Potential reversal of epigenetic age using a diet and lifestyle intervention: a pilot randomized clinical trial

Project 1

Aleksandra Domagała, Aleksandra Mach, Zuzanna Ostas

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Outline

Introduction

- Advanced age is the largest risk factor for impaired mental and physical function and many non-communicable diseases like cancer or type 2 diabetes.
- Delaying aging by 2.2 years could save $7 trillion over fifty years, making it a better approach than disease-specific spending.
- Therefore, increasing longevity in human population is becoming a trendy topic among the research community.
- The basic concept is that individuals with a biological age lower than the respective chronological age are expected to have increased longevity when compared to individuals with a higher biological age than the chronological age.

Methylation data

DNA methylation is a process by which methyl groups are added to the DNA molecule. Methylation can change the activity of a DNA segment without changing the sequence. In mammals, DNA methylation is almost exclusively found in CpG dinucleotides.

Figure: CpG site

Horvath's epigenetic clocks

An epigenetic clock is a biochemical test that can be used to measure age. The test is based on DNA methylation levels, measuring the accumulation of methyl groups to one's DNA molecules.

The first and the most studied multi-tissue epigenetic clock, Horvath's epigenetic clock, was developed by Steve Horvath. It calculates the biological age based on beta values for 353 CpGs.

$$\beta = \frac{\text{methylated signal intensity}}{\text{methylated signal intensity} + \text{unmethylated signal intensity}}$$

A value of zero indicates that all copies of the CpG site in the sample were completely unmethylated and a value of one indicates that every copy of the site was methylated.

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Research Paper

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Kara N. Fitzgerald[1], Romilly Hodges[2], Douglas Hanes[3], Emily Stack[4], David Cheishvili[5], Moshe Szyf[6], Janine Henkel[7], Melissa W. Twedt[7], Despina Giannopoulou[7], Josette Herdell[7], Sally Logan[7], Ryan Bradley[7,8]

[1]Institute for Functional Medicine, Federal Way, WA 98003, USA
[2]American Nutrition Association, Hinsdale, IL 60521, USA
[3]Helfgott Research Institute, National University of Natural Medicine, Portland, OR 97201, USA
[4]Helfgott Research Institute, National University of Natural Medicine, Portland, OR 97201, USA
[5]HKG Epitherapeutics (Hong Kong), Department of Molecular Biology, Ariel University, Israel, Gerald Bronfman Department of Oncology, McGill University, Montreal, Quebec, Canada
[6]Department of Pharmacology and Therapeutics, McGill University, Montreal, QC H3G 1Y6, Canada
[7]Helfgott Research Institute, National University of Natural Medicine, Portland, OR 97201, USA
[8]Division of Preventive Medicine, University of California, San Diego, CA 92023, USA

Correspondence to: Kara N. Fitzgerald; **email:** kf@drkarafitzgerald.com
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About the trial

- Randomized, controlled clinical trial
- 8-week treatment program proceeded by a one-week education period and 3-week washout period
- Intervention in treatment group: diet and lifestyle
- Endpoint: Horvath's DNAmAge clock – to see if it could be potentially slowed or go back in time
- Expectations: influence the DNA methylome using diet and lifestyle should lead to set back DNAmAge and a healthier, more 'youthful' metabolism
- The first randomized controlled study to suggest that specific diet and lifestyle interventions may reverse Horvath DNAmAge epigenetic aging in healthy adult males

Study population

Inclusion criteria	Exclusion criteria
• Males • Age 50-72 • Willing to adhere to 9 weeks of a dietary and lifestyle program including specific nutrition and exercise guidelines • Willing to avoid any over-the-counter medications, supplements or herbal products	• Use of nutrition supplements or herbal products • Currently following a prescribed dietary/lifestyle program or initiated within the 30 days prior to baseline • Use of nicotine, marijuana, or recreational drugs/substances, excessive alcohol consumption • Have a diagnosis of cardiovascular disease, kidney disease, liver disease, diabetes, autoimmune disease, high blood pressure, or cancer

Table: Some of the inclusion and exclusion criteria.

Characteristics	Treatment group n = 21		Control group n = 22	
	Value	%	Value	%
Age, years (mean±SD)	58.5 ± 6.12		60.3 ± 6.68	
Race				
Black or African American	0	0	2	9.1
American Indian or Native Alaskan	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Asian or Asian American	3	13.6	1	4.5
White, Caucasian or European American	18	81.8	18	81.8
Caribbean Islander or African National	0	0	0	0
More than one race	0	0	1	4.5
Unknown	0	0	0	0
Education Level				
Some high school	0	0	0	0
High school	0	0.00	3	0.14
Some university	1	0.05	1	0.05
2 year university	2	0.09	1	0.05
4 year university	6	0.27	4	0.18
Some graduate school	2	0.09	4	0.18
Graduate degree	11	0.50	9	0.41

Figure: Baseline characteristics.

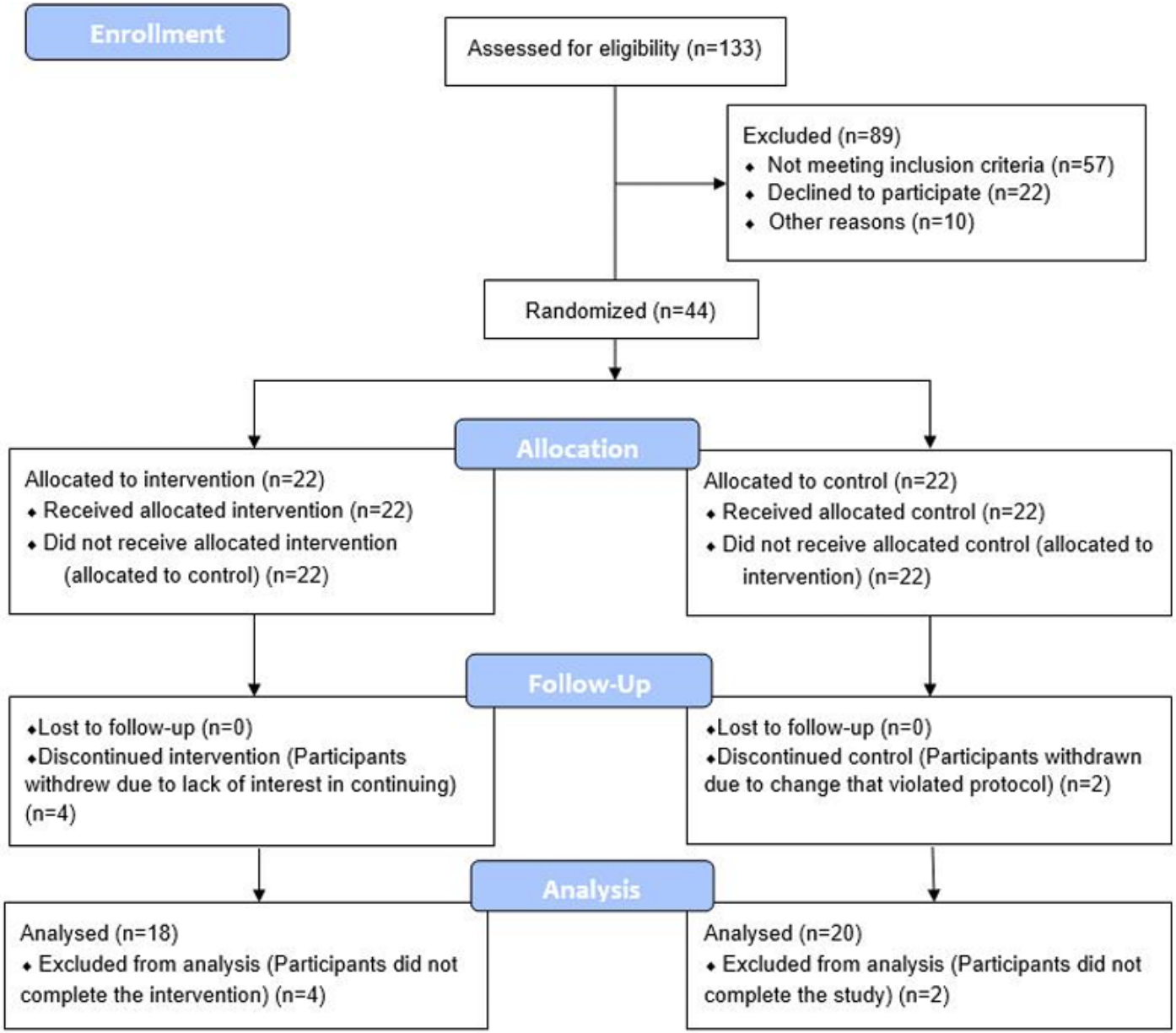

Figure: Participant flow.

Intervention

Breathing exercises
20 minutes twice a day
for stress reduction

Supplements
PhytoGanix, 2 servings daily, divided
UltraFlora Intensive Care, 2 capsules daily, divided

Sleep
average at least 7 hours per night

Exercises
min. 30 minutes of exercise per day
at least 5 days per week
at an intensity of $60 - 80\%$
of maximum perceived exertion

Diet
based largely on biochemistry
and generalized measures of health

This multimodal intervention combines individual interventions, each with evidence of beneficial effects on the DNA methylome and backed by clinical experience. Such interventions likely produce synergistic effects.

- A 3-week washout period was initiated for all participants.
- Study participants were randomized at the baseline visit.
- They also got initial instructions, including a recorded instructional webinar and electronic technology webinar.
- The 8-week intervention began one week after the baseline visit.
- Participants were supported by coaches and electronic technology coaching tool.

Materials and methods

- Epigenetic age was determined using saliva samples, collected at each of the three study visits.
- DNAmAge was calculated using the online Horvath clock (`https://dnamage.genetics.ucla.edu/`).
- Analysis of epigenetic age was performed, blinded, on the final 38 participants.
- P-values were computed as an unpaired 2-tailed t-test between the experimental group and the control group, using the individual score differences.

DNA methylation clock

At the end of the program:

- participants in the treatment group scored an average of 3.23 years younger on the Horvath DNAmAge clock than the control group (p=0.018).
- treatment group participants was on average 1.96 years younger (p=0.066),
- control group participants averaged 1.27 years older (p=0.153).

Metabolic measures

- mean triglycerides (−25%, p=0.009),
- mean serum 5-methyltetrahydrofolate (+15%, p=0.004).

Emotional measures

No significant results

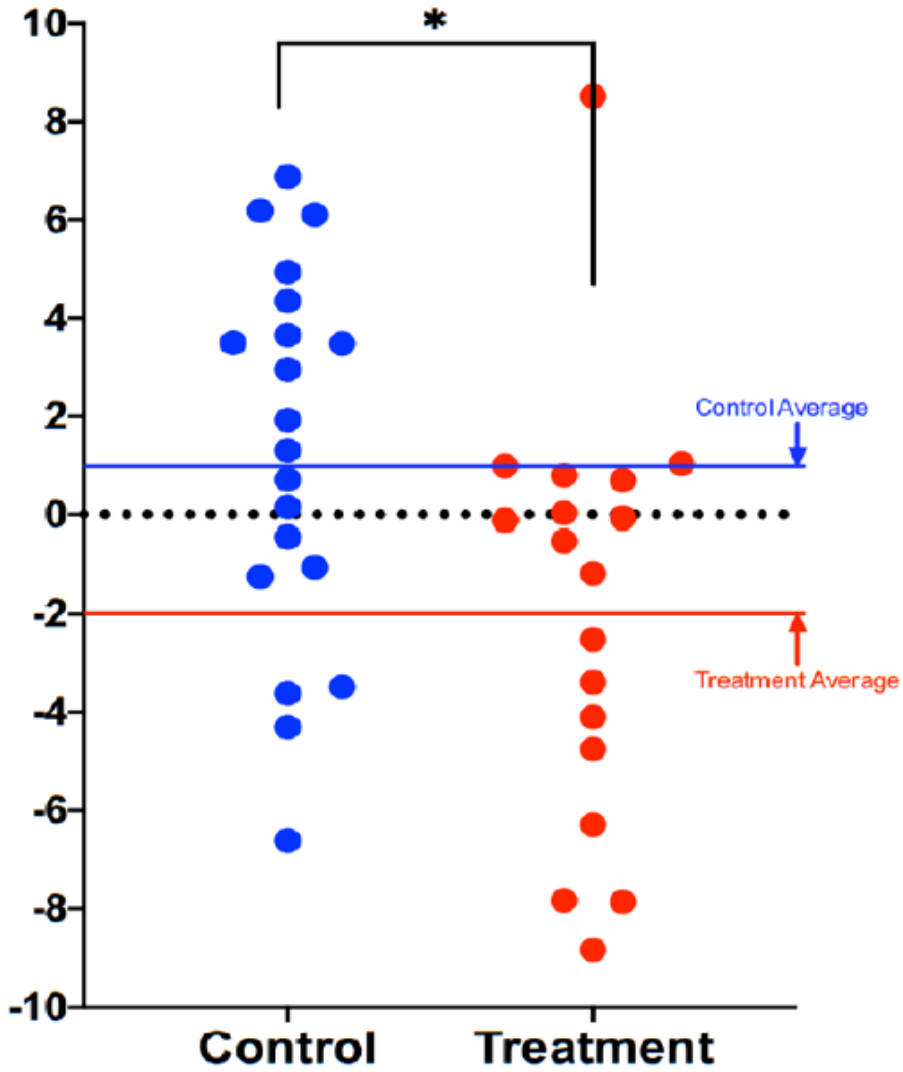

Figure: Comparison of DNAmAge change between treatment and control groups.

Estimation of the biological age using Horvath and Hannun epigenetic clocks

	ID_REF	A.MDL106_v2_5418.Unmethylated.signal	A.MDL106_v2_5418.Methylated.signal
1	cg00000029	1423	388
2	cg00000103	550	2286
3	cg00000109	411	2879
4	cg00000155	462	5247
5	cg00000158	417	6083
6	cg00000165	2499	688
7	cg00000221	577	2437
8	cg00000236	1276	4065
9	cg00000289	289	1554
10	cg00000292	4393	8631
11	cg00000321	2283	1169
12	cg00000363	5979	1992
13	cg00000540	1292	7084
14	cg00000579	1227	5536

Figure: Methylation data sample

Treatment group vs control group

	Before (N=22)	Midpoint (N=21)	After (N=20)	Overall (N=63)
Age				
Mean (SD)	60.3 (6.68)	60.5 (6.80)	60.5 (6.98)	60.4 (6.70)
Median [Min, Max]	61.0 [50.0, 72.0]	61.0 [50.0, 72.0]	61.0 [50.0, 72.0]	61.0 [50.0, 72.0]
mAgeHorvath				
Mean (SD)	52.8 (7.19)	52.2 (6.69)	52.8 (5.57)	52.6 (6.44)
Median [Min, Max]	52.9 [40.8, 63.8]	53.8 [40.8, 62.0]	54.7 [41.9, 60.3]	54.1 [40.8, 63.8]
diff_horvath				
Mean (SD)	7.51 (4.93)	8.31 (4.39)	7.66 (4.52)	7.82 (4.57)
Median [Min, Max]	7.78 [-2.84, 19.6]	8.40 [-1.02, 16.8]	6.61 [0.716, 18.2]	7.92 [-2.84, 19.6]

	Before (N=22)	Midpoint (N=18)	After (N=19)	Overall (N=59)
Age				
Mean (SD)	58.5 (6.12)	57.1 (5.40)	57.8 (6.27)	57.8 (5.88)
Median [Min, Max]	57.5 [50.0, 72.0]	56.0 [50.0, 70.0]	56.0 [50.0, 72.0]	56.0 [50.0, 72.0]
mAgeHorvath				
Mean (SD)	54.4 (5.19)	51.9 (6.15)	52.6 (5.21)	53.1 (5.52)
Median [Min, Max]	54.1 [41.4, 65.9]	51.2 [41.1, 64.1]	53.0 [40.6, 63.1]	53.3 [40.6, 65.9]
diff_horvath				
Mean (SD)	4.06 (4.57)	5.14 (4.25)	5.25 (4.91)	4.77 (4.54)
Median [Min, Max]	3.04 [-4.55, 14.6]	4.52 [-2.81, 13.7]	4.13 [-2.26, 15.4]	4.13 [-4.55, 15.4]

Figure: Characteristics of the control group (at the top) and the treatment group (at the bottom).

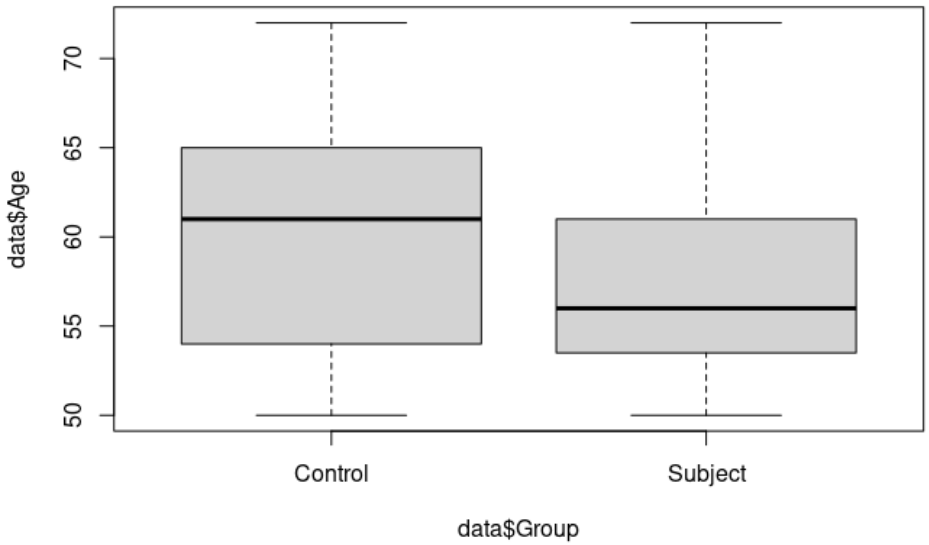

Figure: Age distributions in both groups

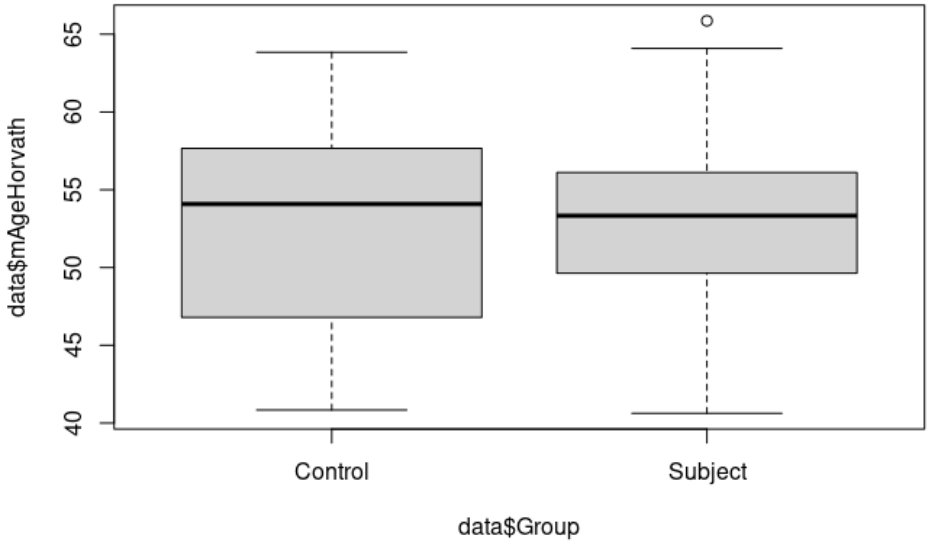

Figure: DNAmAge distributions in both groups

Testing age difference between groups

- For both groups (Treatment and Control) DNAmAge difference was calculated as DNAmAge after intervention minus DNAmAge before intervention for each individual.
- 2-sided t-test was performed on these vector of differences.
- We also performed 1-sided t-test because it seems more reasonable as we want to check whether treatment group has better results (i.e. lower change).

2-sided t-test	**1-sided t-test**
0.0834	0.0417

Table: P-values of the performed tests

- For 2-sided t-test there is no significant change between two groups. For 1-sided t-test treatment group has significantly lower change of age.

Statistical power of the observed differences in mean biological ages

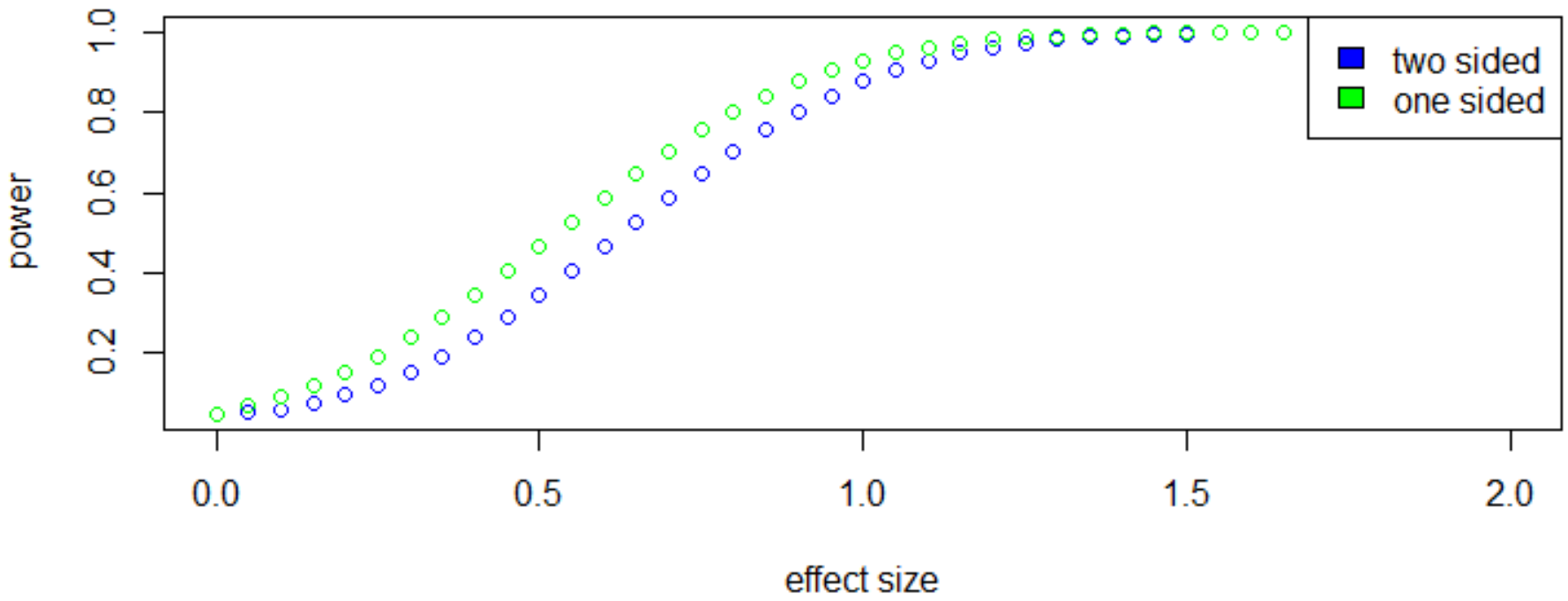

Figure: Power of the test depending on effect size.

Statistical power of the observed differences in mean biological ages

We calculated the effect size based on Cohen's d formula:

$$d = \frac{\overline{x}_1 - \overline{x}_2}{s},$$

where

- x_1 - differences between Horvath age before and after treatment for treatment group
- x_1 - differences between Horvath age before and after treatment for control group
- s - pooled standard deviation, $s = \sqrt{\frac{(n_1-1)s_1^2+(n_2-1)s_2^2}{n_1+n_2-2}}$

Results:
power (two-sided) = **0.425**
power (one-sided) = **0.55**

Recommendations for the continuation of the trial to a phase III

1. **Larger sample size** – to improve statistical power and reliability of the findings.
2. **More diverse population** in terms of age, gender, ethnicity – to see whether the findings are applicable to the general population.
3. **Longer duration of the intervention and follow-up period** – to assess the long-term effects.
4. **Similar age distribution in both groups** – because in this trial age distributions were quite different.
5. **Improving the combination of interventions** – the intervention is very complicated and included many restrictions and it might be too difficult for some individuals to follow all the steps.

Thank you!