Physical Activity as a Predictor of Clinical Trial Outcomes in Bipolar Depression: A Subanalysis of a Mitochondrial-Enhancing Nutraceutical Randomized Controlled Trial

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Physical activity as a predictor of clinical trial outcomes in bipolar depression: a sub-analysis of a mitochondrial-enhancing nutraceutical randomised controlled trial

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Abstract

Objectives: Individuals with bipolar disorder (BD) generally engage in low levels of physical activity (PA), and yet few studies have investigated the relationship between PA and change in BD symptom severity. The aim of this sub-analysis of an adjunctive nutraceutical randomised controlled trial for the treatment of bipolar depression was to explore the relationship between PA, the active adjunctive treatments (a nutraceutical "mitochondrial cocktail"), and clinical outcomes.

Methods: Participants with bipolar depression were randomised to receive either N-acetylcysteine alone, N-acetylcysteine with a combination of nutraceuticals (chosen for the potential to increase mitochondrial activity), or placebo for 16 weeks. Participants (n=145) who completed the International Physical Activity Questionnaire - Short Form (IPAQ-SF; measured at Week-4) were included in this exploratory sub-analysis. Assessments of BD symptoms, functioning and quality of life were completed at monthly visits up until Week-20. Linear mixed models were used to explore if IPAQ-SF scores were a moderator of treatment received on outcomes of the study.

Results: Week-4 PA was not related to changes in Montgomery Åsberg Depression Rating Scale scores across the study until Week-20. However, participants who engaged in more PA and who received the combination treatment were more likely to have a reduction in scores on the Bipolar Depression Rating Scale (p=0.03). However, this was not consistent in all domains explored using the IPAQ-SF. Participants who engaged in higher levels of PA also experienced greater improvement in social and occupational functioning, and less impairment in functioning due to their psychopathology and quality of life at Week-20, irrespective of treatment.

Conclusions: This study provides novel evidence of the association between PA and reduction in BD symptoms in a nutraceutical clinical trial. However, further research assessing the potential synergistic effects of PA in BD is required.

(Trial registered at ANZCTR registry: ACTRN12612000830897)

Keywords: physical activity, exercise, bipolar disorder, bipolar depression, mitochondrial agents, nutraceuticals, N-acetylcysteine

Introduction

Bipolar depression is often difficult to treat. One approach to optimise the effects of current therapeutics may be through lifestyle interventions such as engagement in physical activity. Despite many known benefits of physical activity in the general population[1] and increasing evidence that individuals with other serious mental disorders such as schizophrenia[2] and major depression[3; 4] can also benefit, limited research has investigated physical activity and symptom severity in bipolar disorder (BD; for reviews[5; 6]).

To date, the literature is largely based on cross-sectional, prospective cohort or small pilot studies, all of which suggest that engagement in physical activity improves mood and quality of life, but the evidence-base is limited [5; 7-9]. Individuals with BD engage in lower levels of physical activity, are less likely to meet recommended International guidelines for exercise (World Health Organisation; WHO[10]) and are more likely to be sedentary versus age and sex matched controls[11]. Therefore, not surprisingly, people with BD demonstrate lower levels of cardiorespiratory fitness compared to healthy controls[12,13]. Previous research has suggested that increased physical activity is associated with better cognition in euthymic females with a diagnosis of BD[14]. Achieving an adequate level of physical activity has been included in the current NIH guidelines for treating BD, but only in the broad sense of improving general health[15]. In the general population, it is recommended that individuals achieve 150 minutes of moderate or 75 minutes of vigorous physical activity per week [10]. The literature to date in the general population has found that both continuous and interval aerobic physical activity at a moderate to high intensity can improve mitochondrial function[16-19]. An emerging evidence base also advocates that resistance training, specifically targeting the loading and strengthening of skeletal muscles can also improve mitochondrial function[19-20]. Whilst people with BD may have mitochondrial dysfunction[21], it is unclear if physical activity at moderate to high intensity at recommended guidelines such as those recommended by WHO[10] can influence

mitochondrial function. Physical activity is low-cost, safe and tolerable and therefore could be an effective adjunct to improve response to treatment in BD, however this has been largely unexplored. Therefore, we aimed to investigate whether physical activity was is associated with changes in symptoms, functioning and quality of life in BD. This study was embedded in a double-blind randomised controlled trial (RCT) evaluating the efficacy of adjunctive nutraceuticals for the treatment of bipolar depression. The adjunctive nutraceuticals were specifically selected due to their potential mitochondrial enhancing properties,[22] and there may be a relationship between physical activity and the nutraceuticals via mitochondrial biogenesis [23]. There were three arms of the RCT: N-acetylcysteine (NAC) alone, a combination treatment (CT) of nutraceuticals including NAC and placebo.

We hypothesised that reported physical activity would be an effect modifier for the relationship between those receiving NAC-alone or the CT, and an improvement on depression, functioning and quality of life outcomes. We also hypothesised that physical activity in categorical terms, according to the scoring guide of the physical activity scale (low, moderate, high) would be an effect modifier for the relationship between those receiving NAC-alone or CT, and outcomes (detailed above). Finally, when utilising data categorised by WHO recommendations, we hypothesised that physical activity (according to WHO recommendations)would be an effect modifier for the relationship between treatment with NAC-alone or CT, and outcomes (detailed above).

Methods

Ethics

The study was run in accordance with International Council for Harmonisation Good Clinical Practices Guidelines[24]. Ethics approval was granted from Barwon Health Human

Research and Ethics Committee (HREC), Northern Sydney Local Health District HREC, The Melbourne Clinic Research Ethics committee and Deakin University HREC. The study is registered on the Australian and New Zealand Clinical Trial Registry (ACTRN12612000830897)

Trial study design

Participants (n = 181) were randomised received the study medication for 16 weeks and visited study sites (Melbourne, Geelong, Sydney) every four weeks for clinical interviews with a research assistant up until Week 20. Inclusion criteria included a diagnosis of BD, determined by the Mini International Neuropsychiatric Interview (MINI) 5.0[25] and a current moderate to severe depressive episode measured by a score ≥20 on the Montgomery Åsberg Depression Rating Scale (MADRS)[26]. Full study protocol[16] and primary results[20] have been published previously.

The primary aim of the trial was to assess the efficacy of the two active arms of the study (NAC-alone and CT) compared to placebo for treating depressive symptoms (measured by the MADRS) at week 16. Primary results of the study at the primary endpoint were not significant at Week 16[27]. However, at Week-20 (4 weeks post-study medication discontinuation), CT was superior to placebo at improving the following outcome measures; changes in depression symptoms measured by the MADRS which was the primary outcome measure in the study; Bipolar depression symptom severity measured by the Bipolar Depression Rating Scale (BDRS)[28]; Social and Occupational Functioning Assessment Scale (SOFAS)[29], a clinician-rated measure of functioning; The Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning (LIFE-RIFT)[30], a clinician-rated measure of impairment in functioning from psychopathology and the Clinical Global Impressions Scales Bipolar version – Improvement (CGI-I)[31], a one-item clinician-rated scale measuring

improvement. Participants also completed The Quality of life Enjoyment and Satisfaction Questionnaire – Short Form (Q-LES-Q-SF)[32], a self-report measure of quality of life. There was no significant relationship between CT vs placebo in regards to Q-LES-Q-SF scores, but this outcome was included in the sub-analysis because of the association between physical activity and quality of life in bipolar disorder[13]. Total possible scores for each outcome measure, and indication of direction for improvement, can be found in Supplementary Table 1.

Physical Activity

The International Physical Activity Questionnaire Short Form (IPAQ-SF)[33] was administered at Week 4 to measure each participant's general level of physical activity. The IPAQ-SF is a 10-item self-report questionnaire where participants recall the number of days and minutes of vigorous activity, moderate activity, walking and sitting time, over the past seven days. The IPAQ-SF has been used extensively in other mental health disorder populations and has acceptable validity and reliability[34]. The IPAQ-SF was administered at Week 4 to reduce participant burden at the baseline visit and to coincide with collection of dietary intake data. The IPAQ-SF was administered as secondary outcomes data and has been included in the protocol[16]; however, this measure was inadvertently omitted from the trial registry.

Data was cleaned using IPAQ-SF recommendations[33] that includes removing cases with missing values and removing cases with values too low (less than 10 minutes of activity per day). There was no missing data for vigorous, moderate activity or walking. Two participants had missing values for the 'time spent sitting' item. These participants both remained in the analysis as this item is not used to calculate total scores or categorical scores. Minimum and maximum values were implemented to remove outliers. As a result, one participant was removed for too few minutes (6 minutes) of activity. To normalize the data,

the protocol suggests truncating each daily activity time to no more than 180 minutes. This rule was employed for five participants reporting vigorous activity, seven for moderate activity and six for walking. Of note, one participant filled in the IPAQ-SF questionnaire at Week-8, not Week-4 but remained in the analysis.

Weekly Metabolic Equivalent of Task (MET)-minute scores for each activity type were first calculated as follows:

- Vigorous activity *minutes/week* = total minutes per week of vigorous activity * 8.0 METs
- Moderate activity *minutes/week* = total minutes per week of moderate activity * 4.0 METs
- Walking *minutes/week* = total minutes per week of walking * 3.3 METs

Each total activity-MET score was then summed to create a continuous total physical activity score.

In addition to total physical activity scores, a categorical value was produced for each participant. The categories were low, moderate or high physical activity and were calculated for each participant in accordance with IPAQ-SF scoring protocol[33]. Within this protocol, participants' activity levels were deemed *high* if they engaged in at least three days of vigorous activity and achieving a total activity of at least 1,500 MET-minutes/week, or a combination of all intensity levels for seven days or more and achieving a total activity of at least 3,000 MET-minutes/week. *Moderate* activity category was achieved if participants engaged in at least 20 minutes of vigorous activity for three days, or at least 30 minutes of walking and/or moderate activity for five days, or a combination of any activity level for five or more days and achieving a total activity of at least 600 MET-minutes/week. Lastly, participants' activity was

categorised as *low* if they did not fit into either of the above categories. A summary of categorical scores for the sample can be found in Table 1.

The last item of the IPAQ-SF is 'time spent sitting' and is used to assess participants' rates of sedentary behaviours. Sitting has been presented as a separate variable, measured in average minutes per typical weekday.

In addition to the validated exploration of the IPAQ scale, further analysis was conducted using WHO recommendations. This was completed to provide preliminary data for guidelines and clinical practice and to provide real-world advice to patients. To explore these data in relation to WHO recommendations, total physical activity data in MET-minutes/week were categorically sorted. These additional categories were utilised to aid direct interpretation of the results to participant adherence to WHO recommendations as outlined below. This quick interpretation allows results from this study to be easily translated into policy and clinical care.

1. **No physical activity** – all activity <10 minutes duration (equivalent of 0 MET-minutes/week).
2. **Below WHO recommendations** – less than 150 minutes of moderate activity or 75 minutes of vigorous activity per week. Equivalent of energy expenditure between 0 and 600 MET-minutes/week (not inclusive).
3. **Within WHO recommendations** – At least 150 minutes of moderate activity or 75 minutes of vigorous activity per week. Equivalent of energy expenditure between 600 and 1200 MET-minutes/week (inclusive).
4. **Exceeding WHO Recommendations** – WHO recommends for greater health benefits, at least 300 minutes of moderate activity and 150 minutes of vigorous activity. Equivalent of energy expenditure greater than 1200 MET-minutes/week.

Statistical Analysis

Generalised estimating equations (GEE) were used to assess if physical activity (as a total score, categorical value and according to WHO recommendations) were predictors of outcomes from the nutraceutical RCT (MADRS, BDRS, SOFAS, LIFE-RIFT, Q-LES-Q-SF and CGI-I scores). Each predictor was assessed individually including an exploration of each of the treatment arms (NAC-alone or CT) compared to placebo across the study up until Week 20. By using GEE, the analyses are able to take into account the longitudinal nature of data (i.e., measurement autocorrelation in follow-ups). The primary outcome of the study followed a modified intention-to-treat analysis whereby participants with post-baseline data were included in the analysis[27]. First, the original RCT analyses were replicated by including treatment arms as a nominal factor, log of follow-up time as a covariate and the two-way interaction between log(time) and treatment arms was replicated, followed by including each predictor (each physical activity score) in a separate model to evaluate if it is a predictor of outcomes. The latter model contained treatment arms as a nominal factor, log of follow-up time as a covariate, predictor of interest, all possible two-way interactions and the three-way interactions between treatment arms, log of follow-up time and the predictor of interest. Three-way models evaluated the effect of each predictor on the outcome measure, across time in the study, for each treatment arm. Treatment by physical activity two-way interactions explored the role of the predictor for each of the study, independent of time. Each model utilised Baron & Kenny[35] criteria guidelines as first described by Kraemer et al[36]. Each model for each of the predictors are described below.

Categorical physical activity

We took into account the ordinal nature of physical activity categories when modelling the IPAQ-SF as low, moderate, high. The model included a fixed-effect treatment group and

categorical (ordinal) physical activity, and logarithm of time as covariates, all two-way interactions and the three-way interactions. As above, three-way interactions were then removed to explore two-way interactions. Total physical activity was also assessed as a continuous score, details of which are outlaid in Supplementary Material.

Physical activity according to WHO recommendations

Physical activity according to WHO recommendations was assessed as nominal data and included in the model as a factor. The initial model included a fixed-effect treatment group and physical activity according to WHO recommendations, and logarithm of time as covariate, all two-way interactions and all three-way interactions. After this model was run for each outcome, three-way interactions were then removed to explore all two-way interactions for each outcome.

The *p*-value for all overall three-way interactions were reported alongside Wald χ^2 statistic (used to measure parameter effects). In addition, for each treatment group (NAC-alone or CT), three-way interactions were reported with *p*-value and Wald χ^2 statistic, alongside their corresponding Beta coefficients and 95% confidence intervals (CIs) to measure association.

After examining three-way and two-way interactions of interest for each predictor, the data was then further explored for non-specified predictors. Non-specified predictors demonstrated a relationship with change in the outcome measure independent of what treatment was received and time. Each model for non-specified predictors included the main effects of treatment group the predictor and logarithm of time. This model assesses for non-specified predictors as it explores the predictors response in the sample as a whole (combining all treatment groups).

The GEE technique was implemented for model estimation using an unstructured working correlation matrix and a robust variance estimator[37]. Statistical analyses were completed using IBM® SPSS® Statistics for Windows, version 25[38].

Results

Participants

Of the 181 participants in the clinical trial, 33 participants were excluded from the analysis for not having any post-baseline data, two participants excluded for missing IPAQ-SF data and one participant excluded due to insufficient IPAQ-SF (less than 10 minutes activity). Therefore, 145 participants were included in the current analysis. The average age of the sample was 46.14 years (*SD* = 12.38), ranging from 21 to 72 years of age, and 51% were male. Participants were randomised to receive NAC (*n* = 50), CT (*n* = 46) and placebo (*n* = 49). A full list of study sample characteristics can be found in Table 1.

[Insert Table 1 approximately here]

Analysis of predictors

Change scores were calculated for each outcome measure (except CGI-I which self-evidently had no baseline data available). Mean change (Week 20 minus baseline scores) for each outcome variable per treatment group is shown in Supplementary Table 2. On average, participants in all treatment arms improved across all outcome measures. As CGI-I represents a single score of change from baseline, mean Week-20 CGI-I scores per treatment group are summarised in Supplementary Table 3. On average, research clinicians rated participants as improving across the study. For all models with a significant interaction, age, sex and body

mass index were explored as potential confounders and no factors had a statistically significant impact on the relationships.

Physical Activity as a categorical variable

Physical activity scores on the IPAQ-SF were categorised as low, moderate or high using scale recommendations. From the whole sample, 49.7% of participants were categorised as engaging in low weekly physical activity, 26.2% engaging in moderate weekly physical activity and 24.1% engaging in high weekly physical activity. A visual representation of data has been included in Supplementary Figure 1b.

Categorical physical activity was not significantly associated with scores for MADRS, SOFAS, LIFE-RIFT, Q-LES-Q-SF or CGI-I (see Table 2). There was a three-way interaction between taking NAC and engaging in high exercise and participant's BDRS outcomes. Compared to placebo, participants receiving NAC and engaging in a high amount of exercise showed an increase in BDRS scores, indicating a worsening of symptoms across the trial. For every one-level increase in level of physical activity (i.e., level of physical activity according to IPAQ-SF categorical scores), mean BDRS on NAC further increased by 2.85 (95% CI: 0.03, 5.7) units when compared with placebo group with similar physical activity level. There were no significant two-way interactions between treatment received and categorical physical activity.

[Insert Table 2 approximately here]

WHO Recommendations

Physical activity scores were represented in terms of WHO recommendations. From the whole sample, 11% engaged in no weekly physical activity, 27.6% engaged in weekly physical activity under the WHO recommendations, 15.9% engaged in weekly physical activity within the WHO recommendations and 45.5% engaged in weekly physical activity greater

than, or, exceeding the WHO recommendations. A visual representation of data has been included in Supplementary Figure 1c.

Results of the effect modification analysis is shown in Table 3. Physical activity according to WHO recommendations was not significantly associated with scores for MADRS, SOFAS, LIFE-RIFT, Q-LES-Q-SF or CGI-I. There was a significant three-way interaction between treatment received, physical activity according to WHO recommendations, and time. Participants who were randomised to receive CT and engaged in more physical activity, had a greater reduction in BDRS scores. For every one-level increase in physical activity (i.e., level of physical activity categorised according to WHO recommendations) mean BDRS in the combination therapy group further decreased by 2.15 (95% CI: -4.07, -0.23) units when compared with the placebo group with similar physical activity levels. There were no significant two-way interactions between treatment received and physical activity in terms of WHO recommendations.

[Insert Table 3 approximately here]

Total Physical Activity Scores

Total physical activity, as a continuous score, was not significantly associated with MADRS, SOFAS, LIFE-RIFT, Q-LES-Q-SF or CGI-I scores (see Supplementary Table 4). There was, however, a significant three-way interaction between participants taking CT and engaging in more physical activity and participant's BDRS outcomes. Compared to placebo, participants receiving CT and engaging in a high amount of exercise showed a decrease in BDRS scores at Week 20 indicating an improvement in symptoms across the trial. For every 10% increase in participants' total MET score, BDRS scores decreased by 0.09 (95% CI: -1.8,

-0.1) units. There were no significant two-way interactions between treatment received and log-transformed total physical activity.

Non-specified predictors analysis

Results of the non-specified predictors of outcomes analysis can be found in Table 4. Total physical activity was not significantly related to MADRS, or CGI-I outcomes. Total physical activity was a significant non-specified predictor of SOFAS, LIFE-RIFT and Q-LES-Q-SF scores at Week-20, irrespective of treatment received. For every 10% increase in participants' total met score, SOFAS scores increased by 0.06 (CI: 0.01, 1.31) units, LIFE-RIFT scores decreased by 0.02 (95% CI: -0.41, -0.08) units and Q-LES-Q-SF scores would increase by 0.09 (95% CI: 0.13, 1.80) units.

Categorical physical activity did not significantly predict Week-20 MADRS scores. Higher physical activity categories, according to the IPAQ-SF scoring protocol, was a non-specified predictor of SOFAS, LIFE-RIFT, CGI-I and Q-LES-Q-SF. Higher activity levels were more likely to be associated with slightly improved scores for these measures, regardless of treatment received. For every one-level increase in level of physical activity according to IPAQ-SF categorical scores (i.e. moderate to high), mean SOFAS scores at Week-20 increased by 2.27 (95% CI: 0.24, 4.30) units, mean LIFE-RIFT scores decreased by 0.67 (95% CI: -1.23, -0.11) units, mean Q-LES-Q-SF scores increased by 2.39 (95% CI: 0.03, 4.75) units, and mean CGI-I scores decreased by 0.16 (95% CI: -0.31, -0.01) units.

Physical activity according to WHO recommendations as not associated with Week-20 MADRS or CGI-I scores. Higher physical activity categories, according to WHO recommendations was a non-specified predictor of SOFAS, LIFE-RIFT, and Q-LES-Q-SF. Higher activity levels were more likely to be associated with slightly improved scores for these measures, regardless of treatment received. For every one-level increase in level of physical

activity according to WHO recommendations (i.e. from below to within recommendations), mean SOFAS scores at Week 20 increased by 1.80 (95% CI: 0.35, 3.25) units, mean LIFE-RIFT scores decreased by 0.71 (95% CI: -1.09, -0.34) units and mean Q-LES-Q-SF scores increased by 2.25 (95% CI: 0.45, 4.04) units.

[insert Table 4 approximately here]

Discussion

The aim of this sub-analysis of a nutraceutical RCT was to assess the relationships between physical activity, treatment received, and changes from baseline to Week-20 in outcomes measures for individuals with BD. Results suggest that there may be an association between physical activity and some of the depression and functioning outcomes of the study, but this was not consistent for all outcome measures.

In regard to depression symptoms, physical activity was unrelated to change across the study from baseline to Week-20 on the primary outcome measure, the MADRS. However, for participants receiving CT, total physical activity significantly predicted changes in bipolar depression symptoms (measured by the BDRS). There was a robust relationship between participants receiving CT who exceeded WHO recommendations for physical activity. These participants showed a greater reduction in the BDRS depression symptoms, compared to participants receiving placebo at a similar level of physical activity, in a dose dependent manner; however, the differences between the groups was minimal. In contrast, participants who received NAC and engaged in higher levels of physical activity demonstrated a worsening of their BD symptoms, but this was not consistent across all measures. After some types of strenuous, high intensity or endurance physical activity, there is evidence of a short-term acute inflammatory response in some people[39-42] that adapts over time. Inflammation is a necessary part of muscular recovery from exercise and anti-inflammatory medication such as NAC may

be inhibiting this process[43; 44]. There may be a delicate balance between anti-inflammatory use and benefits of exercise, potentially leading to the need for targeted and timed anti-inflammatory medication[44]. As use of NAC appears to demonstrate a worsening of BD symptoms for those in a high category of the IPAQ compared to placebo, this may be a demonstration of a disruption to this delicate balance, and warrants further investigation. As the CT group demonstrates improvement on this same depression scale, there is potentially an element within the CT which is protective and counter-acting the negative effects of NAC. However, due to the exploratory nature of this sub-analysis, and the low number of participants, cautious interpretation is required.

It is possible that the combination of mitochondrial enhancing physical activity and the mitochondrial enhancing CT, may be an important interaction for improving bipolar depression symptoms. This is in keeping with the hypothesis that bipolar disorder is at its heart a mitochondrial disorder; manifested by decreased biogenesis in depression and excess energy generation in mania[21]. Previous research has also found a reduction of depression (unipolar and bipolar) with physical activity at higher levels[45]. The potential for physical activity in BD is profound, given its positive effects on neuroplasticity[46], hippocampal volume[47], increasing brain derived neurotrophic factor[48], mitochondrial activity and neurogenesis[23] potentially mediated by peroxisome proliferator-activated receptor-gamma coactivator (PGC)-1α[49]. These are all processes that are disturbed in BD, giving rise to the possibility that physical activity may improve symptoms of BD via improving mitochondrial dysfunction and neuroplasticity. The additional benefits of receiving CT and engaging in higher levels of physical activity may be achieved via synergistic effects on the pathway regulating mitochondrial energy generation, such as PGC-1α[49; 50].

There were no significant relationships between participants' physical activity, the treatment they received on the study and functional outcomes (LIFE-RIFT and SOFAS),

quality of life (Q-LES-Q-SF) or clinician-rated improvement (CGI-I). However, there were relationships between the physical activity predictors and outcome measures, irrespective of what treatment they received. Physical activity (including all variations on the scale) was a non-specified predictor of improvement in social and occupational functioning (SOFAS), psychopathology induced impairment of functioning (LIFE-RIFT) and quality of life (Q-LES-Q-SF) at Week 20. These results are in keeping with previous research suggesting improved outcomes for those who engage in more physical activity[51]. One interpretation could be a bidirectional relationship between functioning and physical activity. For instance, if a participant has adequate physical functioning levels then they may have a greater motivation or ability to engage in physical activity. However, as physical activity is only measured once, we cannot determine causality.

Strengths of this study include the design of the double-blind adjunctive RCT adjunctive, allowing for robust clinical trial data. Physical activity has been measured according to a validated scale with two possible outcome measures for interpretation (continuous weekly score and categorical weekly score)[33]. This scale takes a conservative approach in truncating and removing data for less skew. In addition, physical activity has been categorised according to WHO recommendations allowing for real-world, practical interpretations and has implications for public health messages.

Results of this study should be cautiously interpreted due to its limitations. In particular, the phasic nature of BD may interact with physical activity levels of participants. Given the scale was administered at Week 4, we cannot guarantee the phase of BD that participants were in is consistent across the sample. In addition, there is no measure of activity later in the study to assess change in participants' level of physical activity. The disparity of energy expended in different states in BD highlights the potential for a bipolar specific physical activity scale with population specific standards. In terms of the physical activity scale used (IPAQ-SF),

limitations exist due to the nature of self-report and can be prone to error and recall bias[52]. In addition, the IPAQ considers the intensity of physical activity but does not record the types of exercise participants have engaged in. To reduce recall bias and to be able to review types of exercise, actigraphy could be used in addition to physical activity questionnaires[52].

The nature of exploratory sub-analyses in general poses further limitations. The RCT was powered for the primary outcome, that is, change in depression for the active treatment groups, which means the sub-analysis is likely underpowered. Due to the small sample size of the data, there was insufficient power for a robust response to assess the categorical data measured from the IPAQ-SF scoring guide as nominal and as a factor within the model. Physical activity is measured only once and as a covariate that is not directly being intervened which limits interpretability of results. Lastly, the results presented in this sub-analysis are statistically significant, but they represent small changes in outcome and thus, small clinical significance. Future studies directly assessing the impact of physical activity programs should be powered to see greater changes in outcomes. Post hoc analyses always need to be interpreted with caution, as is the case for multiple comparisons.

Conclusion and future directions

Engaging participants to increase their activity may be a cost-effective way of improving treatment outcomes with additional health benefits for comorbid physical disorders This sub-analysis of an adjunctive nutraceutical RCT adds some further support to the association between physical activity and mental health, and in particular, BD. Physical activity measured at the beginning of this study was associated with functioning and quality of life at the end of the study. This sub-analysis suggests that measures of physical activity may be useful when analysing outcomes of a new treatment. Future research may clarify the potential

adjunctive effects of higher physical activity and mitochondrial enhancing therapies in treating bipolar depression symptoms, possibly through mitochondrial biogenesis.

Abbreviations

BD	Bipolar Disorder
BDRS	Bipolar Depression Rating Scale
CGI-BP	Clinical Global Improvement for Bipolar Disorder
CGI-I	Clinical Global Impression Improvement
CT	Combination Treatment
GEE	Generalised estimating equations
HREC	Human Research and Ethics Committee
IPAQ-SF	International Physical Activity Questionnaire - Short Form
LIFE-RIFT	Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning Tool
MADRS	Montgomery Åsberg Depression Rating Scale
NAC	*N*-acetylcysteine
Q-LES-Q-SF	Quality of life Enjoyment and Satisfaction Questionnaire – Short Form
RCT	Randomised controlled trial
SOFAS	Social and Occupational Functioning Scale
WHO	World Health Organisation

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Declaration of conflicting interests

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References

1. Piercy KL, Troiano RP, Ballard RM, Carlson SA, Fulton JE, Galuska DA, George SM, Olson RD. 2018. The physical activity guidelines for americansphysical activity guidelines for americansphysical activity guidelines for americans. JAMA. 320(19):2020-2028.

2. Firth J, Cotter J, Elliott R, French P, Yung AR. 2015. A systematic review and meta-analysis of exercise interventions in schizophrenia patients. Psychol Med. 45(7):1343-1361.

3. Schuch FB, Vancampfort D, Richards J, Rosenbaum S, Ward PB, Stubbs B. 2016. Exercise as a treatment for depression: A meta-analysis adjusting for publication bias. J Psychiatr Res. 77:42-51.

4. Stubbs B, Vancampfort D, Hallgren M, Firth J, Veronese N, Solmi M, Brand S, Cordes J, Malchow B, Gerber M et al. 2018. Epa guidance on physical activity as a treatment for severe mental illness: A meta-review of the evidence and position statement from the european psychiatric association (epa), supported by the international organization of physical therapists in mental health (ioptmh). European psychiatry : the journal of the Association of European Psychiatrists. 54:124-144.

5. Bauer IE, Galvez JF, Hamilton JE, Balanza-Martinez V, Zunta-Soares GB, Soares JC, Meyer TD. 2016. Lifestyle interventions targeting dietary habits and exercise in bipolar disorder: A systematic review. J Psychiatr Res. 74:1-7.

6. Thomson D, Turner A, Lauder S, Gigler ME, Berk L, Singh AB, Pasco JA, Berk M, Sylvia L. 2015. A brief review of exercise, bipolar disorder, and mechanistic pathways. Frontiers Media.

7. Ng F, Dodd S, Berk M. 2007. The effects of physical activity in the acute treatment of bipolar disorder: A pilot study. J Affect Disord. 101(1-3):259-262.

8. Sylvia LG, Nierenberg AA, Stange JP, Peckham AD, Deckersbach T. 2011. Development of an integrated psychosocial treatment to address the medical burden associated with bipolar disorder. J Psychiatr Pract. 17(3):224-232.

9. Sylvia LG, Salcedo S, Bernstein EE, Baek JH, Nierenberg AA, Deckersbach T. 2013. Nutrition, exercise, and wellness treatment in bipolar disorder: Proof of concept for a consolidated intervention. International journal of bipolar disorders. 1(1):24.

10. World Health Organization. 2010. Global recommendations on physical activity for health. Geneva, Switzerland: World Health Organization.

11. Vancampfort D, Firth J, Schuch FB, Rosenbaum S, Mugisha J, Hallgren M, Probst M, Ward PB, Gaughran F, De Hert M et al. 2017. Sedentary behavior and physical activity levels in people with schizophrenia, bipolar disorder and major depressive disorder: A global systematic review and meta-analysis. World Psychiatry. 16(3):308-315.

12. Vancampfort D, Stubbs B, Sienaert P, Wyckaert S, De Hert M, Soundy A, Probst M. 2016. A comparison of physical fitness in patients with bipolar disorder, schizophrenia and healthy controls. Disability and Rehabilitation. 38(20):2047-2051.

13. Vancampfort D, Hagemann N, Wyckaert S, Rosenbaum S, Stubbs B, Firth J, Schuch FB, Probst M, Sienaert P. 2017. Higher cardio-respiratory fitness is associated with increased mental and physical quality of life in people with bipolar disorder: A controlled pilot study. Psychiatry Research. 256:219-224.

14. Fellendorf F, Kainzbauer N, Platzer M, Dalkner N, Bengesser S, Birner A, Queissner R, Rauch P, Hamm C, Pilz R. 2017. Gender differences in the association between physical activity and cognitive function in individuals with bipolar disorder. Journal of affective disorders. 221:232-237.

15. National Collaborating Centre for Mental Health. Bipolar disorder: The nice guideline on the assessment and management of bipolar disorder in adults, children and young people in primary and secondary care. 2018: British Psychological Society.

16. Daussin FN, Zoll J, Dufour SP, Ponsot E, Lonsdorfer-Wolf E, Doutreleau S, Mettauer B, Piquard F, Geny B, Richard R. 2008. Effect of interval versus continuous training on cardiorespiratory and mitochondrial functions: Relationship to aerobic performance improvements in sedentary subjects. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 295(1):R264-R272.

17. Fritzen AM, Thøgersen FB, Thybo K, Vissing CR, Krag TO, Ruiz-Ruiz C, Risom L, Wibrand F, Høeg LD, Kiens B. 2019. Adaptations in mitochondrial enzymatic activity occurs independent of genomic dosage in response to aerobic exercise training and deconditioning in human skeletal muscle. Cells. 8(3):237.

18. Taivassalo T, Shoubridge EA, Chen J, Kennaway NG, DiMauro S, Arnold DL, Haller RG. 2001. Aerobic conditioning in patients with mitochondrial myopathies: Physiological, biochemical, and genetic effects. Annals of neurology. 50(2):133-141.

19. Pesta D, Hoppel F, Macek C, Messner H, Faulhaber M, Kobel C, Parson W, Burtscher M, Schocke M, Gnaiger E. 2011. Similar qualitative and quantitative changes of mitochondrial respiration following strength and endurance training in normoxia and hypoxia in sedentary humans. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 301(4):R1078-R1087.

20. Porter C, Reidy PT, Bhattarai N, Sidossis LS, Rasmussen BB. 2015. Resistance exercise training alters mitochondrial function in human skeletal muscle. Medicine and science in sports and exercise. 47(9):1922.

21. Morris G, Walder K, McGee SL, Dean OM, Tye SJ, Maes M, Berk M. 2017. A model of the mitochondrial basis of bipolar disorder. Neuroscience and biobehavioral reviews. 74(Pt A):1-20.

22. Dean OM, Turner A, Malhi GS, Ng C, Cotton SM, Dodd S, Sarris J, Samuni Y, Tanious M, Dowling N et al. 2015. Design and rationale of a 16-week adjunctive randomized placebo-controlled trial of mitochondrial agents for the treatment of bipolar depression. Revista brasileira de psiquiatria (Sao Paulo, Brazil : 1999). 37(1):3-12.

23. Sylvia LG, Ametrano RM, Nierenberg AA. 2010. Exercise treatment for bipolar disorder: Potential mechanisms of action mediated through increased neurogenesis and decreased allostatic load. Psychother Psychosom. 79(2):87-96.

24. Food Drug Administration (FDA). 1997. International conference on harmonisation: Guidance on statistical principles for clinical trials (ich-e9). Federal Register. 62:25692-25709.

25. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. 1998. The mini-international neuropsychiatric interview (m.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for dsm-iv and icd-10. J Clin Psychiatry. 59 Suppl 20:22-33;quiz 34-57.

26. Montgomery S, Asberg M. 1979. A new depression scale designed to be sensitive to change. The British journal of psychiatry : the journal of mental science. 134:382-389.

27. Berk M, Turner A, Malhi GS, Ng CH, Cotton SM, Dodd S, Samuni Y, Tanious M, McAulay C, Dowling N et al. 2019. A randomised controlled trial of a mitochondrial therapeutic target for bipolar depression: Mitochondrial agents, n-acetylcysteine, and placebo. BMC Med. 17(1):18.

28. Berk M, Dodd S, Dean OM, Kohlmann K, Berk L, Malhi GS. 2010. The validity and internal structure of the bipolar depression rating scale: Data from a clinical trial of n-

acetylcysteine as adjunctive therapy in bipolar disorder. Acta Neuropsychiatr. 22(5):237-242.

29. Morosini PL, Magliano L, Brambilla L, Ugolini S, Pioli R. 2000. Development, reliability and acceptability of a new version of the dsm-iv social and occupational functioning assessment scale (sofas) to assess routine social functioning. Acta Psychiatr Scand. 101(4):323-329.

30. Leon AC, Solomon DA, Mueller TI, Turvey CL, Endicott J, Keller MB. 1999. The range of impaired functioning tool (life-rift): A brief measure of functional impairment. Psychol Med. 29(4):869-878.

31. Spearing MK PR, Leverich GS, Brandt D, Nolen W. 1997. Modification of the clinical global impressions (cgi) scale for use in bipolar illness (bp): The cgi-bp. Psychiatry Res. 73:159-171.

32. Endicott J, Nee J, Harrison W, Blumenthal R. 1993. Quality of life enjoyment and satisfaction questionnaire: A new measure. Psychopharmacol Bull. 29(2):321-326.

33. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF et al. 2003. International physical activity questionnaire: 12-country reliability and validity. Medicine and science in sports and exercise. 35(8):1381-1395.

34. Soundy A, Roskell C, Stubbs B, Vancampfort D. 2014. Selection, use and psychometric properties of physical activity measures to assess individuals with severe mental illness: A narrative synthesis. Arch Psychiatr Nurs. 28(2):135-151.

35. Baron RM, Kenny DA. 1986. The moderator-mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. Journal of personality and social psychology. 51(6):1173-1182.

36. Kraemer HC, Stice E, Kazdin A, Offord D, Kupfer D. 2001. How do risk factors work together? Mediators, moderators, and independent, overlapping, and proxy risk factors. The American journal of psychiatry. 158(6):848-856.

37. White H. 1980. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica. 48(4):817-838.

38. IBM Corp. 2017. Ibm spss statistics for windows, version 25.0. Armonk, NY: IBM Corp.

39. Lira FS, Dos Santos T, Caldeira RS, Inoue DS, Panissa VLG, Cabral-Santos C, Campos EZ, Rodrigues B, Monteiro PA. 2017. Short-term high- and moderate-intensity training modifies inflammatory and metabolic factors in response to acute exercise. Frontiers in physiology. 8:856-856.

40. Monteiro PA, Campos EZ, de Oliveira FP, Peres FP, Rosa-Neto JC, Pimentel GD, Lira FS. 2017. Modulation of inflammatory response arising from high-intensity intermittent and concurrent strength training in physically active males. Cytokine. 91:104-109.

41. Kaspar F, Jelinek HF, Perkins S, Al-Aubaidy HA, deJong B, Butkowski E. 2016. Acute-phase inflammatory response to single-bout hiit and endurance training: A comparative study. Mediators of inflammation. 2016:5474837-5474837.

42. Zwetsloot KA, John CS, Lawrence MM, Battista RA, Shanely RA. 2014. High-intensity interval training induces a modest systemic inflammatory response in active, young men. Journal of inflammation research. 7:9-17.

43. Peake JM, Neubauer O, Della Gatta PA, Nosaka K. 2017. Muscle damage and inflammation during recovery from exercise. Journal of applied physiology (Bethesda, Md : 1985). 122(3):559-570.

44. Chazaud B. 2016. Inflammation during skeletal muscle regeneration and tissue remodeling: Application to exercise-induced muscle damage management. Immunology and cell biology. 94(2):140-145.

45. Schuch FB, Vancampfort D, Firth J, Rosenbaum S, Ward PB, Silva ES, Hallgren M, Ponce De Leon A, Dunn AL, Deslandes AC et al. 2018. Physical activity and incident depression: A meta-analysis of prospective cohort studies. The American journal of psychiatry. 175(7):631-648.

46. Phillips C. 2017. Physical activity modulates common neuroplasticity substrates in major depressive and bipolar disorder. Neural Plasticity. 2017:7014146-7014146.

47. Firth J, Stubbs B, Vancampfort D, Schuch F, Lagopoulos J, Rosenbaum S, Ward PB. 2018. Effect of aerobic exercise on hippocampal volume in humans: A systematic review and meta-analysis. Neuroimage. 166:230-238.

48. Kerling A, Kuck M, Tegtbur U, Grams L, Weber-Spickschen S, Hanke A, Stubbs B, Kahl KG. 2017. Exercise increases serum brain-derived neurotrophic factor in patients with major depressive disorder. J Affect Disord. 215:152-155.

49. Granata C, Jamnick NA, Bishop DJ. 2018. Principles of exercise prescription, and how they influence exercise-induced changes of transcription factors and other regulators of mitochondrial biogenesis. Sports Medicine. 48(7):1541-1559.

50. Nierenberg AA, Ghaznavi SA, Sande Mathias I, Ellard KK, Janos JA, Sylvia LG. 2018. Peroxisome proliferator-activated receptor gamma coactivator-1 alpha as a novel target for bipolar disorder and other neuropsychiatric disorders. Biol Psychiatry. 83(9):761-769.

51. Sylvia LG, Friedman ES, Kocsis JH, Bernstein EE, Brody BD, Kinrys G, Kemp DE, Shelton RC, McElroy SL, Bobo WV et al. 2013. Association of exercise with quality of life and mood symptoms in a comparative effectiveness study of bipolar disorder. Journal of Affective Disorders. 151(2):722-727.

52. Paul DR, McGrath R, Vella CA, Kramer M, Baer DJ, Moshfegh AJ. 2018. Understanding the nature of measurement error when estimating energy expenditure and physical

activity via physical activity recall. Journal of physical activity & health. 15(7):543-549.

Table 1: Study participants characteristics

		Placebo (n = 49)	*NAC (n = 50)*	*CT (n = 46)*	*Total (n=145)*	*Range*	*Median cut-point*
Male gender	*n* (%)	17 (34.7)	18 (36.0)	16 (34.8)	51 (35.2)		
Age	*m* (*SD*)	45.88 (11.9)	45.0 (12.1)	47.7 (13.3)	46.1 (12.4)	21.3-72.0	
BMI	*m* (*SD*)	30.3 (7.9), *n* = 47	27.9 (6.3)	28.2 (6.8)	28.8 (7.0), *n* = 143	16.82-52.8	
Total weekly physical activity (MET- Minutes)	*m* (*SD*)	2024.4 (2477.8)	1766.3 (2433.2)	1603.1 (1718.9)	1801.8 (2239.3)	0-11118.0	990.0
IPAQ Categorical							
Low	*n* (%)	22 (44.9)	27 (54.0)	23 (50.0)	72 (49.7)		
Moderate	*n* (%)	13 (26.5)	11 (22.0)	14 (30.4)	38 (26.2)		
High	*n* (%)	14 (28.6)	12 (24.0)	9 (19.6)	35 (24.1)		
WHO recommendations							
No physical activity	*n* (%)	7 (14.3)	4 (8.0)	5 (10.9)	16 (11.0)		
Below WHO recommendations	*n* (%)	9 (18.4)	17 (34.0)	14 (30.4)	40 (27.6)		
Within WHO recommendations	*n* (%)	9 (18.4)	10 (20.0)	4 (8.7)	23 (15.9)		
Above WHO recommendations	*n* (%)	24 (49.0)	19 (38.0)	23 (50.0)	66 (45.5)		
Minutes spent sitting per weekday.	*m* (*SD*)	421.9 (236.6)	427.6 (251.6)	441.8 (239.9), *n* = 44	430.0 (241.4), *n* = 143	21-1260	360.0

*p<0.05; **p<0.01

Abbreviations: BMI: Body Mass Index; CT: Combination Treatment; IPAQ: International Physical Activity Questionnaire; NAC: N-acetylcysteine.

Table 2: IPAQ scores categorised into low, moderate and high, according to IPAQ-SF guidelines as a predictor of mean change scores for each treatment arm.

	Placebo	*CT*	*NAC*	*Placebo*	*CT*	*NAC*	*Placebo*	*CT*	*NAC*	*CT Interaction*[a] *β Coefficient (95% CI)*	*Interaction test p-value*	*NAC Interaction*[a] *β Coefficient (95% CI)*	*Interaction test p-value*
MET-Categorical	*Low*			*Moderate*			*High*						
MADRS change													
Mean (SD)	-12 (10.1)	-14.1 (9.5)	-15.1 (10.8)	-4.9 (11.2)	-18.7 (7.6)	-15.4 (10.8)	-14.8 (12.2)	-22.5 (3.5)	-10.6 (8.1)	-0.02	$\chi^2(1)<0.01$	2.8	$\chi^2(1)=3.8$
n	20	18	19	10	11	7	10	2	9	(-2.9, 2.8)	$p=0.987$	(-0.01, 5.6)	$p=0.051$
BDRS change mean *(SD)*	-9.5 (9.8)	-10.2 (10.3)	-13.4 (9.7)	-2.1 (12.4)	-17.1 (7.9)	-13.8 (9.3)	-11.7 (9.3)	-21.0 (2.8)	-9.2 (7.2)	0.2	$\chi^2(1)=0.01$	2.9	$\chi^2(1)=3.9$
n	19	16	19	8	11	6	10	2	9	(-2.7, 3.0)	$p=0.917$	(0.03, 5.7)	**$p=0.047$**
SOFAS change mean *(SD)*	12.7 (12.9)	17.3 (12.7)	13.3 (10.6)	2.6 (12.7)	17.5 (14.5)	14.5 (14.5)	14.7 (12.0)	12.5 (7.8)	13.0 (11.2)	-0.7	$\chi^2(1)=0.1$	-0.2	$\chi^2(1)=0.01$
n	19	18	18	9	11	6	10	2	9	(-4.8, 3.4)	$p=0.736$	(-3.8, 3.5)	$p=0.932$
LIFE-RIFT change mean *(SD)*	-2.3 (4.4)	-3.7 (5.0)	-3.7 (3.7)	-0.8 (3.6)	-5.6 (3.8)	-4.7 (2.5)	-3.7 (4.2)	-4.0 (1.4)	-3.6 (2.0)	-0.1	$\chi^2(1)=0.01$	0.5	$\chi^2(1)=0.7$
n	19	16	19	8	11	6	10	2	9	(-1.5, 1.3)	$p=0.909$	(-0.7, 1.8)	$p=0.417$
Q-LES-Q change Mean (SD)	12.7 (23.4)	16.7 (18.0)	18.1 (18.1)	<0.01 (15.0)	23.1 (19.8)	17.1 (10.0)	21.8 (18.0)	26.8 (12.6)	15.9 (23.7)	-1.8	$\chi^2(1)=0.5$	-5.2	$\chi^2(1)=3.1$
n	19	18	19	9	11	7	9	2	9	(-6.7, 3.2)	$p=0.486$	(-11.0, 0.6)	$p=0.077$
CGI-I week 20 [b]										0.2	$\chi^2(1)=0.4$	0.05	$\chi^2(1)=0.03$
n	20	18	19	9	11	7	10	2	9	(-0.4, 0.8)	$p=0.546$	(-0.5, 0.6)	$p=0.864$

[a]three-way interaction between potential predictor, time and treatment group, reference group was placebo

[c] *As CGI-I is not administered at baseline, mean score change has not been measured. High and low levels of each predictor were determined by median split*

Abbreviations: BDRS: Bipolar Depression Rating Scale; CGI-I: Clinical Global Impression Improvement; CT: Combination Treatment; LIFE-RIFT: Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning Tool; MADRS: Montgomery Åsberg Depression Rating Scale; NAC: N-acetylcysteine; SOFAS: Social and Occupational Functioning Scale.

Table 3: Physical activity according to WHO recommendations as a predictor of mean change scores for each treatment arm.

	Placebo	*CT*	*NAC*	*Placebo*	*CT*	*NAC*	*Placebo*	*CT*	*NAC*	*Placebo*	*CT*	*NAC*	*Overall three-way interaction test P-value*	*Interaction[a] β Coefficient*	*Interaction test*	*Interaction[a] β Coefficient*	*Interaction test*
MET - WHO	*No physical activity*			*Below WHO recommendations*			*Within Who recommendations*			*Exceeding WHO recommendations*				*(95% CI)*	*p-value*	*(95% CI)*	*p-value*
MADRS change *Mean (SD)*	-11.3 (14.5)	-9.0 (13.9)	-15.0 (7.2)	-14.1 (5.0)	-14.8 (9.1)	-14.9 (10.1)	-3.0 (11.2)	-15.3 (7.1)	-18.0 (13.7)	-13.0 (11.1)	-19.9 (7.0)	-10.4 (8.5)	χ^2 (2) = 3.3	-0.8	χ^2 (1) = 0.6	1.1	χ^2 (1) = 1.0
n	7	3	4	8	12	12	8	4	7	17	12	12	p = 0.19	(-2.8, 1.2)	p = 0.440	(-1.1, 3.2)	p = 0.319
BDRS change mean *(SD)*	-14.1 (11.0)	<0.01 (14.1)	-14.5 (11.1)	-12.1 (7.5)	-9.9 (9.8)	-12.9 (9.3)	-0.6 (11.1)	-16.8 (5.1)	-15.0 (11.1)	-8.5 (10.2)	-18.1 (7.5)	-9.8 (10.5)	χ^2 (2) = 6.2	-2.2	χ^2 (1) = 4.8	-0.1	χ^2 (1) < 0.01
n	7	2	4	7	11	12	7	4	6	16	12	12	**p = 0.046**	(-4.1, -0.2)	**p = 0.028**	(-2.2, 2.0)	p = 0.951
SOFAS change mean *(SD)*	15.3 (16.0)	7.0 (15.9)	19.8 (10.1)	13.9 (11.3)	18.0 (11.3)	12.9 (8.9)	1.6 (11.3)	16.0 (15.4)	9.7 (16.8)	12.1 (12.4)	18.9 (13.4)	13.7 (10.5)	χ^2 (2) = 1.4	1.7	χ^2 (1) = 1.4	0.8	χ^2 (1) = 0.4
n	7	3	4	7	12	11	8	4	6	16	12	12	p = 0.504	(-1.1, 4.5)	p = 0.243	(-1.8, 3.4)	p = 0.546
LIFE-RIFT change mean *(SD)*	-3.0 (4.5)	<0.01 (7.1)	-3.5 (5.1)	-3.7 (3.5)	-3.7 (4.2)	-3.4 (4.0)	-0.4 (4.7)	-5.0 (4.1)	-4.7 (2.0)	-2.3 (4.2)	-5.7 (4.2)	3.9 (4.0)	χ^2 (2) = 0.8	-0.4	χ^2 (1) = 0.8	-0.1	χ^2 (1) = 0.03
n	7	2	4	7	11	12	7	4	6	16	12	12	p = 0.666	(-1.3, 0.5)	p = 0.382	(-1.0, 0.8)	p = 0.866
Q-LES-Q change Mean (SD)	13.8 (22.2)	10.1 (14.5)	24.6 (11.4)	19.4 (23.7)	18.8 (16.2)	16.4 (18.9)	-3.1 (16.0)	17.0 (11.9)	13.8 (15.8)	14.2 (20.9)	23.7 (22.8)	17.9 (21.1)	χ^2 (2) = 1.3	1.3	χ^2 (1) = 0.5	-1.0	χ^2 (1) = 0.2
n	7	3	4	7	12	12	7	4	7	16	12	12	p = 0.512	(-2.5, 5.1)	p = 0.496	(-5.1, 3.1)	p = 0.640
CGI-I week 20 [b]													χ^2 (2) = 0.8	-0.2	χ^2 (1) = 0.7	<0.01	χ^2 (1) <0.01
n	7	3	4	8	12	12	8	4	7	16	12	*12*	p = 0.67	(-0.6, 0.2)	p = 0.402	(-0.4, 0.4)	p = 0.999

[a]three-way interaction between potential predictor, time and treatment group, reference group was placebo

[b] *As CGI-I is not administered at baseline, mean score change has not been measured. High and low levels of each predictor were determined by median split*

Abbreviations: BDRS: Bipolar Depression Rating Scale; CGI-I: Clinical Global Impression Improvement; CT: Combination Treatment; LIFE-RIFT: Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning Tool; MADRS: Montgomery Åsberg Depression Rating Scale; NAC: N-acetylcysteine; SOFAS: Social and Occupational Functioning Scale.

Table 4: Total weekly physical activity, IPAQ categorical scores and physical activity categorised by WHO recommendations as non-specified predictors of outcomes

Predictor	*β Coefficient (95% CI)*	*Main effect*
Total weekly physical activity		
MADRS	-0.2 (-0.6, 0.3)	χ^2 (1) = 0.6 p = 0.458
BDRS	-0.1 (-0.5, 0.2)	χ^2 (1) = 0.5 p = 0.498
SOFAS	0.7 (0.01, 1.3)	χ^2 (1) = 4.0 **p = 0.046**
LIFE-RIFT	-0.2 (-0.4, -0.1)	χ^2 (1) = 8.5 **p = 0.004**
Q-LES-Q	1.0 (0.1, 1.8)	χ^2 (1) = 5.2 **p = 0.023**
CGI-I	-0.01 (-0.1, 0.04)	χ^2 (1) = 0.1 p = 0.709
IPAQ scores in Categorical		
MADRS	-1.1 (-2.2, 0.03)	χ^2 (1) = 3.7 p = 0.056
BDRS	-0.7 (-1.9, 0.4)	χ^2 (1) = 1.5 p = 0.218
SOFAS	2.3 (0.2, 4.3)	χ^2 (1) = 4.8 **p = 0.028**
LIFE-RIFT	-0.7 (-1.2, -0.1)	χ^2 (1) = 5.6 **p = 0.018**
Q-LES-Q	2.4 (0.03, 4.8)	χ^2 (1) = 3.9 **p = 0.047**
CGI-I	-0.2 (-0.3, -0.01)	χ^2 (1) = 4.4 **p = 0.036**
Physical activity categorised by WHO recommendations		
MADRS	-0.6 (-1.5, 0.3)	χ^2 (1) = 1.5 p = 0.219
BDRS	-0.4 (-1.3, 0.4)	χ^2 (1) = 1.0 p = 0.311
SOFAS	1.8 (0.3, 3.3)	χ^2 (1) = 5.9 **p = 0.015**
LIFE-RIFT	-0.7 (-1.1, -0.3)	χ^2 (1) = 13.7 **p < 0.001**
Q-LES-Q	2.2 (0.5, 4.0)	χ^2 (1) = 6.0 **p = 0.014**
CGI-I	-0.1 (-0.2, 0.1)	χ^2 (1) = 1.1 p = 0.286

Abbreviations: BDRS: Bipolar Depression Rating Scale; CGI-I: Clinical Global Impression Improvement; CT: Combination Treatment; LIFE-RIFT: Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning Tool; MADRS: Montgomery Åsberg Depression Rating Scale; NAC: N-acetylcysteine; SOFAS: Social and Occupational Functioning Scale.

Supplementary Material

For manuscript: *Physical activity as a predictor of clinical trial outcomes in bipolar depression: a sub-analysis of a mitochondrial-enhancing nutraceutical randomised controlled trial*

Index

Supplementary Table 1: MITO-NAC study outcome measures scores

Scale	**Range**	**Direction of improvement**
Montgomery Åsberg Depression Rating Scale (MADRS)	0-60	Lower scores = less symptoms of depression
Bipolar Depression Rating Scale (BDRS)	0-60	Lower scores = less symptoms of depression
Social and Occupational Functioning Scale (SOFAS)	1-100	Higher scores = higher levels of functioning
Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning (LIFE-RIFT)	4-20	Lower scores = less impairment of functioning
Quality of life Enjoyment and Satisfaction Questionnaire – Short Form (Q-LES-Q-SF)	1-100	Higher scores = higher life enjoyment and satisfaction
Clinical Global Impression Improvement (CGI-I)	1-7	Lower scores = greater general improvement

Supplementary Table 2: mean change of outcomes from baseline to Week 20 visit for each treatment arm.

	Placebo				*CT*				*NAC*		
Outcome	*n*	*Mean (SD)*	*Range*	*Median*	*n*	*Mean (SD)*	*Range*	*Median*	*n*	*Mean (SD)*	*Range*	*Median*
MADRS	40	-10.9 (11.3)	-30.0, 17.0	-10.5	31	-16.3 (8.8)	-31.0, 7.0	-18.0	35	-14.0 (10.1)	-35.0, 11.0	-13.0
BDRS	37	-8.5 (10.6)	-28.0, 19.0	-9.0	29	-13.6 (9.7)	-28.0, 10.0	-15.0	34	-12.4 (9.0)	-27.0, 7.0	-12.0
SOFAS	38	10.8 (13.2)	-17.0, 45.0	9.0	31	17.0 (12.8)	-10.0, 46.0	15.0	33	13.4 (11.1)	-15.0, 37.0	12.0
LIFE-RIFT	37	-2.4 (4.2)	-11.0, 7.0	-2.0	29	-4.5 (4.4)	-12.0, 5.0	-4.0	34	-3.8 (3.7)	-11.0, 3.0	-3.5
Q-LES-Q	37	11.8 (21.4)	-28.6, 66.1	10.7	31	19.6 (18.2)	-21.4, 67.9	17.9	35	17.3 (18.0)	-25.0, 58.9	14.3

Percentage change was calculated as week 20 score-baseline score divided by week 20 scores.
NB: As CGI-I is not administered at baseline, calculating percentage change was not possible.
Abbreviations: BDRS: Bipolar Depression Rating Scale; CGI-I: Clinical Global Impression Improvement; CT: Combination Treatment; IPAQ: International Physical Activity Questionnaire; LIFE-RIFT: Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning Tool; MADRS: Montgomery Åsberg Depression Rating Scale; NAC: N-acetylcysteine; SOFAS: Social and Occupational Functioning Scale.

Supplementary Table 3: Table of mean Week 20 CGI-I scores for each treatment arm.

Predictor	*Placebo*		*CT*		*NAC*	
	m	*SD*	*m*	*SD*	*m*	*SD*
IPAQ Scores [a]						
Low	2.6	1.1	2.3	1.0	2.1	0.9
High	2.7	1.5	2.4	0.8	1.9	0.7
IPAQ Categorical						
Low	2.5	1.0	2.3	1.1	2.1	1.0
Moderate	3.0	1.5	2.1	0.7	2.0	0.6
High	2.5	1.7	2.6	0.7	1.5	0.7
WHO recommendations						
No physical activity	2.4	1.3	2.3	0.5	2.7	2.1
Below WHO recommendations	2.1	0.8	2.3	1.0	2.0	0.6
Within WHO recommendations	3.6	1.2	2.3	1.3	1.8	0.5
Above WHO recommendations	2.4	1.4	2.4	0.8	2.0	0.7

[a] Median split has been utilised to visualise data only.

Abbreviations: *IPAQ: International Physical Activity Questionnaire*

Supplementary Material: Exploration of total physical activity score explored as a continuous variable.

Statistical Analysis

Due to the skewness of the data, total score of physical activity scores (measured in MET-minutes/week), were log-transformed prior to running any analysis. The model to predict log of physical activity included a fixed-effect treatment group as a factor, logarithm of time and logarithm of total physical activity score were entered as covariates, all two-way interactions and the three-way interactions were then included. After evaluating the three-way interaction between treatment group, logarithm of total physical and logarithm of time for each outcome measure, this interaction was removed to explore two-way interactions between treatment group and logarithm of total physical activity for each outcome measure. Due to the logarithmic transformation, beta coefficients were multiplied by a log of change and interpretations are given as percentages. For example, to determine the effect of 10% change in predictor will have on the outcome measure, beta is multiplied by log(1.1) to find the log transformed beta coefficient.

Results

Total physical activity scores converted into MET-minutes ranged from 0 to 11,118 MET-minutes/week, average 1801.75 MET-minutes/week (*SD* = 2239.29). Total physical activity data was later categorised as low or high according to a median split (median = 990, interquartile range = 264 to 2,376) for visual representation of the data only (see Supplementary Figure 1a).

Supplementary Table 4: Total weekly physical activity (continuous score) as a predictor of mean change scores for each treatment arm.

Predictor	*Placebo*	*CT*	*NAC*	*Placebo*	*CT*	*NAC*	*CT*		*NAC*	
Total weekly physical activity (MET- Minutes)[a]		*Low*			*High*		*Interaction*[b] *β Coefficient (95% CI)*	*Interaction test p-value*	*Interaction*[b] *β Coefficient (95% CI)*	*Interaction test p-value*
MADRS change										
Mean (SD)	-10.9 (10.5)	-13.2 (9.4)	-15.9 (10.6)	-11.0 (12.2)	-20.0 (6.6)	-10.4 (8.5)	-0.5	$\chi^2(1) = 1.0$	0.3	$\chi^2(1) = 0.3$
n	20	17	23	20	14	12	(-1.4, 0.5)	$p = 0.324$	(-0.7, 1.2)	$p = 0.571$
BDRS change										
mean *(SD)*	-10.4 (9.8)	-9.5 (10.4)	-13.8 (9.7)	-6.7 (11.3)	-17.7 (7.0)	-9.8 (7.2)	-1.0	$\chi^2(1) = 4.9$	0.03	$\chi^2(1) = 0.01$
n	18	15	22	19	14	12	(-1.8, -0.1)	$\mathbf{p = 0.028}$	(-0.9, 1.0)	$p = 0.946$
SOFAS change										
mean *(SD)*	11.0 (13.6)	14.8 (12.1)	13.3 (11.7)	10.7 (13.1)	19.8 (13.5)	13.7 (10.5)	0.8	$\chi^2(1) = 1.4$	-0.1	$\chi^2(1) = 0.02$
n	19	17	21	19	14	12	(-0.5, 2.1)	$p = 0.236$	(-1.1, 1.0)	$p = 0.903$
LIFE-RIFT change										
mean *(SD)*	-2.8 (4.3)	-3.1 (4.3)	-3.8 (3.6)	-2.0 (4.2)	-5.9 (4.2)	-3.9 (4.0)	-0.2	$\chi^2(1) = 0.6$	0.02	$\chi^2(1) = 0.01$
n	18	15	22	19	14	12	(-0.6, 0.3)	$p = 0.449$	(-0.3, 0.4)	$p = 0.920$
Q-LES-Q change										
Mean (SD)	13.8 (21.3)	16.4 (15.0)	17.0 (16.7)	10.0 (21.9)	23.5 (21.5)	17.9 (21.1)	0.8	$\chi^2(1) = 0.8$	-0.7	$\chi^2(1) = 0.7$
n	18	17	23	19	14	12	(-0.9, 2.4)	$p = 0.366$	(-2.3, 0.9)	$p = 0.389$
CGI-I Week 20 [c]										
n	20	17	23	19	14	12	-0.1 (-0.2, 0.1)	$\chi^2(1) = 0.6$ $p = 0.433$	-0.02 (-0.2, 0.1)	$\chi^2(1) = 0.04$ $p = 0.840$

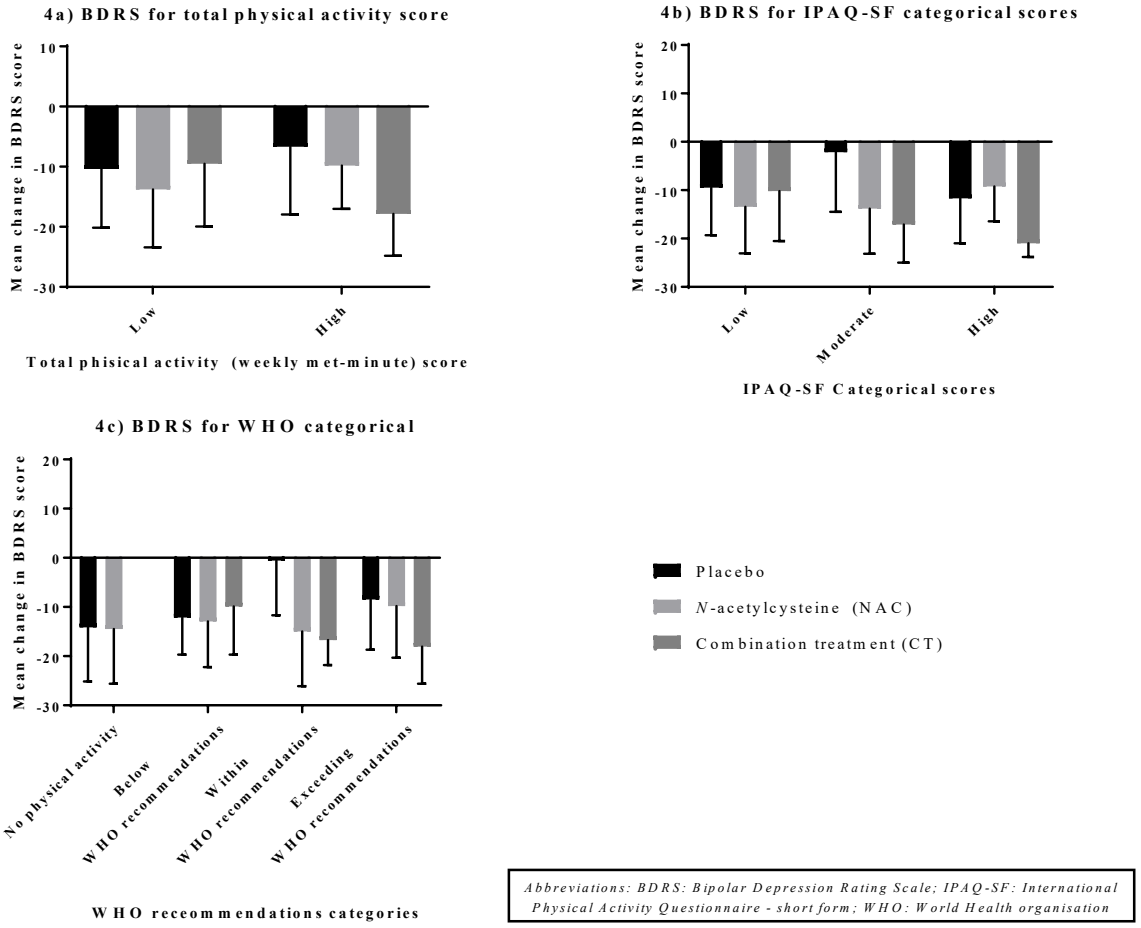
[a] Median split has been utilised to visualise data. Data has been analysed as a continuous score.

[b] Three-way interaction between potential predictor, time and treatment group, reference group was placebo

[c] *As CGI-I is not administered at baseline, mean score change has not been measured. High and low levels of each predictor were determined by median split*

Abbreviations: BDRS: Bipolar Depression Rating Scale; CGI-I: Clinical Global Impression Improvement; CT: Combination Treatment; LIFE-RIFT: Longitudinal Interval Follow-Up Evaluation - Range of Impaired Functioning Tool; MADRS: Montgomery Åsberg Depression Rating Scale; NAC: N-acetylcysteine; SOFAS: Social and Occupational Functioning Scale.

Supplementary Figure 1: Graphs of mean change in BDRS scores from baseline to Week 20 for each treatment arm and reported physical activity



Abbreviations: BDRS: Bipolar Depression Rating Scale; IPAQ-SF: International Physical Activity Questionnaire - short form; WHO: World Health organisation