Protocol #015078

Centre for Brain Research

University of Auckland

Note: This protocol follows the 2013 SPIRIT guidelines (Chan et al., BMJ, 2013). doi: https://doi.org/10.1136/bmj.e7586. It was modified on August 19, 2015, after securing ethics approval at the University of Auckland, New Zealand.

1. Author

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2. Title

High-Intensity Training to Enhance Cognition: A randomized, controlled, 6-week intervention in children

3. Funding

Campus Link Foundation; Kelliher Trust; Perpetual Guardian.

4. Roles and responsibilities

Dr. David Moreau: Design, methods, data collection, analysis, software, writing.
Prof. Ian Kirk: Supervision, funding acquisition, review.
A/Prof. Karen Waldie: Supervision, funding acquisition, review.

5. Background and rationale

Aerobic exercise is the form of exercise typically associated with neural changes and cognitive enhancement (Hillman et al., 2008; Thomas, Dennis, Bandettini, & Johansen-Berg, 2012). This is based on early findings in the animal literature, which typically investigated the effects of physical exercise in rodents–animals who naturally favor aerobic forms of exercise (Gould, Beylin, Tanapat, Reeves, & Shors, 1999; Shors et al., 2001), and because the most dramatic gains in cognition have been observed in the elderly (Erickson, Hillman, & Kramer, 2015; but see also Etnier, Nowell, Landers, &

Sibley, 2006), a population for which moderate-intensity exercise is seemingly most adequate.

Current trends of research suggest other promising directions. A compelling body of research in the field of exercise physiology indicates that interventions based on short, intense bursts of exercise can induce physiological changes that mirror those following aerobic exercise on a wide variety of outcomes. These include measures of cardiovascular function (Chrysohoou et al., 2015), overall fitness (Benda et al., 2015), and general health (Milanović, Sporiš, & Weston, 2015). In some cases, physiological improvements following high-intensity training (HIT) can even go beyond those typically following aerobic regimens (Rognmo, Hetland, Helgerud, Hoff, & Sigrdahl, 2004).

A few studies have directly tested this idea. HIT has been shown to alleviate some of the typical difficulties of attention associated with Attention-deficit/hyperactivity disorder (ADHD) in children (Piepmeier et al., 2015), demonstrating the potency of this type of intervention to mold behavior. HIT therefore has the potential to influence cognition in a meaningful manner, with the added benefit of being time-efficient (Costigan, Eather, Plotnikoff, Taaffe, & Lubans, 2015). Several studies have focused on the brain-derived neurotrophic factor (BDNF) val66met polymorphism, given its direct influence on serum BDNF concentration (Lang, Hellweg, Sander, & Gallinat, 2009). BDNF is known to support neuronal growth and has been shown to facilitate learning, a process that in turn induces BDNF production (Berchtold, Kesslak, Pike, Adlard, & Cotman, 2001; Cotman & Berchtold, 2002; Kesslak, So, Choi, Cotman, & Gomez-Pinilla, 1998). This dynamic coupling makes BDNF an important underlying factor of exercise-induced cognitive improvements. Consistent with this idea, it has been proposed that individuals whose particular BDNF polymorphism is associated with lower production of BDNF (met66 carriers) might benefit from exercise interventions to a greater extent than individuals whose BDNF production is higher (val66 homozygotes). Similarly, a few studies have shown that individuals with lower cardiovascular function might maximize benefits from physical exercise interventions designed to improve cognitive function (Sofi et al., 2011; Strong et al., 2005).

6. Objectives

The present study is intended to test the viability of HIT as a substitute for aerobic exercise to induce cognitive improvements in school populations. In particular, we postulate that HIT will result in improvements in measures of cognitive control and working memory, as both constructs have been linked to fitness levels (Pontifex et al., 2011) and appear to be malleable via aerobic regimens (Erickson et al., 2013). The choice of these constructs is also motivated by previous research showing the malleability of both cognitive control and working memory in training studies, thus providing theoretical and empirical support for the plausibility of expected improvements (Hampshire, Highfield, Parkin, & Owen, 2012; Mishra, de Villers-Sidani, Merzenich, & Gazzaley, 2014). The present study also intends to address interindividual variability so as to isolate the underlying factors of improvement. Based on previous literature (Erickson et al., 2013; Moreau et al., 2015), we hypothesize that exercise training will elicit substantially larger cognitive benefits in individuals whose cardiovascular fitness is low, and in BDNF met66 carriers, whose BDNF production is naturally limited. Finally, we expect physiological improvements with exercise, as typically induced from aerobic interventions (see for a review Gomez-Pinilla & Hillman, 2013).

7. Design

Parallel design (random allocation to HIT and Control groups); superiority framework (HIT > Control).

8. Setting

This will be a multicenter study, across schools in New Zealand.

9. Eligibility

Participants will be between 7-14 years of age. Three signed assent/consent forms (child/parent/school principal) will be required for each individual to participate in the study.

10. Intervention

The high-intensity workout will include the following:

- warm-up (2 min)
- core session (5 x 20 sec, interleaved with incremental breaks (30 sec, 40 sec, 50 sec, 60 sec, and a shorter 20 sec break after the last workout period)
- stretching (2 min).

There is no particular requirement in terms of previous experience or knowledge (i.e. basic fitness movements). Gaze will be fixed on screen at all times. All instructions will be verbal (audio recording) and visual (on-screen captions). Complete details and script can be found in the online repository. A complete session lasted 10 min, and was scheduled every morning on weekdays.

The control condition will consist of a blend of board games, computer games, and trivia quizzes This is in line with current recommendations regarding active control groups (Boot et al., 2013). It is also consistent with findings showing that aerobic exercise interventions typically do not differ from other regimens with respect to participants' expectations (Stothart et al., 2014).

Frequency and duration will be matched between conditions. The intervention is 6-weeks long, with five sessions per week, for a total of 30 sessions. Due to the nature of the intervention, class size will be limited to 20 participants in both conditions. Participants will be supervised at all times, for safety reasons and to ensure a high degree of fidelity to the intended protocol.

11. Outcomes

This section presents the cognitive, physiological, genetic and questionnaire information that will be collected.

Cognitive (Flanker – Go/no-go – Stroop – Backward digit span – Backward Corsi blocks – Visual 2-back). These are the primary measures of interest. They make up two cognitive constructs, cognitive control and working memory capacity, which will be confirmed by an exploratory factor analysis (see "Statistical methods"). HIT is expected to elicit greater improvements in these measures.

Physiological. The following will be collected, throughout the intervention: include minutes of activity, calories burned, intensity, intensity range (sedentary, lightly active, fairly active, very active), steps and heart rate (measured by changes in blood

volume using PurePulseTM LED lights). HIT is expected to elicit greater improvements in these measures.

Genetic. Participants will provide a saliva sample to determine their BDNF val/met 66 polymorphism.

Questionnaire. Participants will also provide information about the following: ethnic background, age, gender, handedness, height, weight, diagnosis of learning disorder, brain trauma or epileptic seizures, current or past enrolment in a remediation or a cognitive training program, and whether English is their first language. In addition, self-reported information will be gathered to quantify videogaming and physical exercise habits (4-point Likert scale in both cases), as well as to evaluate overall health, happiness, sleep quality, and mindset (6-point Likert scale for each item). The latter is intended to capture beliefs about the malleability of cognitive ability in the context of schoolwork, that is, the extent to which students perceive academic achievement in a predominantly fixed or malleable manner. All measures will be collected prior to the intervention, but variables susceptible to change over time will be reassessed post-intervention.

12. Timeline

This section concerns the timeline, exclusively. Details about the particular assessments and measurements used can be found in the "Outcomes" and the "Data collection" sections.

Pre-intervention. Battery of tests (cognitive, genetic, physiological, questionnaire), after participants are deemed eligible for the trial.

Intervention: 6 weeks, with five sessions per week, for a total of 30 sessions. Random assignment to the exercice group (HIT) and the control group.

Post-intervention. Battery of tests (cognitive, physiological, questionnaire).

13. Sample size

An a priori power analysis based on previous studies (Erickson et al., 2013; Moreau et al., 2015) indicated the need for a minimum N of 129 participants per group to detect an effect of $d = 0.35$, with $1 - \beta = .80$ and $\alpha = .05$. The actual sample size is expected to be larger, given that a) we will seek higher statistical power, and b) the intended analyses are

Bayesian, a framework in which the present power analysis is not meaningful. In this statistical framework, a larger sample size will lead to more confidence in an effect, expressed probabilistically, given than it is present.

14. Recruitment

Recruitment will be from our database of interested schools, who have made contact through email, or our website contact box. This is a nationwide trial in New Zealand. After ensuring the interested parties understand the expectations and commitment inherent to the trial, we will work toward an agreement regarding schedule and timeline.

15. Allocation

Randomized (1:1 allocation).

16. Sequence generation

Participants will be randomized using R (R Core Team, 2015). Randomization will be performed after participants have enrolled, via an online link including basic information and demographics, and after baseline measurements. This will ensure concealment of group assignment at the time of testing, before the intervention starts. Specifically, we will use the sample() function in R:

```
function (x, size, replace = FALSE, prob = NULL)
{
   if (length(x) == 1L && is.numeric(x) && x >= 1) {
      if (missing(size))
         size <- x
      sample.int(x, size, replace, prob)
   }
   else {
      if (missing(size))
         size <- length(x)
      x[sample.int(length(x), size, replace, prob)]
   }
```

}

17. Implementation

Sequence generation will be implemented by the first author, following the procedure aforementioned.

18. Blinding

Testers will be blind to group allocations. Participants will not be informed about the particular hypotheses of the intervention, that is, which group is expected to show larger improvements. Note that expectations will be directly measured (see Outcomes section).

19. Data collection

Cognitive measurements

Testing will be conducted on school premises. All cognitive assessments will be computer-based, administered in groups of a maximum of 15 students. This limit on the number of participants tested at a given time is meant to minimize potential averse effects of group testing. For each task, we will measure accuracy and response time. Different stochastic variations of all tasks will be used at pretest and posttest. Unless specified otherwise, the number of trials varied based on individual performance to allow reaching asymptotes, with a minimum and a maximum specified for each task. The reliability of this method for each task will be assessed from a separate sample, by comparing test scores on the asymptotic version vs, the maximal-length version, for each task. Reliability indices will be shared online. The order at pretest and posttest will be the following: Flanker – Go/no-go – Stroop – Backward digit span – Backward Corsi blocks – Visual 2-back. Both testing sessions will be scheduled at the same time of the day. Testing sessions are expected to last approximately an hour.

Flanker. Participants will view a series of arrows, either pointing to the left of the right of the screen. They will be instructed to ignore all stimuli but the arrow at the center of the screen (target), and to respond by pressing the left or right key when presented with arrows pointing left or right, respectively. For any given trial, the number of arrows displayed will range from three to 25, with equal probability for congruent and

incongruent trials. All sessions will include 20 trials. We will record accuracy and response time for both congruent and incongruent trials.

Go/no-go. Participants will be presented with a series of circles, either uniform or patterned. The uniform circle requires a key response ("go") whereas the other requires no response ("no-go"). If response is required, the stimulus will remain visible indefinitely, until a response is made. When the stimulus requires no response, it will disappear after 2000ms. A self-paced button press will trigger the start of the next trial. The interval from the button press to the presentation of the stimulus will range from 500ms to 2000ms (randomly jittered).

Stroop. Participants will be presented with a series of color words, in a colored font either congruent or incongruent, drawn with equal probability. They will be instructed to attend to the color of the font, and to respond by pressing the key corresponding to the appropriate color on the keyboard. Stimuli will remain visible until a response is made. We will record accuracy and response time for both congruent and incongruent trials.

Backward digit span. Participants viewed a series of digits from 1 to 9 presented sequentially for 1000ms, with 500ms intertrial intervals. They will be instructed to respond by entering the corresponding digits on the keyboard at the end of each trial. Hierarchical item randomization will allow the presentation of a maximum of two identical digits consecutively. For each trial, answers can be corrected until submitted. A self-paced button press will trigger the start of the next trial.

Backward Corsi blocks. Participants will be presented with a series of locations on a block, sequentially for 1000ms, with 500ms intertrial intervals. They will be instructed to respond by clicking on the corresponding locations at the end of each trial. Hierarchical item randomization will not allow the presentation of identical locations consecutively. For each trial, no correction will be allowed once submitted. A self-paced button press will trigger the start of the next trial.

Visual 2-back. Participants will view a series of pictures presented sequentially for 2000ms, with 500ms intertrial intervals. They will be instructed to press a key to signal a match, that is, two identical pictures interleaved with one stimulus in between

(i.e., 2-back). No action will be required in the absence of match. The number of matches will range from 20 to 35 per session, randomized.

Physiological measurements

All physiological measures will be collected using FitbitChargeHRTM, powered by the MEMS tri-axial accelerometer. Measures will include minutes of activity, calories burned, intensity, intensity range (sedentary, lightly active, fairly active, very active), steps and heart rate (measured by changes in blood volume using PurePulseTM LED lights).

Questionnaire

Participants will also provide information about the following: ethnic background, age, gender, handedness, height, weight, diagnosis of learning disorder, brain trauma or epileptic seizures, current or past enrolment in a remediation or a cognitive training program, and whether English is their first language. In addition, self-reported information will be gathered to quantify videogaming and physical exercise habits (4-point Likert scale in both cases), as well as to evaluate overall health, happiness, sleep quality, and mindset (6-point Likert scale for each item). The latter is intended to capture beliefs about the malleability of cognitive ability in the context of schoolwork, that is, the extent to which students perceive academic achievement in a predominantly fixed or malleable manner. All measures will be collected prior to the intervention, but variables susceptible to change over time will be reassessed post-intervention.

Genotyping

Participants will provide a small saliva sample, collected via Oragene-DNA Self Collection kits following the instructions of the manufacturer. DNA extraction from the blood samples will follow the method given by Nishita et al. (2009). DNA samples will be resuspended in Tris-EDTA buffer and quantified using Nanodrop ND-1000 1-position spectrophotometer (Thermo Scientific). Samples of DNA will be diluted to 50ng/μL, and DNA amplification will be carried out following a modified version of that described by Erickson et al. (2008).

Amplification will be carried out on the 113 bp polymorphic BDNF fragment, using the primers BDNF-F 5-GAG GCT TGC CAT CAT TGG CT-3 and BDNF-R 5-CGT GTA CAA GTC TGC GTC CT-3. Polymerase chain reaction (PCR) will be

conducted using 10X Taq buffer (2.5L μL), Taq polymerase (0.125 μL), dNTPs (5 nmol), primers (10 pmol each), Q solution (5 μL), and DNA (100 ng) made up to 25 μL with dH2O. The PCR conditions will consist of denaturation at 95°C for 15 min, 30 cycles on a ThermoCycler (involving denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s) and a final extension at 72°C.

PCR product (6.5 μL) will be incubated with Pm1l at 37°C overnight. The digestion products will be analyzed using a high-resolution agarose gel (4%) with a Quick Load 100 bp ladder (BioLabs) and a GelPilot Loading Dye (QIAGEN). After immersion in an ethidium bromide solution for 10 min, DNA will be visualized under ultraviolet light. Enzyme digestion will result in a 113 bp fragment for the BDNF met66 allele, and 78 and 35 bp fragments for the val66 allele. This procedure is consistent with the one described by Erickson et al. (2008).

20. Data management

Data will be secured on University of Auckland servers. Anonymized, partial data sets will be released with publication of the study. This is to maintain confidentiality, given the personal nature of several variables collected (e.g. genetic information, clinical diagnoses, etc.).

21. Statistical methods

The statistical methods are described in details in the appended statistical analysis plan (SAP). The SAP includes detailed and commented code. Statistical analyses will be performed in R (R Core Team, 2015). We will report all packages used in our analyses. R code will be shared on GitHub. The repository includes data sets, R scripts, details and script of the HIT workout, the CONSORT flow diagram and the CONSORT checklist. We will report descriptive statistics for both groups, on age, gender, sample size, handedness, BMO, previous clinical diagnosis, previous remediation, experience with videogaming, physical exercise, and self-reported health, sleep quality and happiness.

We will report Bayesian model comparisons, to allow quantifying the degree of evidence for a given model compared to other models tested, combined with Bayesian

parameter estimations when relevant. We will also report the equivalent frequentist analyses for transparency, and in order to facilitate reading.

We will examine normality of distribution for all continuous variables. If distributions are skewed, we will compare results using non-corrected vs. log-transformed data, and will look for discrepancies. If distributions are leptokurtic or platykurtic, we will identify the source of extreme kurtosis, but note that the analyses we will present are extremely robust to outliers, as priors can be adapted to reflect deviations from normality. Regardless, we will check consistency using standard approaches to outlier exclusion, to facilitate direct comparisons with frequentist tests. Outliers will be defined as values more than 3/2 times the upper quartile or less than 3/2 times the lower quartile of a given distribution, and systematically checked consistency of our results with and without inclusion.

Outcomes

Physiological

Physiological improvements will provide corroborating evidence for the hypothesized changes associated with exercise, and they will allow identifying idiosyncratic parameters often characteristic of training interventions. We will conduct an (Bayesian) ANCOVA on change in resting heart rate, with Condition (HIT vs. Control) as a fixed factor and baseline heart rate as a covariate. We will report the main group effect, and test for homogeneity of variance (Levene's test). This will be complemented by a Student/Welch (Bayesian) two-sample t-test on resting heart rate change, by Condition (HIT vs. Control), after a split on resting heart rate at pretest. We will use (Bayesian) linear regression (after checking assumptions) to model effort and workout load across training sessions.

Exploratory factor analysis

An exploratory factor analysis using principal component extraction and promax rotation will be performed on all six cognitive measures at pretest (Flanker – Go/no-go – Stroop – Backward digit span – Backward Corsi blocks – Visual 2-back). Promax allows factors to correlate; this property is especially appropriate when the factors extracted are assumed to be correlated to some degree. This is to be expected in the proposed design, because of the positive manifold (Spearman, 1904). We will inspect the corresponding scree plot and

eigenvalues to determine the number of components, and will report factor loadings for all constructs. We will confirm the structure of the model with the corresponding chi-squared value and Bayesian Information Criterion. The factors extracted from the EFA will be used for subsequent group comparisons at the construct level.

Priors and robustness

We will use default prior scales across analyses (Morey & Rouder, 2015). For Bayesian repeated measures ANOVA and ANCOVA, the prior scale on fixed effects will be set to 0.5, the prior scale on random effects to 1, and the prior scale on the covariate to 0.354. The latter will also be used in Bayesian Linear Regression. The Bayesian t-test uses a Cauchy prior with a width of $\sqrt{2}/2$ (~ 0.707), i.e. half of parameter values lies within the interquartile range [-0.707; 0.707]. For transparency, we will plot the prior and posterior distribution for the comparison between Conditions (HIT vs. Control) for all constructs, to be published in the paper or as online supplemental material.

Markov Chain Monte Carlo (MCMC) parameters

Where appropriate, we will provide details or relevant references about the MCMC algorithm we use. MCMC will be used to generate posterior samples via the Metropolis-Hastings algorithm (see for details Rubinstein & Kroese, 2011). All analyses will be set at 10,000 iterations, with diagnostic checks for convergence. One chain per analysis will be used for all analyses reported in the paper, with a thinning interval of 1 (i.e., no iteration to be discarded).

Cognitive

We will perform the following analyses on cognitive measures:

- Repeated measures (Bayesian) ANOVA on each factor score with Session (pretest vs. posttest) as a within factor and Condition (HIT vs. Control) as a between factor. Test of the interaction. Test for homogeneity of variance (Levene's test).
- (Bayesian) regression analysis on factor score gains with change in resting heart rate as a predictor, for each group.
- (Bayesian) regression analysis on factor score gains with baseline resting heart rate as a predictor (HIT group only).

- Repeated measures (Bayesian) ANOVA on each factor score with Session (pretest vs. posttest) as a within factor and BDNF polymorphism (val vs. met) as a between factor Test of the interaction. Test for homogeneity of variance (Levene's test).
- Student/Welch two-sample (Bayesian) t-test on each factor score at pretest, by BDNF polymorphism (val66 vs. met66).
- Repeated measures (Bayesian) ANOVA on cognitive scores (Flanker – Go/no-go – Stroop – Backward digit span – Backward Corsi blocks – Visual 2-back) with Session (pretest vs. posttest) as a within factor and Condition (HIT vs. Control) as a between factor. Test of the interaction. Test for homogeneity of variance (Levene's test).

Additional analyses

We will also report analyses for which our *a priori* hypotheses were null effects. These variables will be collected either to control for potential confounds, or for exploratory purposes. Testing for differences will be conducted using (Bayesian) t-tests. We will ensure there is no difference between groups regarding self-reported enjoyment or motivation, to control for expectation effects (Boot, Simons, Stothart, & Stutts, 2013; Stothart, Simons, Boot, & Kramer, 2014). We will also make sure there is no group difference regarding self-reported belief about cognitive malleability (i.e., mindset), ethnic background, age, gender, handedness, height, weight, diagnosis of learning disorder, brain trauma or epileptic seizures, current or past enrolment in a remediation or a cognitive training program, English as first language, videogaming experience, physical exercise, self-reported happiness, sleep quality, or general health.

22. Harms

The intervention is not expected to result in any particular harm to individuals. Because the intervention involves random assignment to conditions, full access to the content of the experimental condition will be given to all participants after completion of the trial, inclusive of the initial control group.

23. Ethics approval

Ethics approval was obtained from the University of Auckland ethics committee on August 19, 2015, for a 3-year duration.

24. Amendments

Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the participant or may affect participant safety, including changes of study objectives, study design, patient population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be agreed upon all authors listed here, and approved by the Ethics Committee prior to implementation.

[Updated 08/19/15] Note that no amendments to the protocol were submitted after the date of approval.

25. Consent

Participants, parents and school principals will give their informed consent for inclusion in this study. The Ethics Committee at the University of Auckland has approved all procedures (protocol #015078).

26. Confidentiality

All study-related information will be stored securely. Consent forms and participant information will be stored in locked file cabinets in areas with limited access. All laboratory specimens, reports, data collection, process, and administrative forms will be identified by a coded ID number to maintain participant confidentiality. All records that contain names or other personal identifiers, such as locator forms and informed consent forms, will be stored separately from study records identified by code number. All local databases will be secured with password-protected access systems. Forms and lists, or any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access.

27. Declaration of interests

No competing interests exist.

28. Access to data

All investigators will have internal access to the data, without restrictions. This includes cleaned and raw data sets, for all measures collected.

29. Dissemination policy

Code for analyses will be shared on the first author's Github account. We will also share the CONSORT flow diagram and CONSORT checklist, as per the 2010 CONSORT guidelines (http://www.consort-statement.org/consort-2010). We will seek publication in an open-access journal, to promote visibility to communities outside science.