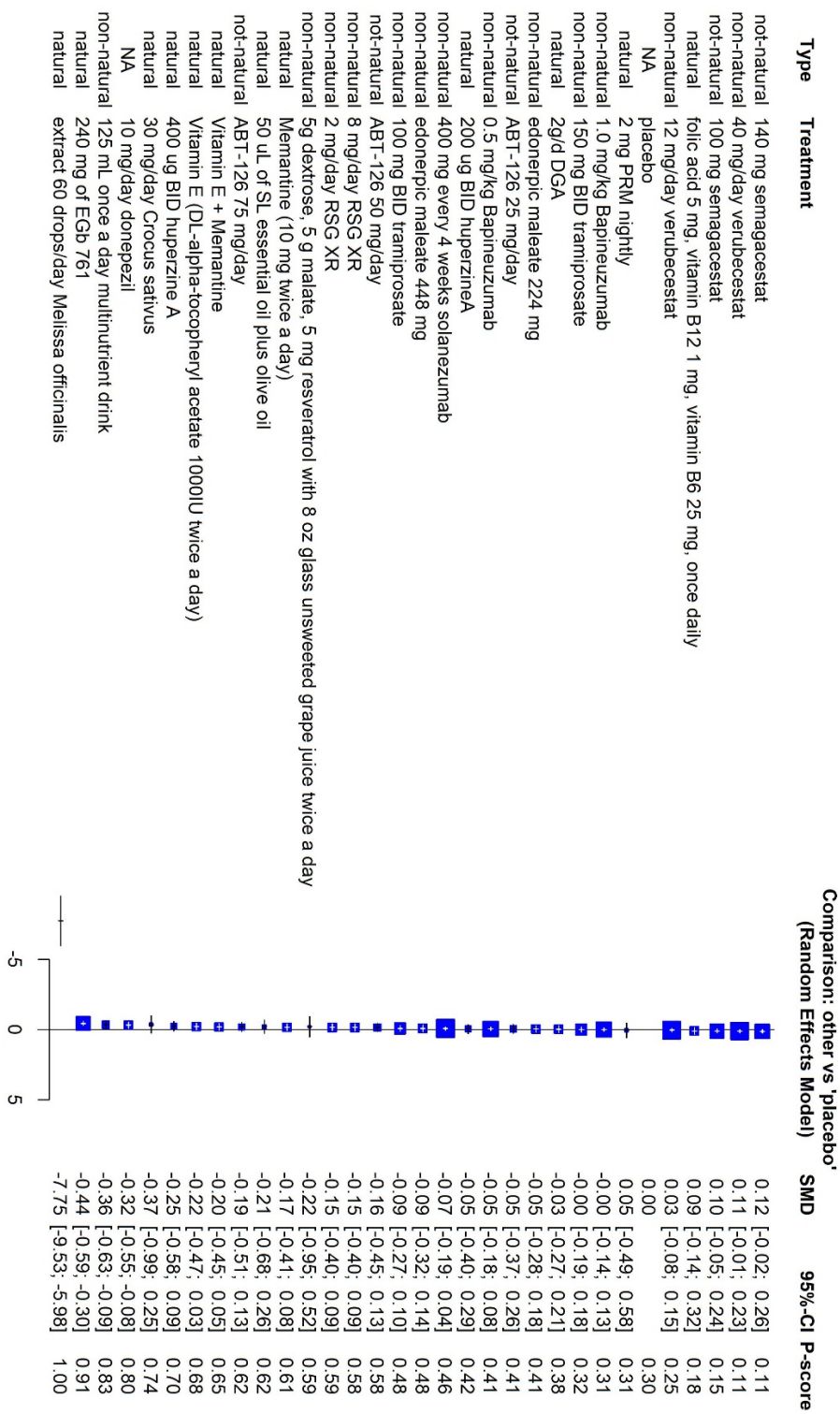


Figure 9. Higher-is-Worse random effects model forest plot.
 Note: donepezil is non-natural but listed as NA because some studies used this as a control.
 Interventions on the bottom are the highest performing treatments for the random effect model.

Treatment Outcomes & Adverse Effects

Figure 11 shows the boxplots of the percentage of patients with the top 10 adverse effects by the type of treatment, natural vs. non-natural. Among treatment patients, natural and non-natural treatments result in a comparable percentage of patients with at least one adverse effect, however, more patients had at least one serious adverse effect and fall on natural treatments. The remaining adverse effects showed little difference among treated patients. Figure 12 shows that overall, more studies involving natural treatments found that the treatments were superior. In contrast, fewer studies involving non-natural treatments found that the treatments were superior.

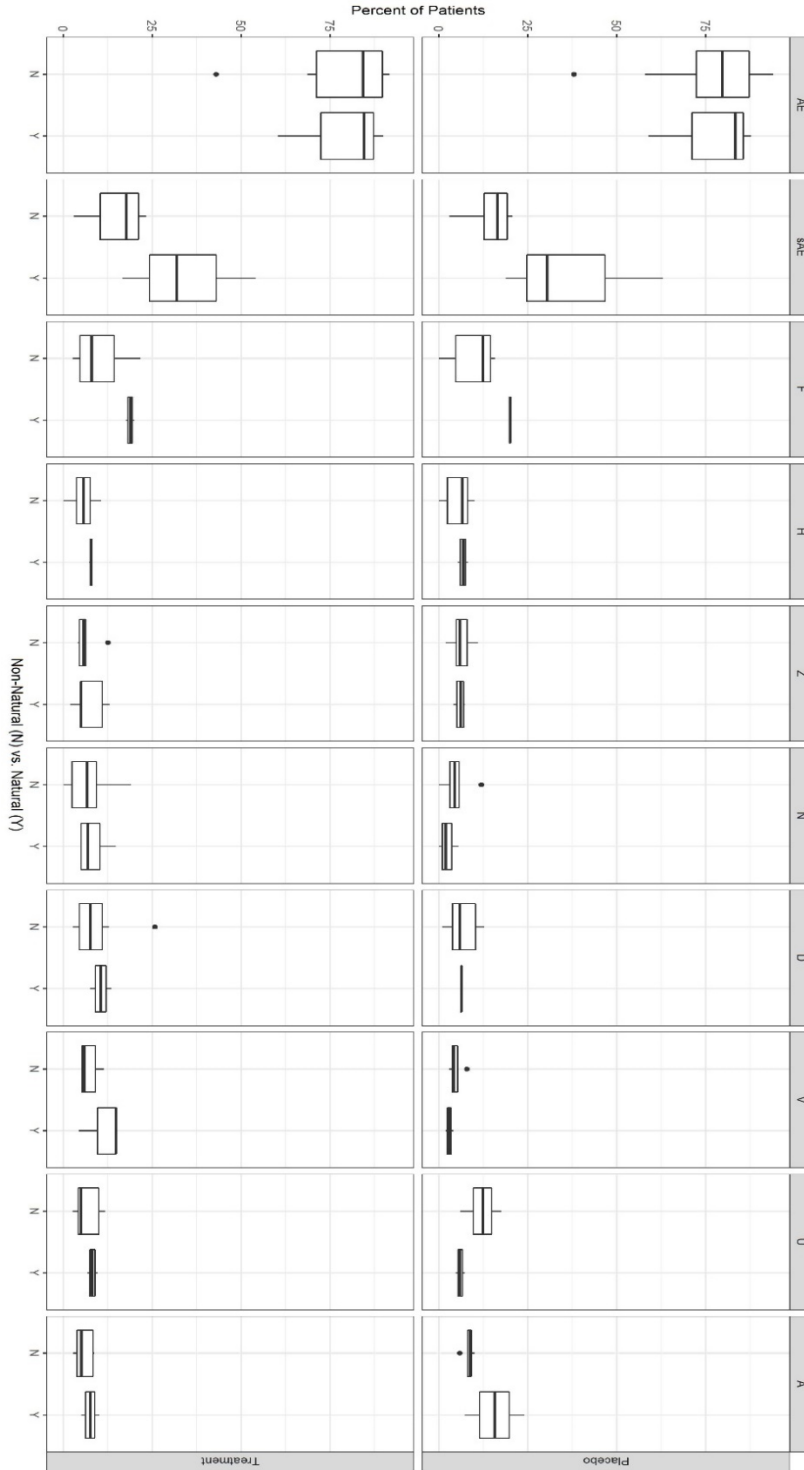


Figure 11. Comparison of top 10 adverse effects by effect and type of treatment. Treatments are abbreviated as AE=at least one adverse event; sAE=at least one serious adverse event; F=Fall; H=Headache; Z=Dizziness/ Vertigo; N=Nausea; D=Diarrhea; V=Vomiting; U=UTI; A=Agitation). The patterns of adverse effects were consistent when comparing natural and non-natural—no significant findings.

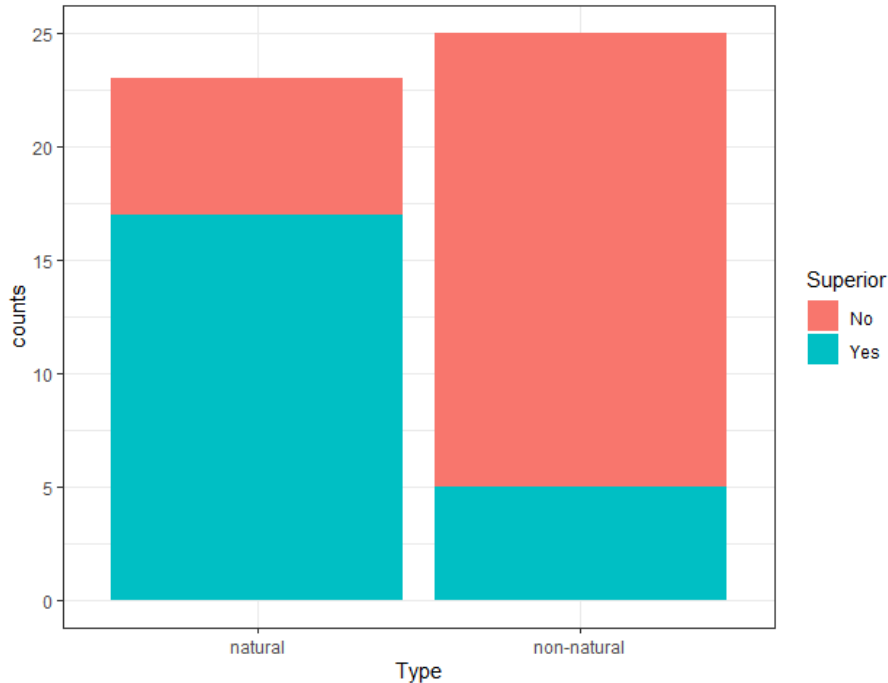


Figure 12. Overall effectiveness (natural; non-natural).

This plot included more studies than used in the Meta-Analysis (49 studies). This plot was done by evaluating the abstract/conclusion section of the studies to determine if an intervention was effective. More natural intervention clinical trials show an overall positive effect on a patients

Treatment Effects – Pairwise Meta-Analysis

Higher-is-Better Scales. The fixed effect SMD was 0.039 ($z = 1.69, p = 0.0901$) - Figure 13. The random effect SMD estimate was 0.061 ($z = 1.32, p = 0.187$) - Figure 13. $Q = 45.34$ supported heterogeneity ($p = 0.0001$) in the model, as did $\tau^2 = 0.0176$ and $I^2 = 64.7\%$. The funnel plot for Higher-is-Better pairwise Meta-Analysis is in Figure 14. The rank correlation test of funnel plot asymmetry ($z = 1.8125, p = 0.07$) and linear regression test of funnel plot asymmetry ($t = 1.33, df = 15, p = 0.204$) did not support asymmetry in the plot. The method of moments test did support asymmetry in the funnel plot ($t = 2.36, df = 15, p = 0.032$). Subgroup analysis yielded an SMD for natural treatments of 0.184 and an SMD for non-natural treatments of -0.002 – Figure 15. The test of subgroup differences supported between-group differences ($Q = 4.72, df = 1, p = 0.0298$).

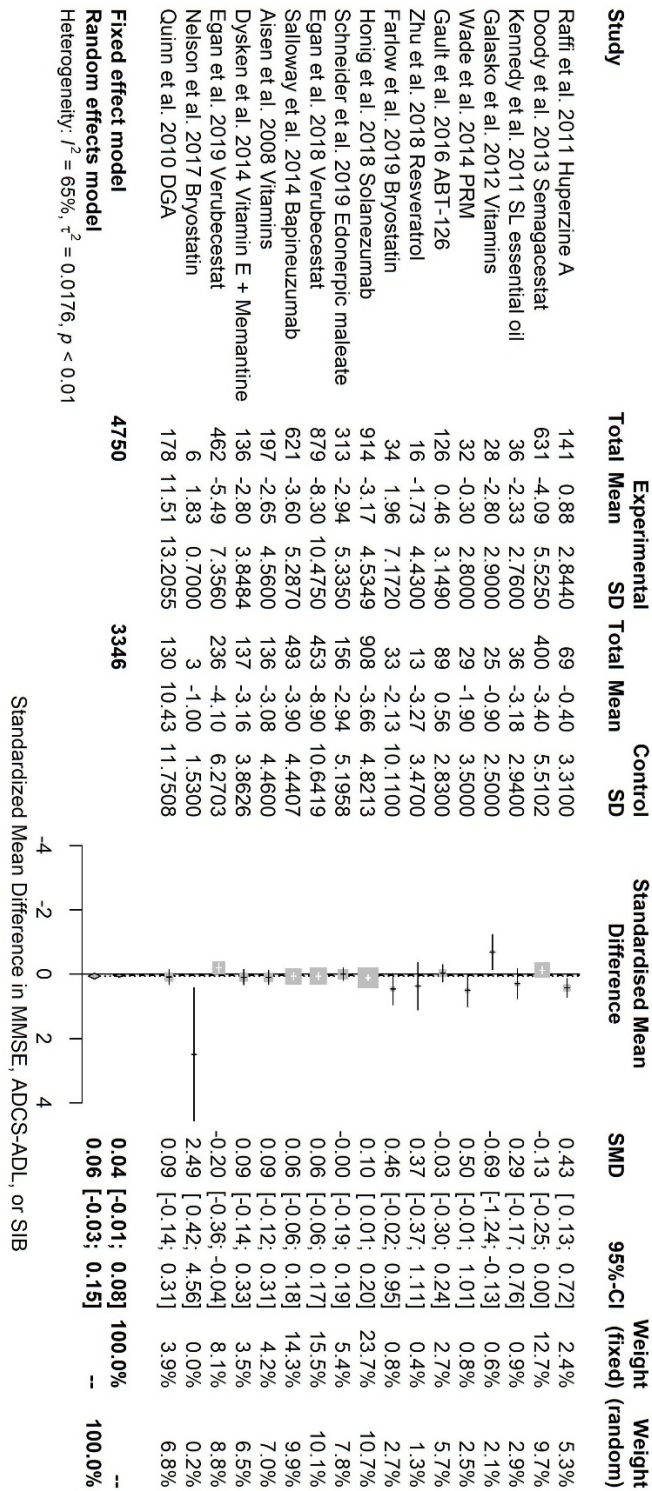


Figure 13. Forest plot for the paired outcomes analysis. This is for scales that indicate better function at higher scores.

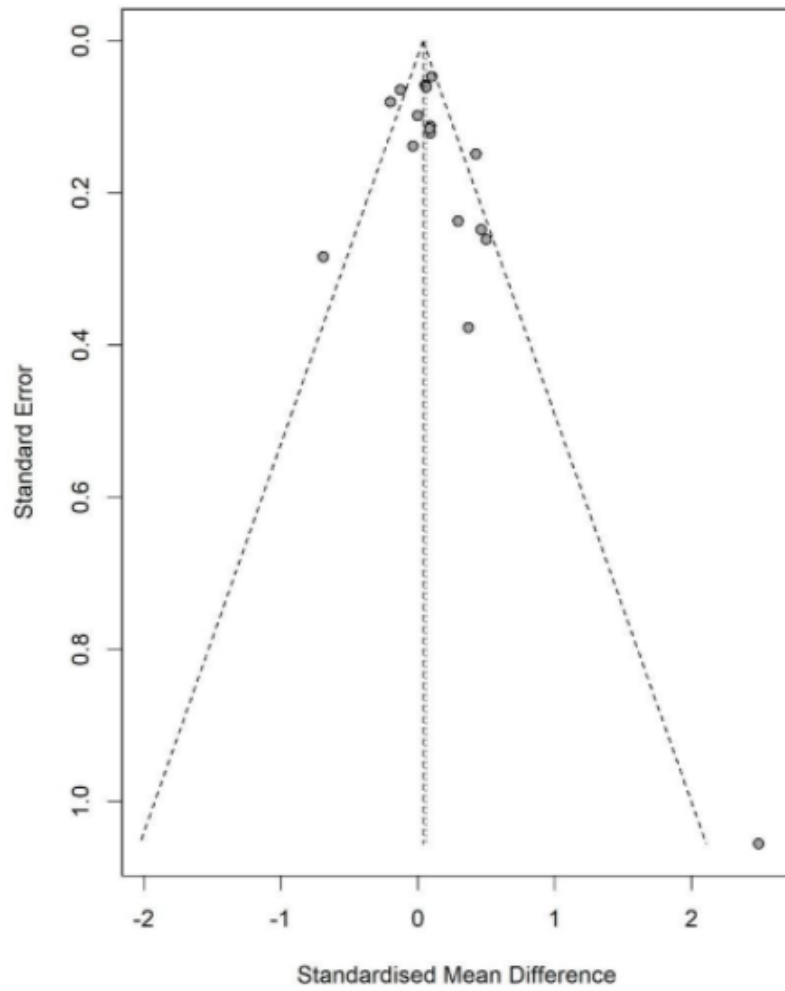


Figure 14. Funnel plot for the paired outcomes analysis of scales that indicate better function at higher scores.

The plot demonstrated mild asymmetry (not a favorable result).

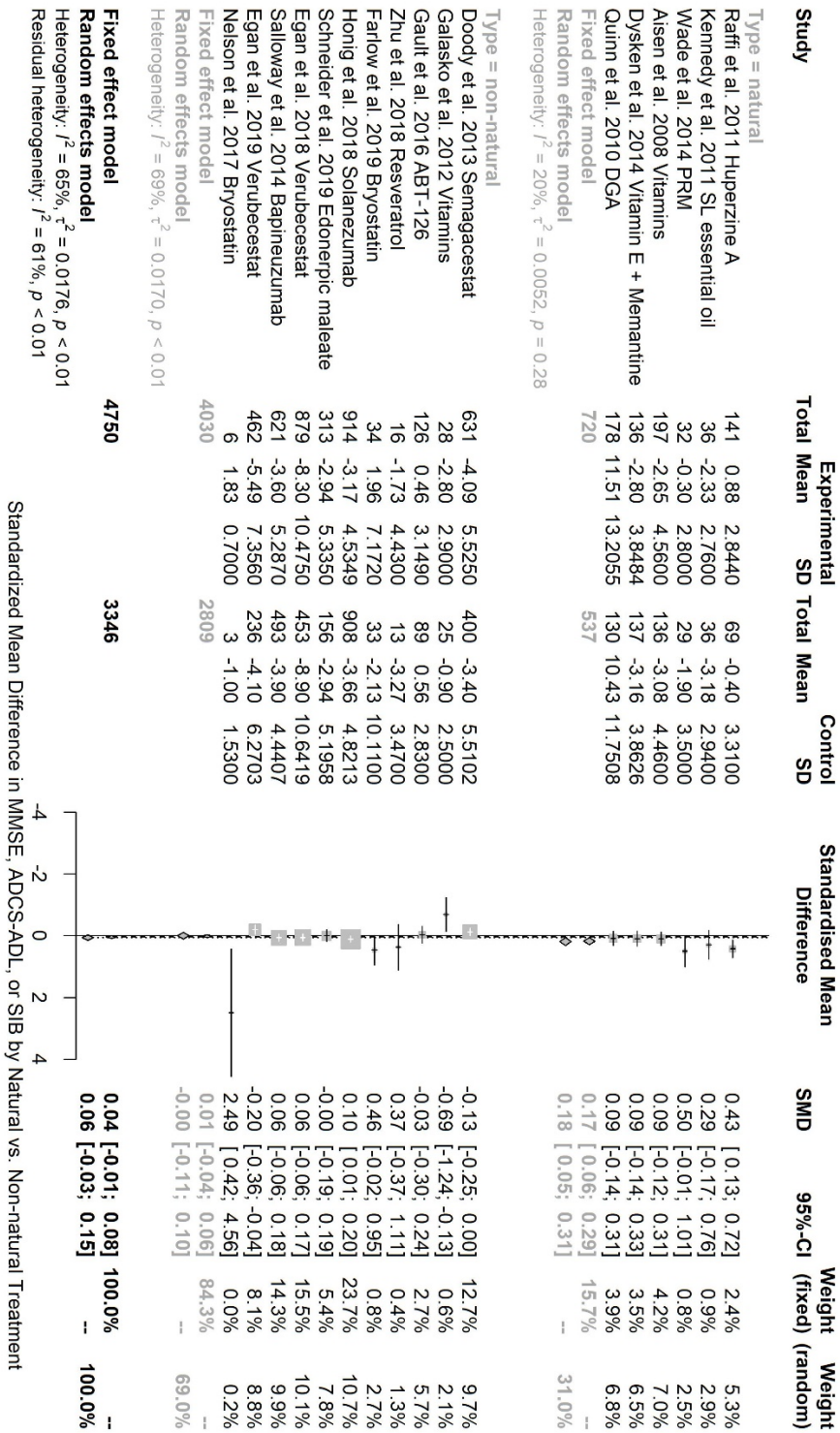


Figure 15. Subgroup analysis for the Meta-Analysis of outcomes indicating better function at higher scale scores.

Higher-is-Worse Scales. The fixed effect SMD was -0.055 ($z = -2.81, p = 0.0049$) and the random effects SMD was -0.126 ($z = -2.26, p = 0.0237$) – Figure 16. $Q = 131.41$ supported heterogeneity ($p < 0.0001$) in the model, as did $\tau^2 = 0.0476$ and $I^2 = 84.8\%$. Funnel plot for the Higher-is-Worse paired comparisons model shows mild asymmetry (Figure 17). The rank correlation test of funnel plot asymmetry supported asymmetry in the funnel plot ($z = -2.05, p = 0.04$), as did the linear regression test ($t = -2.92, df = 19, p = 0.009$) and the method of moments test ($t = -5.77, df = 19, p = 1.45e-05$). The trim and fill method identified 7 additional studies needed for funnel symmetry. The linear regression method for bias adjustment adjust the random effect SMD estimate to 0.10 (95% CI 0.01, 0.18) ($z = 2.25, p = 0.0243$). Subgroup analysis yielded an SMD for natural treatments of -0.225 and an SMD for non-natural treatments of -0.015 (Figure 18). The test of subgroup differences supported between-group differences ($Q = 4.01, df = 1, p = 0.0453$).

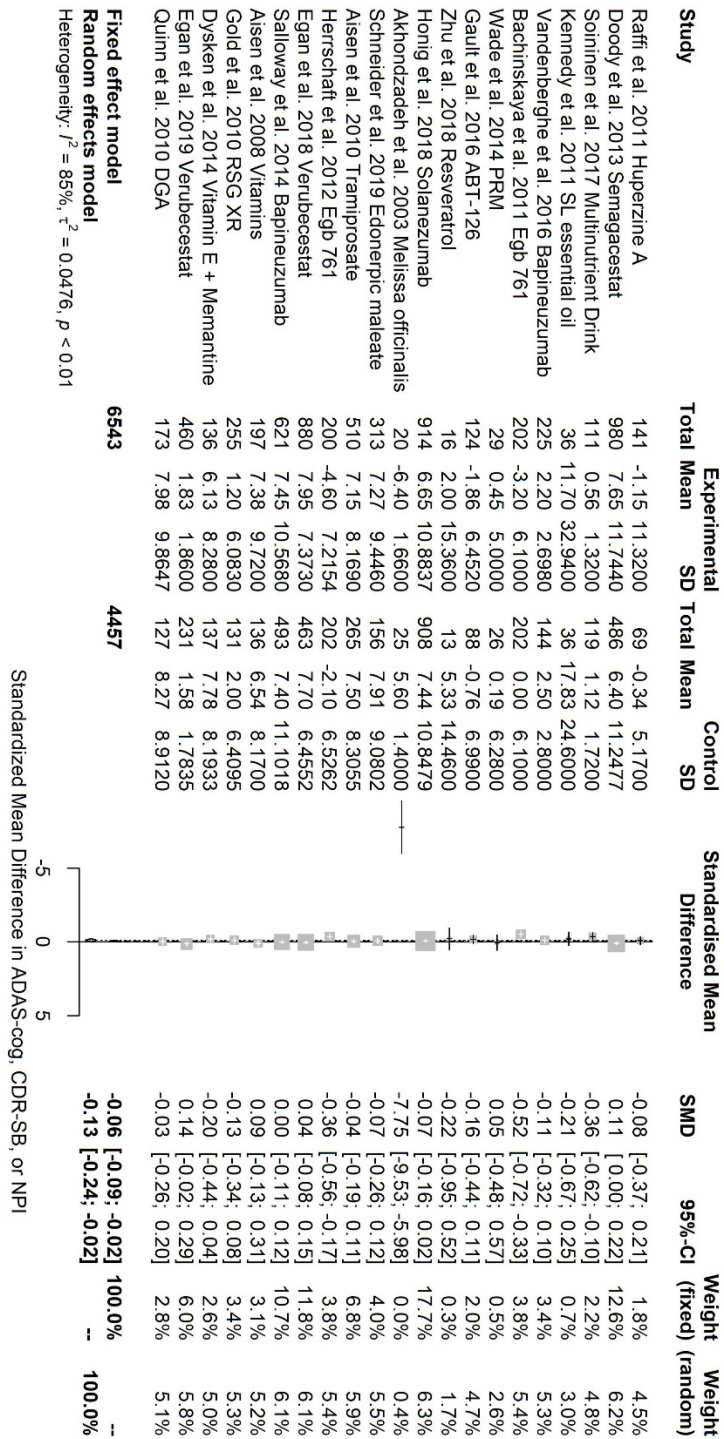


Figure 16. Forest plot for the Higher-is-Worse paired comparisons model with fixed and random effects.

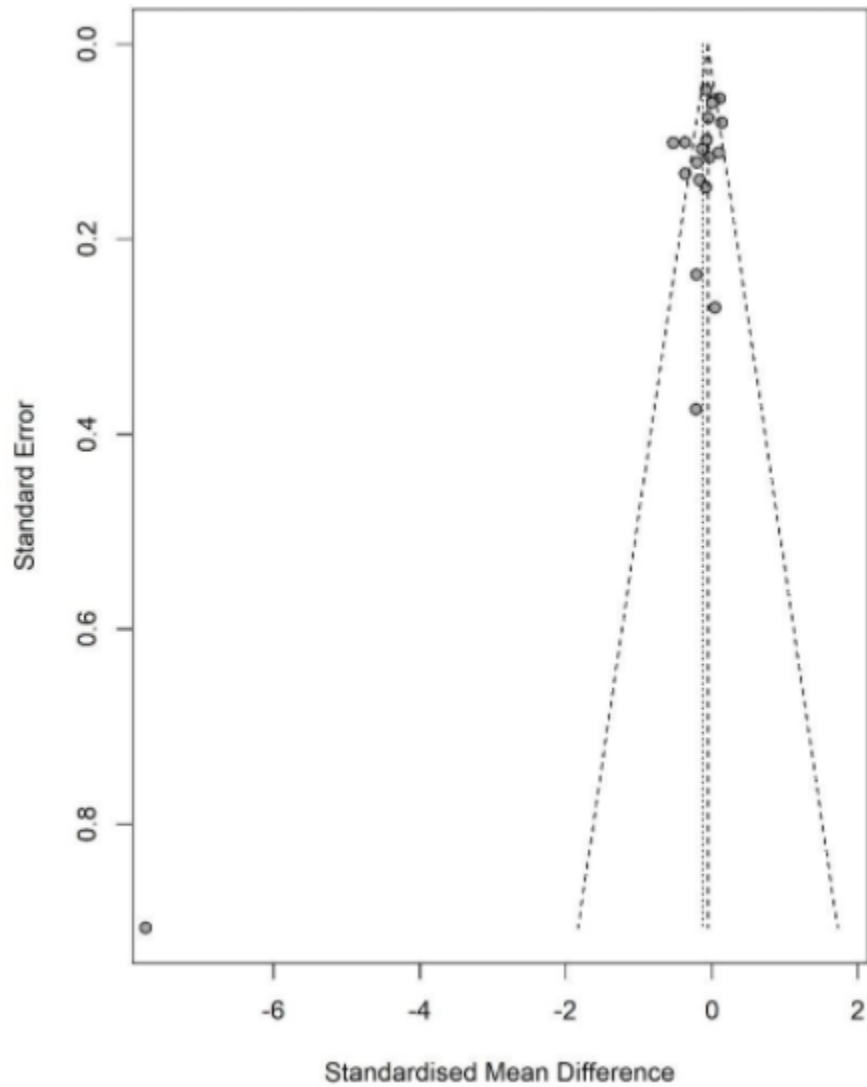


Figure 17. Funnel plot for the Higher-is-Worse paired comparisons model. The plot demonstrated high asymmetry mostly generated by a single outlier, indicating this outlier had a very substantial impact on publication bias (not a good result).

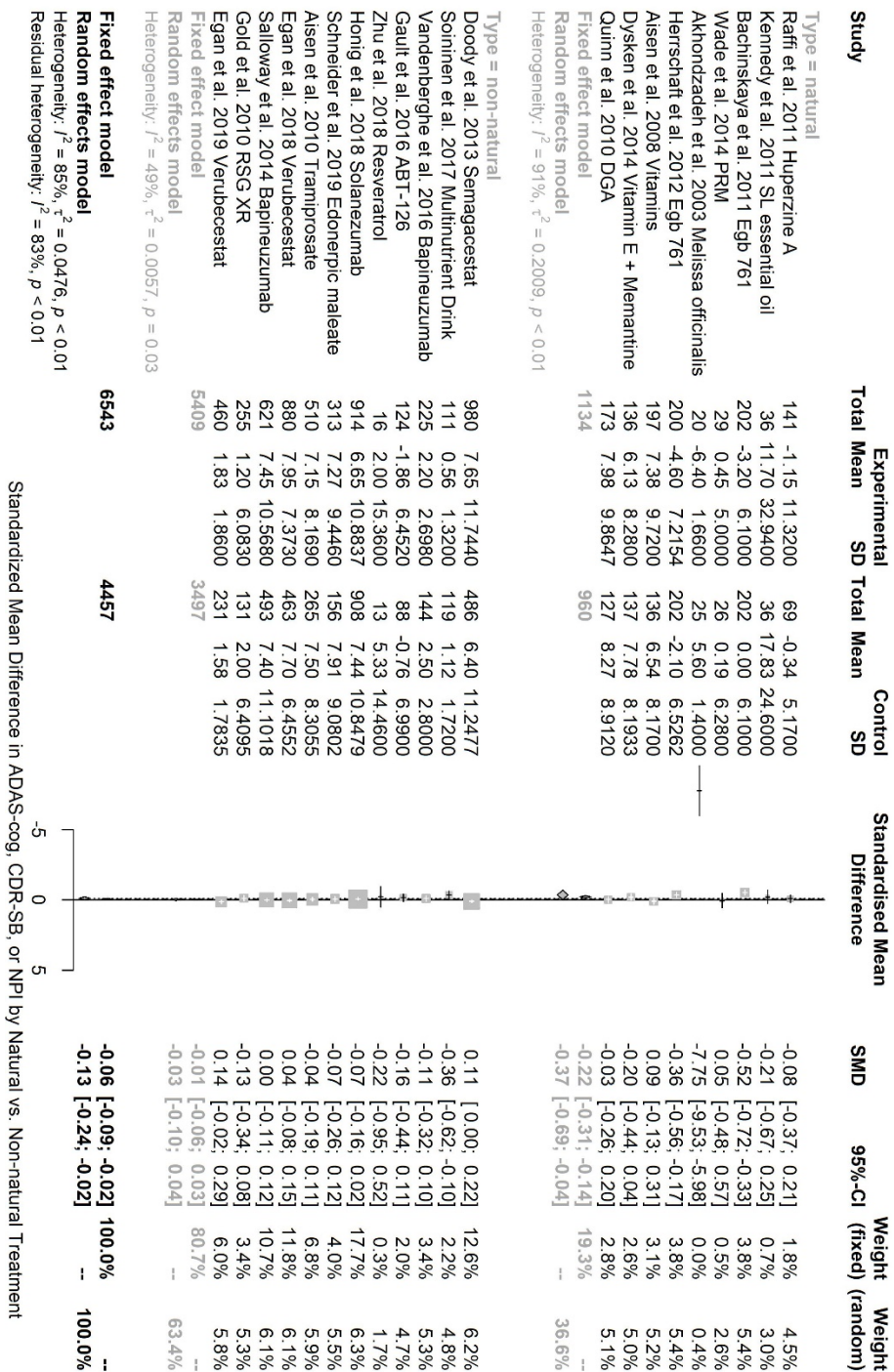


Figure 18. Subgroup analysis for the Higher-is-Worse paired comparisons model.

Discussion

Models Assessing Better Function at Higher Scores

In the Network Meta-Analysis fixed and random effects models, the highest positive effect was achieved by Bryostatin, followed by huperzine A, PRM, donepezil, resveratrol, and SL essential oil, in order of decreasing effect (Fig. 5-6). It is important to note that the sample size for Bryostatin was very small (9 patients total), and thus, the result may not be statistically significant. Smaller studies tend to show larger treatment effects. There was no support for publication bias. With the exception of Bryostatin and resveratrol, the top treatments were natural.

In the Pairwise Meta-Analysis, treatments showing large improvements over placebo included Bryostatin, resveratrol, PRM, SL essential oil, and huperzine A. The overall effect was positive (indicating improvement) but not statistically significant for either the fixed effects or random effects models (Fig. 13). The funnel plot (Fig. 14) demonstrates mild asymmetry, and this, combined with the results of plot asymmetry tests, suggests publication bias is not a serious concern, though note should be taken that the method of moments test was significant for asymmetry. In the Subgroup Meta-Analysis (Fig. 15), natural treatments showed positive overall effects (0.17 for fixed effects and 0.18 for random effects) that were statistically significant ($p < 0.05$), whereas the non-natural treatments showed lower positive overall effects (0.01 and 0 for fixed and random effects, respectively) that were not statistically significant ($p > 0.05$).

Consequently, it may be concluded that the natural treatments outperformed the non-natural treatments as assessed by scales that reflect better function at higher scores. Furthermore, non-natural treatments had an overall effect of 0, indicating no improvement at all over

placebo/control, whereas natural treatments did have a positive impact. This surprising result may be significant for the Alzheimer's patient and physician communities.

Models Assessing Poorer Function at Higher Scores

In the Network Meta-Analysis fixed and random effects models, the highest negative effect (indicating improvement) was attained by Melissa officinalis, EGb 761, multi-nutrient drink, donepezil, Crocus sativus, huperzine A, Vitamin E, and Vitamin E and Memantine, in decreasing order of improvement. With the exception of the multi-nutrient drink, all of these treatments are natural (Fig. 8-9). There was no support for publication bias (Fig. 10).

In the Pairwise Meta-Analysis, treatments showing large improvements over placebo included Melissa officinalis, EGb 761, a multi-nutrient drink, resveratrol, and vitamin E + memantine. The overall effect was negative (indicating improvement) and statistically significant ($p < 0.05$) for both the fixed and random effects models (Fig. 16). The funnel plot (Fig. 17) demonstrates high asymmetry mostly generated by a single outlier, indicating this outlier had a very substantial impact on publication bias. The linear regression method for bias adjustment adjusts the random effect SMD estimate to 0.10 (95% CI 0.01, 0.18) ($z = 2.25, p = 0.0243$), indicating worsening of the condition rather than improvement. Consequently, results from these models should be interpreted with caution. Subgroup Meta-Analysis showed that natural treatments had statistically significant and more negative (indicating greater improvement) fixed and random effects overall estimates, -0.22 and -0.37, respectively, than the non-natural treatments (-0.01 and -0.03 for overall fixed and random effects, respectively, and non-significant) (Fig. 18). Overall, natural treatments outperformed non-natural treatments.

The confidence intervals and overall effect of the Melissa officinalis study look suspiciously perfect. The Melissa study reported that the change in ADAS-cog at endpoint compared to baseline was -6.40 (1.66) and 5.60 (1.40) the Melissa and placebo, respectively (SD is in parentheses). So ADAS-cog decreased for Melissa while it increased for placebo by almost as much. The sample size is 20/25, which is low, but it is not the lowest seen in the Meta-Analysis. Based on this information, the treatment is actually not an outlier.

Using both types of scales (Higher-is-Worse/Higher-is-Better), either one or more natural treatments generated the greatest differences and improvements in outcome from placebo. The overall descriptive view of the studies shown in Figure 12 supports this outcome. The difference in most commonly reported adverse effects does not appear to be related to the type of treatment (natural vs. non-natural), as patterns were generally consistent across placebo and treatment groups (Fig. 11).

Study Limitations

Out of 63 studies considered for this Meta-Analysis, only 26 were incorporated into the Meta-Analysis. This includes studies that reported least-squares means with standard error and/or confidence intervals, which may introduce additional bias into the results. These studies were only used to include as many studies in the Meta-Analysis as possible. Additionally, many studies had multiple arms and multiple outcome measures. Outcome measures were divided into two categories, based on whether the scale followed a ranking such that higher scores were better or higher scores were worse cognitive function. These outcome measures could not be pooled as their effects measure improvement in opposite directions, and the results would be confounded. As a result, one Meta-Analysis was performed for each group, (1) a higher score indicates better cognitive development and (2) a higher score indicates poorer cognitive development. The most

common scales were selected for each group. Only one outcome per study per group (Higher-is-Better, Higher-is-Worse) was selected to adhere to independence assumptions. Because outcome measures were not consistent, the standardized mean difference was used as the effect measure across analyses.

Given that many studies were multi-arm studies, either a network or combined groups approach is the recommended method for Meta-Analysis [12]. Network Meta-Analysis makes the most efficient use of the data but does not provide a clear answer regarding the efficacy of natural vs. non-natural treatments. As a result, the combined groups approach was used as a supplementary analysis. To combine groups, if the arms included different treatments (rather than different doses), one or more arms were removed. Studies that did not use a clear placebo were also removed to prevent the entry of additional bias into the Meta-Analysis. If there were more than two treatment groups with the same intervention and different doses, the two most extreme doses (highest and lowest) were used to provide a view of the average effect. The combined groups approach subjected to a subgroup analysis yielded natural treatments as the clear winner, though with substantial information discarded. However, the results were consistent with those from network Meta-Analysis.

Ideally, a network Meta-Analysis should be done for each outcome independently, as there are clearly scale-based differences in outcome. However, this would reduce the number of studies entering the Meta-Analysis. A Network Meta-Analysis was selected to make the most efficient use of the information presented in the studies. The multiarm tolerance was relaxed to prevent estimates of 0 heterogeneity and removal of studies, which resulted in the need to fit meta-analytic models to account for separate sub-networks.

Conclusion

Natural treatments generated greater improvements in Alzheimer's Disease as measured by the SIB, ADAS-cog, MMSE, ADCS-ADL, CDR-SB, and NPI. The most effective non-natural treatment was Bryostatin, whereas the most effective natural treatments were huperzine A, Melissa officinalis, and EGb761. There was no evidence of higher adverse effects due to natural vs. non-natural treatment in placebo or treatment groups.

As mentioned in the introduction, when describing the statistical models, the fixed effect model of this research should not be the basis of any medical conclusions or interventions. All of the studies selected are random, and there is no way the "one population" assumption could be valid. The fixed effect model was only used as a sanity check to see heterogeneity measurement and to see the overall difference between random effect vs fixed effect. If the analysis approach used in this research were to be repeated with a larger dataset to provide real answers in medical studies, only the random effect model should be considered.

For Alzheimer's Disease clinical trials, the Network Meta-Analysis, together with Combined Groups Meta-Analysis is a robust way to measure the effectiveness of interventions. While performing Meta-Analysis of multiple studies requires more effort than assessing individual studies, it provides a more significant scientific value. The more interventions you can compare, the more confident you could be when choosing the "best" or most promising for further drug development. That is why some drugs now require a comprehensive Meta-Analysis for further certification. FDA provides a guidance document for applicants submitting investigational new drug applications [52].

There are many patients that prefer not to take additional medication to avoid side effects or to avoid mixing new pills with daily prescriptions. If natural treatments are proving to be more efficient in this small-scale study, then this could be a "breakthrough" for the Alzheimer's community. Most natural treatments (vitamins and plant extracts) are much cheaper than pharmaceutical solutions and would not cause severe side effects. If natural treatments are proven to help improve Alzheimer's symptoms, this will significantly impact the projected \$1.1 trillion dollar spending on AD healthcare in 2050. The global population is aging, and robust solutions are needed now! Besides the substantial financial burden, there will be an even greater impact on the caregiving community.

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Appendix

Clinical Trials Used

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