Evaluating the effectiveness of innovative psychological intervention tools in optimizing health outcomes: A multimethod approach

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Optimizing health outcomes in response to immune-related and psychosocial challenges by an e-health psychological intervention: A randomized controlled trial

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Schakel L, Veldhuijzen DS, van Middendorp H, Prins C, Drittij AMHF, Vrieling F, Visser LG, Ottenhoff THM, Joosten SA, Evers AWM. An e-health psychological intervention to optimize health outcomes in response to immunological and psychosocial challenges: a randomized controlled trial

Abstract

Psychological interventions have shown promise in promoting health outcomes. Recently, internet-based cognitive behavioral therapy (e-health CBT) and serious gaming interventions have been suggested to enhance accessibility and engagement in such interventions. Few studies, however, have investigated their effectiveness in the context of simulated real-life challenges. We performed a randomized trial to examine the effectivity of an e-health CBT combined with serious gaming intervention in optimizing self-reported psychophysiological and immunological health outcomes in response to psychophysiological as well as *in vitro* and *in vivo* immune-related challenges. Sixty-nine healthy males were randomly assigned to the intervention condition, receiving e-health CBT combined with serious gaming for six weeks, or the control condition, receiving no intervention. Self-reported vitality and other self-reported, psychophysiological and immunological outcomes were assessed in response to various challenges including a BCG-vaccination evoking pro-inflammatory responses, one and four weeks after the intervention period. Although the intervention did not affect vitality associated parameters, self-reported sleep problems and bodily sensations were lower directly after the intervention compared to controls. Furthermore, well-being was higher in the intervention group after the psychophysiological challenges. Although no significant group differences were found for the psychophysiological and immunological outcomes, the data provided preliminary support for optimized outcomes on heart rate variables as well as increased IgG antibody responses at follow-up time-points. Differential chemokine outcomes were observed at the end of the test day in the intervention compared to the control condition. The present study provides some support for optimizing health outcomes with an e-health CBT combined with serious gaming intervention. Future research should replicate and further extend the present findings by consistently including challenges and a wide range of immune parameters into the study design.

Introduction

The effectiveness of psychological interventions in optimizing health outcomes has been studied extensively in the last few decades. Psychological interventions have shown to be effective in optimizing self-reported health outcomes (299, 300) and to improve immune status (200, 301, 302). For example, modest support for the effectiveness of psychological interventions in optimizing immune function was found in two meta-analytic reviews (2, 303). The large heterogeneity in the incorporated interventions (i.e., various types of relaxation, conditioning, disclosure and stress management interventions) and immunological outcomes (i.e., quantitative and qualitative immunological outcomes) contributed to the difficulty in providing a conclusive view on these findings. It is important to examine whether recent developments in psychological treatments may further enhance the effectiveness of psychological interventions in optimizing both self-reported health outcomes as well as immunological measures.

A rather novel development focuses on providing psychological interventions based on cognitive behavioral therapy (CBT) via the internet. A meta-analysis showed that the effectiveness of guided internet-based (i.e., e-health) CBT interventions is comparable with the effectiveness of face-to-face interventions in patients with chronic somatic conditions (10). Advantages of e-health interventions over face-to-face interventions are the increased convenience for users and enhanced flexibility of the specific location and time where the intervention sessions are completed (304). In view of the lower adherence rates in e-health interventions compared to face-to-face treatments, engagement should be taken into account (19, 305). Engagement can be enhanced by applying persuasive e-health technologies, such as serious gaming. Serious gaming is able to provide education in an entertaining manner and is therefore intrinsically motivating (25, 26). A meta-analysis provided evidence for the effectiveness of serious gaming in promoting a healthy lifestyle (28). Since behavior change strategies that can be targeted with serious gaming are not restricted to explicit behavior change strategies (e.g., goalsetting and transferring knowledge), but can also imply more implicit behavior change strategies (e.g., priming and evaluative conditioning), serious games are able to tap into multiple learning processes. Although further investigation is required, serious gaming could be added onto e-health interventions to optimize their effectiveness.

To gather more insights in the external validity of a psychological intervention, research should not only assess basal health outcomes, but should preferably also assess health outcomes in situations that challenge actual health status (303). Immunological and psychophysiological challenges that approximate stressful situations that people can face in everyday life provide insights into the effectiveness of psychological interventions in

handling daily life hassles. However, few studies so far have incorporated immunological and psychophysiological challenges in their study design. Immunological challenges may comprise *in vitro* exposure to a chemical substance (e.g., to lipopolysaccharide or to pokeweed mitogen (236, 245)), to obtain insights in the cellular responses after a psychological intervention. Furthermore, immunological challenges can also be applied *in vivo* to observe subsequent responses. For example, antibody responses can be measured upon vaccination (199), or the healing process of experimentally created wounds (306) can be monitored. Moreover, psychophysiological challenges can provide insights in participants' responses to stress after a psychological intervention (e.g., exposure to a social evaluative stressor). A recent systematic review focusing on studies that evaluated wound healing after a psychological intervention provided some support for the effectiveness of psychological interventions in optimizing immunological markers, including wound healing (174). However, due to the small number of studies performed and the large heterogeneity in psychological interventions, more research is needed. Moreover, most studies that incorporated challenges focused on incorporating one specific challenge and did not yet combine and compare effects on both *in vitro* and *in vivo* immunological as well as psychophysiological challenges (303).

The aim of this randomized controlled trial was to investigate whether an e-health CBT combined with serious gaming intervention can effectively optimize self-reported, psychophysiological and immunological health outcomes in response to *in vitro* and *in vivo* immunological as well as psychophysiological challenges (256, 303). Participants were randomized to either a 6-week e-health CBT combined with serious gaming intervention or a control condition, receiving no intervention. In the week following completion of the intervention or control condition, participants received a live BCG-vaccination, which is a controlled human infection, which has good safety records and is known to induce pro-inflammatory cytokine responses (256, 275). One day post-vaccination, psychophysiological challenges were performed (e.g., a social evaluative stressor). Furthermore, *in vitro* stimulation of whole blood with lipopolysaccharide (LPS) took place before and after BCG-vaccination. Vitality was included as a primary outcome, as this construct encompasses a dynamic reflection of physical as well as mental health and well-being (291). It was hypothesized that participants in the intervention condition would show higher self-reported vitality and related health outcomes after the intervention compared to the control condition. In addition, optimized self-reported, psychophysiological and immunological health outcomes after the *in vitro* and *in vivo* immunological as well as psychophysiological challenges were expected in the intervention condition compared to the control condition. Finally, basal self-reported, psychophysiological and immune outcome measures were explored at a four-week follow-up.

Methods

The study protocol was approved by the Medical Ethical Committee of Leiden University Medical Centre (registration number P15.099/NL52434.058.15) and preregistered at the Netherlands National Trial Register (NTR5610). The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation (ICH) Guidelines on Good Clinical Practice (GCP). Details on the study protocol and design have been published previously (256) and are described in short below.

Study population

Healthy male participants were recruited from February 2016 until April 2018. Participants were recruited through digital and printed flyers at various faculties of Dutch universities. Healthy males between 18 and 35 years of age without any somatic or psychological conditions interfering with the study protocol were eligible to participate in the study.

Procedure

The flowchart of the study has been published previously (256). Participants received an information letter prior to participation. After signing informed consent, participants completed self-reported and psychophysiological outcomes, and venous blood was collected. Participants who met the inclusion criteria were randomly assigned to the intervention or control condition. In the week following the 6-week intervention or control period (ranging from 1 to 7 days after completion of the intervention period), all participants again completed self-reported and psychophysiological outcomes, and blood was collected. Directly afterwards, participants were vaccinated with BCG. One day later, they were invited for a test day with psychophysiological stress challenges (i.e., PASAT, CPT, and TSST). At the start and end of the test day, self-reported and psychophysiological outcome measures were assessed, and blood was again collected. Four weeks later, participants received a follow-up measurement, including self-reported outcomes as well as psychophysiological outcome measures and collection of a blood sample. Total time investment was around 15 to 20 hours, including 4 visits to the study center and participants received €200 for their participation. See Appendix 2 for the details of the self-reported, psychophysiological and immune outcome measures on each measurement point.

Randomization and blinding

Participants were randomized to the intervention or control condition based on a 1:1 allocation ratio. The test leader on the test day was blinded for group allocation. A block randomization was performed with random.org (block size = 4) in order to control for seasonal influences (256).

Intervention

Participants in the intervention group received a guided e-health CBT intervention for 6 weeks (256). The intervention contained an adjusted version of the e-health CBT intervention for chronic somatic diseases developed in our research group (285, 307). The intervention was based on 6 modules (goal setting, healthy food and exercise, relaxation, sleep, cognitions and worldview, and long-term goals) that were guided by a therapist from whom participants received homework assignments and feedback messages. In addition, participants in the intervention condition played a serious game (ViaNova©), which incorporated comparable modules as the guided intervention (i.e., healthy food and exercise, sleep, relaxation, and long-term goals) as part of the e-health CBT. A subset of these games that focused specifically on food-related health behavior was tested in a previous study that demonstrated preliminary support for the effectiveness of a single serious gaming session in optimizing virtual food choice and implicit food preference (161). Two weeks after the intervention, participants received a booster session by telephone which focused on relapse prevention. The control condition did not receive any training.

Challenges

In vitro and in vivo immunological challenges

As an *in vitro* immunological challenge, heparinized whole blood samples were stimulated *in vitro* with lipopolysaccharide (LPS) at baseline (before the intervention), at the start of the vaccination day, and one day later at the start of the test day (256). One ml of sodium-heparinized blood (BD vacutainer) was stimulated with LPS (*E. Coli*, ultra-pure, Invivogen, Toulouse, France) at a final concentration of 100 ng/ml or as a control without LPS, and samples were incubated at 37°C for 6 hours. Tubes were spun at 3400 rpm for 10 minutes and plasma was collected and stored until testing at -80°C.

In addition, in the week following the intervention (or similar time frame for the control arm), all participants were vaccinated with *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG), a live-attenuated vaccine used against tuberculosis. This vaccine was incorporated as an *in vivo* challenge to the immune system. BCG (Intervax, via RIVM, Bilthoven, The Netherlands) was administered by intradermal injection (0.1 ml) in the upper arm.

Psychophysiological challenges

The day post-vaccination, participants were exposed to three psychophysiological challenges: a modified version of the Paced Auditory Serial Addition Task (PASAT) (288), the Cold Pressor Test (CPT) (308), and the Trier Social Stress Test (TSST) (51), in this order. All challenges are known to reliably induce psychophysiological stress responses (51, 154, 289, 308).

Outcome measures

Self-reported outcome measures

The Subjective Vitality Scale (SVS) (291) and Checklist Individual Strength (CIS-20) (292, 294) were used to measure self-reported vitality. The SVS consists of 7 items on a 7-point rating scale ranging from 1 (*not at all true*) to 7 (*very true*). The CIS-20 contains 20 items on a 7-point rating scale ranging from 1 (*yes, that is true*) to 7 (*no, that is not true*). The composite score of the SVS and CIS-20 was used as a primary outcome in this study. This composite score was determined by subtracting the standardized sum score of the CIS-20 from the standardized sum score of the SVS. Scores on the composite scale can be interpreted as higher scores representing higher self-reported vitality. The SVS and CIS-20 have shown to be reliable and valid in previous research (309, 310), and had a good internal reliability in the present study (Cronbach's alpha = .84 and .87, respectively).

In addition, the RAND-36 was used to assess physical and mental health-related quality of life by determining sum scores of the subscales physical functioning and emotional well-being (311), which has shown to be reliable and valid in previous literature (312). The physical functioning scale contains 10 items on a 3-point scale ranging from 1 (*yes, seriously limited*) to 3 (*no, not at all limited*), on which participants are asked to consider the past 4 weeks on both scales. The emotional well-being scale contains 5 items on a 6-point scale ranging from 1 (*constantly*) to 6 (*never*). Standardized T-scores were computed for both scales, with higher scores representing higher self-reported quality of life.

Bodily sensations were measured with the Pennebaker Inventory of Limbic Languidness (PILL) (313). The 54 items on this scale represents bodily sensations, including head ache, nausea and other types of sensations that are usually experienced as being annoying, on a 5-point scale ranging from 1 (*never or almost never*) to 5 (*more than once a week*). Participants are asked to consider the past 4 weeks, with higher scores representing a higher level of self-reported bodily sensations. The PILL shows a good internal reliability in the present study (Cronbach's alpha = .89).

Sleep problems were assessed with 9 items of the Medical Outcomes Study Sleep Scale (MOS Sleep) (314), which showed good internal reliability previously (314). One item ('How long did it usually take to fall asleep in the past 4 weeks') was presented on a 5-point scale from 1 (*0 – 15 minutes*) to 5 (*more than 60 minutes*). All other items were presented on a 6-point scale from 1 (*always*) to 6 (*never*), also considering the past 4 weeks. Higher scores on this scale represent lower levels of self-reported sleep problems. Although this questionnaire yielded sufficient internal reliability at follow-up (Cronbach's

alpha = .73), the internal reliability in the present study was low at baseline and after intervention (Cronbach's alpha = .45 and .36, respectively), and therefore the results on this scale in the present study should be interpreted with caution.

Well-being was assessed using the 20-item Positive and Negative Affect Schedule (PANAS) (158) and a 7-item Numeric Rating Scale (NRS) on well-being (315). The PANAS was subdivided into the positive affect scale and the negative affect scale, which both showed good reliability and validity in previous literature (316), as well as good reliability in the present study (Cronbach's alpha = .88 and .70, respectively). On the NRS that was used to measure well-being, scores ranged from 0 (*not at all*) to 10 (*very much*) and participants completed questions such as '*How stressed do you feel at this moment?*'. Higher scores on this questionnaire represent higher levels of self-reported well-being. The present incorporated NRS showed a good internal reliability in the present study (Cronbach's alpha = .80).

Psychophysiological outcome measures

Heart rate, heart rate variability and skin conductance were assessed with a BIOPAC MP150® system using Acknowledge software version 4.1.1. Recording of the electrocardiogram (ECG) signal was performed with an ECG100C module set at 1000Hz. The high pass filter was set at 0.05Hz and the low pass filter at 35Hz. For heart rate, electrodes were attached at the sternum and somewhat below the left lower rib. To measure skin conductance, Ag/Agcl electrodes were attached at the medial phalange of two fingers of the non-dominant hand, i.e., the middle and index finger. A GSR100C module was used to measure skin conductance, set at 1000Hz. Gain was set at 5µ℧/V and the low pass filter at 10Hz. The Physio Data Toolbox Version 0.4 was used for visual inspection of the data as well as for calculating the mean heart rate, heart rate variability and skin conductance levels for each time point (317).

In addition, saliva samples were collected to measure cortisol and alpha amylase. Samples were stored at -80°C until analyzed. Cortisol was assessed in saliva with a competitive electrochemiluminescence immunoassay using a Modular Analytics E602 immunoanalyzer (Roche Diagnostics, Mannheim, Germany). Cortisol activities are measured and expressed in nanomoles per liter (nmol/L). Determination of salivary alpha amylase was performed using a kinetic colorimetric assay for total amylase activity (Cat Nr. 03183742, Roche Diagnostics, Mannheim, Germany) on a routine clinical chemistry analyzer. Amylase activity is measured and expressed in units per liter (U/L).

Immune outcome measures

Blood samples were collected in cloth activating tubes (BD vacutainer) at baseline, after the intervention/ pre-vaccination, post-vaccination and at four weeks follow-up. Samples were clotted for an hour at room temperature before centrifugation at 2500 rcf for 10 minutes, serum was collected and aliquoted for storage at -80°C.

The list of cytokines and chemokines that were analyzed is specified in Appendix 1. Cytokine and chemokine levels were measured in serum as well as in stimulated or control plasma samples using the multiplex bead array (Bio-Plex Pro™ Human Chemokine Panel, 40-Plex #171AK99MR2, Bio-Rad laboratories, Veenendaal, The Netherlands (318)). CRP concentrations were determined in serum by ELISA according to the instructions of the manufacturer (Abnova, Heidelberg, Germany) at baseline, at the start of the vaccination day, at the start of the test day and at follow-up.

In addition, IgG antibody levels were evaluated at baseline and 4 weeks after vaccination. PPD (5 µg/ml, Statens Serum Institute, Copenhagen, Denmark) was coated to 96 well Microlon plates (Greiner, Alphen aan den Rijn, The Netherlands). Sera were diluted 1 to 25 and incubated overnight. IgG antibody binding was detected using HRP-labelled polyclonal rabbit anti-human IgG (Dako, Glostrup, Denmark), staining with TMB substrate buffer (Sigma Aldrich, Zwijndrecht, The Netherlands), stop with H2SO4 and OD450 reading (319).

Statistical analyses

As described in our design paper (256), a total sample size of 60 was deemed sufficient to detect scientifically and clinically relevant differences in the incorporated primary outcome. An Analysis of Covariance (ANCOVA) with condition (intervention vs control) as between subjects factor, vitality after the intervention as dependent variable and baseline vitality as covariate was conducted to assess the primary hypothesis that participants in the intervention condition would show higher self-reported vitality after the intervention (pre-vaccination) compared to the control condition. In addition, when a significant effect was found on the ANCOVA, it was investigated whether the effects were also present at the other time points. This was done by a repeated measures Analysis of Variance (RM ANOVA) with condition (intervention vs control) as between subjects factor and time (i.e., baseline, after intervention (pre-vaccination), after vaccination, follow-up) as within subjects factor. For the RM ANOVAs, we were specifically interested in the interaction effects between time and condition, as well as in the main effects of time, which are therefore specified in the results section. To examine at which time point(s) groups differed on vitality, represented by a significant interaction effect between time and condition on the RM ANOVA, Holm's corrected ANOVAs were performed to compare the intervention condition with the control

condition at specific time intervals by calculating difference scores between baseline and each of the other time points. Since we did not observe substantial missing data or deviations from the actual timeline within participants, we decided to test the secondary outcomes in a similar way (RM ANOVA) as done for the primary outcome measure instead of the preplanned multilevel analyses for the secondary outcomes (256). The results for bodily sensations, quality of life and sleep problems were analyzed as described above, although these analyses yielded three time points (i.e., baseline, after intervention (pre-vaccination), follow-up). As the items on these questionnaires were based on experiences of the last four weeks, these questionnaires were not completed post-vaccination.
In order to test any group differences for well-being and positive and negative affect in response to the test day, RM ANOVAs were performed for well-being and positive affect and negative affect with condition (intervention vs control) as between subjects factor and four time points (i.e., baseline, start of the test day, end of the test day, follow-up) as within subjects factor. Data on cortisol, alpha amylase, heart rate, heart rate variability, and skin conductance were analyzed in a similar way.

For both serum and LPS whole blood stimulation assay, principal component analysis (PCA) was performed to identify and subsequently exclude extreme outliers. IL-6 and IL-8 were excluded from the LPS whole blood stimulation analysis. For each time point comparison, two types of linear models were fitted: 1) linear multiple regression model using Δ-cytokine concentrations at different time points (i.e., pg/ml at start test day – pre-vaccination, pg/ml at end test day – pre-vaccination, and pg/ml at follow-up – baseline) as dependent variables to estimate the effect of intervention as independent variable on changes in cytokine concentrations while correcting for age; 2) linear mixed model with random intercept per subject to estimate the effect of time on cytokine levels in either the control or intervention group while correcting for age. Resulting *p*-values were false discovery rate (FDR) corrected to obtain *q*-values. Data were mean centered and scaled to standard deviation units for the generation of volcano plots. Finally, PCA, fitting of multiple linear regression models and linear mixed models and plotting of analysis results were performed using R version 3.5.0 with the following packages: 'mixOmics' (320), 'lme4' (321), 'lmerTest' (322), and 'ggplot2' (323).

Results

Sixty-nine participants were included in the present study (see Figure 1). Three participants dropped out of the study, one in the control condition and two in the intervention condition. Additionally, one participant did not start in the intervention condition after group allocation, due to time constraints. Due to global production problems of the

BCG-vaccine, two participants in the intervention condition and two participants in the control condition dropped out of the study after completion of the primary outcome measurement. Furthermore, one participant in the intervention condition dropped out of the study after completion of the intervention, as this participant was no longer able to complete the vaccination day, test day and four-week follow-up due to time constraints. This resulted in 31 participants in the control condition and 29 participants in the intervention condition that completed all visits. Analyses were performed for available data. No significant differences were found in age or BMI between the participants in the control condition (age: M = 22.9, SD = 4.1; BMI: M = 23.0, SD = 2.8) and the intervention condition (age: M = 22.5, SD = 2.3, p = .67; BMI: M = 22.5, SD = 2.4, p = 46).

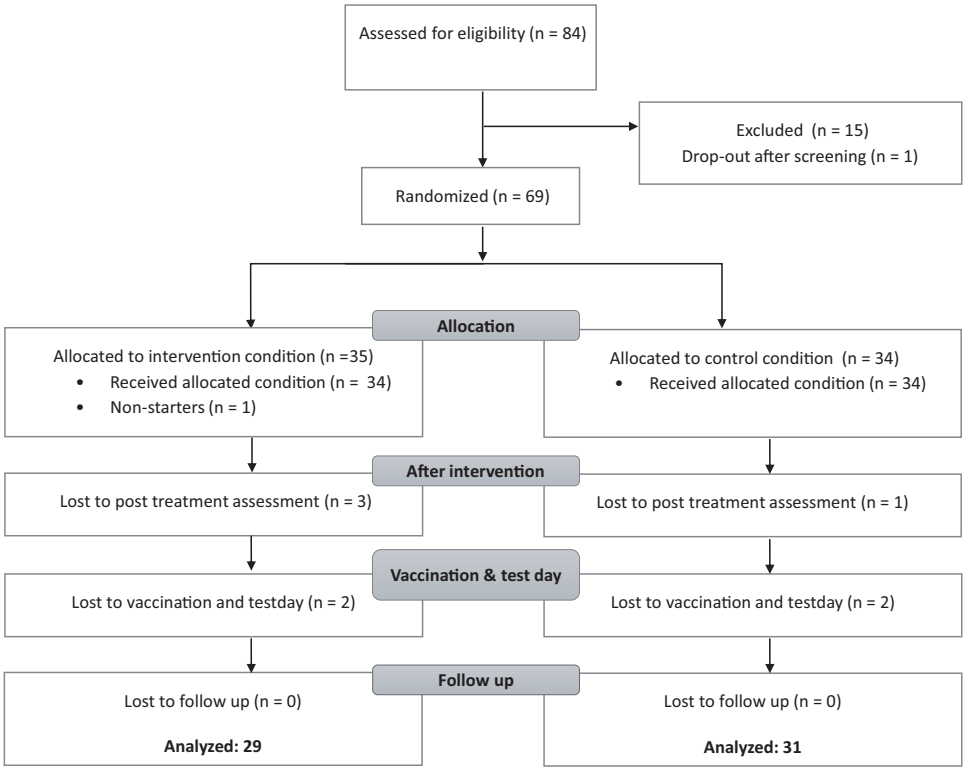

Figure 1. Flow diagram

Vitality

No significant differences were found between the groups for self-reported vitality within one week after the intervention (pre-vaccination) (F(1, 62) = 0.63, p = .43. The descriptive results for vitality on all time points are displayed in Figure 2.

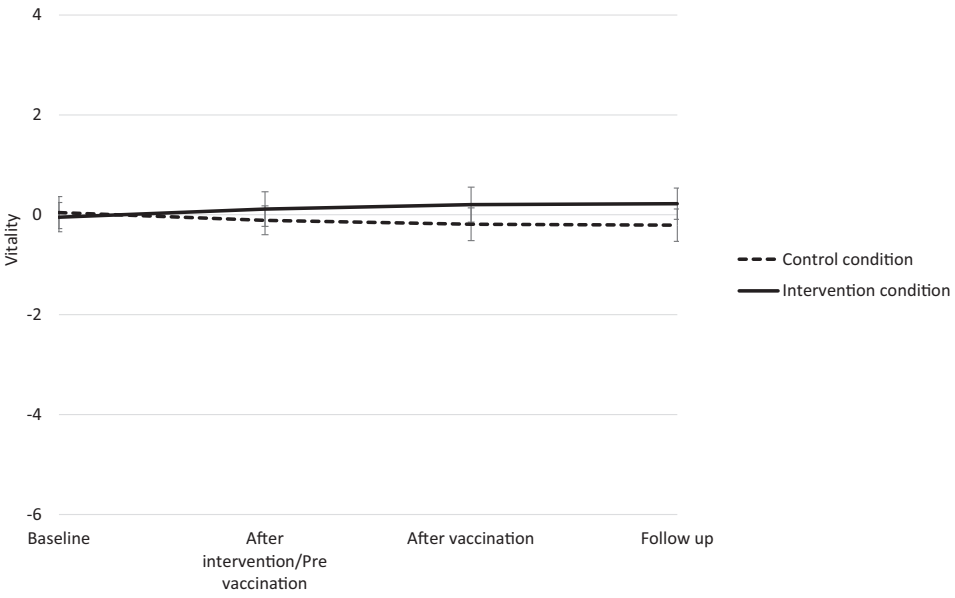

Figure 2. Mean and standard error of self-reported vitality at baseline, after intervention (pre-vaccination), after vaccination, and at follow-up, separately for the control condition and the intervention condition.

The y-axis represents a composite score of the Subjective Vitality Scale (SVS) and Checklist Individual Strength (CIS-20). Scores are standardized z-scores (vitality minus fatigue) with higher scores representing higher self-reported vitality levels.

Self-reported quality of life, bodily sensations, sleep, positive and negative affect, and well-being

In supplementary Figure 1, the results on quality of life are shown, for the physical (1A) and the mental (1B) quality of life subscale. Both ANCOVAs did not yield any significant group differences ($F(1, 62) = 0.01$, $p = .92$; $F(1, 62) = 1,42$, $p = .24$, respectively).

Figure 3 depicts the results on bodily sensations. An ANCOVA yielded a significant main effect for condition, $F(1, 62) = 4.30$, $p = .04$, $n^2 = .56$, indicating less bodily sensations for the intervention condition compared to the control condition directly after the intervention (pre-vaccination). The RM ANOVA yielded a significant main effect of time ($F(1.65, 79.03) = 7.30$, $p = .002$). Irrespective of condition, Holms corrected pairwise comparisons showed a significant decrease from baseline to after intervention (pre-vaccination) ($t(64) = 3.16$, *p adjusted* = .004), as well as a significant decrease from baseline to follow-up ($t(49) = 2.43$, *p adjusted* = .019). No significant interaction effect between time and condition was found ($F(1.65, 79.03) = 1.00$, $p = .36$).

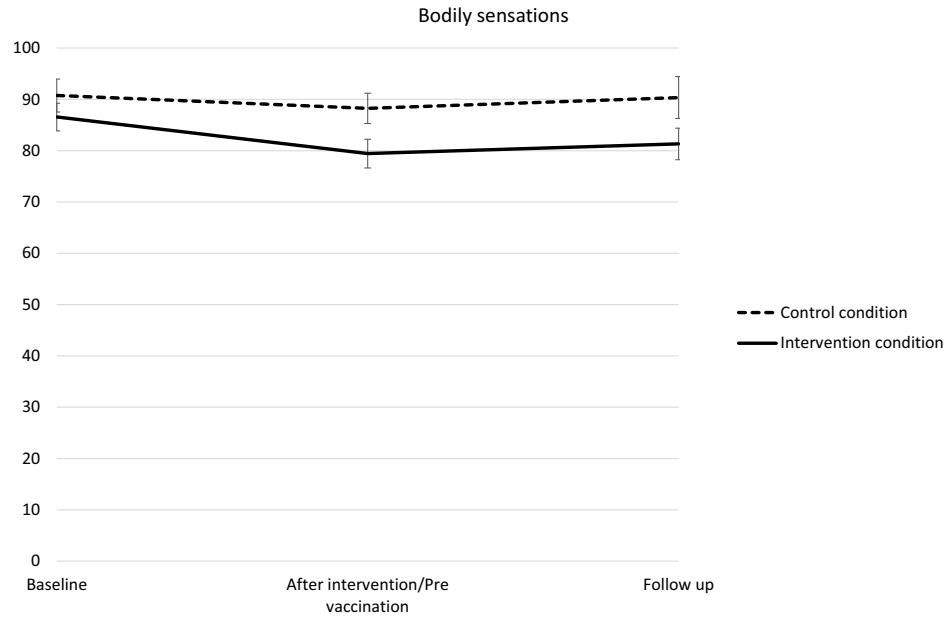

Figure 3. Mean and standard error of the mean of self-reported bodily sensations at baseline, after intervention (pre-vaccination), and at follow-up, separately for the control condition and the intervention condition.

Higher scores represent a higher frequency of experienced bodily sensations.

The results on sleep problems are presented in Figure 4. An ANCOVA showed a trend for an effect of the intervention, $F(1, 62) = 3.30$, $p = .07$, $n^2 = .44$. The RM ANOVA did not yield a significant effect of time ($F(1.66, 104.74) = 1.81$, $p = .18$), but showed a significant interaction between time and intervention ($F(1.66, 104.74) = 4.02$, $p = .03$, $n^2 = .06$). Holms corrected pairwise comparisons showed a significant difference between the intervention condition and the control condition from baseline to after intervention (pre-vaccination) ($F(1, 63) = 4.60$, *p adjusted* = .04, $n^2 = .07$), as well as from baseline to follow-up ($F(1, 63) = 6.23$, *p adjusted* = .03, $n^2 = .09$), indicating fewer sleep problems directly after the intervention (pre-vaccination) and also at follow-up for the intervention condition compared to the control condition.

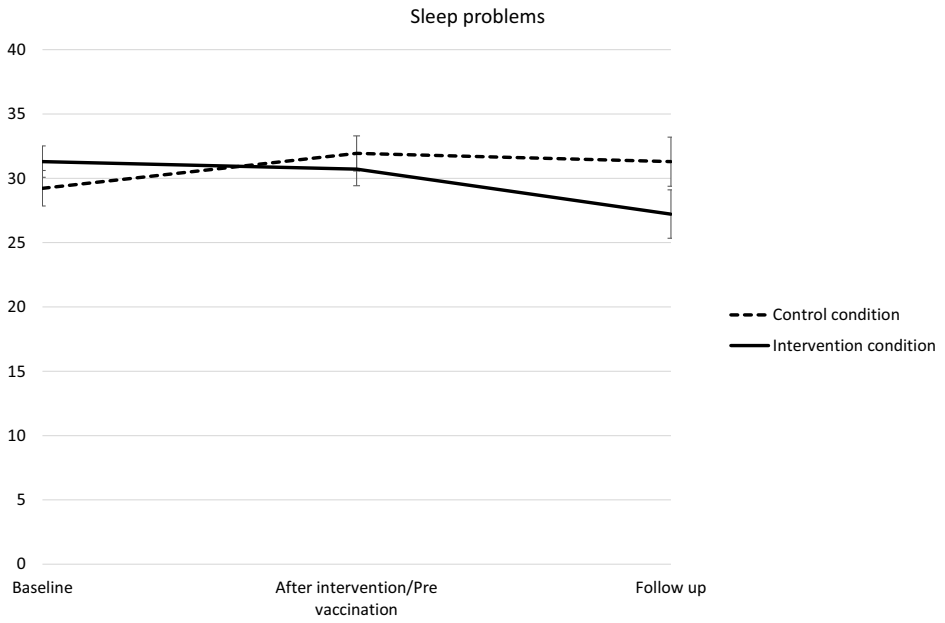

Figure 4. Mean and standard error of the mean of sleep problems at baseline, after intervention (pre-vaccination), and at follow-up, separately for the control condition and the intervention condition.

Higher scores represent a higher level of experienced sleep problems.

The results for positive and negative affect are shown in supplementary Figure 2A and 2B, respectively. For positive affect, the RM ANOVA yielded a significant main effect of time (F(2.98, 173.08) = 24.90, p < .001). Irrespective of intervention, Holms corrected pairwise comparisons showed a significant decrease in positive affect from baseline to the end of the test day (t(59) = 7.17, *p adjusted* < .001) (see supplementary Figure 2A). No significant interaction effect between time and condition was found, F(2.98, 173.08) = .48, p = .69. Negative affect was significantly influenced by time (F(2.00, 115.98) = 24.18, p < .001). Irrespective of condition, Holms corrected pairwise comparisons yielded a significant difference from baseline to the start of the test day (t(59) = 4.99, *p adjusted* < .001), the end of the test day (t(59) = -3.71, *p adjusted* < .001), and follow-up (t(59) = 2.29, *p adjusted* = .026) (see supplementary Figure 2B). No significant interaction between time and condition was found, F(2.00, 115.98) = 1.96, p = .15.

For well-being the results are shown in Figure 5. The RM ANOVA yielded a significant main effect of time (F(2.14, 124.22) = 70.84, p < .001), and also a significant interaction effect between time and intervention (F(2.14, 124.22) = 3.22, p = .04, n^2 = .05). Holms corrected

pairwise comparisons showed a significant difference between the intervention condition and the control condition from baseline to the end of the test day (F(1, 58) =7.45, p *adjusted* = .024, n^2 = .11), indicating a lower decrease in self-reported well-being from baseline to the end of the test day for the intervention compared to the control condition.

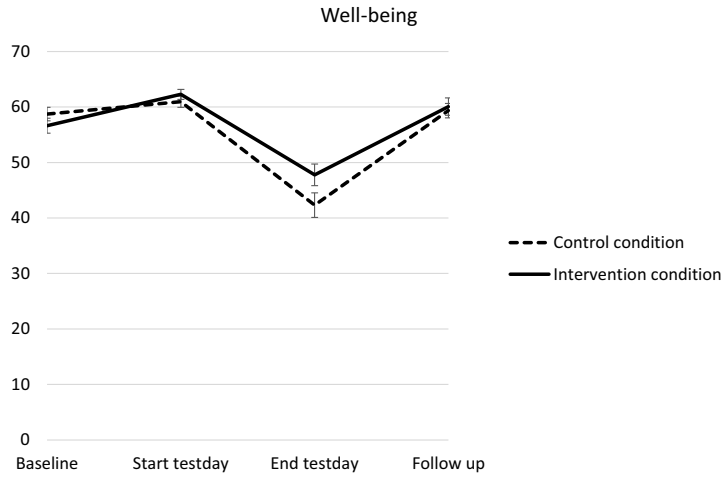

Figure 5. Mean and standard error of the mean of self-reported well-being at baseline, the start of the test day, the end of the test day, and at follow-up, separately for the control condition and the intervention condition.

Higher scores represent a higher level of experienced sleep problems.

Psychophysiological outcomes

Table 2 shows the descriptive statistics for heart rate, skin conductance, heart rate variability, as well as cortisol and alpha amylase, for the control and the intervention groups. For cortisol, the RM ANOVA showed a significant main effect of time (F(2.35, 131.57) = 35.28, p < .001). Holms corrected pairwise comparisons showed a significant increase in cortisol from baseline to the end of the test day (t(58) = -7.42, p *adjusted* < .001). No significant interaction between time and condition was found (F(2.35, 131.57) = 2.21, p = .11). Similar results were found for alpha amylase, as the RM ANOVA showed a significant main effect of time (F(2.26, 131.25) = 23.25, p < .001). Holms corrected pairwise comparisons showed a significant increase from baseline to after the intervention (t(60) = 4.25, p *adjusted* < .001), a significant decrease from baseline to the start of the test day (t(59) = 4.98, p *adjusted* < .001), and a significant increase from baseline to follow-up (t(59) = 4.18, p *adjusted* < .001), although no significant differences were found from baseline to the end of the test day (p *adjusted* = .20). Moreover, alpha amylase yielded no significant interaction effect between time and condition (F(2.26, 131.25) = .14, p = .90).

For heart rate, a significant main effect of time was found ($F(2.30, 132.23) = 11.37$, $p < .001$). Irrespective of the conditions, Holms corrected pairwise comparisons showed a significant decrease from baseline to the end of the test day ($t(56) = -3.78$, p *adjusted* $< .001$). A trend was found for an interaction effect between time and condition ($F(2.30, 132.23) = 2.44$, $p = .08$), indicating a lower heart rate at follow-up in the intervention condition compared to the control condition. For heart rate variability, a significant main effect of time was found ($F(1.49, 80.29) = 4.74$, $p = .02$), which varied over time (see Table 2). Holms corrected pairwise comparisons indicated no significant differences over time. No significant interaction effect was found between time and condition, $F(1.49, 80.29) = 2.00$, $p = .15$. For skin conductance, no significant main effect of time ($p = .46$) neither an interaction effect between time and condition was found ($p = .26$).

Table 2. Means and standard deviations for heart rate, skin conductance, heart rate variability, as well as cortisol and alpha amylase, separately for the control condition and the intervention condition.

		Baseline	After intervention / pre-vaccination	Start test day	End test day	Follow-up
HR						
	CC	68.3 (8.1)		67.2 (9.1)	63.5 (9.2)	72.8 (13.4)
	IC	66.6 (7.8)		65.7 (9.0)	64.1 (7.6)	67.6 (9.4)
SC						
	CC	4.5 (2.3)		3.8 (1.5)	4.3 (1.7)	4.3 (2.1)
	IC	4.2 (2.2)		4.7 (2.4)	5.2 (3.7)	4.9 (5.2)
HRV						
	CC	55.9 (38.4)		54.4 (43.8)	79.5 (79.5)	44.1 (27.8)
	IC	54.9 (25.1)		55.0 (26.0)	66.1 (35.0)	58.7 (35.8)
Cortisol						
	CC	5.5 (4.1)	6.2 (5.0)	4.9 (2.6)	7.9 (4.7)	7.7 (6.7)
	IC	4.8 (1.9)	6.0 (4.8)	4.6 (1.3)	6.7 (3.4)	5.2 (1.6)
Alpha Amylase						
	CC	2180.8 (1891.4)	1248.1 (900.7)	1155.7 (791.6)	1810.6 (1740.5)	1433.0 (1062.0)
	IC	2360.1 (2144.0)	1348.6 (744.4)	1211.2 (772.6)	1709.1 (1149.0)	1449.7 (1052.4)

Note. CC = control condition, HR = heart rate, HRV = heart rate variability, IC = intervention condition, SC = Skin conductance. Cortisol is expressed in nanomoles per liter (nmol/L) and skin conductance is expressed in units per liter (U/L).

Immune outcomes

Figure 6 shows volcano plots of significantly upregulated and downregulated serum analytes between pre-vaccination to the end of the test day. The multivariate linear regressions yielded no significant differences between the intervention and control group at any time point. However, within the control or intervention group significant changes over time were identified for unique sets of analytes. For the control condition, significant

increases for various cytokines and chemokines (i.e., IL-2, IL-10, CCL1, CCL17, CCL19, CCl23, CCL25, CCL26, CXCL2, CXCL6, CXCL13, CX3CL1, GM-CSF), as well as significant decreases for other chemokines (i.e., CCL2, CCL15, CCL21, CCL27; all FDR-corrected *p-values* < .05) between pre-vaccination and end of the test day were found. For the intervention condition, also significant increases were found for various cytokines and chemokines from pre-vaccination to end of the test day (i.e., IL-1β, IL-2, IL-8, IL-10, IL-16, CCL1, CCL8, CCL11, CCL17, CCL19, CCL22, CCL23, CCL25, CCL26, CXCL1, CXCL2, CXCL5, CXCL6, CXCL9, CXCL11, CXCL13, MIF, TNF-α,) and a significant decrease for CCL15 (all FDR-corrected *p-values* < .05). The results for the upregulated IL-8, CXCL5 and TNF-α, as well as for the downregulated CCL15 are shown in supplementary Figure 3, as these analytes showed the most prominent group differences. Similar results were found from start of test day to end of test day. No significant differences were found from baseline to follow-up in the control condition, although the intervention condition showed significant increases in serum IL-10, CCL19, and CXCL9 concentrations, as well as a significant decrease for CCL15 (all FDR-corrected *p-values* < .05).

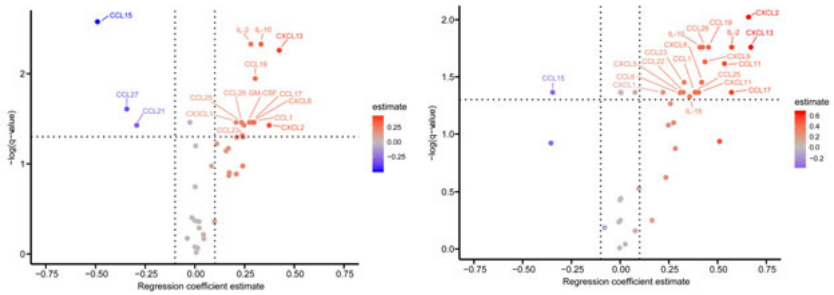

Figure 6. Vulcano plots for the control (upper graph) and intervention condition (lower graph) separately for the comparison pre-vaccination to the end of the test day. Significance is displayed on the y-axis and estimate of variance on the x-axis.

Negative values indicate analytes that are downregulated at the end of the test day compared to pre-vaccination, positive values indicated upregulated analytes at the end of the test day compared to pre-vaccination. Analytes with an estimated effect < 0.1 were not considered, since those estimates frequently represent very small changes in cytokine levels below the detection limits of variation in technical duplicates.

The results for the IgG antibody levels are displayed in supplementary Figure 4. The multivariate linear regressions yielded no significant differences between the intervention and control group at any time point for IgG antibody levels. However, when looking at changes over time separately for the intervention and control condition, no significant differences were found from baseline to follow-up in the control condition, whereas the intervention condition showed significant increases in PPD specific IgG levels (FDR-corrected *p-value* < .05).

Serum CRP levels were not significantly different between groups (data not shown).

LPS stimulation of whole blood samples, did not induce significant differences between the intervention and control groups (all FDR-corrected *p-values* > .05). In an explorative analysis, we investigated the intervention condition and control condition separately for the different time ranges. For the control condition from baseline to test day, we found significant increases for IL-1β and TNF-α (both FDR-corrected *p-values* < .05), whereas no significant differences were found for the intervention condition.

Discussion

The aim of the present study was to investigate the effects of an e-health CBT combined with serious gaming intervention on optimizing self-reported, psychophysiological and immunological outcomes in response to *in vitro* and *in vivo* immunological as well as psychophysiological challenges. The present study was the first to incorporate an e-health CBT combined with serious gaming intervention. No significant differences between the intervention and control condition were found for self-reported vitality. The intervention group did show fewer bodily sensations and fewer sleep problems after the intervention. Furthermore, the intervention group showed higher self-reported well-being after different psychophysiological stressors compared to the control group. No significant group differences were found for the psychophysiological and immunological outcomes, although some preliminary support was found for optimized outcomes on heart rate variables as well as increased IgG antibody responses at follow-up and differential chemokine outcomes at the end of the test day in the intervention compared to the control condition. The present study thus provides a first step towards unraveling the effectiveness of an e-health psychological intervention combined with serious gaming elements on optimizing various self-reported, psychophysiological and immunological health outcomes.

Concerning vitality, although the intervention condition showed a rise in self-reported vitality and the control condition did not, no significant group differences were found. Also, no significant group differences were found for quality of life, however, these scores were already rather high at baseline for both groups. We included a healthy population, which presumably already possessed a good quality of life that could not be maximized further by our psychological intervention. In contrast, bodily sensations, including head ache, itch, and other negative sensations, and sleep problems were significantly decreased after the intervention, compared to the control condition. As bodily sensations and sleep problems affect general health outcomes (324, 325), the intervention was effective in

optimizing precursors of health. Due to the heterogeneous findings for bodily sensations, sleep problems, quality of life, and vitality, no conclusive view on the effectiveness of the intervention in optimizing self-reported health outcomes can be formulated. Since the present study was one of the first incorporating self-reported vitality as an outcome measure for health condition by combining two questionnaires into a composite score, more research on the external validity of this composite score is needed. Furthermore, as participants already yielded high baseline scores for physical and mental quality of life, and vitality comprises a comparable construct, it would be interesting to further investigate whether a sample at risk for low vitality or quality of life could benefit from the psychological intervention.

The present study also investigated the results of self-reported outcomes in response to *in vitro* and *in vivo* immunological as well as psychophysiological challenges. Although no significant differences were found between conditions in positive and negative affect, a higher self-reported well-being was found at the end of the test day for the intervention condition compared to the control condition. This provides some preliminary support for optimized resilience in response to psychophysiological stressors by an e-health CBT combined with serious gaming intervention. The present psychological intervention focused on healthy participants to see if it was possible to improve health outcomes by optimizing skills to cope with daily stressors. Possibly, the population included here already possessed sufficient resilience and skills to handle the immunological and psychophysiological challenges applied. Future studies should therefore also include participants at risk for health problems, including participants with chronic somatic conditions or with (sub) clinical levels of anxiety and/or depression to see whether they may also benefit from such a psychological intervention (326).

When specifically assessing the psychophysiological health outcomes, i.e., heart rate, heart rate variability, skin conductance, cortisol and alpha amylase, no strong evidence was found for the effectiveness of the intervention. Some preliminary evidence for optimized outcomes after the intervention was found. Particularly, the intervention condition had a lower heart rate at follow-up as compared to the control condition. Although not significant, the results for heart rate variability showed a similar pattern, in that heart rate variability at follow-up appeared to be higher for the intervention condition compared to the control condition. As a lower heart rate and higher heart rate variability can be seen as biomarkers for better stress-related health outcomes (327-329), these data cautiously support the effectiveness of the psychological intervention in optimizing health. However, no significant effects were found for skin conductance, cortisol and alpha amylase. The results therefore provide limited support for optimizing the response of the sympathetic-

adrenal-medullar (SAM) axis, but no support for influencing the hypothalamic-pituitary-adrenal (HPA) axis, whereas the SAM- and HPA-axis are known to interact with each other in order to maintain homeostasis (330). In addition, as no indications were found for group differences on the test day for heart rate, heart rate variability, cortisol and alpha amylase, more research is needed on the external validity and clinical relevance of the present findings on psychophysiological health outcomes.

For the immune outcomes, the between-group analyses yielded no significant findings. The explorative analyses showed significant alterations in several cytokines and chemokines from baseline to follow-up in the intervention condition, whereas no significant alterations were found in the control condition between these time points, providing some cautious support for higher responses for most analytes at the follow-up in the intervention condition. Previous literature on the effectiveness of psychological interventions on optimizing immune function did not yet focus specifically on cytokines and chemokines (2). Cytokines and chemokines are known to have a significant influence on inflammatory processes, as they provide directional cues for the movement and tissue homing of leukocytes (331, 332). To make more conclusive statements on the effectiveness of psychological interventions in optimizing chemokine functioning, future research should incorporate a wide range of analytes with varying immunological characteristics into the study design, in order to replicate the present findings and to gather more insights in the mechanisms underlying differential immune responses after a psychological intervention. Concerning the *in vivo* challenge (i.e., the BCG-vaccination), we found increased IgG antibody levels from baseline to follow-up for the intervention condition, whereas no such significant differences were observed in the control condition. This finding provides some preliminary support for an altered host response to the BCG-vaccine after the intervention. This preliminary finding is in line with a previous study from Petrie and colleagues (1995) who found higher antibody levels in response to a Hepatitis B vaccine in the intervention condition receiving an emotional disclosure intervention compared to a control condition receiving no intervention (199). In contrast to a Hepatitis B vaccine, the BCG-vaccine, being a live vaccine, actually is a human challenge model and as such approximates immune responses that are observed after natural infections (256). Since antibody titers in the present study were not different in the between groups analyses, the findings need to be interpreted with caution. The present study was the first to incorporate BCG-vaccination, and future studies incorporating BCG into the study design should provide further insights into the effects of training towards this infectious challenge.

When looking at the *in vitro* immunological challenge, the between-group analyses on LPS-stimulated cytokines and chemokines yielded no significant differences. In exploratory

analyses, we found that IL-1β, IL-8, CXCL5 and TNF-α were significantly increased from pre-vaccination to start of the test day in the intervention but not in the control group. Furthermore, CCL2, CCL21 and CCL27 were significantly decreased from pre-vaccination to end of the test day, only in the control group, but not in the intervention group. Those findings suggest differential immune activation between the groups. However, the data do not support altered immune function following a psychological intervention in response to LPS as *in vitro* immunological challenge. Moreover, LPS is a rather strong immune-activator, possibly having masked subtle immunologic differences between the intervention and control groups.

After intervention, but before the vaccination and test day, no significant differences between unstimulated immune outcomes were found. Therefore, incorporation of *in vivo* immunological as well as psychophysiological challenges may be needed to identify more subtle immune alterations after a psychological intervention in healthy participants. However, whether one single challenge or a combination of several challenges caused the findings cannot be disentangled by the present study, due to the fact that the test day comprised multiple challenges. Furthermore, due to logistic restrictions, we did not incorporate an *in vitro* LPS-stimulation after the BCG-vaccination and psychophysiological challenges. More informative results on the *in vitro* immunological challenge might have been gathered when this stimulation had also been performed after the psychophysiological challenges, as this could provide more insights in the possible interaction between the psychophysiological challenges and the *in vitro* LPS stimulation.

Besides the innovative features of the present study, i.e., the combination of innovative intervention components directed at both automatic and conscious information processing and behavior change, multiple *in vitro* and *in vivo* immunological and psychophysiological challenges, as well as the inclusion of a wide range of self-reported and psychophysiological outcome measures, the present study has some limitations that should be mentioned as well. First of all, the present incorporated study population consisted of healthy males between 18 and 35 years of age. Although we were able to thoroughly investigate the effectiveness of a psychological intervention on health outcomes by incorporating *in vitro* and *in vivo* immunological as well as psychophysiological challenges in a homogeneous healthy sample, future research should investigate whether the intervention might be (more) effective in other populations, including patients with chronic somatic conditions and/or patients in need of a psychological intervention due to (sub) clinical levels of stress. Second, the present incorporated study design does not allow us to unravel the effectiveness of the separate intervention components. A first step towards disentangling the effectiveness of serious gaming on health outcomes was performed in a study on

the effects of a subset of the serious games on food outcomes that found preliminary support for the effectiveness of serious games on virtual food choice and implicit food preference (333). Third, although we tried to keep track of the time participants spent on the serious game by saving log files of the gaming activity, those log files were saved offline by participants themselves and we did not receive log files from each participant, making that we could not verify whether they actually played the game five days a week. Although the therapist that guided the intervention tried to keep track on the gaming frequency by asking participants to report on their gaming activities in the online e-health intervention, future studies should attempt to receive live tracking via online electronic records. Finally, although we asked participants not to use drugs and alcohol 48 hours before each measurement and we checked this by verbally asking them whether they used alcohol or drugs, we cannot be entirely sure that participants have not violated these rules. As consumption of alcohol and drugs can alter cytokine responses (334), future research should include quantification of alcohol and drug consumption with objective tests.

In conclusion, although the present study did not find support for the optimization of vitality, it did find some support for the effectiveness of an e-health CBT combined with serious gaming intervention in decreasing bodily sensations and sleep problems. Also, the present study showed that the intervention participants had higher levels of self-reported well-being in response to the psychophysiological challenges than control participants. Additionally, specific IgG antibody levels were increased at four weeks after BCG-vaccination in the intervention condition. As this is one of the first studies incorporating multiple challenges to evaluate the effects of a psychological intervention on health outcomes, the present study provides a first step towards optimizing health outcomes with a psychological intervention even though clearly more research is needed on this topic. Future research should further investigate whether tailoring the intervention to specific populations, including patients with chronic somatic conditions or participants with (sub) clinical levels of stress/anxiety problems, enhances efficacy and impacts relevant disease related parameters and biomarkers. Given the innovative study design, combining multiple new elements, future studies should consistently incorporate challenges and a wide range of immune parameters into the study design in order to get a more complete view on the effects of innovative psychological interventions.

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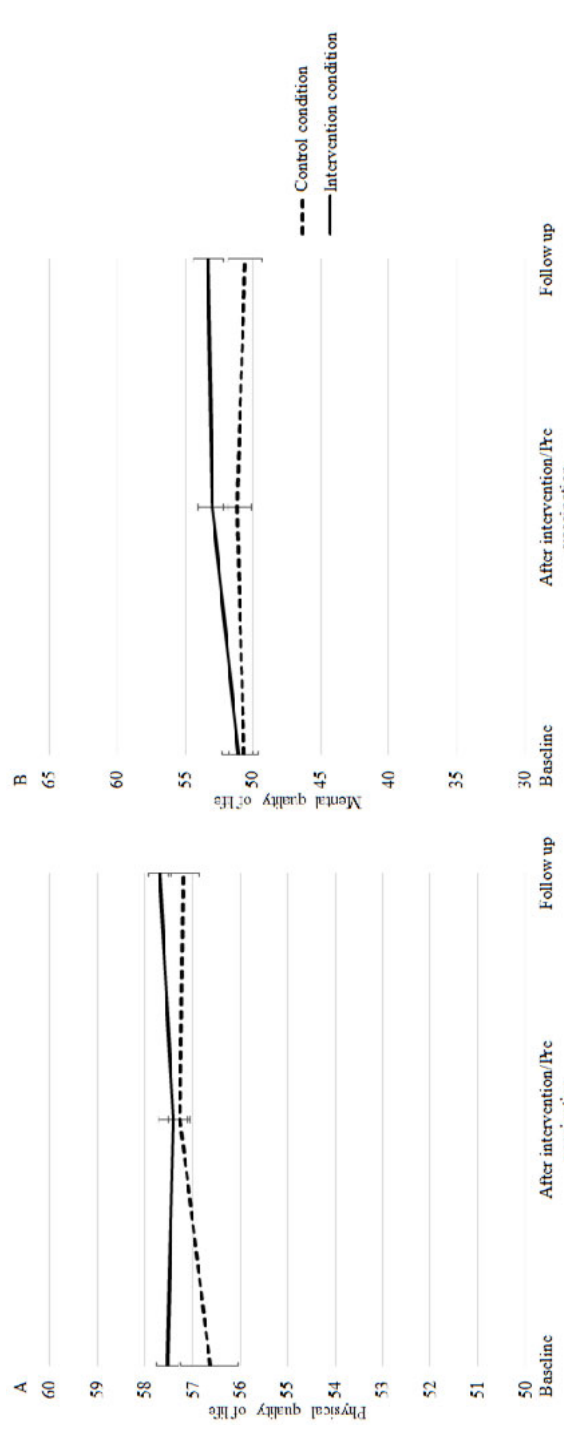
Appendices and supplementary material

Appendix 1. Overview of chemokines and other cytokines that were analyzed in the 40-plex assay.

IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-16	IP-10
CCL1	CCL2	CCL3	CCL7	CCL8	CCL11	CCL13	CCL15
CCL17	CCL19	CCL20	CCL21	CCL22	CCL23	CCL24	CCL25
CCL26	CCL27	CXCL1	CXCL2	CXCL5	CXCL6	CXCL9	CXCL11
CXCL12	CXCL13	CXCL16	CX3CL1	GM-CSF	MIF	TNF-α	IFN-γ

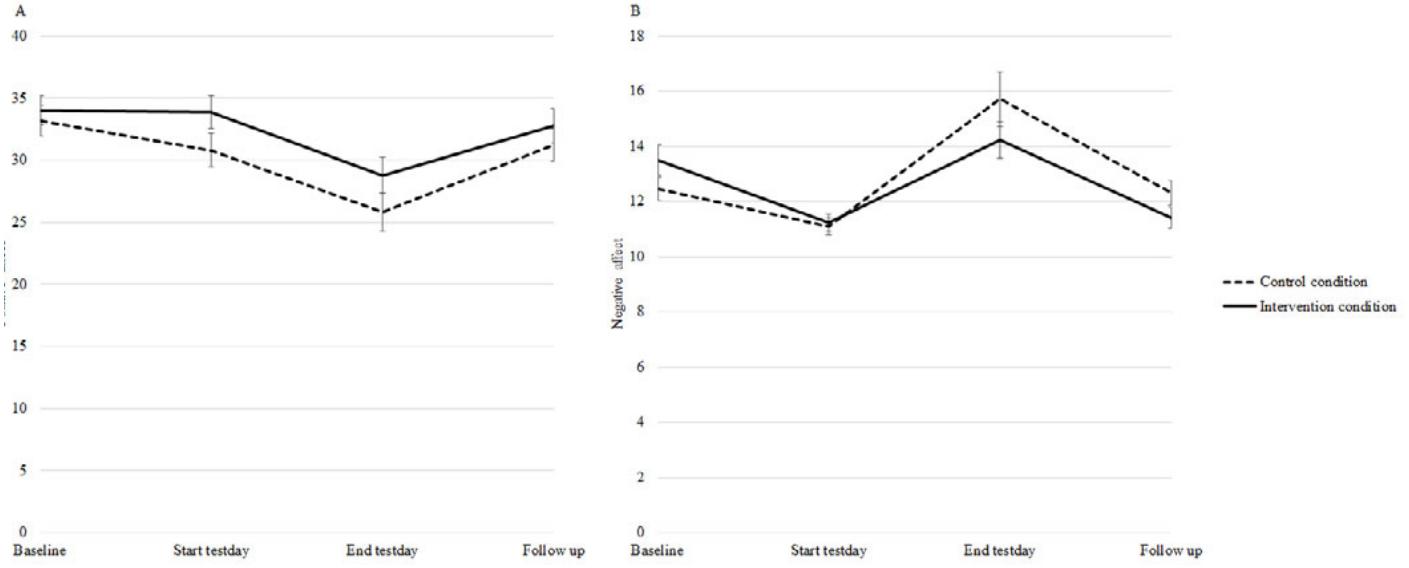
Appendix 2. Details of the self-reported, psychophysiological and immune outcome measures on each measurement point.

	Baseline	After intervention / pre-vaccination	Start test day	End test day	Follow-up
Self-reported outcomes	SVS, CIS-20, RAND-36, PILL, MOS Sleep, PANAS, and NRS	SVS, CIS-20, RAND-36, PILL, MOS Sleep, PANAS, and NRS	SVS, CIS-20, PANAS, and NRS	PANAS and NRS	SVS, CIS-20, RAND-36, PILL, MOS Sleep, PANAS, and NRS
Psycho-physiological outcomes	Heart rate variables, skin conductance, cortisol, and alpha amylase		Heart rate variables, skin conductance, cortisol, and alpha amylase	Heart rate variables, skin conductance, cortisol, and alpha amylase	Heart rate variables, skin conductance, cortisol, and alpha amylase
Immune outcomes	Unstimulated as well as LPS-stimulated serum samples	Unstimulated as well as LPS-stimulated serum samples	Unstimulated as well as LPS-stimulated serum samples	Unstimulated serum sample	Unstimulated serum sample



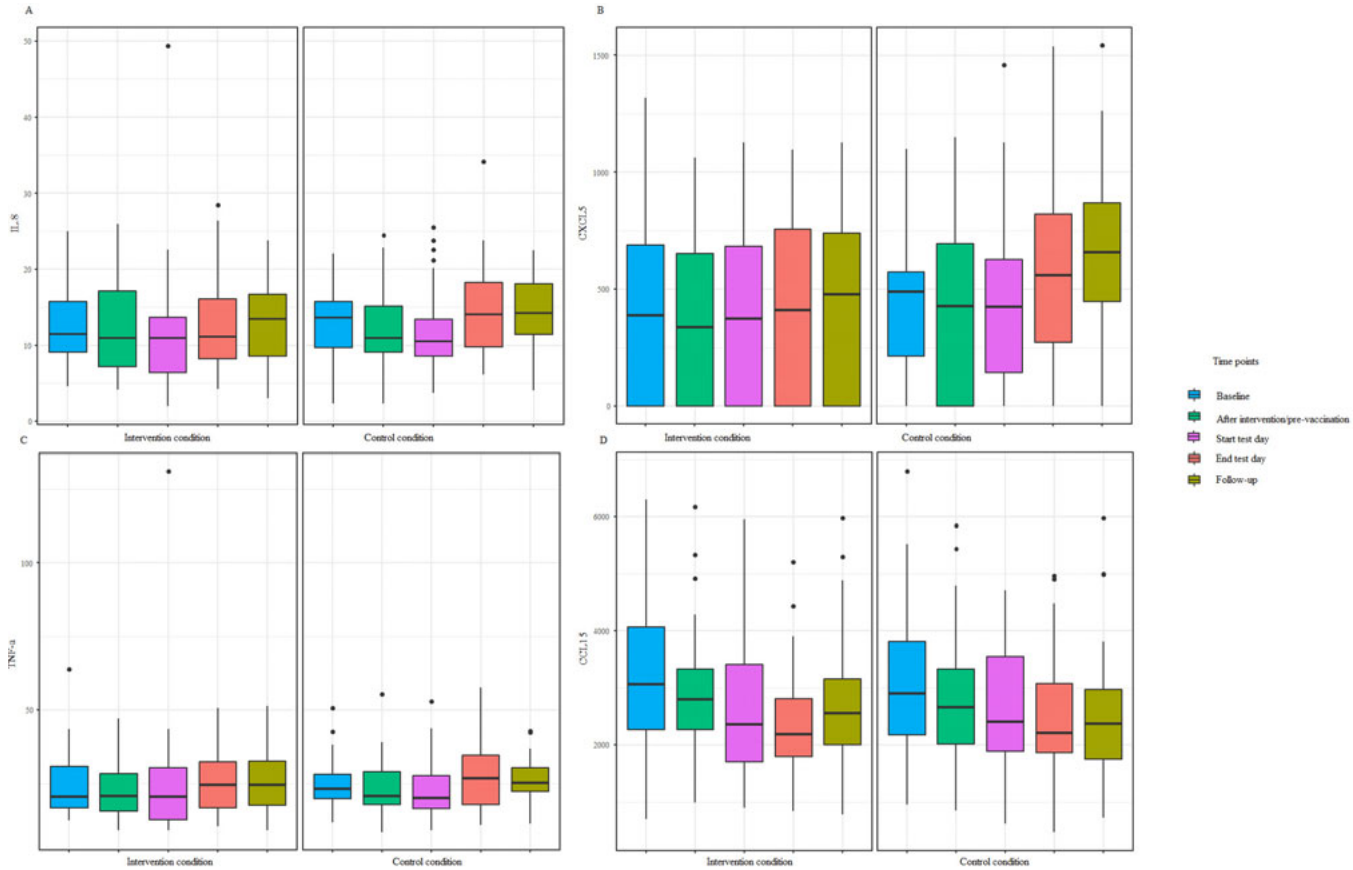
Supplementary Figure 1. Mean and standard error of the mean of self-reported physical quality of life (A) and mental quality of life (B) T-scores at baseline, after intervention (pre-vaccination), and at follow-up, separately for the control condition and the intervention condition.

A higher score on the y-axis represents a higher quality of life.



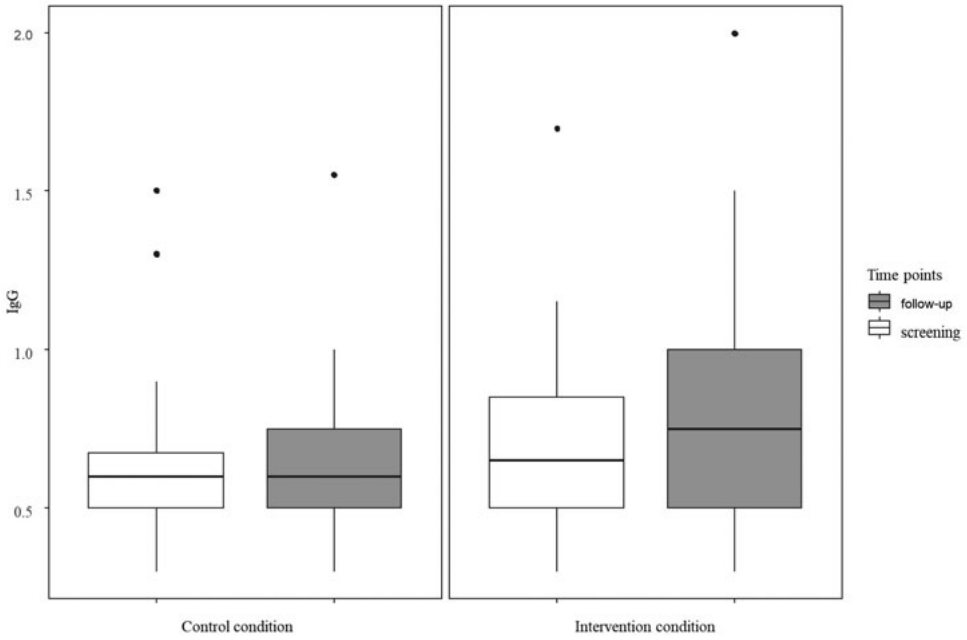
Supplementary Figure 2. Mean and standard error of the mean of the standardized scores for self-reported positive affect (A) and negative affect (B) at baseline, the start of the test day, the end of the test day, and at follow-up, separately for the control condition and the intervention condition.

A higher score on the y-axis represents a higher level of self-reported positive affect and negative affect, respectively.



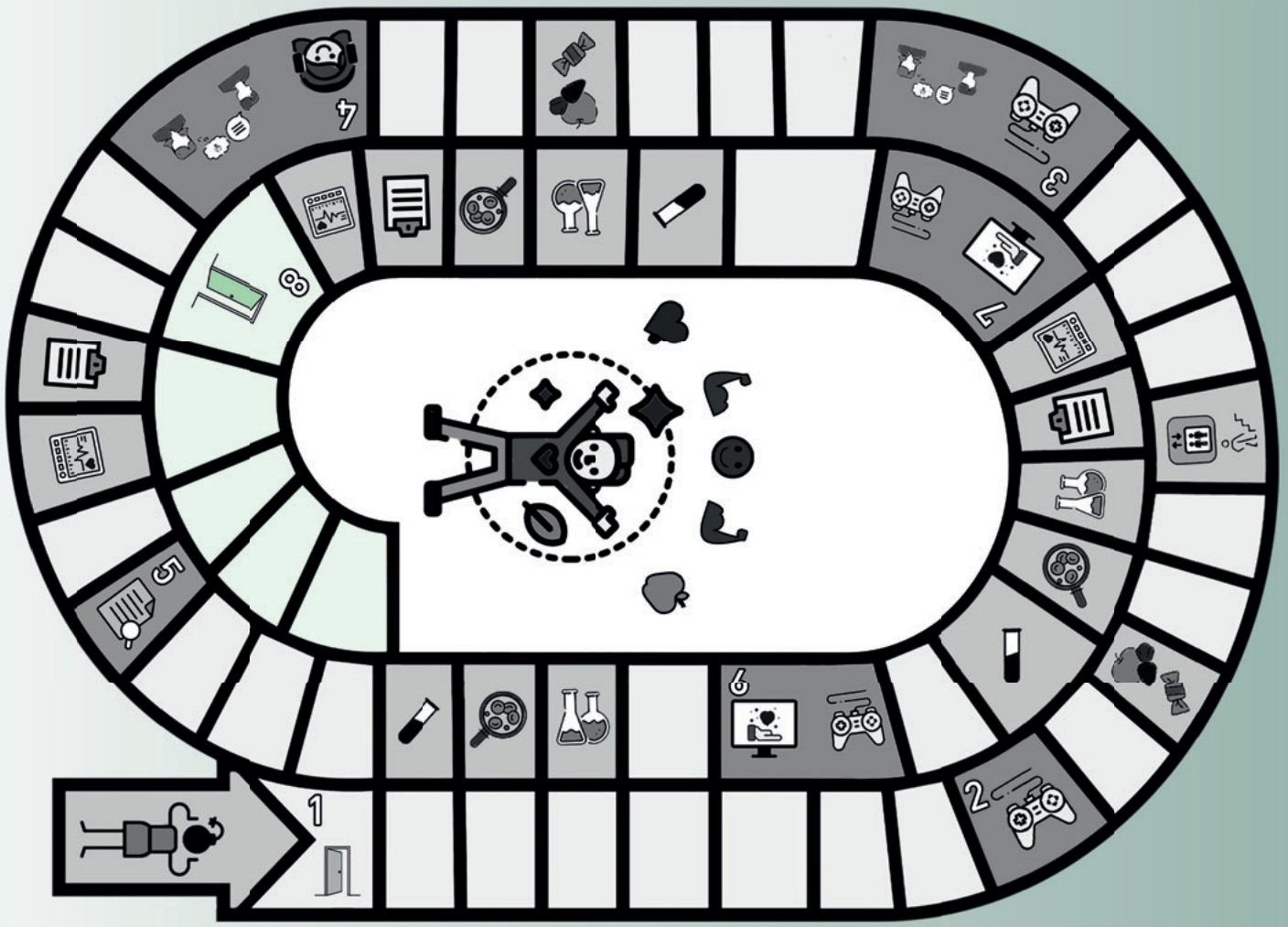
Supplementary Figure 3. Boxplots for the upregulated IL-8 (A), CXCL5 (B) and TNF- α (C), as well as for the downregulated CCL15 (D) for the control condition (left graph) and intervention condition (right graph) separately at baseline, pre-vaccination, start test day, end test day and follow-up.

A higher level in pg/ml on the y-axis represents a higher cytokine/chemokine level.



Supplementary Figure 4. Boxplots with the OD450 readings for the control condition (left graph) and intervention condition (right graph) separately with the PPD specific IgG antibody levels at baseline and follow-up.

A higher OD450 reading on the y-axis represents a higher IgG antibody level.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