EVALUATING THE EFFECTIVENESS OF INNOVATIVE PSYCHOLOGICAL INTERVENTION TOOLS IN OPTIMIZING HEALTH OUTCOMES: A MULTIMETHOD APPROACH

LEMMY SCHAKEL

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

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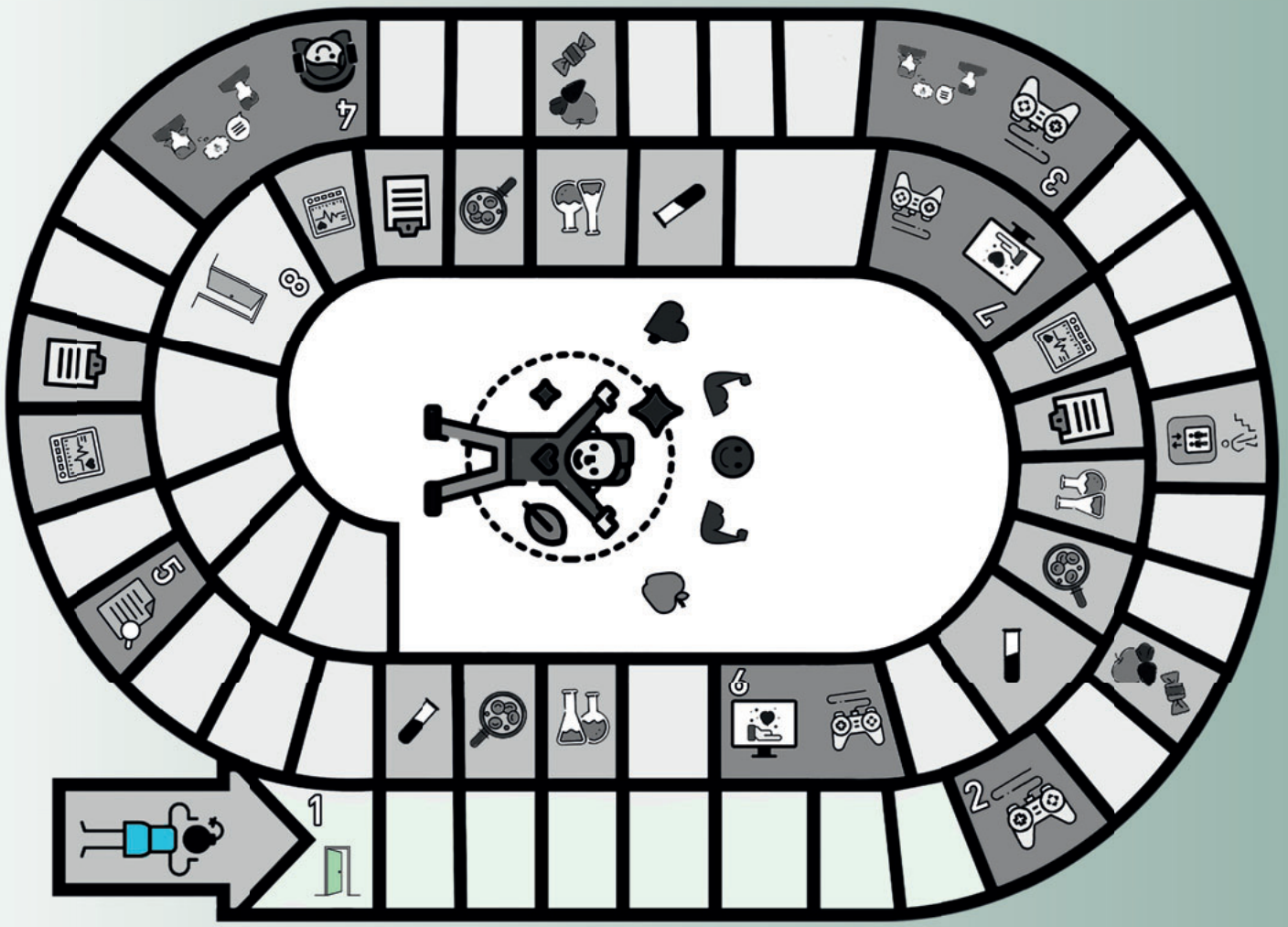
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General introduction

1

Psychological interventions entail therapeutic strategies to improve physical and/or psychological well-being by means of modifying emotions, cognitions and/or behaviors (1, 2). One of the most well-researched psychological interventions is Cognitive Behavioral Therapy (CBT) (3), which is based on challenging dysfunctional beliefs and cognitive distortions by various cognitive and behavioral strategies (3, 4). CBT has been demonstrated to be effective in optimizing physical and psychological health in various populations, including patients with type 2 diabetes (5), cancer (6), chronic fatigue (7), and chronic pain (8). Despite the generally positive effects of CBT for those patient populations, there are a number of barriers for traditional face-to-face CBT, including limitations concerning accessibility, such as mobility, treatment costs, and the limited number of qualified therapists, resulting in longer waiting lists (9, 10). Second, a substantial number of participants in treatment programs drop out during the treatment program, primarily due to a lack of motivation and engagement with the intervention (11). Finally, not only the actual experience of success of an intervention is of importance, also the expectancies people have concerning the outcomes of the intervention prior to starting the intervention predict the actual effectiveness of psychological interventions (12). From the placebo literature, positive outcome expectancies are already known to be an important component to maximize treatment outcomes, for example by means of verbal suggestions (13). Therefore, there is a need for innovative tools that can optimize accessibility, engagement, and positive outcome expectancies in psychological interventions.

Innovative tools to optimize psychological interventions

Optimizing accessibility: delivering interventions via the internet

Web-based tools offer great potential for enhancing the accessibility of psychological interventions. Electronic interventions aiming to optimize health, i.e., e-health interventions, are always and anywhere accessible from a networked device, and can thereby overcome the barrier of accessibility (14). Furthermore, they can reduce intervention costs and as more patients can be treated simultaneously, they can also reduce waiting lists (14, 15). However, the drop-out rates in internet-based interventions can be even higher as compared to those of face-to-face interventions; non-familiarity with use of a computer and/or internet is considered to be an important reason (16, 17). Adherence to e-health interventions seems to increase when they are tailored to the specific needs of an individual, and it is well known that therapeutically guided e-health interventions are more effective than non-guided interventions (17, 18). Although internet-based interventions offer fewer key features for developing and maintaining a therapeutic relationship as compared to traditional face-to-face interventions, they are

better able to strengthen independent problem-solving skills, as well as self-determination and empowerment (19, 20). Another strength of guided e-health interventions compared to face-to-face interventions is that the former optimizes accessibility not only for the patient, but also for the therapist, as it costs less time for the therapist to carry out an internet-based intervention compared to a face-to-face intervention (17). A meta-analytic review already provided support for the effectiveness of guided e-health-based CBT in improving psychological and somatic outcomes in patients with chronic somatic conditions (10). Additionally, a systematic review provided support for guided e-health interventions outperforming unguided internet-based interventions in optimizing treatment outcomes (21). Therefore, guided e-health interventions can optimize the accessibility of psychological interventions.

Optimizing engagement: implementing serious gaming elements

Engagement with the e-health interventions, i.e., the process of involving users in health education in such a way that it enhances users' motivation and leads to behavioral changes, is a key factor in enhancing the effectiveness of psychological interventions (22, 23). Engagement therefore can reduce drop-out rates. An innovative e-health method that can be applied to enhance engagement is serious gaming. Serious games are interventions that at least partially exist of gaming elements as a way to accomplish a serious goal, i.e., health optimization (24). It is a rather promising tool to enhance engagement as serious games are entertaining, rewarding and reinforcing, resulting in intrinsic motivation (24-26). Previous studies have provided support for the potential effectiveness of serious gaming in reducing symptoms of mental health disorders (25, 27). Additionally, serious games can promote health behaviors (28, 29) and are therefore a promising tool to provide added value to internet-based CBT interventions.

Optimizing positive outcome expectancies: providing verbal suggestions

In addition to innovative e-health tools, enhancing positive outcome expectancies concerning an intervention have the potential to maximize treatment effects (30, 31). The effectiveness of inducing positive outcome expectancies has already been demonstrated by a large body of placebo studies, showing that providing people with verbal suggestions can optimize perceived treatment credibility and health outcomes (32). For instance, interventions based on expectations have shown to be effective in relieving itch in healthy participants (33), and to effectively relieve pain in various patient populations (34). Concerning psychological interventions, a study on the effectiveness of a smoking cessation intervention found that the efficacy of tailored messages in this intervention was higher when positive outcome expectancies were raised for this intervention (30). Additionally,

a meta-analytic review on the effectiveness of outcomes expectancies in altering the effectiveness of psychotherapy provided evidence for the effects of positive outcome expectancies in optimizing treatment outcomes (35). Positive outcome expectancies do not only increase adherence to the therapy, but can also improve outcomes by increasing feelings of hope and alleviation of symptoms (36). As positive outcome expectancies have shown promising results in optimizing treatment outcomes in various somatic conditions, it would be interesting to explore whether those outcome expectancies are also effective in maximizing the effectiveness of psychological interventions directed at optimizing health outcomes.

Evaluating the effects of psychological interventions on health outcomes

Evaluating basal health outcomes

Health can be defined as a dynamic state of well-being, consisting of physical and psychological potential that can handle challenging demands, such as physical stress (e.g., a wound) or psychological stress (e.g., daily hassles) (37, 38). There are numerous ways to evaluate health. Most studies so far that evaluated health outcomes are based on observing basal levels of health outcomes by including self-reporting outcome measures concerning mental and physical well-being. Those self-reporting outcomes can provide valuable information concerning the participants' view on his/her health status. Although self-reporting outcomes are often well validated and reliable, there are discrepancies between self-reported outcomes and actual health outcomes. Therefore, in order to acquire more insights in actual health outcomes, these outcomes should be evaluated next to self-report measures whenever possible and preferably using multiple independent methods. Self-reporting outcomes, for example, could be supplemented with observations of behavioral outcomes and psychophysiological measures to gather a more complete view on the effectiveness of psychological interventions. Behavioral outcomes can be assessed, e.g., by exposing people to a choice between healthy and unhealthy food products and subsequently observing their actual choice. Besides behavioral outcomes, psychophysiological outcomes could also complement knowledge on self-reported outcomes, including evaluations of the HPA-axis (e.g., measuring cortisol in saliva), Sympathetic Adrenal Medullary (SAM)-axis (e.g., measuring heart rate) and immunological outcomes (e.g., measuring various cytokines in blood serum) in order to acquire a multi parameter view of health-related determinants. Besides, measuring these outcomes in the presence of circumstances that challenge the health status could provide meaningful additional insights on whether individuals can handle such situations adequately.

Evaluating health outcomes in the context of a challenge

A factor that significantly challenges individuals' health status in everyday life is stress, which is determined by the interaction between characteristics of the individual such as appraisal (i.e., interpretations of the situation, event and/or behavior), the environment in which the event occurs, and the internal and external resources a person encompasses (37, 39). Due to unsuccessful cognitive appraisal and coping strategies, stress can have adverse effects on health, including adverse effects on the immune system (40, 41). Chronic stress, for instance, can suppress protective immune responses and promote pathological inflammatory immune responses (42-44). These immune alterations can result in slower wound healing processes (43, 44), impaired responses to vaccines (45), and progression of infectious and immune-mediated diseases (42, 46). Stress does not only have direct detrimental health consequences but can also indirectly affect health. For example, stress can increase health-risk behaviors, including unhealthy eating behaviors, cigarette smoking, and alcohol use (47-50). Those health-risk behaviors, in turn, can have physiological consequences, such as weight gain and even chronic somatic conditions. As psychological interventions are mainly aimed at optimizing health, it would be interesting to investigate the effects of a psychological intervention in the context of a challenge that directly targets the factors that can negatively affect health.

To evaluate the effectiveness of a psychological intervention, participants can be exposed to a situation that simulates the target of the interventions, such as psychophysiological or physical challenges. In the case of psychological interventions that aim to optimize coping with stress, it is, for example, possible to provide participants with challenges that are known to increase stress, in order to observe participants' self-reported as well as psychophysiological responses to the controlled stressful situation. A well-known test to induce psychological stress is the Trier Social Stress Test, which involves a mental arithmetic test and a job interview in front of jury members who do not give any personal feedback (51). This test reliably affects self-reported state anxiety, the HPA-axis, the SAM-axis as well as the immune system (52). Additionally, physical challenges can also result in insightful evaluations of health outcomes, including providing participants with experimentally created wounds and subsequently observing the wound healing process (53). By incorporating those challenges into the study design, more information can be acquired on the effectiveness of psychological interventions in adequately handling with stress and subsequently on optimizing health outcomes. In summary, a model that can be proposed is shown in Figure 1.

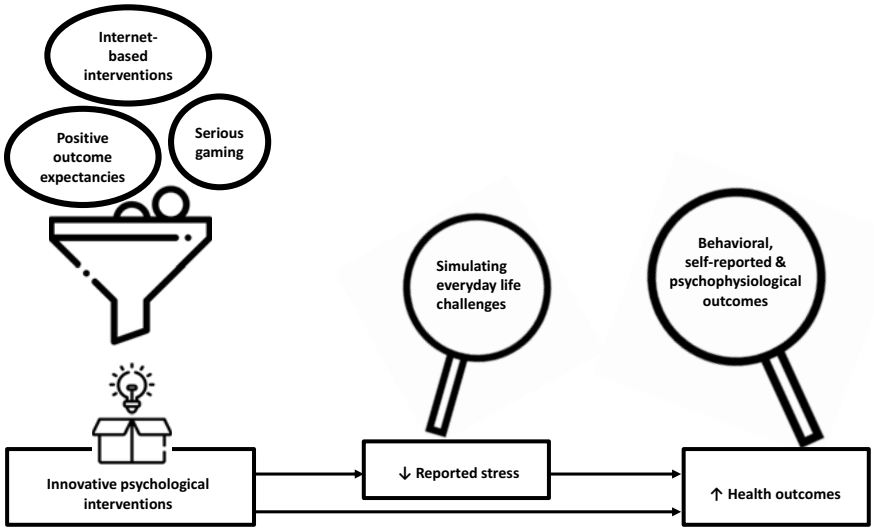


Figure 1. Hypothetical model of how to develop innovative psychological interventions and subsequently assess their effectiveness in optimizing health outcomes.

Aims and outline of the thesis

The main aim of this thesis is to examine the effectiveness of innovative psychological interventions on health optimization by (1) evaluating the effectiveness of innovative psychological tools, i.e., serious gaming, verbal suggestions, and internet-based interventions, to optimize various health behaviors and psychophysiological outcomes; (2) providing a concise overview of the currently existing evidence of psychological interventions in optimizing immune function in response to *in vitro* or *in vivo* immunological as well as psychophysiological challenges; and (3) incorporating various self-reporting, behavioral and psychophysiological outcome measures, but also physical and psychophysiological challenges, including psychophysiological, physical and/or cognitive stressors, to evaluate the effectiveness of psychological interventions on health outcomes. In Figure 2, a visual overview of the content of the present thesis is displayed.

Chapters 2 – 4 describe the results of innovative psychological intervention tools to optimize health outcomes. **Chapter 2** describes the results of a study on the effectiveness of serious gaming in optimizing health behaviors. In this study, health behaviors were evaluated through self-reporting, and by behavioral observations of participants' choices between unhealthy and healthy food products, as well as choosing between using stairs or elevator. Subsequently, **Chapter 3 and 4** report on the possible independent or additional role of innovative intervention tools. Specifically, **Chapter 3** reports on the effectiveness

of serious gaming and verbal suggestions in optimizing self-reported and actual food outcomes, and **Chapter 4** shows the results of a relaxation intervention accompanied or not with verbal suggestions in optimizing self-reported (e.g., state anxiety), as well as psychophysiological stress outcomes (e.g., heart rate and cortisol). **Chapter 5** provides a thorough meta-analytic review on the effectiveness of psychological interventions in optimizing immune function. This review summarized studies that incorporated an *in vitro* immunological, *in vivo* immunological or psychophysiological challenge into the study design and studied the effectiveness of psychological interventions in optimizing immune function. **Chapters 6** and **7** provide the design and results for a study on the effectiveness of a psychological intervention, based on a combination of multiple evidence-based innovative tools, on optimizing self-reported (e.g., vitality) and psychophysiological (e.g., immune outcomes, heart rate, cortisol) health outcomes. The effectiveness of psychological interventions on various health outcomes was evaluated by including *in vitro* and *in vivo* immunological as well as psychophysiological challenges into the study design. **Chapter 6** provides the rationale and design for this psychological intervention study and **Chapter 7** reports on the results of this study. Finally, **Chapter 8** provides an overarching overview and discussion of the findings from the present thesis by focusing on the scientific and clinical implications.

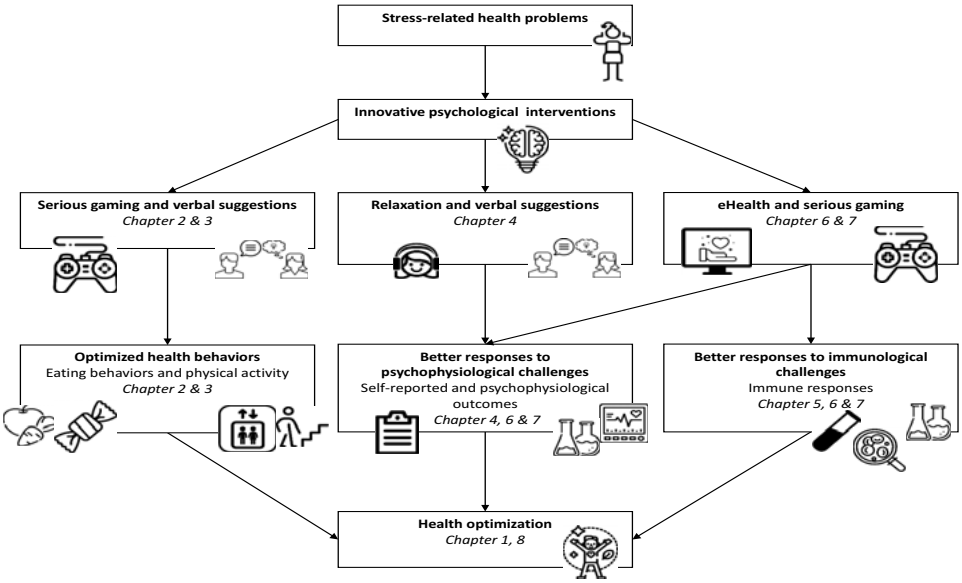
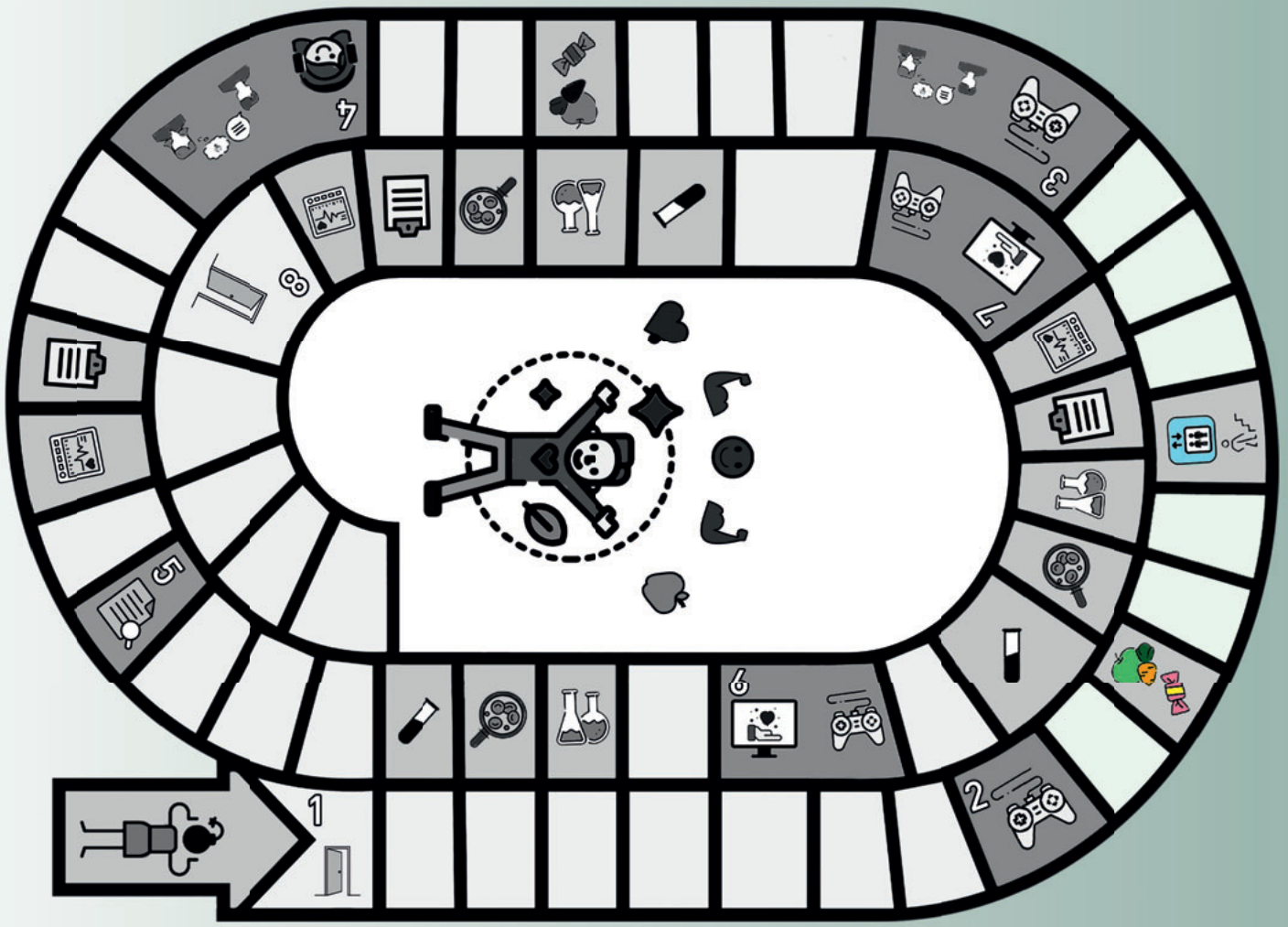


Figure 2. Visual overview of the thesis.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Optimizing self-reported healthy food preferences by serious gaming

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Abstract

Serious gaming is an upcoming and promising tool in healthcare practice. The aim of this experimental study was to examine whether health-related serious gaming could optimize food-related outcomes and physical activity. Eighty-one healthy participants (80% female) were randomly allocated to an experimental condition, in which participants played serious games based on transferring information, priming and evaluative conditioning, for half an hour, or a control condition, in which participants played non-health-related computer games. The primary study outcome was self-reported food preference and self-reported food choice, assessed by the Food Choice Task with food pairs differing in healthiness, or in both healthiness and attractiveness. Secondary outcomes were actual food choice and physical activity. A significantly healthier food preference for pairs differing in healthiness was found on the Food Choice Task in the experimental compared to the control condition. No significant differences were found on the other outcomes. This study provides preliminary support for the effects of serious gaming based on optimizing food preferences. More research is needed to confirm the present findings and to further elucidate and optimize the effects of serious gaming on health behaviours.

Introduction

When people are trying to adopt a healthy lifestyle by, for example, improving diet and/or physical activity, they often encounter barriers such as a lack of awareness, knowledge, motivation, and available facilities (54, 55). To overcome some of these barriers, serious gaming, an innovative approach directed at optimizing a healthy lifestyle, can potentially be helpful. Serious gaming has the purpose of educating and motivating users to change behaviours, doing so in an entertaining and engaging manner (25, 26), and distinguishes itself from traditional interventions by the combination of a serious component with a gaming component. Promising aspects of serious games are that they can be used to model positive health behaviours, provide opportunities to virtually engage in practicing health behaviours, transmit information about health, and provide immediate feedback on performance (26, 56). Recently, systematic reviews have provided preliminary support for the effectiveness of serious games on health behaviours. For example, serious games have been shown to increase knowledge about alcohol and other drugs (57) and to improve knowledge and self-management skills in various populations with chronic conditions, such as diabetes, asthma, and cancer (29). One review reported small positive effects of serious gaming on healthy lifestyle promotion and determinants of a healthy lifestyle such as knowledge. However, these results were heterogeneous: The largest effect sizes were found for increased knowledge, whereas smaller effect sizes were found for optimized behaviour change intentions, self-efficacy, and behaviour (28). Overall, these findings suggest that serious games are a useful tool to optimize knowledge concerning health behaviours, though the effects of serious gaming on behavioural outcomes, such as intentions and actual health behaviours, are less conclusive. There is no consensus concerning the duration of a serious gaming session or the number of sessions required to affect health behaviours, since the number and length of gaming sessions varies widely over studies. It has been shown that serious gaming can already be effective in improving health outcomes after one single session, although repeated exposure may lead to stronger effects and, previously, shorter interventions were found to be of lower quality generally (58).

Serious games for mental health often rely on cognitive behavioural therapy (CBT) related principles, which have been shown to be effective in changing health behaviours in adolescents and adults (59, 60). CBT related principles comprise various techniques directed at challenging cognitions underlying dysfunctional behaviours (3), and are furthermore possibly also effective in improving health behaviours such as diet and/or physical activity (61, 62). Serious games for mental health frequently make use of CBT based behaviour change techniques that particularly focus on modifying conscious processes, including education (25). In contrast, innovative behaviour change techniques

that fall outside the awareness of the participant have less often been incorporated in serious gaming paradigms in order to improve diet and/or physical activity. Some attempts show promising effects for some studies, especially in the field of cognitive training for health purposes (i.e., working memory, cognitive inhibition training) as well as cognitive bias modification. One study already combined implicit and explicit behaviour change techniques according to dual processing to improve adolescents' snacking habits, but was not able to improve snack choices (63). Potentially promising techniques for influencing behaviours more automatically are priming and evaluative conditioning. Priming involves exposing people to stimuli without them actually being aware of the influence these stimuli have on their subsequent judgments and behaviours (64, 65). Previous research has demonstrated that participants who were primed with motivating sentences about physical activity showed increased motivation to be physically active and exercised for a longer period (64, 65). In addition, evaluative conditioning involves changing the valence of a stimulus by repeatedly pairing this stimulus with other positive or negative stimuli (66). There is preliminary support for the effect of retraining automatic action tendencies towards unhealthy food items by means of evaluative conditioning with an approach-avoidance training. In this training, participants were repeatedly required to approach healthy food products and avoid unhealthy food products. In a subsequent computer task involving images of chocolate, the trained group showed faster avoidance responses than the control group (67-70). Although the effects of evaluative conditioning and priming on food consumption and physical activity are preliminary and have not yet been evaluated in the context of serious gaming, initial findings are promising. The beneficial effects of altering approach tendencies through evaluative conditioning have also been found in the alcohol domain, in hazardous drinkers as well as in alcoholic patients (71, 72). However, an attempt to incorporate attentional bias retraining into a serious game did not show any effects on self-reported drinking behaviour in heavy-drinking college students (73). In the field of healthy lifestyle, a pilot study investigated the add-on effectiveness of an approach avoidance training on an inpatient childhood obesity program did not find support for the effectiveness for an approach avoidance training in optimizing obesity treatment (74). Further research is needed to investigate the potential of applying evaluative conditioning techniques in serious gaming in combination with other techniques, as combining various behavior change techniques based on dual processing may optimize the effectiveness of serious gaming.

There are various ways to evaluate the effects of serious gaming on health behaviours. Previous research has primarily relied on self-reported outcome measures; however, observations of actual health behaviours could provide valuable additional information about health behaviours, since they are less influenced by demand characteristics than

is the case with self-reported outcome measures (75). Furthermore, previous studies have reported a discrepancy between self-reported intentions to change behaviours and actual health behaviour changes (76, 77). For example, although participants reported that they would choose a healthy snack, when confronted with the actual food choice they only actually chose a healthy snack in 27% of cases (78). For physical activity, also a gap exists between intentions and actual behaviour (79). Factors such as self-efficacy, self-control, and goal-setting are known to play a role in the decision-making processes of health behaviours such as food intake and physical activity: Higher levels of self-control, self-efficacy and goal-setting are related to increased levels of health-promoting behaviours (80-84). As self-reported outcome measures can provide valuable information on the mechanisms preceding an actual choice and observations of actual health behaviors evaluate the actual choice, including factors that play a role in the decision-making process, both self-reported outcome measures and observations of actual health behaviours should be assessed.

The aim of the present study was to evaluate the effects of health-related serious gaming on food-related outcomes and physical activity. In an experimental study, participants were randomly allocated to an experimental condition playing serious games, or a control condition playing non-health-related games for one half-hour session. The primary aim was to investigate whether health-related serious gaming influenced self-reported measures of food preference and food choice. It was hypothesized that participants who played the serious games would have a healthier food preference and make a healthier food choice than the participants who played the non-health-related games. The secondary aim was to explore whether health-related serious gaming influenced actual food choice (i.e., choosing a healthy or unhealthy option) and physical activity (i.e., taking the stairs or the elevator). It was hypothesized that participants who played the serious games would choose a healthy food option more often than participants who played the non-health-related games, and would more often take the stairs rather than the elevator. Factors previously found to be associated with food choice and physical activity, such as self-efficacy, self-control, and goal setting (80-84), were assessed with questionnaires and added as covariates in the analyses where appropriate.

Material and Methods

Participants

Eligible participants were recruited from Leiden University via written and online advertisements from February to April 2016. As the present study is a first proof-of-concept study, we included a rather homogeneous student sample to restrict possible alternative

explanations for the results of serious gaming on food-related outcomes and physical activity. Inclusion criteria were: Being between 18 and 35 years of age and speaking Dutch fluently. Exclusion criteria were: Severe physical or psychiatric conditions (e.g., heart disease, diabetes, and other serious conditions; or Diagnostic and Statistical Manual of Mental Disorders Fourth Edition Text Revision (DSM-IV-TR) psychiatric disorders), body mass index (BMI) ≥ 30 (since obesity is known to be significantly associated with an unhealthy lifestyle (85, 86)), and/or having any food allergies/intolerances. Participants were compensated with either €10 or course credits for their participation.

Design

Participants were randomized to an experimental condition or a control condition. Participants allocated to the experimental condition played serious games, whereas participants allocated to the control condition played non-health-related games. Both gaming conditions were similar in design and all games were provided in three different levels of difficulty. For the experimental condition, games pertaining to food and physical activity domains were used. Those games were derived from a 6-week serious game that was focused on optimizing health outcomes (87). These games (ViaNova©) were specifically developed for this purpose through a partnership between students of Delft University of Technology which were specialized in serious games and game technology, and researchers of the Health, Medical and Neuropsychology Unit of Leiden University. As the 6-week serious game did not only focus on lifestyle factors, but also on other health domains, including relaxation, sleep, cognitions and worldview, only a subset of this 6-week serious game pertaining to food and physical activity was used. Screenshots of each of the serious games and non-health-related games are presented in Appendix 1.

Games

In both conditions, each game started with an instruction screen presenting the aim of the game. This instruction screen could also be consulted at any time during the game. In total, participants played six different games. Those games were played during two sessions of 15 minutes each, separated by a five-minute break. Since the games vary in duration, some of the games were repeated more than once in order to keep the duration of the game sessions half an hour. The order in which participants played the games was random in both conditions for each participant and the number of games participants repeated depended on the speed with which participants read the instructions and completed the games. As an additional reinforcement for motivation during playing the games, participants were always rewarded with a virtual golden, silver or bronze medal at the end of each game depending on their performance.

In the experimental condition**,** participants performed several serious games with different intended strategies, i.e., transferring information, priming and principles of evaluative conditioning. First, to transfer information about health behaviour, participants had to play a game in which health-related facts appeared after making progress in the game. This serious game was repeated three times, with participants receiving a maximum of 12 facts in between playing the game (four facts during each game, e.g., 'being physically active each day for 30 minutes is beneficial for your health' with some examples of how to achieve that goal, such as taking the bicycle instead of the car in order to go to work) in total. Facts were formulated according to the guidelines of the Dutch Health Council. Additionally, two other serious games were directed at priming with healthy items, in which participants had to match three or more of the same healthy food products in a row, and healthy items and words based on food and physical activity had to be found in a newspaper. Finally, three serious games were directed at principles of evaluative conditioning, in which participants had to focus on healthy food products by collecting those items in one game, or had to push and click unhealthy items food and physical activity items away in the two other games. The food products included a broad range of items, with the healthy products including different types of fruits and vegetables (e.g., tomatoes, apples) and the unhealthy products including various high-caloric products (e.g., chips, chocolate). For the items related to physical activity, healthy items were related to exercising (e.g., pictures of people performing exercise) and unhealthy items were related to physical inactivity (e.g., people lying on a couch watching tv). The amount of items presented to participants was fixed in one game (40 items in total; see Appendix 1A), but depended on the speed with which they completed the game, as well as on the level of difficulty in the other games. In the control condition, participants performed six non-health-related games, in which they had to find the exit in a labyrinth and collect some neutral objects on the way to the exit, had to find similar non-health-related pictures, had to fill horizontal lines with different shaped blocks that fell down, had to find a wolf in a crowd of sheep where the wolf was only different from the sheep by a pointed nose, had to break a color code by guessing the pattern of colors, and, finally, had to reach the finish by moving around obstacles and collecting coins on the way to the finish.

Measures

Self-reported food preference and choice

The self-reported food preference and food choice were measured by a computerized Food Choice Task, which was adopted from a previous study (88), in which two products were presented each time. This Food Choice Task consisted of the same food product pairs as used in the study of Salmon and colleagues. These food products were presented

in two different types of tradeoff pairs; three product pairs solely differed in healthiness (i.e., chocolate versus grapes, chocolate cookie versus fruit biscuit, and Dutch caramel waffle versus banana) and three other food product pairs differed in healthiness as well as attractiveness in order to represent a self-control conflict by pairing one tasty, unhealthy food product with a healthy, less palatable food product (i.e., chocolate bar versus cereal cookies, crisps versus rice crackers with peanuts, and crisps versus mixed nuts and raisins) (88). First, participants had to rate for each product of the presented pair how strong their preference was for that specific product on a 7-point scale ranging from 1 (*not at all*) to 7 (*very much*) and subsequently, participants had to indicate which food product they would choose at that moment for each product pair.

Actual food choice

The actual food choice task was based on a previous study in which participants had to choose a snack from a set of four snacks (two healthy and two unhealthy snacks) (78). Participants were told that they could choose a food product that was introduced to them as a gift. A basket with tangerines, apples, candy bars, and pink glazed cakes was presented to the participant. Their food choice was recorded and registered as either healthy (i.e., tangerine or apple) or unhealthy (i.e., candy bar or pink glazed cake).

Physical activity

Physical activity was measured by observing participants' choice between the stairs and the elevator (89). Participants were instructed to move from the first to the fifth floor in order to receive their money or course credits. The stairs and elevator were both located in the same open area in close proximity to each other and were both easily accessible and visible. Their choice in taking the stairs or the elevator in order to move to the fifth floor was recorded. When participants reached the fifth floor, they had to indicate their motivation and intention to take the stairs in the future.

Self-efficacy

The 7-item healthy food factor of the Healthy Eating and Weight Self-Efficacy scale (HEWSE) was used to measure individual differences in self-efficacy (90). Items were judged on a 5-point scale from 1 (*strongly disagree*) to 5 (*strongly agree*), such as 'I am able to consume fruits and vegetables in most of my meals'. Scores can range from 7 to 35, with higher scores indicating higher levels of self-efficacy. The original English version of this questionnaire was translated to Dutch by two independent translators using a forward-backward translation method. A similar internal reliability was found in this study as in the original study (Cronbach's alpha = .81 (90)).

Self-control

The 13-item Brief Self-Control Scale (SCS) was assessed to measure individual differences in self-control on a 5-point scale ranging from 1 (*not at all*) to 5 (*very much*), such as 'I am good at resisting temptation' (81). Scores on this questionnaire can range from 13 to 65, with higher scores indicating higher levels of self-control. The Dutch translation of this questionnaire was used (82), for which a good internal reliability was found in the present study (Cronbach's alpha = .83).

Health behaviour goals and hunger

Healthy eating goal, goal to be physically active, and current level of hunger were measured by three separate items on a 7-point scale ranging from 1 (*not at all)* to 7 (*very much*) (88). Self-reported intention and motivation to take the stairs in future occasions were asked by filling out a 7-point scale from 1 (*totally disagree)* to 7 (*totally agree*) (91).

Procedure

The study procedure was approved by the local psychological ethics committee of Leiden University (CEP16-0222/78) and the study followed the rules stated in the Declaration of Helsinki (2013). Participants provided written informed consent prior to participation. They were told that the experiment consisted of a combination of three independent studies, namely a questionnaire study, a game study, and a food marketing study. This cover story was provided in order to keep participants naive for the actual purpose of the study and to minimize any influence of demand characteristics. Interested participants first completed several online questionnaires considering the inclusion and exclusion criteria, demographics, as well as some other questionnaires that were not relevant for this study aim. If participants were eligible to participate in the study, they were invited for a single lab session guided by a first test leader, which took place at the Faculty of Social and Behavioural Sciences of Leiden University, the Netherlands. First, baseline assessments were made of multiple psychological characteristics, including self-reported self-efficacy, self-control and health behavior goals, as well as some personality questionnaires that were not relevant for this study aim as these were used for educational purposes. Subsequently, participants were randomly allocated, based on a 1:1 allocation ratio as generated by an online random number generator (www.random.org), to the experimental condition or control condition. Participants were unaware of randomization or any differences between conditions during the experiment. During the gaming sessions, the test leader observed whether participants understood the instructions of the games and provided additional explanation concerning the instructions if necessary. After playing the games, participants had to complete the food choice task and completed some personality questionnaires that

were not relevant for this study aim as these were used for educational purposes. Next, participants were instructed to go from the first to the fifth floor of the building in order to receive money or course credits for their participation. Participants were unaware that a second test leader observed participants' choice in taking the stairs or the elevator. When they reached the fifth floor, they had to fill out two questions regarding motivation and intention to take the stairs in the future. They were also told that the study was sponsored by the marketing study and, therefore, they could choose one of the free food products. In fact, participants' choice between a healthy or an unhealthy food product was observed and recorded. At the end of the session, participants were debriefed about the actual purpose of the study and were asked whether they had heard details of the study beforehand other than the details delivered by the study personnel. All participants provided permission to use the observed data.

Data preparation and statistical analyses

Data were analyzed with IBM SPSS Statistics version 23 for Windows (IBM Corporation, Armonk, NY, USA) using a two-tailed significance level of $\alpha < .05$. To test the first hypothesis that participants playing the health-related games would report a healthier food preference compared to the participants playing the non-health-related games, analyses of covariance (ANCOVAs) were performed with food preference as a dependent variable, condition (experimental or control) as a between-subjects factor, and self-efficacy, self-control, and healthy eating goal as covariates. Since ratings of food preference were asked by presenting pairs of products, a relative food preference was computed by subtracting the unhealthy food preference rating from the healthy food preference rating for each product pair and subsequently calculating a sum score for the three pairs. Scores can range from -18 till 18, in which higher scores indicate a healthier food preference. Separate ANCOVAs were conducted for healthiness tradeoff pairs and pairs differing in healthiness as well as attractiveness in which covariates were included. Exploratory, analyses were repeated without inclusion of the covariates in order to elucidate the influence of the covariates. To test the effects of playing serious games on self-reported food choice, a similar approach was used. Analyses were performed with self-reported food choice (number of healthy food choices) as the dependent variable and condition as the between-subjects factor, again separately for healthiness tradeoff pairs and pairs differing in healthiness as well as attractiveness and with and without the above-mentioned covariates. In order to test the second hypothesis that participants playing the health-related games would more often choose the healthy food option than participants playing the non-health-related games, a logistic regression analysis was performed with actual food choice (healthy or unhealthy) as the dependent variable and condition as the between-subjects factor. Self-efficacy, self-control, and healthy eating goal were entered as covariates. To test whether the effects

were comparable after removing the covariates, a Chi square test was conducted with food choice as dependent variable and condition as between-subjects factor. In order to test the third hypothesis that serious gaming will result in taking the stairs more often compared to playing non-health-related games, a logistic regression analysis was performed with physical activity (taking the stairs or the elevator) as the dependent variable and condition as the between-subjects variable. Goal to be physically active was entered as a covariate. To test whether the effects were similar after removing the covariate, a Chi square test was conducted. Furthermore, to test whether intention and motivation to take the stairs in the future differed between the two conditions, an analysis of variance (ANOVA) was performed with the summed score of the two items regarding intention and motivation to take the stairs in the future as a dependent variable and condition as a between-subjects factor. Finally, since the two conditions differed significantly at gender, this factor was incorporated as a covariate in the subsequent analyses.

Results

Baseline characteristics

In total, 104 participants completed the online questionnaire. Twenty-one of them did not meet the eligibility criteria and therefore were excluded from further participation. Two participants did not show up for the lab session. In total, 81 participants (65 females, 80%), with an average age of 21.9 years (*SD* = 2.6; range 18 – 33) completed the study.

Forty participants were allocated to the experimental condition and 41 participants were allocated to the control condition. Gender significantly differed between the two conditions: 30% of the participants in the experimental condition were male compared to 10% in the control condition, χ^2 (1, *N* = 81) = 5.24, *p* = .02. Gender was therefore taken into account as a covariate and significant differences due to gender are reported below. Mean age, BMI, and hunger did not differ between the conditions. Also, no significant baseline differences between the two conditions were found for self-efficacy, self-control, healthy eating goal, and goal to be physically active (all $p > .20$). The outcomes for the experimental and control condition on the above-mentioned baseline measurements are presented in Table 1.

Table 1. Outcomes of age, gender, body mass index (BMI), self-control, self-efficacy, hunger, healthy eating goal and goal to be physically active, separately for the control condition and experimental condition.

	Control condition	Experimental condition	Significance (*p*)
Age, M (SD)	21.78 (2.41)	22.06 (2.72)	.63
Gender, *n* female (%)	37 (90.20)	28 (70.00)	.02
BMI, M (SD)	21.90 (2.88)	22.16 (2.99)	.69
Self-control, M (SD)	41.22 (8.39)	41.23 (8.38)	.99
Self-efficacy, M (SD)	24.85 (5.17)	24.15 (5.78)	.57
Hunger, M (SD)	2.88 (1.54)	2.58 (1.57)	.38
Healthy eating goal, M (SD)	5.46 (1.00)	5.20 (1.18)	.28
Goal to be physically active, M (SD)	5.20 (1.21)	5.15 (1.37)	.88

Note. M = Mean, SD = Standard Deviation

Self-reported food preference

The results for the experimental and control condition on all outcome measures are presented in Table 2. A significant main effect of condition for healthiness tradeoff pairs was found when controlling for self-efficacy, self-control, healthy eating goal, and gender (F (1, 75) = 5.02, p = .03, η^2 = .23). This effect indicated that the experimental condition showed a healthier food preference (M = 3.45, SD = 6.30) compared to the control condition (M = 1.20, SD = 5.54). The covariate self-efficacy turned out to be significant (F (1, 75) = 9.03, p = .004). A significant positive relation between self-efficacy and food preference was found (r = .41, p < .001). Exploratory, after exclusion of the covariates, a marginally significant main effect of condition remained (F (1, 79) = 2.93, p = .09, η^2 = .04; see Figure 1 left panel). No significant main effect of condition for pairs differing in healthiness as well as attractiveness was found on self-reported food preference when controlling for self-efficacy, self-control, healthy eating goal, and gender (F (1,75) = 1.36, p = .25). Exclusion of the covariates yielded similar results (F (1, 79) = .24, p = .63; see Figure 1 right panel).

Table 2. Outcomes on self-reported food preference, self-reported food choice, actual food choice and physical activity, separately for the control condition and experimental condition, without correction for the covariates.

	Control condition	Experimental condition	Significance (*p*)
Food preference H tradeoff, M (SD)	1.20 (5.54)	3.45 (6.30)	.09
Food preference H+A tradeoff, M (SD)	-2.41 (6.85)	-1.63 (7.71)	.63
Food choice H tradeoff, M (SD)	1.88 (0.84)	2.08 (0.83)	.29
Food choice H+A tradeoff, M (SD)	1.39 (0.95)	1.35 (0.98)	.85
Actual food choice, *n* healthy choices (%)	18 (46.15)	21 (55.26)	.42
Actual physical activity choice, *n* taking stairs (%)	17 (41.46)	16 (40.00)	.54

Note. M = Mean, SD = Standard Deviation, H tradeoff = healthiness tradeoff, H+A tradeoff = healthiness as well as attractiveness tradeoff.

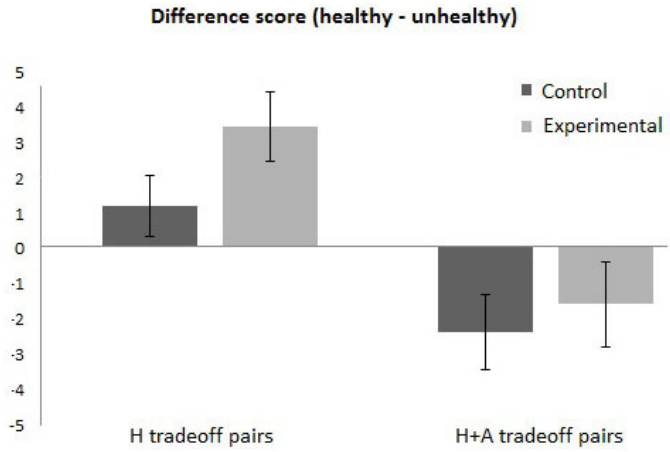

Figure 1. Mean and standard error of the mean for the relative food preference (a higher score on the y-axis represents a more healthy food preference) on healthiness tradeoff pairs (H tradeoff pairs; left panel) and pairs differing in healthiness as well as attractiveness (H+A tradeoff pairs; right panel) in the control condition versus the experimental condition.

In this figure, the experimental condition shows a trend towards a more healthy food preference, compared to the control condition on H tradeoff pairs, but no significant differences are shown for H+A tradeoff pairs.

Self-reported food choice

No significant main effect of condition for healthiness tradeoff pairs was found on self-reported food choice, when controlling for self-efficacy, self-control, healthy eating goal, and gender (F (1,75) = 1.57, p = .22). Exclusion of the covariates yielded similar results (F (1, 79) = 1.13, p = .29; see Figure 2 left panel). No significant main effect of condition was found on self-reported food choice for pairs differing in healthiness as well as

attractiveness on self-reported food choice when controlling for self-efficacy, self-control, healthy eating goal, and gender (F (1,75) = .14, p = .71). An explorative analysis excluding the covariates yielded similar findings (F (1, 79) = .04, p = .85; see Figure 2 right panel).

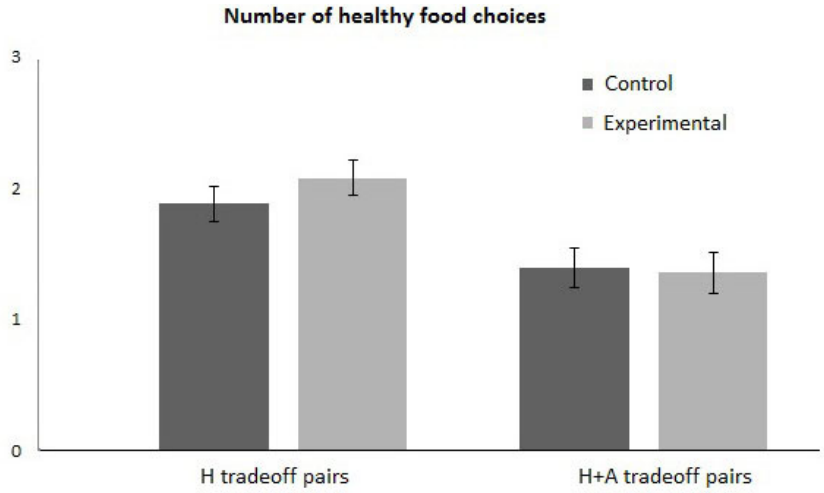

Figure 2. Mean and standard error of the mean for number of self-reported healthy food choices on healthiness tradeoff pairs (H tradeoff pairs; left panel) and pairs differing in healthiness as well as attractiveness (H+A tradeoff pairs; right panel) in the control condition versus the experimental condition.

In this figure, no significant differences are shown between the experimental and control condition in number of healthy food choices on H tradeoff pairs and H+A tradeoff pairs.

Actual food choice

Four participants did not make an actual food choice and were excluded from further data analyses concerning this outcome measure. No significant main effect of condition was found for actual food choice when controlling for self-efficacy, self-control, healthy eating goal, and gender (p = .41). An explorative analysis excluding the covariates also did not find a significant main effect of condition on actual food choice (X^2 (1, N = 77) = .64, p = .42).

Physical activity

No significant main effect of condition was found on physical activity when controlling for goal to be physically active and gender (p = .49). An explorative analysis excluding the covariates yielded a similar finding (X^2 (1, N = 81) = .018, p = .89). An ANOVA on intention and motivation to be physically active revealed no significant main effect of condition (F (1, 79) = .001, p = .98).

Discussion

Serious gaming is an upcoming and promising tool with widespread potential for application in health care. The present study is among the first to incorporate innovative behaviour change techniques of priming and evaluative conditioning into serious gaming in order to investigate the effects on self-reported as well as actual food choice and physical activity. The results provide support for the positive influence of a brief (30-minute) serious gaming session on self-reported food preference with regard to food pairs that differ in healthiness. No significant effects were found after a serious gaming session, however, in relation to food pairs that differ in both healthiness and attractiveness. The present study also yielded no effects on self-reported food choice, actual food choice, actual physical activity, and self-reported intention or motivation to be physically active. The results therefore provide limited support for the use of serious games based on CBT-based behaviour change techniques in the optimization of health behaviours.

In this study, participants reported a healthier food preference after playing serious games than participants playing non-health-related games. Significant results were found for healthiness tradeoff pairs when taking self-efficacy, self-control, healthy-eating goal, and gender into account as covariates. A trend for this finding remained when the analysis was performed without the covariates. Results on the pairs that differed in both healthiness and attractiveness were in the same direction, but no significant effects were found on these pairs after the participants had played either serious games or non-health-related games. Such pairs present a palatable, tasty option that is satisfactory in the short-term in opposition to a healthy option that is beneficial in the longer term (88), giving rise to a self-control conflict. This result suggests that playing brief serious games may not be effective in overcoming a self-control conflict. In addition, no significant differences between the two conditions were found for self-reported food choice. These results suggest that a healthier self-reported food preference does not necessarily translate into a healthier self-reported food choice after participants have played the serious games, which is in line with previous research (78). Also, no significant differences were found for actual food choice or physical activity. These results are in line with the results from a review by DeSmet and colleagues (2014) that show that optimized behaviour changes are more difficult to accomplish than intentions. The finding that playing the serious games does not affect actual behavioural outcomes contrasts with a review by Primack and colleagues, however, that shows that health outcomes can already be improved after a single session of gameplay (58). An important distinction between the present study and the review by Primack and colleagues lies in the population studied and the outcome measures. The present study involved healthy students, who were assessed on health behaviours through self-report as well as observations of actual health behaviours, whereas the review by Primack and colleagues primarily included individuals with various

somatic and psychological conditions, who were assessed on health behaviours with various outcome measures. This may clarify the discrepancy and future research should therefore also evaluate the effects of health-related serious gaming in target populations, such as people with overweight. Though the present study tried to optimize the effectiveness of serious gaming by combining multiple behaviour change techniques based on dual processing, no effects were found on behavioural outcomes. Those limited results are similar to those of a comparable study in the alcohol domain, involving a gamified attentional bias retraining (92) and a pilot study in the domain of optimizing lifestyle factors in children (74). The effects of a brief session of serious gaming based on a combination of behaviour change techniques, therefore, appear to be limited to self-reported food preference in healthiness tradeoff pairs. This is the first variable on which a change can be expected, since it can be seen as a first step towards a healthy food choice. Although the serious games that were played in the present study did not influence participants' actual food choice, previous literature has found that food preference plays an important role in actual food intake, in that the two are highly correlated (93). Possibly, transfer to actual health behaviour changes will take place after repeatedly performing serious gaming sessions, as is also underlined by research stating that learning effects are dependent on practice (94). The present study could therefore serve as a first step forward for future research on the effects of serious gaming incorporating various durations and number of sessions on health behaviours. The finding that self-efficacy was significantly related to self-reported food preference in healthiness tradeoff pairs indicates that participants with a stronger belief in their ability to engage in healthy eating behaviours also tend to have healthier food preferences. This is in line with previous literature demonstrating that self-efficacy is an important predictor of healthy food behaviours and weight control (95-97). Self-control was not significantly related to food preference. Due to the absence of a self-control conflict in the healthiness tradeoff pairs, it can be assumed that self-control does not significantly influence this relationship. This is in accordance with previous research suggesting that the presence of a self-control conflict is required in order to observe the role of self-control in food choices (80, 88). Furthermore, it should be noted that participants in the present study tended overall to have a strong healthy eating goal. This may have limited the role of the healthy eating goal in the relation between serious gaming and food preference.

The present study added several innovative features that are worth highlighting. First, the serious games in the present study consisted of a combination of providing information, priming and evaluative conditioning, which all have shown to be promising in changing health behaviours. By combining those techniques, the present serious gaming sessions relied on a rather strong empirical basis. The present study advances scientific knowledge regarding the effectiveness of serious gaming on health behaviours by combining various

behaviour change techniques based on dual processing, such as priming and evaluative conditioning and including such techniques, for the purpose of strengthening the effects of psychological interventions. Another strength of the present study is that self-reported outcome measures were implemented in combination with observations of actual behavioural outcome measures. By combining different methods of measuring health behaviours, the present study provides a more elaborated view of health behaviour change, contributing to the effects of serious gaming on health behaviours.

It is important also to note some limitations of the present study. First, the study involved a rather highly educated student sample with a high goal to eat healthily, including a large proportion of women. Possibly, by including a less healthy population, the effects of the intervention are more valid. Furthermore, although we aimed to include a rather homogeneous sample to rule out possible alternative explanations for our findings, the present sample was based on students from Leiden University and is therefore not representative for the general population regarding social economic status nor for the target population that needs the actual intervention. Therefore, future studies should also include other populations in order to test the generalizability of the results. Second, as the present study attempted to optimize the effectiveness of serious gaming by combining innovative CBT-based behaviour-change techniques based on dual processing, the current study design does not allow us to disentangle the effectiveness of the separate serious components of the serious gaming sessions. Since serious gaming include both a serious component as well as a fun component, future research should further evaluate the effectiveness of those separate components. Moreover, although beyond the scope of the present study, future research should further elucidate the effectiveness of the fun component of the serious gaming sessions by incorporating a non-gaming control group next to a non-health-related gaming group. Third, the serious games used in the present study is not yet evidence based and did not incorporate certain aspects, such as a storyline and personalization, that have been suggested in the literature to increase the effectiveness of serious games (92); possibly by incorporating these elements into the intervention, this could have been strengthened the effectiveness of the intervention. However, the literature regarding the effective components of serious gaming is still very much in its infancy. Fourth, in addition to the previous limitation, although we do not have indications that the games were not enjoyable for participants, in future studies it should be assessed whether people are engaged when playing the games since engagement is considered to be a major aspect of the effectiveness of these interventions. Nevertheless, it is difficult to properly assess the level of engagement; this field is currently developing rapidly and hopefully we will have good measures available in the nearby future. Fifth, future studies should further vary study duration as well as session frequency in order to see whether serious games could be a

promising stand-alone or add-on tool to CBT-based interventions in healthcare practice. Sixth, although the manipulation of actual physical activity is based on a manipulation used in a previous study (89), the present study is one of the first to incorporate such a manipulation of physical activity into the study design; future research therefore should further examine the psychometric properties of this manipulation. Moreover, it might be that the choice that people made regarding taking the stairs or the elevator affected their subsequent actual food choice, as this choice influences their calorie consumption. Although the calorie consumption may not be substantially altered by this physical activity choice, future studies should take the influence of physical activity on subsequent food choices into account. Seventh, although we have no indications that the cover story was not plausible for participants, filling in the questions regarding physical activity before the actual food choice manipulation may have influenced their food choice by making them aware that the study aimed to evaluate health behaviours. Eighth, although multiple serious games related to food as well as physical activity, the focus of the serious games lays more on food; this may have led to less effective results on physical activity. Ninth, although we controlled for self-reported self-efficacy, self-control and health behavior goals in all analyses, the present study does not have a baseline measurement of food choice and physical activity in order to compare pre to post intervention improvements. Since incorporating these measures could have given away too much information of the actual study aim and could have subsequently affected the outcomes, we chose to not include a baseline assessment on these measures in the current study. As we did not observe any significant differences between the two groups at baseline on self-reported self-efficacy, self-control and health behavior goals, we expect no differences in outcomes when incorporating baseline measurements. Finally, the present study incorporated a question regarding hunger, but in order to more profoundly control for level of hunger in future studies, it would be wise to instruct participants to refrain from eating and drinking for several hours before participation.

In conclusion, our findings cautiously suggest that a short session of serious gaming that incorporates multiple behaviour change techniques including priming and evaluative conditioning can serve as a first step forward in the optimization of health behaviours in healthy participants, by influencing their food preferences. However, taking into account that this study is a first proof-of-concept study, future research should confirm the present findings in order to further elucidate and optimize the effects of serious gaming. Moreover, future studies should evaluate various durations and frequencies of game play, as well as the effects of serious gaming in target populations. In addition, besides self-report outcome measures, it should structurally incorporate actual behavioural outcome measures in order to obtain more insight into the underlying mechanisms and to further optimize the effectiveness of serious games for healthcare practice.

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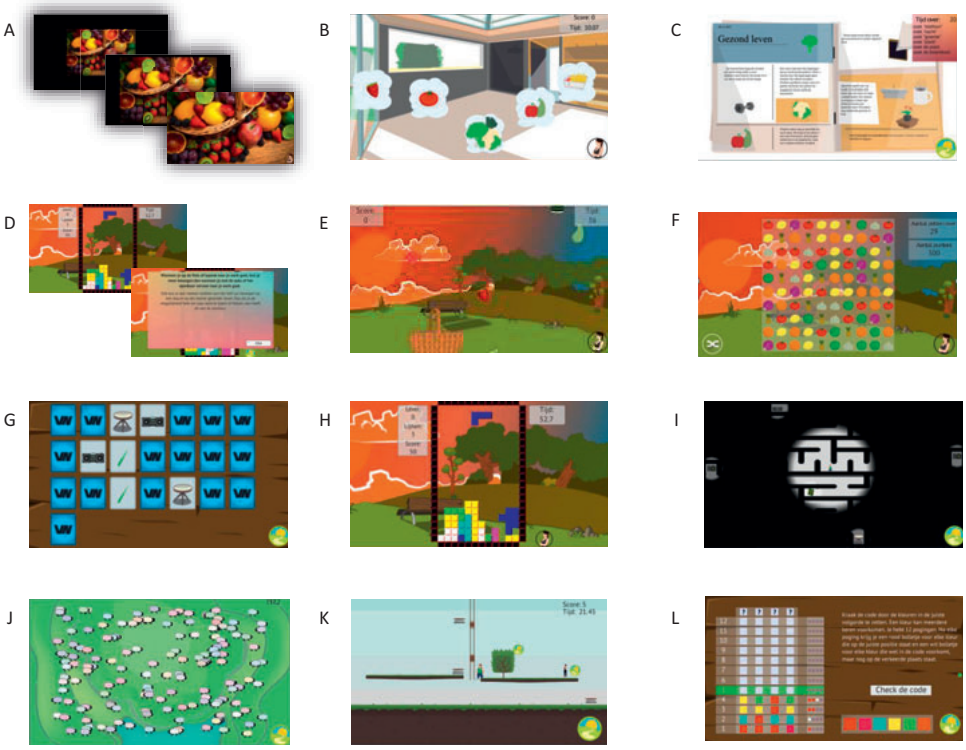
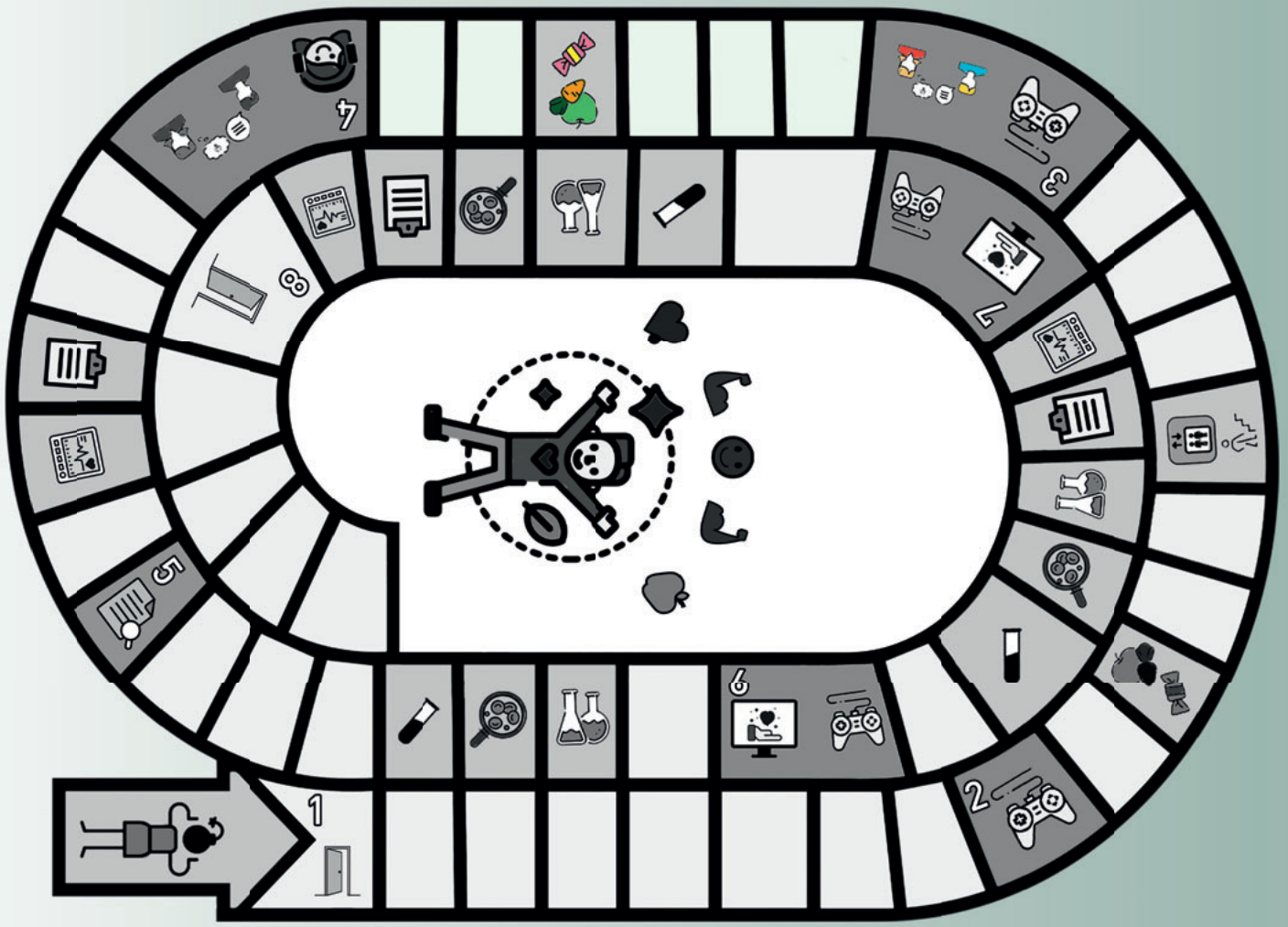
Appendix 1



Screenshots of each of the serious games and non-health-related games. (A) Serious game in which participants have to approach healthy items and avoid unhealthy items by using the corresponding arrows on the keyboard; (B) Serious game in which participants had to focus on healthy items by clicking unhealthy items away; (C) Serious game in which participants had to find healthy items and words in a newspaper; (D) Serious game in which participants had to fill horizontal lines with different shaped blocks that fell down and received health-related facts after filling multiple rows; (E) Serious game in which participants had to collect healthy items in a basket; (F) Serious game in which participants had to match three or more of the same healthy food products in a row; (G) Non-health-related game in which participants had to find similar non-health-related pictures; (H) Non-health-related game in which participants had to fill horizontal lines with different shaped blocks that fell down; (I) Non-health-related game in which participants had to find the exit in a labyrinth; (J) Non-health-related game in which participants had to find a wolf in a crowd of sheep where the wolf was only different from the sheep by a pointed nose; (K) Non-health-related game in which participants had to reach the finish by moving around obstacles and collection coins on their way to the finish; and (L) Non-health-related game in which participants had to break a color code by guessing the pattern of colors.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The effects of a gamified approach avoidance training and verbal suggestions on food outcomes

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Abstract

There is initial support for the effectiveness of approach-avoidance trainings in altering food-related health behaviors. Furthermore, outcome expectancies induced by verbal suggestions might optimize the effectiveness of these interventions, as shown in placebo research. The present study investigated the effectiveness of a gamified approach-avoidance training on food-related outcomes and whether verbal suggestions could strengthen those effects. A total of 120 participants were randomly assigned to 1 of 4 conditions: serious gaming only, verbal suggestions only, serious gaming combined with verbal suggestions, or a gaming control condition. Virtual food preference and food choice were assessed with a food choice task, with pairs differing in healthiness or in healthiness and attractiveness. Implicit food preference was assessed with an Implicit Association Test and food intake with a bogus taste test. Participants in both serious gaming conditions made healthier food choices for pairs differing in healthiness and attractiveness and had healthier implicit food preferences compared to gaming control. No effects were found on food intake. These findings provide the first preliminary support for the effects of a gamified approach-avoidance training on virtual food choice and implicit food preference. Future studies should further elucidate these effects, also in other health domains such as physical activity.

Introduction

Repeated exposure to appetitive food-related cues can result in approach biases towards unhealthy food products. These biases, in turn, can translate into unhealthy food behaviors (98-102). Such cognitive biases can be altered by applying approach-avoidance interventions (i.e., repeatedly approaching or avoiding certain stimuli) (103). There is some initial support for the effectiveness of approach-avoidance interventions in altering food-related stimulus evaluations, as reflected in reduced approach biases towards unhealthy food stimuli (101, 104-106). The results of approach-avoidance interventions on actual health behaviors such as food consumption are, however, less conclusive. More specifically, one study did find positive effects of an approach-avoidance intervention on actual food consumption (101), whereas several other studies reported non-significant effects on actual food consumption (99, 104, 107). A possible explanation for these inconclusive results on actual health behaviors comes from a qualitative study showing that a lack of excitement is often experienced in approach-avoidance interventions due to its repetitive nature (108).

Serious gaming can potentially be a useful tool to enhance the engagement of approach-avoidance interventions. Serious gaming is an umbrella term for computer-delivered interventions that provide training and education in an entertaining way (26). This innovative tool is increasingly applied in healthcare practice (26, 109), and recent studies in the food-related health domain have provided preliminary evidence for its effectiveness in optimizing food-related outcomes, including food intake (110-112). A meta-analysis on the effects of serious gaming on healthy lifestyle indicated heterogeneous results, however, which can be at least partially due to the fact that serious games often lack evidence-based interventions (109). A gamified approach-avoidance training has been investigated in one study so far, in the alcohol domain, which showed that a gamified approach-avoidance training produced similar results as a more traditional approach-avoidance training paradigm (113). Although the effects of non-gamified approach-avoidance training have been investigated before in various domains, including healthy food behavior [2, 4, 7, 10], the effects of gamified approach-avoidance training have not yet been investigated in healthy food behavior.

Besides the lack of excitement that people often experience when completing approach-avoidance interventions, it was shown that those interventions are often faced with a lack of perceived credibility towards the helpfulness of such interventions (108). Verbal suggestions can possibly optimize the effectiveness of gamified approach-avoidance trainings, since verbal suggestions are able to optimize perceived treatment credibility and health outcomes, as shown previously particularly in placebo research (32, 114). First,

this might be accomplished by influencing expectancies regarding the effectiveness of an intervention, i.e., outcome expectancies (114, 115). Prior studies have demonstrated the effectiveness of outcome expectancies induced by verbal suggestions in relieving itch and pain in healthy participants (33, 116), and have shown that verbal suggestions are able to induce analgesic effects in various clinical patient populations, including patients with irritable bowel syndrome and patients undergoing thoracotomy (34, 117, 118). A second way to influence health outcomes is by means of verbal suggestions that influence specific actions of approaching and avoiding certain stimuli without actually performing these actions, i.e., stimulus-response contingency instructions (119-122). Verbal suggestions concerning stimulus-response contingencies were recently shown to alter evaluations of fictitious social groups or meaningless words (120-122). These findings suggest that the effectiveness of gamified approach-avoidance trainings might be strengthened by verbal suggestions.

The present study aimed to investigate the effects of gamified approach-avoidance training on food-related outcomes and whether verbal suggestions could strengthen those effects. In this study, four conditions were compared: a gaming control condition, a serious gaming only condition, a verbal suggestions only condition, and a combined serious gaming and verbal suggestions condition. Virtual food preference and food choice, as assessed by a food choice task, were the primary study outcomes. Secondary outcomes were implicit food preference, as measured by an Implicit Association Test (IAT), and actual food intake, which was measured by a bogus taste test. It was hypothesized that both serious gaming conditions combined (i.e., with or without verbal suggestions) would show improved food-related outcomes compared to the gaming control condition. It was further explored whether the combined serious gaming and verbal suggestions condition would outperform the serious gaming only condition as well as the verbal suggestions only condition. The role of possible moderating factors such as self-control, self-efficacy and healthy eating goal was also explored (123-127).

Methods

Ethics statement

The protocol was approved by the local psychological ethics committee of Leiden University (registration code: CEP16-0728/261) and was preregistered at the Netherlands Trial Register (registration code: NTR6198). The study was performed according to the Declaration of Helsinki (2013).

Design

The present study used a randomized experimental study design. Participants were randomly allocated, based on a 1:1:1:1 allocation ratio as generated by an online random number generator (www.random.org), to one of the four conditions, stratified for gender. During the experiment, participants were unaware of the existence of four different conditions and therefore blinded for randomization.

Participants

A total of 120 participants were included in this study. Eligible participants were recruited by written and online flyers which were distributed from September to November 2016 at the campus of Leiden University. Participants had to be fluent in Dutch and between 18 and 35 years old. Exclusion criteria were: (a) severe physical or psychiatric conditions (e.g., chronic somatic diseases affecting daily life or Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition Text Revision [DSM-IV-TR] psychiatric disorders) that interfered with the study protocol, (b) body mass index (BMI) ≥ 30 (given the significant association of obesity with unhealthy lifestyles (128, 129)), and/or (c) having any food restrictions.

Experimental conditions and control condition

The serious games and control games were developed in collaboration with Delft University of Technology (ViaNova©). See Figure 1 for screenshots of all games.

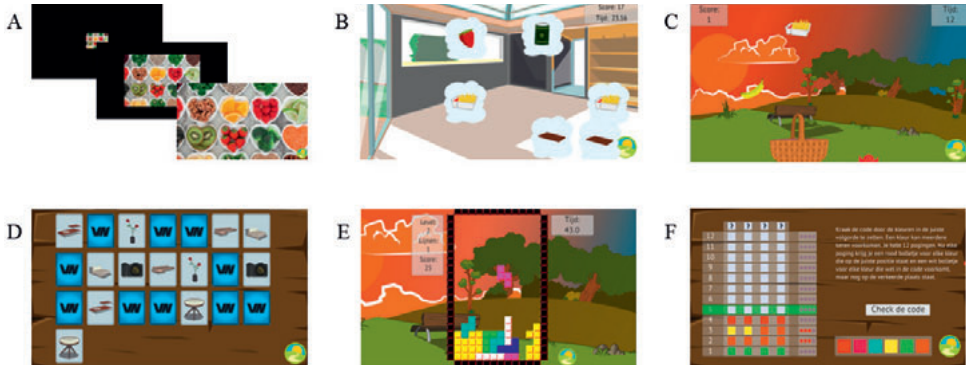

Figure 1. Screenshots of all games.
(A) Serious game in which participants were instructed to approach healthy items and avoid unhealthy items by pressing the corresponding arrows on the keyboard; (B) Serious game in which participants were instructed to click away the unhealthy items; (C) Serious game in which participants were instructed to collect the healthy items in a basket; (D) Non-health-related game in which participants were instructed to find and match similar pictures; (E) Non-health-related game in which participants were instructed to complete horizontal lines with various shaped blocks that fell down; (F) Non-health-related game in which participants were instructed to guess a color code by identifying the color pattern.

In all gaming conditions, participants first saw an instruction screen that informed them about the aim of the game. All games were comparable in their appearance and were provided with three different levels of difficulty. All games had a duration around one minute for each game and participants played all three games on the three levels of difficulty twice. In total, participants played 18 games for half an hour, divided into two sessions of 15 minutes, each with a 5-minute break in-between. To motivate participants, they were always rewarded with a virtual medal (golden, silver or bronze) at the end of each game, depending on their performance. In most of the games, participants could earn points and keep track of their performance through a score bar presented at the top of the screen. Accuracy and reaction times were tracked during the games to match the rewards with the performance of participants (note that they were not saved in a log file). In both serious gaming conditions, participants performed a gamified approach-avoidance intervention pertaining to food. Participants were exposed to three different games (see Figure 1A, 1B and 1C) that were all based on the same principle (i.e., approach-avoidance training) aiming to keep participants motivated in playing the games. The food products included in the games entailed various healthy food items, including different types of fruits and vegetables (e.g., pineapple, paprika) and unhealthy food items, including various high-caloric products (e.g., fries, cookies). In all three serious games, participants had to approach healthy items and avoid unhealthy items in an object-referenced way. In two games, participants had to push or click away (avoid) unhealthy items (see Figure 1A and 1B), and in one other game, participants had to focus on healthy items by collecting (approaching) these items and avoiding unhealthy items (see Figure 1C). In the gaming control condition, participants performed three non-health-related computer games, in which the instruction was to match similar non-health-related pictures in one game (see Figure 1D), to complete horizontal lines with different shaped blocks that fell down in another game (see Figure 1E), and to unlock a color code by guessing the color pattern in a third game (see Figure 1F). All games were presented to participants on a computer screen and participants could use the computer mouse and keyboard to play the games.
In the condition that was provided solely with verbal suggestions as well as in the combined serious gaming and verbal suggestions condition, participants received verbal suggestions. The verbal suggestions focused on the effectiveness of the serious games in order to induce outcome expectancies, and also informed participants about stimulus-action contingencies of the approach-avoidance training in the serious games. More specifically, participants were provided with the following verbal suggestions (translated from Dutch):

> *"You will play mini games for 15 minutes. After that you will have a break for a few minutes and then you will play the mini games for another 15 minutes.*

There are three different mini games. In each of these mini games, you will repeatedly respond to healthy and unhealthy stimuli. In the first mini game, you will see healthy and unhealthy food images. Your task is to pull images of healthy food products towards you and to push images of unhealthy food products away. In the second mini game, you will see images of healthy and unhealthy food products flying over. Your task is to keep healthy food products and click unhealthy food products away. In the third mini game, you will learn to make healthy choices. You will do this by catching healthy food products in a picnic basket and avoid the unhealthy food products.

Prior research has shown that playing each of these mini games is effective in improving dietary habits."

The verbal suggestions were followed by the information that some other tasks first had to be completed before the games would be played (verbal suggestions only condition) or that the games would be played immediately (combined serious gaming and verbal suggestions condition). In the verbal suggestions only condition, it was emphasized to participants that they had to make sure they would not forget the instructions in order to play the games accurately later on. Thereafter, they were exposed to the food-related outcome tasks. After completion of each task, the verbal suggestions regarding the instructions of the games were repeated. Although participants in the verbal suggestions only condition were told that they would play the games after those outcomes, they did not play the games anymore. In the combined serious gaming and verbal suggestion condition, participants were only provided once with the verbal suggestions.

Food-related outcomes

Food Choice Task

During a computerized food choice task adapted from a previous study (126), participants were presented with seven food product pairs (including one example pair) each containing one healthy food item and one unhealthy food item. As already determined in the previous study of Salmon and colleagues (2014), there were two different types of food product tradeoff pairs of which the first type of product pairs differed in healthiness (i.e., chocolate versus grapes, chocolate cookie versus fruit biscuit, and Dutch caramel waffle versus banana) and the second type of product pairs differed in attractiveness as well by pairing one tasty, unhealthy food product with a healthy, less palatable food product (i.e., chocolate bar versus cereal cookies, crisps versus rice crackers with peanuts, and crisps versus mixed nuts and raisins) (126). For each pair, participants had to rate how strong their preference was separately for the healthy and unhealthy food product on 7-point scales ranging from 1 (*not at all*) to 7 (*very much*) and they had to indicate which of the two food products they would choose at that moment. A relative food preference

was computed for each food product pair by subtracting the unhealthy food preference rating from the healthy food preference rating and subsequently calculating a sum score. Separate scores were determined for the healthiness tradeoff pairs and the healthiness and attractiveness tradeoff pairs. Scores can range from -18 to 18, with higher scores indicating healthier food preferences. Food choice was determined by summing the healthy food choices, with scores ranging from 0 to 3.

Implicit Association Test

The food-related IAT used in the present study was based on a previously validated task (130) with slight changes to some items within categories as to fit the content of the task to the present study purpose. In this task, participants were instructed to categorize pleasant (i.e., happy, smile, peace, joy, pleasure) and unpleasant (i.e., pain, death, poison, sickness, vomit) words, next to healthy (i.e., fruits, banana, vegetables, salad, water) and unhealthy (i.e., chocolate, candy, cake, pastry, cookie) food-related words. The IAT consisted of five blocks. It started with a practice block of ten trials in which food-related words were presented and participants were asked to label these words as either unhealthy (left label) or healthy (right label). Thereafter, another practice block was presented to participants with ten trials in which pleasant and unpleasant words were each presented and participants were asked to assign these words to either positive (left label) or negative (right label) categories. The third and fifth block were test blocks consisting of 40 trials each in which participants had to assign both healthy and unhealthy food-related words, as well as pleasant and unpleasant words to different evaluative categories labeled with 'unhealthy or positive' (left label Block 3) or 'healthy or positive' (left label Block 5). In the fourth block, consisting of 10 trials, participants again had to categorize food-related words either as healthy or unhealthy, but now with reversed category locations as compared to Block 1 (i.e., left label = 'healthy' and right label = 'unhealthy'). In order to measure the strength of the association between healthy and unhealthy food-related words and the positive and negative valence, participants were instructed to perform the task as fast and accurately as possible. The IAT has been shown be a reliable measure with good predictive validity in measuring behavioral preference towards healthy and unhealthy food items (130). Implicit food preference was calculated using the D4-algorithm (131), in such a way that higher scores indicate a healthier food preference.

Bogus taste test

In order to measure actual food consumption, a bogus taste test was adopted from previous research (132). Participants were presented with three different unhealthy food products (i.e., 75 grams of crisps, 225 grams of mini Dutch cookies, and 325 grams of M&Ms). These products were presented in separate bowls. For each food product,

two identical bowls were presented to participants, who were informed that there were small differences between the food products, whereas these were actually identical. Participants were asked to rate the products from both bowls on various characteristics (e.g., sweetness, crispness) regarding any differences of the food products. Participants were informed that they could eat as much as they wanted and were given 10 minutes to complete their ratings. Unbeknownst to the participants, all bowls were weighed before and after the test in order to explore the total food consumption. Total food consumption was computed by subtracting the weight of all bowls after finishing the taste test from the weight before the start of the taste test.

Possible moderating factors

Self-control

Self-control was measured by the 13-item Brief Self-Control Scale (SCS). Participants completed items on a 7-point scale ranging from 1 (*not at all*) to 5 (*very much*) (127). Scores on this questionnaire can range from 13 to 65, with higher scores representing higher levels of self-control. The Dutch translation of this questionnaire was used (123), which was found to have a good internal reliability (Cronbach's alpha = .84).

Self-efficacy

The healthy food factor of the Healthy Eating and Weight Self-Efficacy scale (HEWSE) was used to measure self-efficacy. This questionnaire consists of 7 items (133). Participants completed items on a 5-point scale from 1 (*strongly disagree*) to 5 (*strongly agree*). Scores can range from 7 to 35, with higher scores representing higher levels of self-efficacy. A Dutch translation of the original English version was made by two independent translators applying a forward-backward translation method. A good internal reliability was found in the present study (Cronbach's alpha = .81), comparable to the original study (133).

Healthy eating goal, hunger and appetite

Healthy eating goal was measured by a single item ('*To what extent do you have the goal to eat healthily?*') on a 7-point scale ranging from 1 (*not at all*) to 7 (*very much*) (126). In addition, hunger, appetite and 'feeling like a bite' were measured by separate single items ('*To what extent are you hungry / do you experience appetite / do you feel like a bite at the moment?*') on a 7-point scale ranging from 1 (*not at all*) to 7 (*very much*).

Procedure

Prior to participation, participants were informed that the experiment was about games and food, without further detailed information about the actual study purpose, and

written informed consent was provided. First, several online questionnaires considering the inclusion and exclusion criteria, demographics, and some other questionnaires not related to the present study aim were completed. If participants were eligible to participate in the study, they were invited for a single lab session that took place at the Faculty of Social and Behavioural Sciences of Leiden University, the Netherlands. Participants were instructed to refrain from eating and drinking except for water for two hours prior to the lab session. At the start of the lab session, baseline psychological characteristics, including self-control, self-efficacy and healthy eating goal, were assessed. After randomization to one of the four conditions, participants were subjected to the food choice task, followed by the IAT and bogus taste test. The order of the IAT and bogus taste test was counterbalanced across participants. In the verbal suggestions condition, the verbal suggestions were repeated after each task. After the tasks, participants had to complete some questionnaires regarding psychological characteristics, which are not described here since they are unrelated to the present study aim. At the end of the session, participants were debriefed about the actual study purpose and received compensation for their participation (€10 or course credits).

Data preparation and statistical analyses

Data were analyzed using IBM SPSS Statistics for Windows (Version 23; IBM Corporation, Armonk, NY, USA) with a two-tailed significance level of $\alpha < .05$. The sample size calculation was performed in G*power 3.1 (134). Based on an effect size f of .31 from a previous study on the effects of serious gaming on virtual food preference and food choice (135), a total sample size of 30 participants in each group, including 5 drop-outs (120 in total), was deemed sufficient to obtain a power of .80 with an $\alpha = .05$. Two participants were excluded from the data analyses due to protocol deviations during the lab session (i.e., incorrect sequence of task completion). The data on the covariates were not processed adequately for one participant due to technical problems and for one participant the data on the food choice task were not processed adequately. Furthermore, actual food consumption was not weighed correctly for one participant and one participant did not want to eat one of the food products. Therefore, data of 117 participants were available for analyses on virtual food preference and food choice, as well as for implicit food preference, whereas data of 116 participants were available for analyses on virtual food preference and food choice and data of 115 participants were available for analyses of actual food consumption.

Concerning the food choice task, separate analyses were conducted for pairs differing in healthiness and pairs differing in both healthiness and attractiveness. Because the primary hypothesis of the study was that serious gaming, with or without verbal suggestions,

would optimize food outcomes, we performed Analyses of Covariance (ANCOVAs) that tested the effect of the between-subjects factor of Type of Game (serious game vs. control game) by comparing each of the different outcomes for participants in the serious gaming conditions combined, i.e., with and without the verbal suggestions, with food preferences for participants in the gaming control condition. In case there was a significant effect of Type of Game, Holm's corrected pairwise comparisons were carried out to compare each of the four study conditions (gaming control, serious gaming only, verbal suggestions only, and combined serious gaming and verbal suggestions) separately, in order to receive more insights in the possible effective components of serious gaming (and verbal suggestions). Self-efficacy, self-control, and healthy eating goal were entered as covariates in all analyses.

Results

Participant characteristics

151 participants completed the online questionnaire. The eligibility criteria were not met by 26 participants and they were therefore not included in the present study. Five participants did not show up for the lab session. In total, 120 participants (97 women; 80.8%), with an average age of 21.3 years (*SD* = 2.4; range 18 – 31), completed the study. Baseline characteristics for the four conditions are presented in Table 1. Mean age, gender, BMI, hunger, appetite and feeling like a bite did not differ between the conditions. Also, no significant baseline differences for self-efficacy, self-control, and healthy eating goal were found (all *p*-values > .05).

Table 1. Descriptives for the four conditions separately

	Gaming control (*N* = 28)	**Serious gaming** (*N* = 29)	**Verbal suggestions** (*N* = 30)	**Serious gaming + verbal suggestions** (*N* = 31)
Age	20.89 (1.85)	20.72 (2.42)	22.13 (2.91)	21.32 (2.18)
Body Mass Index	22.53 (2.61)	21.99 (1.78)	22.07 (2.79)	22.21 (2.59)
Sex, *n* female (%)	24 (85.70)	24 (82.80)	23 (76.70)	25 (80.60)
Hunger	3.82 (1.98)	3.97 (1.61)	3.77 (1.85)	4.20 (1.38)[1]
Appetite	4.18 (2.04)	4.66 (1.57)	4.00 (1.74)	4.67 (1.40)[1]
Feeling like a bite	4.36 (2.09)	4.76 (1.41)	4.23 (1.89)	4.97 (1.43)[1]
Self-control (SCS)	38.50 (7.95)	37.21 (9.14)	42.10 (6.61)	39.03 (8.51)
Self-efficacy (HEWSE)	24.07 (5.02)	23.83 (4.72)	24.60 (4.53)	24.70 (5.19)[1]
Healthy eating goal	5.21 (1.07)	4.90 (1.08)	5.10 (0.96)	5.37 (0.85)

[1]*N* = 30.
Note. SCS = Self-Control Scale, HEWSE = Healthy Eating and Weight Self-Efficacy scale

Virtual food preference

The results for food-related outcome measures are presented in Table 2. The virtual food preference for the different conditions is presented in Figure 2. No significant differences between conditions were found for the healthiness tradeoff pairs nor the healthiness and attractiveness tradeoff pairs (both *p*-values > .05).

Table 2. Means and standard deviations of food-related outcome measures per condition.

	Gaming control (*N* = 28)	**Serious gaming** (*N* = 29)	**Verbal suggestions** (*N* = 30)	**Serious gaming + verbal suggestions** (*N* = 30)
Food preference H tradeoff	1.50 (5.32)	1.03 (5.52)	4.53 (6.62)	2.38 (7.82)[1]
Food preference H+A tradeoff	-2.36 (5.06)	-3.93 (5.03)	-2.93 (7.89)	-0.66 (6.61)[1]
Food choice H tradeoff	1.89 (0.79)	1.83 (0.89)	2.10 (0.66)	1.90 (1.05)[1]
Food choice H+A tradeoff	0.79 (0.79)	0.97 (0.78)*	1.03 (0.81)	1.34 (0.90)*[1]
Implicit food preference	0.51 (0.64)	0.84 (0.49)*	0.79 (0.50)	0.88 (0.46)*
Food Consumption	58.71 (23.96)	66.04 (26.43)	59.02 (23.18)[2]	66.33 (25.88)

*indicates a significant difference between both serious gaming conditions combined, i.e., with and without the verbal suggestion, and the gaming control condition
[1]*N* = 29.
[2]*N* = 28.
Note. H tradeoff = Healthiness tradeoff, H+A tradeoff = Healthiness and Attractiveness tradeoff

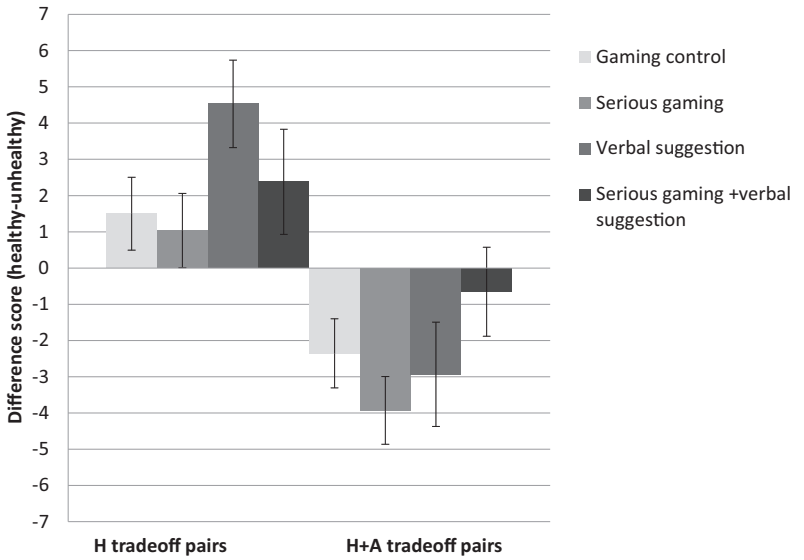

Figure 2. Means and standard errors of the mean for virtual food preference.

H tradeoff pairs = Healthiness tradeoff pairs; H+A tradeoff pairs = Healthiness and Attractiveness tradeoff pairs. A higher score on the y-axis represents a more healthy food preference. No significant differences were found in relative food preference between the four conditions on H tradeoff pairs and H+A tradeoff pairs.

Virtual food choice

In Figure 3, virtual food choice for the different conditions is presented. We did not find a significantly healthier virtual food choice on healthiness tradeoff pairs in the serious gaming conditions (p = .95).

On pairs differing in healthiness as well as attractiveness, however, a significantly healthier food choice was found for both serious gaming conditions combined, i.e., with and without the verbal suggestions, compared to gaming control, F (1,81) = 4.54, p = .036, η^2 = .13. Pairwise comparisons did not yield any significant differences between the groups (all adjusted p-values > .05).

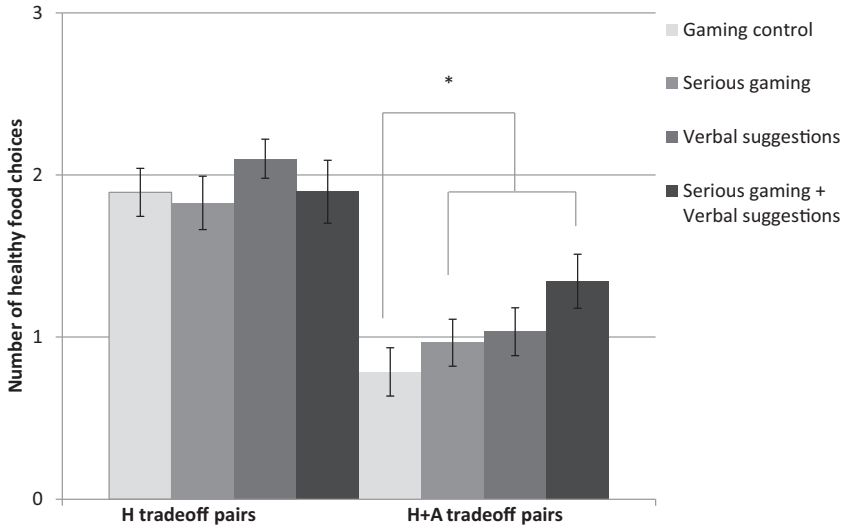

Figure 3. Means and standard errors of the mean for number of virtual healthy food choices.

H tradeoff pairs = Healthiness tradeoff pairs; H+A tradeoff pairs = Healthiness and Attractiveness tradeoff pairs. A significant difference was found in that the two serious gaming conditions combined, i.e., with and without the verbal suggestion, showed a higher mean of healthy food choices on H+A tradeoff pairs compared to gaming control. No significant differences between the four conditions were found for H tradeoff pairs.

Implicit food preference

Implicit food preference outcomes are presented in Figure 4. A significantly healthier implicit food preference was found for both serious gaming conditions combined, i.e., with and without the verbal suggestions, compared to the gaming control condition, F (1, 82) = 8.09, p = .006, η^2 = .14. Pairwise comparisons showed a trend towards a healthier implicit food preference for the combined serious gaming and verbal suggestions condition compared to gaming control, F (1, 53) = 6.383, adjusted p = .090, η^2 = .14.

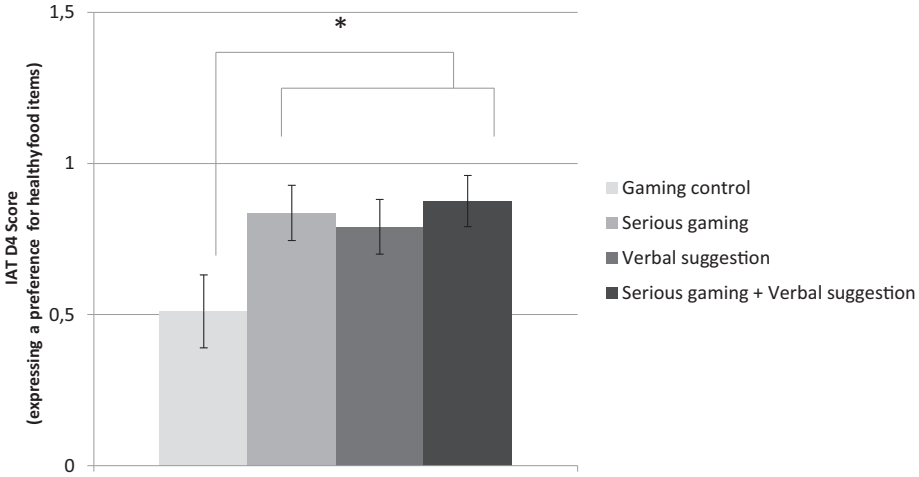

Figure 4. Mean and standard error of the mean for implicit food preference.

A higher score on the y-axis represents a more healthy implicit food preference. A significant difference was found in that the two serious gaming conditions combined, i.e., with and without the verbal suggestions, showed a higher implicit preference for healthy food items compared to gaming control.

Food consumption

The results for the four conditions on implicit food preference are presented in Figure 5. No significant differences between conditions were found on the bogus taste test (p = .17).

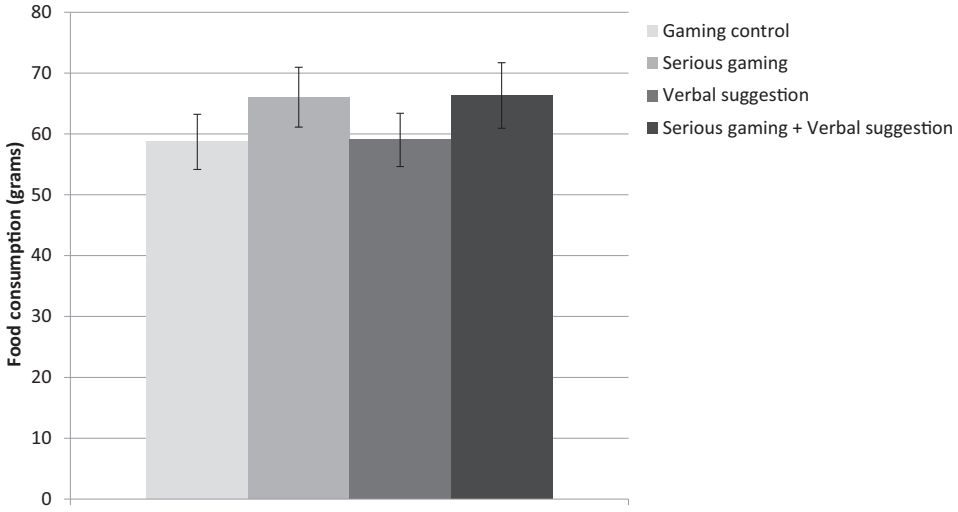

Figure 5. Mean and standard error of the mean for total amount of food consumption.

No significant differences between the four conditions were found in total amount of food consumption.

Discussion

The present study used an innovative approach of combining a gamified approach-avoidance training and verbal suggestions to optimize food-related outcomes. A gamified approach-avoidance training resulted in more healthy virtual food choices for pairs differing in both healthiness and attractiveness, and also in a healthier implicit food preference, compared to a non-health-related gaming session. No effects were found on actual food consumption. By investigating the effectiveness of a gamified approach-avoidance training and the add-on effects of verbal suggestions on multiple food-related outcome measures, this study extends current literature on the effectiveness of approach-avoidance trainings in optimizing food outcomes and shows that a gamified approach-avoidance training with or without verbal suggestions affect both virtual and implicit food preference.

Concerning virtual food preference and food choice, the gamified approach-avoidance training resulted in more healthy virtual food choices on pairs differing in healthiness as well as attractiveness compared to playing non-health-related games. On healthiness tradeoff pairs, however, we did not find a healthier virtual food preference and choice after serious gaming. Because the present study included a sample with a relatively high healthy eating goal (i.e., M = 5.1 on a 7-point scale), the tradeoff pairs solely differing in healthiness possibly were not challenging enough to optimize these goals even further by means of serious gaming with or without the add-on of verbal suggestions. Instead, opportunities for optimization of food choices are provided by administering a more challenging tradeoff of food pairs differing in healthiness as well as attractiveness, which is intended to generate a self-control conflict (126).

Implicit food preference was also optimized after playing the serious games, in that participants in the serious gaming conditions showed a higher preference for healthy food products over unhealthy food products compared to the gaming control condition. Actual food intake was, however, not affected by playing the serious games and/or providing verbal suggestions. Hence, in our data, the effects of the gamified approach-avoidance training and the add-on of verbal suggestions are restricted to indirect measures of food behaviors, such as virtual food choice and implicit food preference. These discrepant results for direct and indirect measures of food behaviors are partially in line with previous studies on the effects of standard approach-avoidance interventions that also did not find any effects on food consumption (99, 104, 107). In one study in which participants were trained to approach or avoid chocolate, an effect on food consumption was found in the group that was instructed to avoid chocolate, in that this group ate less of a chocolate muffin, but not of a blueberry muffin, in a subsequent taste test (101). The results therefore seemed to be restricted to the trained stimulus and did not generalize to other

stimuli. Although the present study was not designed to measure possible transfer effects to other food stimuli, future studies should aim to explore possible transfer effects after serious gaming and verbal suggestions by incorporating various food stimuli.

In the present study, the gamified approach-avoidance training was based both on approaching healthy food items and avoiding unhealthy food items. However, the incorporated bogus taste test only consisted of high caloric snack foods, withholding participants from making a healthy food-related choice. Although the majority of bogus taste tests applied in experimental studies are restricted to high caloric snack foods (136), future studies should look into the possibility to develop a more ecologically valid reflection of food consumption, in which healthy food choices can be made as well.

Verbally induced expectancies have been shown to affect health outcomes and combining multiple learning strategies can possibly result in optimized effectiveness of learning processes (114, 137). For example, the placebo literature demonstrated that strengthening positive expectations towards interventions by verbal suggestions can enhance treatment effectiveness (34, 114, 138). The verbal suggestions that were incorporated in the present study could have altered participants' expectations and thereby strengthened the effectiveness of those games. In line with this reasoning the present study found a trend on the IAT indicating more positive implicit food preference for the combined condition compared to the gaming control condition. This trend was not found for the serious gaming only condition or the verbal suggestions only condition, providing some (very preliminary) evidence that playing serious games accompanied by verbal suggestions might be more effective than only playing serious games. Future research should further investigate the added effectiveness of incorporating verbal suggestions in serious gaming.

Interestingly, the results of the verbal suggestions only condition on food-related outcomes seemed comparable with those of the serious gaming only condition and the combined serious gaming and verbal suggestions condition (see Table 2). The fact that the serious games in the present study were primarily based on approach-avoidance training does not mean that effects were the result of low-level mental processes such as the automatic formation of stimulus-response associations on the basis of repeated pairings of stimuli and responses. In accordance with an inferential account of approach-avoidance training effects [46], the training (or even the instructions about stimulus-response contingencies before the training) could also have allowed participants to make inferences about the foods or about the outcomes of food-related actions (e.g., expectancies of positive effects of eating healthy). This could also, at least partially, provide an explanation for the similar effectiveness of verbal suggestions and serious gaming conditions, as solely

providing participants with verbal suggestions concerning the actions of serious gaming might be sufficient to make similar inferences that influence food-related outcomes. This observation is in line with previous literature, which already demonstrated that verbal suggestions can change stimulus evaluations not only by repeatedly performing approach and avoidance actions, but also by providing people with verbal suggestions concerning these actions (120-122). In the present study, however, no significant effects were found for the verbal suggestions only condition. It would nevertheless be interesting to further investigate the effects of verbal suggestions in future research with a specific aim to address the efficacy of verbal suggestions only in optimizing food outcomes which was not a predefined aim of the present study.

Next to the innovative approaches used in the present study to examine the combined effect of a gamified approach-avoidance training and verbal suggestions by incorporating multiple food-related outcome measures, there are several limitations that should be noted as well. First of all, recruitment of a predominantly highly educated student population who had rather high healthy eating goals precludes generalization of findings to a broader population. It is recommended that future studies incorporate other (target) populations in order to further examine the effectiveness of serious gaming and verbal suggestions in optimizing health behaviors. Second, one of the factors contributing to the effectiveness of serious gaming is enhanced engagement (139). In this study, serious gaming was used in order to enhance engagement of approach-avoidance interventions; however, formal testing of engagement was not included due to the fact that formal indices of engagement are generally lacking. Future studies should aim to develop and incorporate assessments of engagement. Third, although we do not have indications that there was a lack of perceived credibility concerning the provided verbal suggestions, future studies should incorporate a manipulation check (as this might be an important determinant of the effects of verbal suggestions). Additionally, future studies might include a measure of demand compliance because this might provide an explanation of our study results. Note, however, that this explanation does not fit well with the observation that most of the participants were surprised by hearing the study aim. Fourth, previous research provided support for the moderating effects of contingency awareness on implicit and explicit stimulus evaluations (120, 140). In future research, it would therefore be interesting to evaluate whether contingency awareness moderates the effects of gamified approach-avoidance training and verbal suggestions on food-related outcomes. Fifth, although we minimized the information concerning the actual study aim of the experiment prior to participation, it cannot be ruled out that participants' responses on the tasks may have been influenced by informing them beforehand that this experiment was about games and food. Sixth, the present study was designed to investigate the combined effects of

the serious gaming conditions, i.e., with and without the verbal suggestion, compared to the gaming control condition. In order to draw conclusions on the effectiveness of the individual conditions, future studies should include a larger sample size. Finally, the present study only investigated the effects of serious gaming and verbal suggestions directly after the manipulations. Since we do not know whether those effects will be maintained on a longer term, this should be investigated in future studies by including follow-up assessments. In addition, future studies should evaluate important moderators of serious gaming effects by incorporating various durations of the games and amount of sessions.

The present study was the first in investigating the combined effectiveness of serious gaming and verbal suggestions on food-related outcomes and as such integrates the serious gaming research with research on placebo effects. Some initial support was provided for the effects of serious gaming on virtual food choice and implicit food preference, and possibly the add-on effectiveness of verbal suggestions. By combining innovative ways to alter approach and avoidance tendencies, the present study advances scientific knowledge on the effectiveness of approach-avoidance trainings in optimizing food-related outcomes. Future research should further investigate the effectiveness of serious gaming and the role of verbal suggestions in optimizing food-related outcomes in various target populations and other health domains, such as physical activity.

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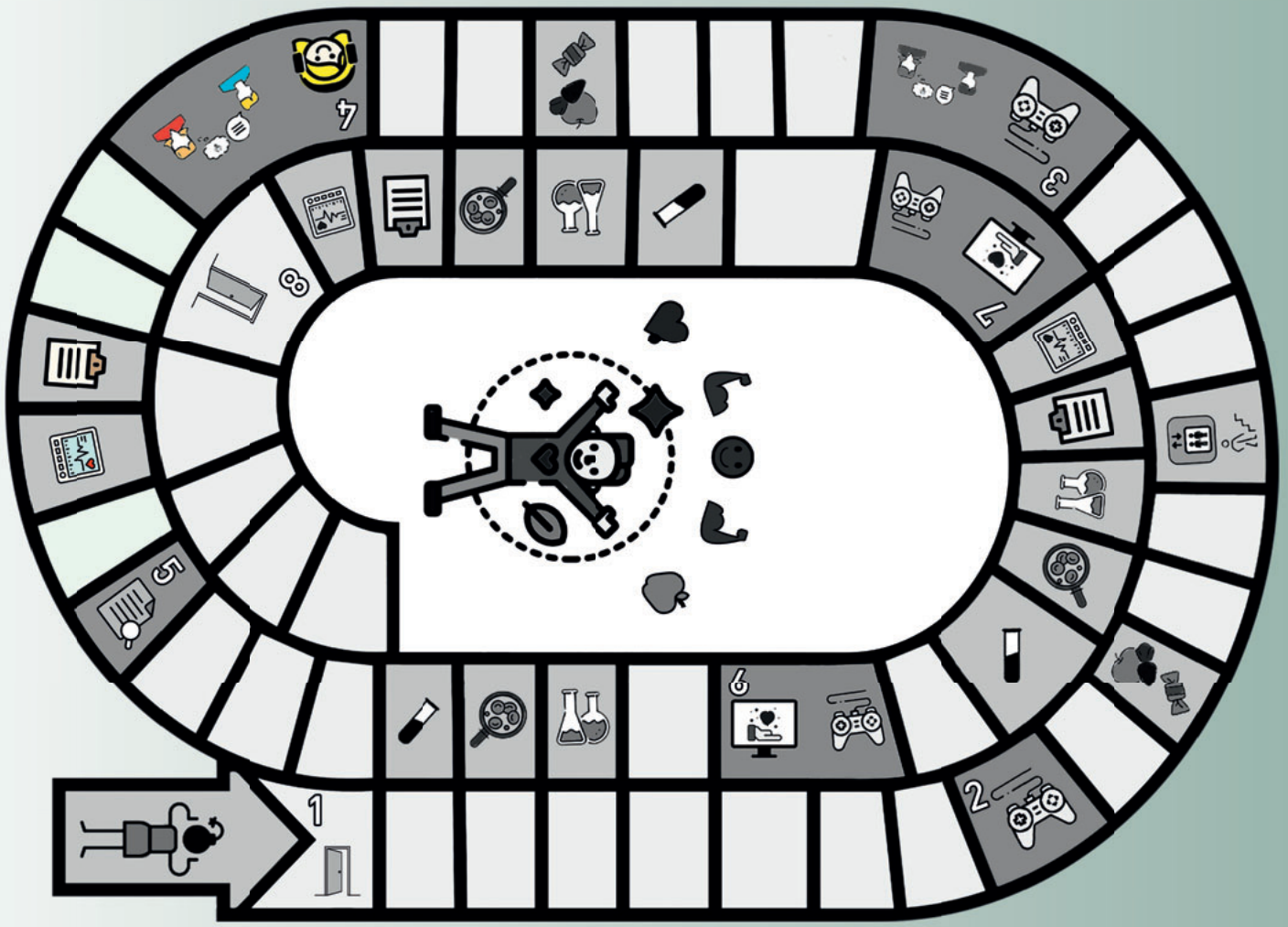
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Can verbal suggestions strengthen the effects of relaxation?

4

This Chapter is accepted for publication in a slightly modified version as:
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Abstract

Short stress management interventions such as relaxation therapy have demonstrated preliminary effectiveness in reducing stress-related problems. A promising tool to strengthen the effectiveness of relaxation-based interventions is the use of verbal suggestions, as previous research provided evidence that verbal suggestions can induce positive outcome expectancies, facilitate adaptive responses to stress and improve health outcomes. The present proof-of-concept study aimed to investigate the effects of a brief relaxation intervention and specifically the role of verbal suggestions on stress-related outcomes assessed by self-report questionnaires and psychophysiological data. 120 participants were randomized to one of four intervention conditions: a brief relaxation intervention plus verbal suggestions condition, a brief relaxation intervention only condition, a verbal suggestions only condition, and a control condition. Afterwards, participants were subjected to a psychosocial stress challenge to assess reactivity to a stressful event. Immediately after both relaxation interventions (with and without verbal suggestions), lower self-reported state anxiety was found compared to the control condition, but no differences were observed in response to the stressor. The verbal suggestions only condition did not impact state anxiety. No significant effects were found for verbal suggestion interventions on cortisol, alpha amylase, heart rate and skin conductance. This is the first study investigating the role of verbal suggestions in the effectiveness of a brief relaxation intervention. Although this proof-of-concept study provides support for the effectiveness of a brief relaxation intervention in lowering state anxiety directly after the intervention, the effects did not impact the response to a subsequent stressor and we did not observe any evidence for the add-on effectiveness of verbal suggestions. The effectiveness of brief relaxation interventions on stress responses should be investigated further in future research by incorporating interventions that are tailored to the specific stress challenge and various types of verbal suggestions.

Introduction

Accumulating findings demonstrate that intense acute stress and prolonged experienced stress can have adverse effects on health (40, 141). Stress management interventions have demonstrated to be effective in reducing stress and are considered beneficial for health (142, 143). Relaxation therapy, i.e., learning to focus on arousal reduction by releasing muscle tension (144), is one of the most commonly used types of stress management interventions (143, 145). Most relaxation interventions involve multiple sessions, but there is also some initial support for the effectiveness of brief (2 to 3 sessions) interventions (146, 147). One study even found preliminary evidence for the effectiveness of a relaxation intervention during one single session of 15 – 20 minutes in reducing acute distress (148). To improve the effectiveness of relaxation-based interventions, it might be useful to investigate potential factors that influence the effects of brief interventions for the treatment of stress consequences.

It has been argued that expectancies towards the outcomes of interventions, e.g., expecting beforehand that an intervention will result in a reduction of stress, might strongly moderate the effects of (stress management) interventions. Because verbal suggestions are thought to provide a good way to steer outcome expectancies (149), they might improve outcomes of relaxation-based interventions. The effectiveness of verbal suggestions is not yet investigated in the context of stress management interventions. However, it has already been shown that verbal suggestions by themselves can alter stress responses. Two experimental studies showed that verbal suggestions regarding biofeedback responses (i.e., informing participants that they have a high heartbeat and are aroused, or that they have a calm heartbeat and are relaxed) altered their psychophysiological stress responses, in that participants became more aroused after being provided with verbal suggestions (150, 151). In addition, studies in the placebo literature have provided strong evidence that verbal suggestions can result in symptom relief in several psychiatric and non-psychiatric conditions, as well as somatic conditions (13, 152). For instance, a study of Skvortsova and colleagues (2018) found that verbal suggestions can reduce levels of self-reported pain (153). Since enhanced outcome expectancies through verbal suggestions can influence psychobiological stress responses as well as various somatic symptoms, they may possibly also effectively optimize the effectiveness of a short stress management intervention.

The present proof-of-concept study investigates the effects of a brief relaxation intervention based on a single session on stress-related outcomes and more specifically assesses whether verbal suggestions can optimize these effects. Participants were randomized to one of four conditions: a brief relaxation intervention plus verbal suggestions condition,

a brief relaxation intervention only condition, a verbal suggestions only condition, and a control condition without any relaxation practice or verbal suggestions. As the aim of the brief relaxation intervention and the verbal suggestions is to optimize coping with everyday life stressors, it is interesting to investigate the effectiveness of these interventions on stress-related outcomes by exposing people to a well-validated real-life psychosocial stress challenge and subsequently evaluate the stress response. We therefore administered a validated stress challenge test to all participants. Our primary study question was whether participants in the brief relaxation intervention conditions (with or without verbal suggestions) would show less self-reported state anxiety immediately after the intervention, as well as after the stress challenge, compared to the control condition. First, we examined whether a brief relaxation intervention would reduce state anxiety by comparing stress responses in the two relaxation conditions (with and without verbal suggestions) to the control condition. When significant group differences were found in this comparison, it was furthermore examined whether (1) the brief relaxation intervention plus verbal suggestions condition outperforms the brief relaxation intervention only condition, and (2) the verbal suggestions only condition outperforms the control condition. Subsequently, we studied effects for secondary self-reported and psychophysiological outcomes, including self-reported well-being, self-reported positive and negative affect, salivary cortisol and alpha-amylase, as well as heart rate and skin conductance.

Methods

Ethics statement

The protocol was approved by the local psychological ethics committee of Leiden University (registration code: CEP17-0102/1). Preregistration of the study was done at the Netherlands Trial Register (registration code: NTR6392) and the study was performed according to the Declaration of Helsinki (2013).

Design

The present study used a randomized experimental study design. Based on a 1:1:1:1 allocation ratio, participants were randomized to one of the four conditions (see above), stratified for gender. The randomization procedure was generated by an online random number generator (www.random.org). During the experiment, participants were blinded for the allocation to one of four different conditions.

Participants

Participants were recruited by written and online flyers that were distributed from March to June 2017 at Leiden University. Inclusion criteria were: (1) being fluent in Dutch and (2) being between 18 and 35 years old. Exclusion criteria were: (a) severe somatic or psychiatric conditions (e.g., chronic somatic diseases that affect daily life or Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition Text Revision [DSM-IV-TR] psychiatric disorders) interfering with the study protocol, (b) current or recent (< 3 months ago) stressful life events interfering with daily life, and/or (c) heavy drug or alcohol (≥ 3 units a day) use.

Experimental conditions and control condition

In both relaxation intervention conditions, i.e., with and without verbal suggestions, participants were provided with two brief relaxation exercises and participants were told that they would perform two relaxation exercises with a short break in between the exercises. Participants were instructed to sit upright in their chair with their feet on the ground and their arms resting on their thighs or on the seat rests. Subsequently, the experimenter provided the participant with Sennheiser HD201headphones and instructed them to start the audio file when they were ready to listen to the relaxation exercises. The experimenter then left the room. The first relaxation exercise focused on progressive muscle relaxation and led participants through a series of practices to tense and relax various muscle groups throughout the whole body. This relaxation exercise took around 16 minutes. Thirty seconds after the end of the first exercise, the second visual imagery-based relaxation exercise automatically started, which was focused on visualization of optimal health. Participants had to visualize that they were feeling healthy and full of energy, and that their body was in an optimal condition. This intervention took around 5 minutes. Total duration of the relaxation intervention was 20 – 25 minutes.

The verbal suggestions focused on the effectiveness of the relaxation intervention in performing the follow-up challenge test with the aim to facilitate more positive outcome expectancies for this task and yielded some cues for better performance on this task. More specifically, the experimenter provided participants with the following verbal suggestions (translated from Dutch):

> *"In a moment, you will complete some tasks, including an arithmetic task. Prior research has shown that you can complete those tasks better when you relax by:*
>
> 1. *Sitting as restful and relaxed as possible;*
> 2. *Keeping in mind that your respiration has to be restful and regular, calm and relaxed.*

It is important that you keep this information in mind. You need this information in order to perform well on the tasks. Therefore, be careful not to forget these instructions."

In the brief relaxation intervention plus verbal suggestions condition, participants were first provided with the verbal suggestions followed by the relaxation intervention. In the verbal suggestions only condition, participants did not receive the relaxation exercises, but were exposed to the stress task directly after receiving the verbal suggestions.
In the control condition, participants completed several neutral word finding puzzles for 25 minutes before they were exposed to the TSST.

Psychosocial stress challenge

In order to provide participants with a psychosocial stress challenge, participants were subjected to the TSST (51). This validated and commonly used task contains a mock job interview and a mental arithmetic task, which have to be performed in front of a two-member panel of judges. In order to induce anticipatory stress, participants were given 5 minutes to prepare a presentation about their preferred future job position. Subsequently, participants had to present themselves in front of a two-member panel of judges. During the presentation, the panel members pretended to take notes and asked some critical questions without providing any personal feedback to the participants. Also, participants were informed that the presentation was recorded with a video-camera and a voice recorder. After 6 minutes, participants were instructed to count backwards in steps of 17 from 1965 to 0. When participants provided the wrong answer or did not answer fast enough, they were told to start over again from 1965. After 2 minutes, all participants were told that they made too many mistakes or that they responded too slowly and that they would be provided with an 'easier' task (count backwards in steps of 13 from 1687 to 0), which actually reflected the same level of difficulty. The duration of this mental arithmetic part was 4 minutes and the total TSST procedure took around 15 minutes. This task has been found to reliably induce psychological, neuroendocrine, and autonomic nervous system responses (52, 154).

Self-reported outcomes

State anxiety

State anxiety was measured by the short state version of the State Trait Anxiety Inventory (STAI-S-s) (155). Participants completed 6 items on a 4-point scale ranging from 1 (*not at all*) to 4 (*very much*), such as 'I am tense'. Scores on this scale can range from 6 to 24, with

higher scores representing higher state anxiety. The Dutch translation was found to have a good internal reliability in previous literature (Cronbach's alpha = .90) (156), as well as in the present study (Cronbach's alpha = .78).

Well-being

Self-reported well-being was measured by a Numeric Rating Scale (NRS) (157). Participants completed 7 items on an 11-point rating scale ranging from 0 (*not at all*) to 10 (*very much*), such as 'How calm do you feel at this moment?'. Total scores on this scale can range from 0 to 70, with higher scores representing higher well-being. The NRS was found to have a good internal reliability in the present study (Cronbach's alpha = .86).

Positive and negative affect

In addition, a Dutch version of the Positive and Negative Affect Schedule (PANAS) was used in order to measure affect (158). Participants completed 20 items on a 5-point scale ranging from 1 (*barely or totally not*) to 5 (*very*), such as 'inspired' or 'nervous'. For each scale, scores can range from 10 to 50 with higher scores on the positive affect scale indicating higher self-reported positive affect and higher scores on the negative affect scale indicating higher negative affect. The PANAS was found to have a good internal reliability in previous literature (Cronbach's alpha positive affect scale = .88; Cronbach's alpha negative affect scale = .87) (159), as well as in the present study (Cronbach's alpha positive affect scale = .85; Cronbach's alpha negative affect scale = .72).

Psychophysiological outcomes

Cortisol and alpha-amylase

Saliva samples were collected with Sarstedt salivettes (Sarstedt, Germany) in order to measure cortisol and alpha-amylase. Saliva was separated from the cotton swab by centrifugation and stored at -80°C until analyzed. Cortisol was determined with a competitive electrochemiluminescence immunoassay ECLIA using a Modular Analytics E602 immunoassay analyzer on the Roche Cobas 8000 from Roche Diagnostics (Mannheim, Germany). Determination of salivary alpha-amylase was performed using a colorimetric method with Ethylidene Protected Substrate (EPS) on the Roche Cobas 8000 C702 and C502 (Mannheim, Germany).

Heart rate and skin conductance

Heart rate and skin conductance levels were measured continuously with a BIOPAC MP150 system® using Acknowledge software version 4.4.1. The electrocardiogram (ECG)

signal was recorded with an ECG100C module set at 1000Hz. The gain was set at 1000, the high pass filter was set at 0.05Hz and the low pass filter was set at 35Hz. For skin conductance level, Ag/Agcl electrodes were attached at the medial phalange of the index and middle finger of the non-dominant hand. Skin conductance was measured using a GSR100C module set at 1000Hz with a gain of 5μ℧/V and a low pass filter at 10Hz. The Physio Data Toolbox Version 0.1 (160) was used for visual inspection of the data as well as for calculating the mean heart rate and skin conductance levels for each of the specific time points. Due to technical problems, data of skin conductance were not reliable for 4 participants and a substantial number of artifacts (e.g., extra systoles, frequent movements during the measurements) in the heart rate data was found for 5 participants, resulting in exclusion of those data from further data analyses.

Procedure

Prior to participation, participants were informed that the experiment was about attention processes and arithmetic skills, without providing further information on the actual study purpose. Participants provided written informed consent. Next, several online questionnaires probing the eligibility criteria, demographics, and participant characteristics not related to the present study aim were completed. If participants were eligible to participate in the study, they were invited for a single lab session that took place at the Faculty of Social and Behavioural Sciences of Leiden University, the Netherlands. Participants were instructed to refrain from using alcohol and drugs 24 hours before the lab session, to refrain from heavy physical activity starting from the evening before the lab session, and to refrain from consuming caffeine, smoking, and consuming warm meals 2 hours before the lab session. All lab sessions were planned in the afternoon, in order to limit the influence of the cortisol awakening response on the neuroendocrine measures.
At the start of the lab session, baseline self-reported state anxiety, well-being, and positive and negative affect were assessed. Thereafter, participants were connected to the equipment during the whole lab session, in order to measure resting state heart rate and skin conductance continuously. In addition, heart rate and skin conductance were also measured in resting state for 5 minutes at 5 different time points: baseline, after the intervention, directly after the TSST, and 10 and 20 minutes after the TSST. For all those measurements, participants were instructed to sit quietly and to move as little as possible. For the saliva sampling, participants were asked to hold the cotton swab in their mouth for one minute and to move it around without chewing on it, since less saliva will remain in the cotton swab after chewing on it. Thereafter, participants were randomized to one of four conditions and received the relaxation or control exercises and/or verbal suggestion, followed by completion of the questionnaires and psychophysiological measures (2nd measurement). Subsequently, participants were exposed to the TSST. Directly after

the TSST, as well as 10 and 20 minutes afterwards, participants again completed the questionnaires and the psychophysiological measures (3rd, 4th, and 5th measurement). At the end of the session, participants were debriefed about the actual study purpose. The total study duration was about 2 hours and participants received compensation for their participation (€15 or course credits).

Data preparation and statistical analyses

Based on a previous study on the effectiveness of a brief stress management intervention on state anxiety, an effect size around f = .27 was expected (148). A sample size of 30 participants in each condition (total 120 participants) allowed sufficient statistical power (power = .80) to detect this effect size with α = .05, as calculated by G*power 3.1 (134). IBM SPSS Statistics for Windows (Version 23; IBM Corporation, Armonk, NY, USA) was used to analyze the data with a two-tailed significance level (α = .05). To test the primary hypothesis that a brief relaxation intervention, with or without verbal suggestions, would result in decreased self-reported state anxiety, mixed repeated measures analyses of variance (RM ANOVAs) were conducted with the within-subjects factor Time (i.e., 5 levels: baseline, post-intervention, and post-, 10 min, and 20 min after TSST) and the between-subjects factor Type of Manipulation (both relaxation intervention conditions vs. control) (161). A check on the stressfulness of the TSST was performed by examining the significance of the factor Time was performed before the primary outcome of interest, i.e., the interaction effect of Time and Type of Manipulation. To examine at which time point(s) groups differed on state anxiety when a significant interaction effect was found between Time and Type of Manipulation, Holm's corrected ANOVAs were performed to compare both relaxation intervention conditions with the control condition at specific time intervals by calculating difference scores between baseline and each of the other time points. Next, to further investigate the role of verbal suggestions, Holm's corrected pairwise comparisons were performed on the time intervals that indicated significant differences to compare all four conditions separately. To investigate the effects of the relaxation intervention, with or without verbal suggestions, for the secondary self-reported outcomes well-being and positive and negative affect, analyses were performed in a similar way as for self-reported state anxiety. For heart rate and skin conductance, the analyses were performed in a similar way as described above, although the within-subjects factor Time contained 7 time points instead of 5 (i.e., baseline, during manipulation, after manipulation, during TSST, after TSST, 10 min after TSST, and 20 min after TSST). For cortisol and alpha-amylase, the area under the curve was calculated with respect to the ground (AUC_g), irrespective of the time distance between measurement points (162). To test the effects on AUC_g cortisol and alpha-amylase, between-subjects ANOVAs (with

the between-subjects factor Type of Manipulation) and subsequent pairwise comparisons were performed in a similar way as described above (without the within-subjects factor Time).

Results

Participant characteristics

Of the 149 participants who completed the online questionnaire, 131 were found eligible to participate in the present study. Eleven participants did not show up for the planned lab session, resulting in 120 participants (97 women; 80.8%) with a mean age of 22.1 years (*SD* = 2.3; range = 18 – 29) who completed the present study (30 in each condition). One participant (in the brief relaxation plus verbal suggestions condition) aborted the study after completion of the TSST and therefore, data of this participant are only available until completion of the TSST. Baseline characteristics of all participants are presented in Table 1. No significant group differences were found on demographics and baseline levels of the self-reported and psychophysiological outcome measures, as indicated by separate ANOVAs (all $p > .05$).

Table 1. Descriptive baseline statistics (mean and standard deviations) for the four conditions separately

	Brief relaxation intervention + verbal suggestions (*N* = 30)	**Brief relaxation intervention only** (*N* = 30)	**Verbal suggestions only** (*N* = 30)	**Control** (*N* = 30)
Sex, *n* female (%)	26 (86.7%)	23 (76.7%)	23 (76.7%)	25 (83.3%)
Mean (SD)				
Age	22.5 (2.1)	22.0 (2.4)	21.7 (2.3)	22.3 (2.2)
State anxiety	8.9 (2.1)	9.3 (3.0)	10.1 (2.7)	9.6 (2.4)
Well-being	56.7 (6.4)	55.9 (9.1)	53.7 (8.6)	54.3 (8.4)
Positive affect	28.4 (6.9)	27.0 (6.1)	26.8 (7.2)	27.6 (6.5)
Negative affect	12.2 (2.5)	12.0 (2.7)	12.4 (2.6)	11.9 (2.3)
Heart rate	74 (10)[1]	70 (8)	74 (9)	73 (8)[2]
Skin conductance	3.5 (1.9)[3]	2.6 (1.0)[1]	3.7 (1.7)[3]	3.3 (1.5)
Cortisol in nmol/L	5.6 (3.1)	4.5 (1.8)[3]	6.3 (2.1)[3]	5.1 (3.0)[3]
Alpha-amylase in U/L	103.4 (66.5)	140.0 (93.8)[3]	117.3 (98.4)[3]	172.1 (126.9)[3]

Note. *SD* = standard deviation, nmol/L = nanomoles/liter, U/L = units/liter.
[1]*N* = 28; [2]*N* = 27; [3]*N* = 29.

State anxiety

The results for self-reported state anxiety are presented in Figure 1. A significant main effect of Time was found ($F(2.76, 240.12) = 63.63$, $p < .001$, $n^2 = .42$), indicating that the TSST was effective in increasing self-reported state anxiety (see Figure 1). In addition, we observed a significant interaction effect between Time and Type of Manipulation ($F(2.76, 240.12) = 7.65$, $p < .001$, $n^2 = .08$), indicating lower self-reported state anxiety after the condition for both intervention conditions combined (with and without verbal suggestions) compared to the control condition (see Figure 1). Holm's corrected ANOVAs showed a significant difference between the combined intervention groups and the control condition post-intervention ($F(1, 88) = 10.56$, p *adjusted* $< .01$, $n^2 = .11$). When investigating all four groups on this time interval, Holm's corrected pairwise comparisons showed significantly smaller increase in state anxiety from baseline to post-intervention state anxiety in both the brief relaxation intervention plus verbal suggestions condition ($M = -1.03$, $SD = 2.62$; $t(58) = 3.07$, p *adjusted* $= .018$) and the brief relaxation intervention only condition ($M = -0.80$, $SD = 2.71$; $t(58) = 2.66$, p *adjusted* $= .050$) as compared to the control condition ($M = 0.93$, $SD = 2.33$). No significant differences were found for the other pairwise comparisons.

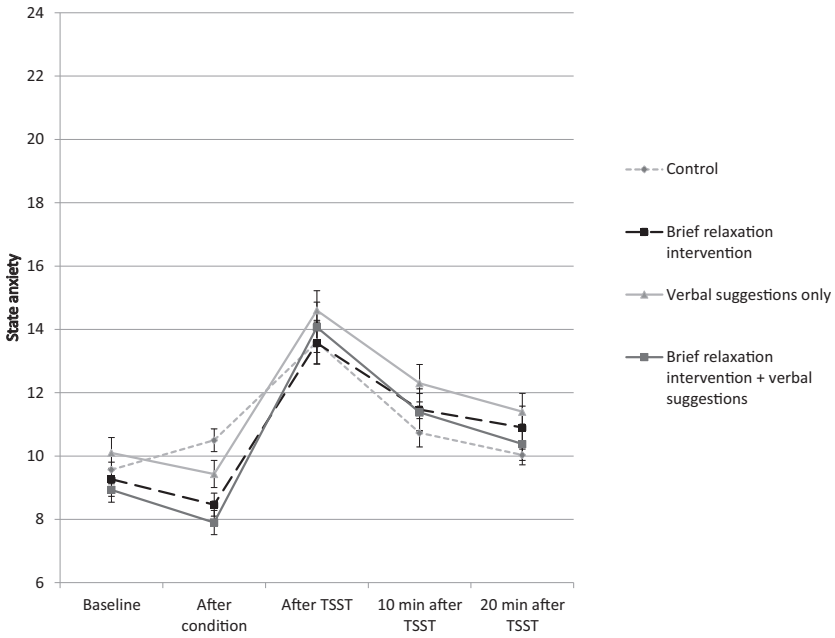

Figure 1. State anxiety levels for the four conditions on the various time points.

The x-axis represents the various time points, whereas the y-axis represents the level of self-reported state anxiety. A higher score on the y-axis represents a higher level of state anxiety.

Well-being

The results for well-being are presented in Figure 2. A significant main effect of Time was found (F(2.11, 183.34) = 62.81, p < .001, n^2 = .42), indicating that the stress manipulation was effective in reducing self-reported well-being (see Figure 2). A trend towards significance was found for the interaction effect between Time and Type of Manipulation (F(2.11, 183.34) = 2.92, p = .05, n^2 = .03), indicating higher self-reported well-being after the condition for both intervention conditions combined (with and without verbal suggestions) compared to the control condition (see Figure 2). Exploratory pairwise comparisons did not yield any significant group differences.

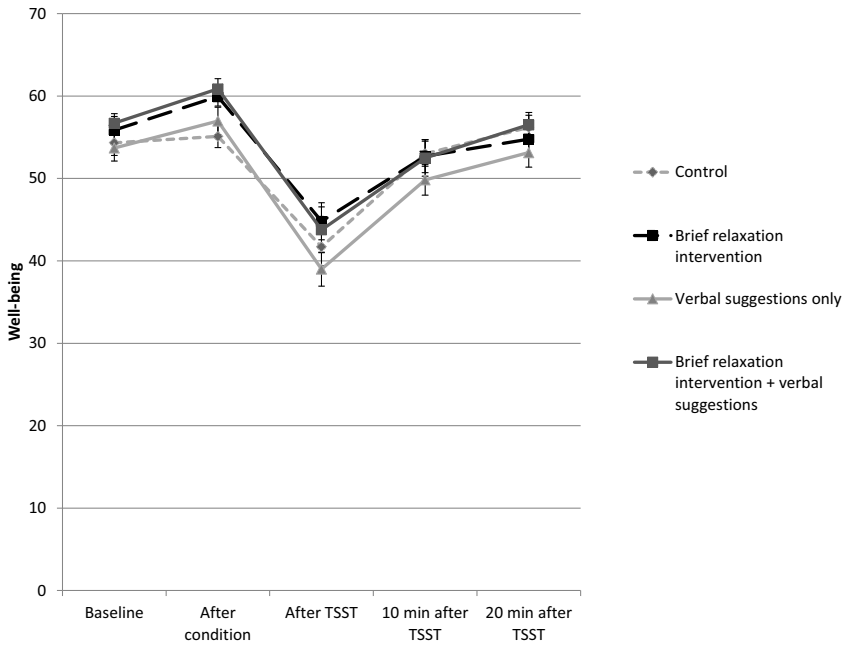

Figure 2. Levels of well-being for the four conditions on the various time points.

The x-axis represents the various time points, whereas the y-axis represents the level of self-reported well-being. A higher score on the y-axis represents a higher level of well-being.

Positive and negative affect

The results for positive and negative affect are presented in Figure 3a and Figure 3b, respectively. For positive affect, a significant main effect of Time was found (F(3.35, 291.70) = 19.75, p < .001, n^2 = .19), indicating that the TSST reduced self-reported positive affect. No significant interaction effect was found between Time and Type of Manipulation, indicating that positive affect was not influenced differentially between conditions.

For negative affect, a significant main effect of Time was found, F(1.83, 158.96) = 39.40, p

< .001, n^2 = .31, indicating that the TSST was effective in increasing self-reported negative affect. No interaction effect was found between time and Type of Manipulation, indicating that negative affect was not influenced differentially between conditions.

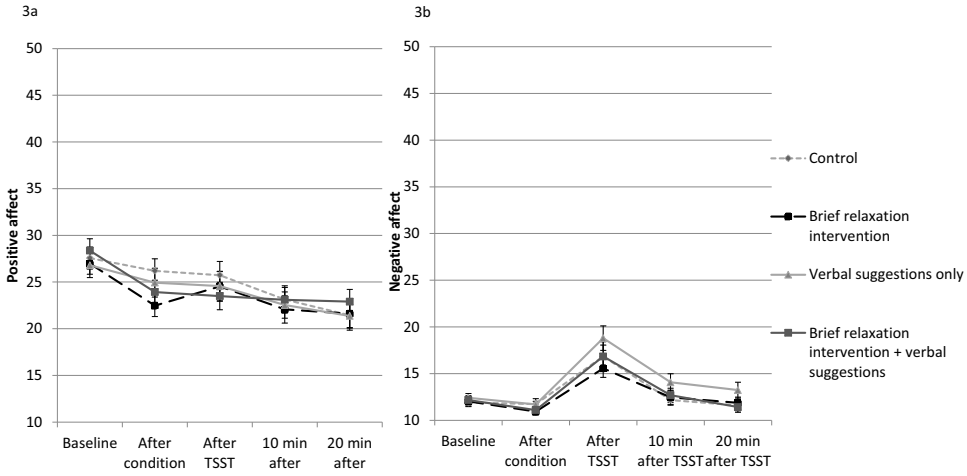

Figure 3. Levels of positive affect (Figure 3a) and negative affect (Figure 3b) for the four conditions on the various time points.

The x-axis represents the various time points, whereas the y-axis represents the level of self-reported positive affect or negative affect, respectively. A higher score on the y-axis represents a higher level of positive affect or negative affect, respectively.

Heart rate and skin conductance

The results for heart rate and skin conductance are presented in Figure 4a and Figure 4b, respectively. For heart rate, a significant main effect of Time was found (F(1.85, 151.50) = 178.10, p < .001, n^2 = .69), indicating that the TSST was effective in increasing heart rate. No significant interaction effect between Time and Type of Manipulation was observed, indicating that heart rate was not affected differentially between conditions.

For skin conductance, a significant main effect of Time was found (F(2.43, 203.70) = 68.68, p < .001, n^2 = .45), indicating that the TSST was effective in increasing skin conductance. In addition, a significant interaction effect between Time and Type of Manipulation was observed (F(2.43, 203.70) = 4.67, p = .007, n^2 = .05), showing that skin conductance was affected differentially between conditions. Holm's corrected ANOVAs showed no significant differences between the combined relaxation intervention groups and the control group on any of the specific time intervals. Exploratory, pairwise comparisons across time points showed a significant difference between the control condition and verbal suggestions only condition (F (2.20, 125.49) = 8.61, p *adjusted* < .001, n^2 = .13), with no other significant group differences found (p-values > .05). Post hoc pairwise

comparisons on the various time intervals showed a significantly higher skin conductance rise in the verbal suggestions versus control condition from baseline to during the TSST (*M* = 3.14, *SD* = 1.10 vs. *M* = 2.27, *SD* = 1.02; *p adjusted* = .01), directly post- TSST (*M* = 1.66, *SD* = 1.31 vs. *M* = 0.89, *SD* = .99; *p adjusted* = .03), 10 minutes after the TSST (*M* = 1.60, *SD* = 1.47 vs. *M* = 0.65, *SD* = .97; *p adjusted* = .02), and 20 minutes after the TSST (*M* = 1.66, *SD* = 1.43 vs. *M* = .62, *SD* = .93; *p adjusted* = .01).

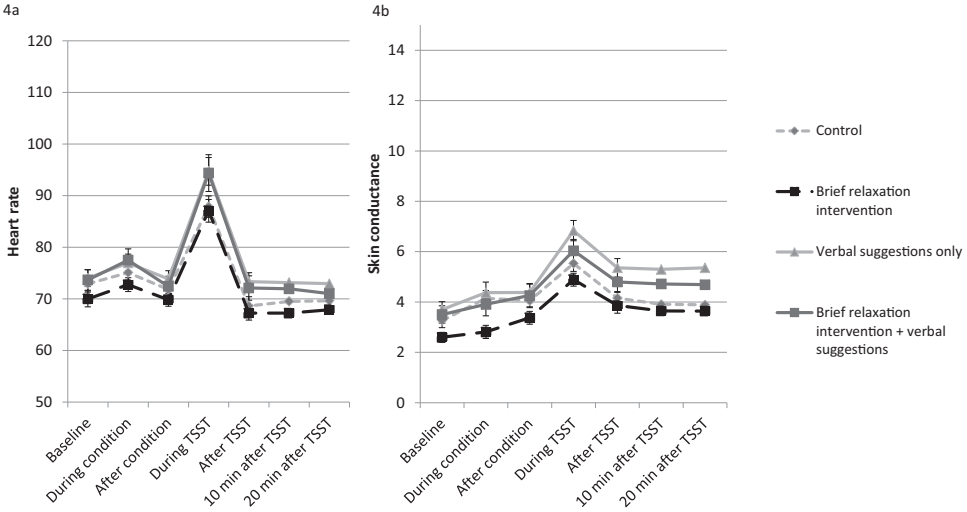

Figure 4. Mean heart rate (Figure 4a) and skin conductance (Figure 4b) for the four conditions on the various time points during the lab session.

The x-axis represents the various time points, whereas the y-axis represents the mean heart rate and skin conductance, respectively. A higher score on the y-axis represents a higher mean heart rate level or skin conductance, respectively.

Cortisol and alpha-amylase

Figure 5a and Figure 5b present the results on cortisol and alpha-amylase, respectively. For cortisol, the AUC_g data were not normally distributed and, therefore, a logarithmic transformation was applied. No significant group differences were found for cortisol ($p > .60$). For the AUC_g data on alpha-amylase, a trend towards significance was found (F (1, 84) = 3.44, p = .07), in that both intervention conditions combined showed a marginally lower level of AUC_g alpha-amylase compared to the control condition.

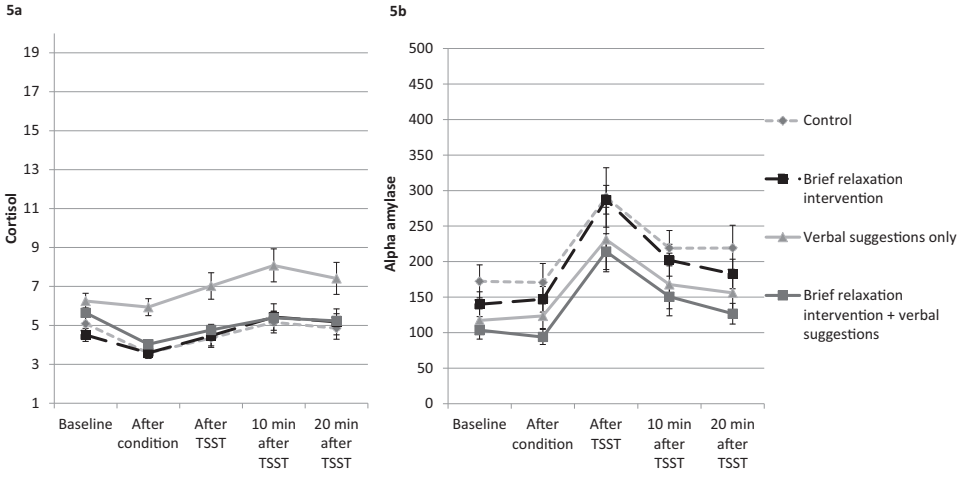

Figure 5. Levels of cortisol (Figure 5a) and alpha-amylase (Figure 5b) for the four conditions on the various time points during the lab session.

The x-axis represents the various time points, whereas the y-axis represents the level of cortisol and alpha-amylase, respectively. A higher score on the y-axis represents a higher level of cortisol or alpha-amylase, respectively.

Discussion

The present proof-of-concept study investigated for the first time the effects of a brief relaxation intervention and the role of verbal suggestions on stress-related outcomes by exposing participants to a psychosocial stress challenge. After the brief relaxation interventions (with or without verbal suggestions), lower self-reported state anxiety was found compared to the control condition. This effect was only seen directly after relaxation, however, and did not impact the subsequent psychosocial stress challenge. No significant effects of the relaxation interventions were found for other self-reported outcomes or the psychophysiological outcome data. In addition, no support was found for the add-on effectiveness of verbal suggestions. By applying an innovative design including four different conditions and evaluating them with various self-reported as well as psychophysiological outcome measures, the present study provides preliminary support for the effectiveness of a brief relaxation intervention on reducing state anxiety.

The present study demonstrated the effectiveness of a brief relaxation intervention in reducing state anxiety and, moreover, was the first in investigating whether verbal suggestions, based on inducing positive outcome expectancies, can strengthen the effects of a relaxation intervention. This study did not find support for the add-on effectiveness of verbal suggestions. Nonetheless, it is premature to conclude that verbal suggestions do

not have any add-on effects on relaxation interventions. As the present proof-of-concept study primarily aimed to evaluate the effectiveness of a brief relaxation intervention on the stress response, the present study had a good power to detect the effects of both brief relaxation interventions and control condition, but lower power to detect effects in the separate conditions. Therefore, more research with larger sample sizes is needed to gain more insight into the specific add-on effects of the verbal suggestions. In the placebo literature, the effectiveness of verbal suggestions on health outcomes is well-described (13, 152). Moreover, the verbal suggestions used in the present study were formulated rather generic (i.e., to address a broad target population) encompassing multiple relaxation components, whereas verbal suggestions in placebo literature are usually focused on a specific sensation or manipulation (163). It could be that participants did not benefit from the verbal suggestions just because they could not pick up the link between the verbal suggestions and the upcoming stress challenge. More specifically, the verbal suggestions were focused on being able to perform an arithmetic task better when participants adequately performed two actions referring to relaxation practice, but participants in the combined relaxation and verbal suggestions condition had to make the link between the provided instructions and relaxation practice themselves, whereas participants in the verbal suggestions only condition could not even make this link. Additionally, the verbal suggestions did only prepare participants on the mental arithmetic task and not on the speech task, in order to avoid any anticipation stress. However, by not telling them about the speech task, participants possibly did not feel able to complete this task better. Future studies should therefore investigate whether formulating the verbal suggestions more specifically (e.g., providing concrete examples of the benefits of stress management and providing more information on the link between the components of the verbal suggestions and the actual relaxation response) can optimize stress responses. In addition, the effectiveness of the verbal suggestions should be further elucidated in future research by incorporating various types of verbal suggestions, e.g., varying in the details that are provided concerning stress-management techniques, the details of the effectiveness of stress management, as well as varying the number of times the verbal suggestions are provided to participants or by providing participants with booster verbal suggestions (e.g., by exposing participants in the verbal suggestions only condition with multiple booster suggestions, as in the present study this condition was provided only once with the verbal suggestion and directly followed by the psychosocial stress challenge). Moreover, as the effectiveness of a combination of associative learning processes such as conditioning and verbal suggestions has been demonstrated in a previous study on placebo effects (33), it would be interesting to investigate whether verbal suggestions can strengthen the effects of learned relaxation responses. As verbal suggestions and conditioning focus on different learning processes, those methods can potentially complement each other (164).

The brief relaxation intervention resulted in lower self-reported state anxiety after both brief relaxation intervention conditions combined (with and without verbal suggestions) compared to the control condition. However, no group differences were observed after the psychosocial challenge. This finding is in accordance with a previous pilot study, also reporting a significantly lower self-reported state anxiety after a stress management intervention, but not after the TSST (148). The results for self-reported well-being were in line with the results for self-reported state anxiety as a trend was found for a higher level of self-reported well-being after both brief relaxation interventions combined (with and without verbal suggestions), compared to the control condition, although pairwise comparisons did not yield any significant differences. As the results on state anxiety were only seen directly after the intervention and not in response to the psychosocial stress challenge, it might be that a brief relaxation intervention is not effective enough to buffer the effects of stress in response to a challenge. An alternative explanation can be found in the type of stressor that was used. The TSST is a well-validated stressor that is commonly used in research (52). It is, however, a rather robust acute stressor and as all participants show increased stress levels by this task, it might therefore be difficult to differentiate between the stress responses of the present incorporated experimental and control conditions after the TSST. A previous study comparing the TSST to another stressor, i.e., a cold pressor test (CPT), demonstrated that the TSST was most effective in activating the hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axis, whereas with the CPT a differential response pattern between various included populations could be identified. However, the CPT concerns a stressor of shorter duration, which can impact the time course of the response (165). Future research might therefore incorporate various stressors (e.g., anticipatory and social evaluative stressors as well as a more physical stressors) in order to investigate the potential generalizing effects of a brief relaxation intervention to other stressors and the potential added value of verbal suggestions thereon. In addition, future studies might consider incorporating participants who are at risk for inadequate coping with stress (e.g., participants with high trait anxiety), as well as participants with a predisposition for high levels of stress in order to see whether the intervention can be used to optimize stress management skills in specific target populations.

For the psychophysiological outcomes, a trend was found for both brief relaxation intervention conditions combined (with and without verbal suggestions) showing a lower overall alpha-amylase concentration as compared to the control condition. As lower concentrations of alpha-amylase are related to lower autonomic nervous system arousal, this finding is in accordance with our expectation that both brief relaxation intervention conditions would result in a lower stress response. However, this finding

should be interpreted with caution, since only a trend was found. Additionally, this trend was not supported by the other psychophysiological outcome measures, as no significant group differences were found for heart rate (also reflecting autonomous nervous system reactivity) and cortisol (reflecting activation of the HPA-axis). Concerning skin conductance level, pairwise comparisons demonstrated a higher level of skin conductance for the verbal suggestions only condition compared to the control condition during and after the TSST, suggesting a higher level of arousal of the sympathetic nervous system in response to acute stress. A higher level of skin conductance is related to higher arousal of the sympathetic nervous system, however, skin conductance is also related to attention (166). Since skin conductance variations can encompass different processes and the findings on the other psychophysiological outcomes were heterogeneous, no unidirectional view can be formed on the effectiveness of a brief relaxation intervention and/or verbal suggestions in altering psychophysiological outcomes. The results on the psychophysiological outcome data in the present study, therefore, warrant further research.

Next to the innovative features incorporated in the present study, i.e., incorporating four different experimental conditions, incorporating a psychosocial challenge to observe the effects of a brief relaxation intervention accompanied or not with a verbal suggestion on state anxiety, and incorporating self-reported as well as psychophysiological outcome measures, there are some limitations that should be noted as well. First of all, the present study was based on a rather highly educated sample, limiting its generalizability. Second, although TSST panel members and test leaders had to behave strictly according to a predefined script and we do not have indications that their behaviors varied along the condition to which participants were randomized, research personnel were not blinded to the allocation procedures. In order to exclude any performance bias, future studies should make both test leaders and TSST panel members blind for allocation by including a third independent researcher that performs randomization and subsequently completes the condition with participants, but is not involved in other parts of the study. Third, we did not include a manipulation check to evaluate whether the verbal suggestions were credible to participants. Finally, the present study did incorporate a brief relaxation intervention that was not necessarily tailored to the specific needs of the participant. Possibly, some participants already possessed the skills that were provided to them in the brief relaxation intervention, whereas others might have had difficulties with acquiring coping skills from the intervention. Therefore, future studies may tailor the intervention by guiding the relaxation practice according to the specific challenge they are faced with and evaluate whether participants actually pick up the link between the instructions and handling with the upcoming stress challenge.

In conclusion, the present proof-of-concept study found some preliminary support for the effectiveness of a brief relaxation intervention, with or without verbal suggestions, in decreasing state anxiety. These effects occurred directly after the condition, but not after a psychosocial challenge. No add-on effect of verbal suggestions was found in the present study. Future studies should further investigate the effectiveness of brief relaxation interventions on stress responses by tailoring the intervention to the specific challenges and by incorporating other (more sensitive) psychosocial challenges. In addition, future research should further elucidate the role of verbal suggestions by incorporating various types of verbal suggestions and manipulate the number of times the verbal suggestions are provided.

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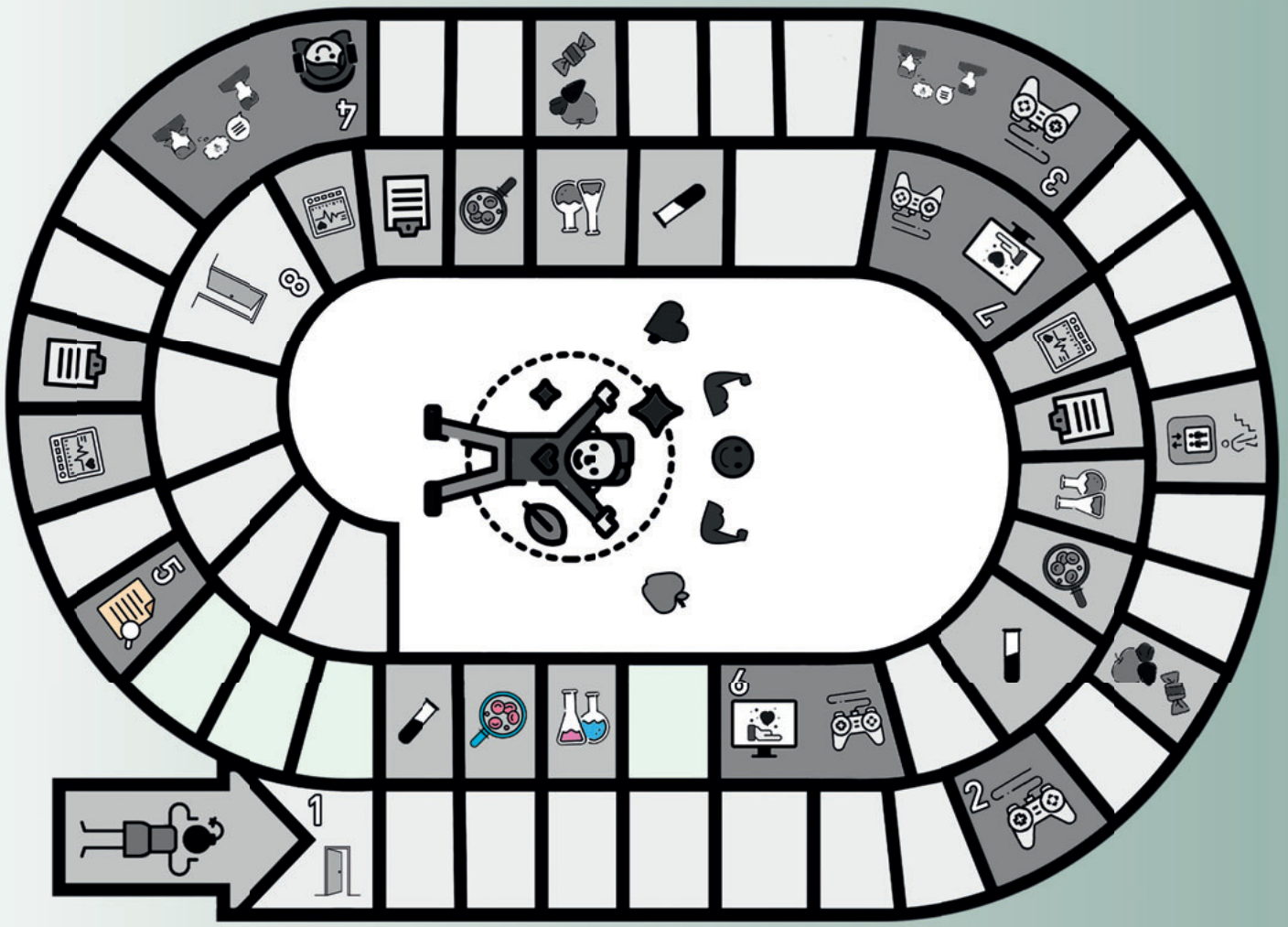
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Effectiveness of stress-reducing interventions on response to challenges to the immune system: A meta-analytic review

5

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Abstract

There is consistent evidence showing an interplay between psychological processes and immune function in health and disease processes. The present systematic review and meta-analysis aimed to provide a concise overview of the effectiveness of stress-reducing psychological interventions on the activation of immune responses in both healthy subjects and patients. Included are three types of challenges: *in vivo*, *in vitro*, and psychophysiological. Such challenges are designed to mimic naturally occurring immune-related threats. A systematic literature search was conducted using PubMed, EMBASE, and PsychInfo, resulting in 75 eligible studies. Risk of bias was assessed with the Cochrane risk of bias tool. Across all studies, a small to moderate effect size was found for the effects of psychological interventions on optimizing immune function (g = .33). While largest effects were found for *in vivo* immune-related challenges (especially on studies that incorporated skin tests and wound healing), studies incorporating psychophysiological challenges and *in vitro* immune-related stimulations similarly suggest more optimal immune responses among those receiving stress-reducing interventions. These findings showed substantial heterogeneity depending on type of challenge, study populations, and intervention types. These data demonstrate support for the effectiveness of stress-reducing psychological interventions in improving immunity in studies that tested immune function by means of incorporating an *in vivo, in vitro,* or psychophysiological challenge.

Introduction

Stressful events can influence functioning of the immune system (41, 167), whereby chronic stress has mostly been found to suppress protective immune responses and promote pathological immune responses (42-44). These immune alterations can be expressed as slower wound healing (43, 44), impaired responses to vaccines (42), and the progression of infectious and immune-mediated diseases (42, 46).

Various psychological interventions have been found to effectively reduce stress, including cognitive behavioral therapy (CBT) (3), mindfulness meditation (168), mindfulness-based stress reduction (MBSR) (142), and relaxation (168). Therefore, it has been argued that such stress-reducing interventions may help to counteract the adverse effects of stress on immune functioning. A previous meta-analysis, however, found little support for an immune-optimizing potential of psychological interventions (169). Some supporting evidence was provided by studies using conditioning and hypnosis interventions, although the results were heterogeneous. Due to substantial variation in immune outcomes, generalizability was uncertain (169). More specifically, the immune outcomes in these studies varied from counting white blood cell subsets, to evaluating cell function by activating the immune system by either *in vitro* methods (i.e., exposing isolated white blood cells to immune-activating stimulus), or *in vivo* (i.e., stimulating an immune response in the intact person, e.g., vaccination). Each of these methods provides a different window and type of information on the functioning of the immune system. Counting cells in resting state provides information on the number of immune cells in the circulation. However, the circulation represents only a small and selective proportion of the total cell population, is highly dynamic within individuals, and the normal range of adequate cell numbers is rather broad. Therefore, in somatically healthy participants cell counts are of uncertain clinical significance. On the other hand, the immune system's response to activating stimuli is considered a more representative estimate of a person's ability to mount an adequate immune response in the face of a natural challenge, and may be considered a more biologically valid marker of immunocompetence (170).

In the studies that are reviewed here, *in vitro* activations include natural killer cell activity, stimulated lymphocyte proliferation response, and stimulated pro-inflammatory and anti-inflammatory cytokine production (i.e., chemical challenges), whereas *in vivo* stimulations include hypersensitivity responses to skin tests, time of healing of a biopsy wound, or the extent to which a vaccine produces antibodies (i.e., physical challenges). In addition to the above-mentioned *in vitro* and *in vivo* activations of the immune system, psychosocial stress can also challenge the immune system (40, 41, 171, 172). Therefore, a number of studies have evoked psychosocial stress in their participants by exposing

them to psychophysiological challenges, i.e., challenges that have the potential to evoke a psychophysiological stress response, including exposure to a psychosocial stress task, to obtain additional information on how stress-reducing psychological interventions may optimize the extent to which the immune system responds to these challenges (173). A recent systematic review provided support for the effectiveness of psychological interventions in optimizing wound healing (174). There is, however, no recent examination of the effectiveness of stress-reducing interventions on a broader range of immune challenges, also taking psychophysiological challenges into account.

In the last few decades, studies have evaluated how the immune system responds to chemical, physical, and psychophysiological challenges after undergoing a stress-reducing psychological intervention. Since there has been no systematic review of this literature, no consensus exists on the effectiveness of stress-reducing psychological interventions on subsequent responses to challenges to the immune system. Therefore, the aim of the current systematic review and meta-analysis is to summarize the effectiveness of stress-reducing psychological interventions directed at optimizing immune function, focusing on studies incorporating various *in vivo* or *in vitro* immune-related and/or psychophysiological stimulations/challenges into the study design. We expected that after a stress-reducing psychological intervention, participants would show a more optimized immune response to challenges as compared to participants that did not receive a stress-reducing psychological intervention. More specifically, after the stress-reducing psychological intervention we expected higher natural killer cell activity, higher anti-inflammatory cytokine responses, lower pro-inflammatory cytokine responses, higher lymphocyte proliferation responses, higher antibody responses, higher delayed-type hypersensitivity responses, as well as faster wound healing. We analyzed the pooled effects of the three types of challenges together, as well as separately.

Methods

This systematic review and meta-analysis was performed according to the PRISMA criteria (175) and was registered in PROSPERO (registration number: CRD42017055722).

Inclusion and exclusion criteria

Studies were included when they met the following inclusion criteria: incorporation of a stress-reducing psychological intervention (which was defined as having cognitive behavior change techniques as the main component, i.e., duration more than 50% of the intervention time, such as psychotherapy, mindfulness or relaxation); inclusion of immune outcome measures assessed in blood or saliva (e.g., quantification of cytokines,

lymphocytes), incorporation of immune-related and/or psychophysiological challenges into the study design which were assessed after the start of the stress-reducing psychological intervention, and incorporation of at least one control group without a stress-reducing psychological intervention. Articles were excluded when they assessed immunological functioning not by objective measurements or parameters, but when they were, for example, solely based on self-report (e.g., self-reported infection), when they were based on case studies, or when they had insufficient methodological or statistical details about the immune or psychophysiological challenges or results (e.g., conference abstracts).

Literature search strategy

A systematic search was conducted using the databases PubMed, EMBASE, and PsychInfo until January 26, 2017. Search terms included Medical Subject Headings (MeSH) and words from title/abstract (tiab) as qualifiers, classified in three categories: stress-reducing psychological interventions, immune function, and immune-related as well as psychophysiological challenges (see Supplemental Table 1 for the search strategy per database). All retrieved references were loaded into Endnote and two independent reviewers (LS and PC) screened the titles, abstracts, and subsequently full texts when appropriate regarding study eligibility and relevance. The reference lists of the included studies were additionally searched for potential eligible studies.

Data extraction

A data extraction form was used to extract relevant data from the eligible studies. Extracted information for each study included: study population (e.g., healthy participants or patients), participant demographics, details of the intervention and control condition, study methodology, incorporated chemical, physical, and/or psychophysiological challenges, immune outcome parameters, relevant outcome data, statistical analyses, and relevant information concerning the methodological quality assessment. The information was extracted by the two reviewers (LS, PC) independently. Discrepancies were identified and resolved through discussion by involving one or more additional reviewer(s) (DV, JB, AE).

Methodological quality assessment in included studies

Two reviewers (LS, PC) furthermore independently assessed risk of bias (RoB) of the included studies using the Cochrane risk of bias tool (176). The biases that were assessed included selection bias (process of randomization and concealment of allocation), performance bias (blinding of participants and research personnel), detection bias (blinding of outcome assessment), reporting bias (handling of missing data), and attrition

bias (description of reasons for withdrawal in all conditions). Biases were classified as being low, high or unclear. Disagreements between the review authors regarding the RoB in particular studies were resolved by discussion, with involvement of a third review author (DV) if necessary.

Data analyses

Data were analyzed using Comprehensive Meta-Analysis software version 3.3.070 (Biostat, Englewood, CO). Hedges *g* was the effect size metric that was applied on the descriptive statistics of the study. The effect size was calculated by subtracting the pre- from the post-immune outcome parameters in the control group and subsequently subtract this difference score from the difference score in the intervention group, divided by the pooled standard deviation and weighted across the number of subjects in each group. Effect sizes of 0.2 can be considered as small, whereas 0.5 and 0.8 can be considered as moderate and large, respectively (177). For the included studies performing within-subjects comparisons, the correlation coefficient could not be derived and therefore a correlation coefficient of $r = .05$ was imputed. In case a study contained multiple conditions with eligible psychological interventions, these groups were combined into one single pairwise comparison, according to the recommendations of the Cochrane handbook (176). The pooled effects were analyzed using a random effects model, since substantial variation was present in research characteristics (e.g., various types of challenges and immune outcomes). Heterogeneity was assessed by evaluating the I^2 statistic and by visual inspection of the forest plot. Values of $I^2 = 25\%$, 50%, and 75% can be interpreted as low, moderate, and high heterogeneity, respectively. In case the results of a study were based on post-intervention scores only (e.g., in case of wound healing studies), the effect size was based on the post-intervention scores. When the descriptive statistics were not available, authors were requested to provide those data and when the data were not provided, alternative methods were used to calculate the effect size (e.g., using reported statistics, reported mean change scores, etc.). When studies reported that the results were not significant, without further specifications of the outcomes, effect sizes were computed assuming no differences between the groups ($r = .00$). Because this is a rather conservative strategy that had to be applied to a substantial proportion of the data (i.e., imputation was used in 23.8% of the cases), meta-analyses were performed with and without those studies in order to evaluate potential bias of this method. All immune outcomes were scaled in the direction of positive Hedges *g* representing an optimized immune function.

The pooled effects of all three different types of challenges (i.e., *in vitro* immune-related stimulations, *in vivo* immune-related challenges, and psychophysiological challenges)

were analyzed together and separately. The *in vitro* immune-related stimulations were subsequently subcategorized into natural killer cell activity (NKCA), stimulated lymphocyte proliferation response (LPR), and stimulated cytokine production. *In vivo* immune-related challenges were subdivided into wound healing, vaccine responses, and hypersensitivity responses after skin tests. *In vivo* psychophysiological challenges were further subdivided into acute and more protracted stress challenges, separately for plasma numbers of lymphocytes (i.e., enumeration of CD4, CD8, and CD56 numbers) and cytokines (i.e., quantification of IL-1ß, IL-6, IL-8, and TNF-α). When the outcomes of *in vitro* stimulations were assessed on multiple concentrations of the stimulus (e.g., multiple effector-to-target ratios to evaluate NKCA or various dilutions to evaluate LPR), the effect size was derived from the concentration that most optimally differentiated conditions (i.e., the stimulus concentrations that showed the largest differences). Planned subset analyses evaluated the effects of different types of challenges within a specific category.

Data of at least three studies had to be available in order to conduct a meta-analysis. Sensitivity analyses were performed concerning the reliability of the results. In order to assess the stability of the overall effect size, it was investigated whether the effects were similar when studies with a substantial risk of bias (i.e., studies containing at least one classification of high risk of bias) were excluded from the analyses. In addition, publication bias was assessed by inspection of the funnel plot and applying the trim and fill method (178).

Results

Search results

Figure 1 shows the flow-chart of the systematic search and study selection. A total of 19,780 studies (including duplicates) were found by searching PubMed, EMBASE, and PsychInfo. After removing duplicates and screening the studies on title and abstract, 138 articles were examined in full text by the two independent reviewers. Of these, 65 articles fulfilled the inclusion criteria. Screening of the reference lists of the included articles yielded 9 additional eligible studies, which were not identified in the primary search as most of these studies did not specify immune outcome measures in the title and/or abstract. In total, 75 studies reported in 74 articles were included.

RoB assessment

Supplemental Figure 2 presents the RoB graph and Supplemental Figure 3 the RoB summary. Of the 75 studies, 68 (90.7%) did not provide sufficient details on the methods used to randomize participants and 71 articles (94.7%) did not sufficiently specify the

methods of allocation concealment (unclear RoB). RoB on performance was low for 2 articles (2.7%), due to adequate blinding procedures. In 9 articles (12.0%), participants and/or personnel were aware of group allocation, which could have led to performance bias (high RoB). For 26 articles (34.7%), the RoB concerning lack of blinding of participants and personnel was low. In 35 articles (46.7%), the drop-out rates and reasons for drop-out were sufficiently described and unrelated to the study outcomes, which resulted in a low RoB evaluation regarding incomplete outcome data. No study protocol was available for 73 articles (96.1%), resulting in an unclear RoB regarding selective reporting.

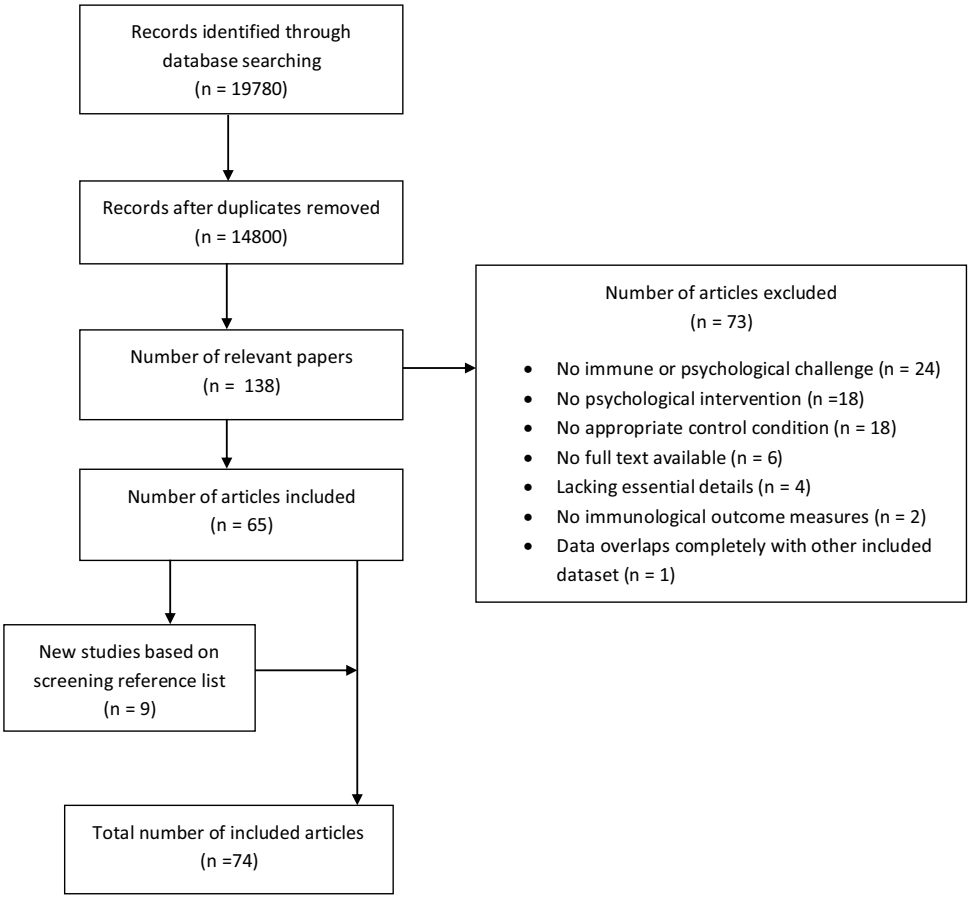

Figure 1. Flowchart of the study design showing the selection process, including reasons for exclusion.

Study selection was done by two independent reviewers.

Study characteristics

A total of 4,141 participants took part in the 75 studies. Detailed information concerning the study characteristics and incorporated psychological interventions are described in Supplemental Table 2. The total individual study sample size varied between N = 12 (179) and N = 252 (180) (*M* = 57, *SD* = 48). In 29 studies (38.7%), healthy volunteers were included as the study population (179, 181-207). Other samples included patients or vulnerable adults, for example with various types of cancer (208-227), patients with HIV infection (180, 228-232), patients with rheumatoid arthritis (173, 233-235), older adults (236-239), patients with asthma/allergies (240-242), widows/ women who lost a close relative to cancer (243, 244), patients with ulcerative colitis (245, 246), women with depression after bypass surgery (247), patients with late life insomnia (248), women suffering from infertility (249), veterans (250), and patients that underwent surgery (251). The mean age of participants varied between 18.5 and 78.8 years. Details on age were not provided in 7 studies (9.3%). Twenty-four studies (32.0%) only included female participants, whereas 9 studies (12.0%) only included male participants. In 36 studies (48.0%), both males and females were included. Details on gender were not reported for 6 studies (8.0%).

Type of stress-reducing psychological interventions

In total, 82 stress-reducing psychological interventions were evaluated in the 75 studies. Most interventions (28 interventions; 34.1%), were based on relaxation or stress management. Multicomponent cognitive-behavioral interventions were also common and assessed in 18 cases (22.0%), including psycho-education and various cognitive and behavioral techniques. Other interventions were based on mindfulness and/or meditation (13 interventions; 15.9%), hypnosis (12 interventions; 14.6%), emotional disclosure (7 interventions; 8.5%), and counseling (4 interventions; 4.9%). The interventions varied in their total duration from 1 single session to multiple sessions over a period of 12 months. Regarding the guidance of the interventions, all interventions included face-to-face or telephone appointments, except for 2 interventions that relied on self-practice. Of the guided interventions, 48 (58.5%) also encouraged self-practice.

Overall immune effects

Detailed information concerning the immune-related challenges and outcomes for each study is presented in Supplemental Table 3.

When performing an overall random-effects meta-analysis on the data, i.e., irrespective of the incorporated challenge, an overall small to moderate effect size was found (k = 84, g = .33 [95% CI .22; .43]) with moderate heterogeneity across the studies (I^2 = 59.41%).

When excluding the studies that were set at r = .00, a slightly higher overall small to moderate effect size was found (k = 64, g = .43 [95% CI .30; .55, I^2 = 67.69%]).

In vitro immune-related stimulations

Of the 75 studies, 52 studies (68.4%) incorporated at least one *in vitro* immune stimulation test, including NKCA (32 studies), LPR (28 studies), cytokine production (10 studies), and monocyte chemotaxis (1 study).

Figure 4 presents the forest plot on the random-effects meta-analysis for *in vitro* immune-related stimulations. Overall, a small effect size was found (k = 52, g = .28 [95% CI .15; .42]), with moderate heterogeneity across the studies (I^2 = 61.43%). After excluding the studies that were set at r = .00, a small to moderate effect size was found (k = 39, g = .39 [95% CI .22; .56], I^2 = 70.75%). When looking at specific subgroups of *in vitro* immune stimulation tests, we found a small effect size for NKCA (k = 31, g = .21 [95% CI .06; .35], I^2 = 40.22%), a small to moderate effect size for LPR (k = 28, g = .35 [95% CI .13; .57], I^2 = 73.07%), and a small to moderate effect size for cytokine production (k = 9, g = .32 [95% CI .14; .51], I^2 < .01%).

In vivo immune-related challenges

In vivo immune-related challenges were incorporated in the study designs of 17 studies (22.4%), including skin testing (8 studies), vaccination (5 studies), and wound healing (4 studies).

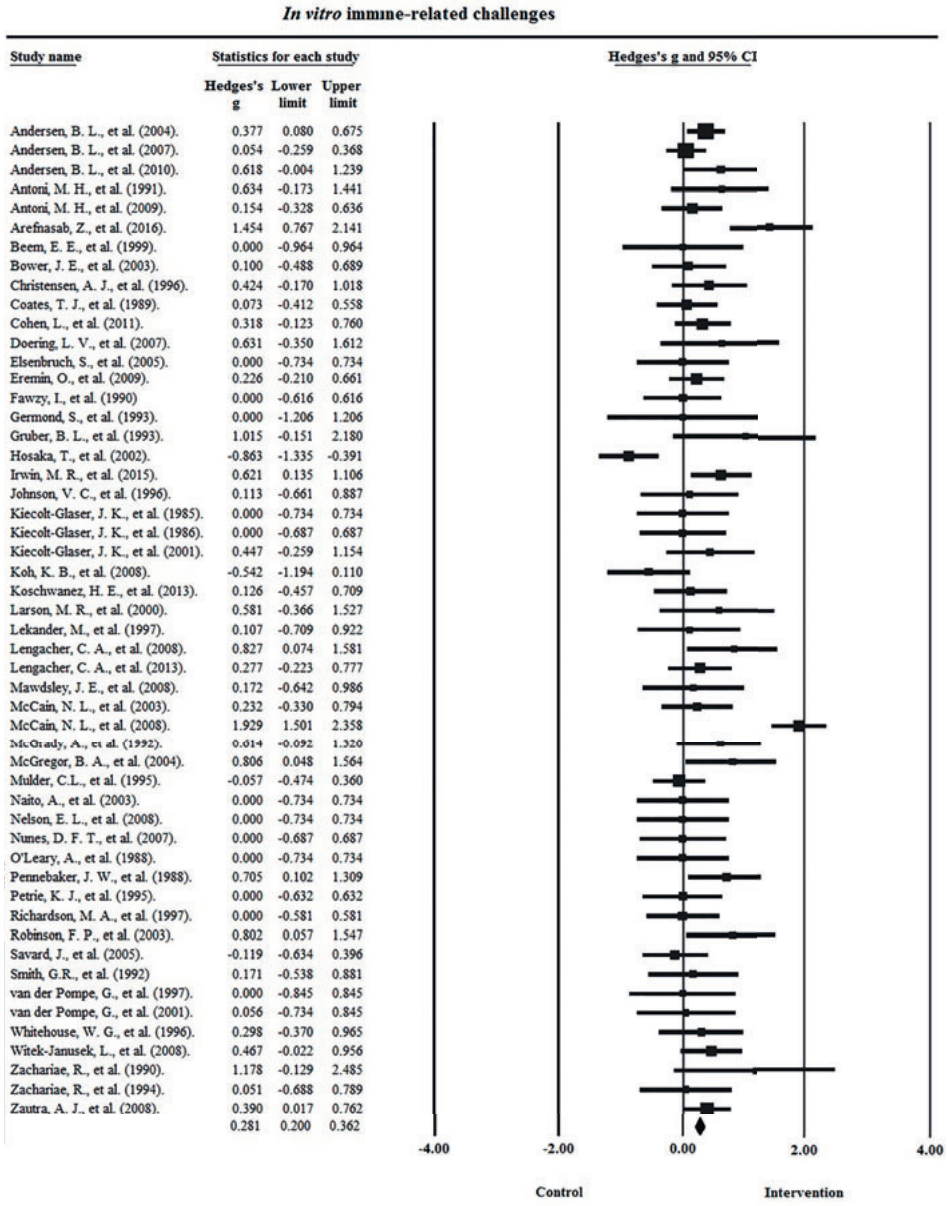

Figure 4. Forest plot of the random-effects meta-analysis on the studies incorporating in vitro immune-related stimulations.

Positive values for g indicate more optimal immune responses in the intervention condition than in the control condition.

Figure 5 presents the results of the random-effects meta-analysis on the pooled effects of *in vivo* immune-related challenges. A moderate effect size was found (k = 17, g = .61 [95% CI .34; .88]), with high heterogeneity across the studies (I^2 = 74.59%). After excluding the studies that were set at r = .00, a similar moderate effect size was found (k = 15, g = .64, [95% CI .35; .92], I^2 = 76.73%). When looking at specific subgroups within the *in vivo* immune-related challenges, a large effect size was found for studies using skin tests (k = 8, g = .80 [95% CI .30; 1.30], I^2 = 80.72%), whereas a small to moderate effect size was found for vaccine studies (k = 5, g = .37 [95% CI -.17; .90], I^2 = 77.69), and a moderate to large effect size for wound healing studies (k = 4, g = .75 [95% CI .45; 1.05], I^2 < 0.01%).

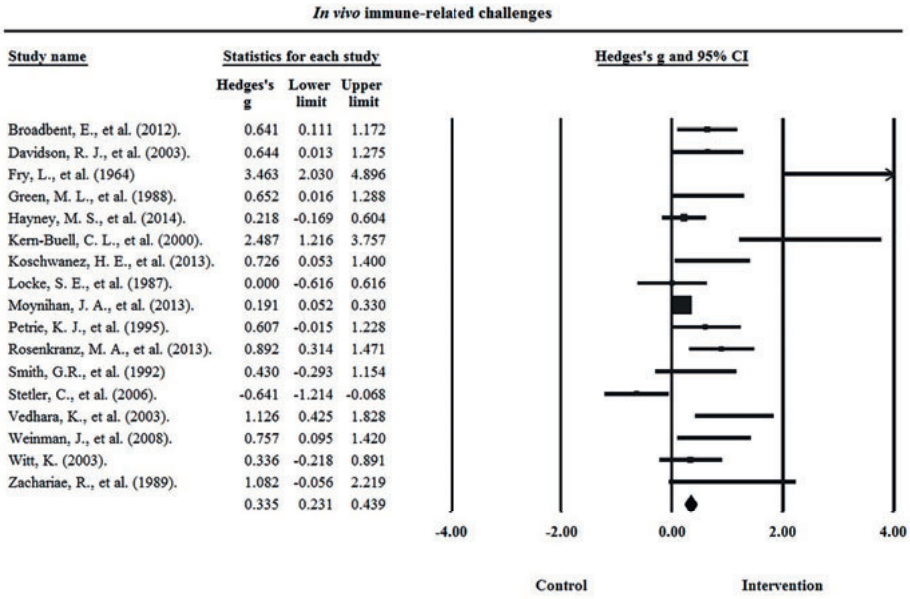

Figure 5. Forest plot of the random-effects meta-analysis on the studies incorporating in vivo immune-related challenges.

Positive values for g indicate more optimal immune responses in the intervention condition than in the control condition.

Psychophysiological challenges

In 16 studies (19.7%), a psychophysiological challenge was incorporated, whereby acute challenges included a speech task, exams, cold pressor test, and treadmill exercise test (10 studies), and challenges of a more protracted character, including academic stress and HIV serostatus notification (6 studies).

In Figure 6, the results of the random-effects meta-analysis on the pooled effects of psychophysiological challenges is shown. One study was not included in the meta-analysis as the outcomes were only based on in vitro LPR stimulation, instead of plasma measurements T-cell enumeration or cytokine quantification. Overall, no effect was found (k = 15, g = .18 [95% CI .01; .35], I^2 < .01), whereas a small effect size was found when excluding the studies that were set at r = .00 (k = 10, g = .28 [95% CI .07; .49], I^2 < .01). When assessing studies that incorporated enumeration of lymphocyte subsets after a psychophysiological challenge (i.e., CD4, CD8, CD56), a small to moderate effect size was found for studies incorporating a more protracted stress challenge (k = 4, g = .33 [95% CI = -0.06; .72], I^2 = 1.68%). For acute stress challenges, there were not enough studies available that had incorporated those markers in order to evaluate the effects after an acute stress challenge (k = 2). For studies that incorporated plasma cytokine measurements (i.e., IL-1ß, IL-6, IL-8, TNF-α) after a psychophysiological challenge, a small effect size was described in studies incorporating an acute challenge (k = 4, g = .22 [95% CI -.04; .49], I^2 < .01%), whereas no studies incorporated those markers to evaluate the effects after a more protracted stress challenge.

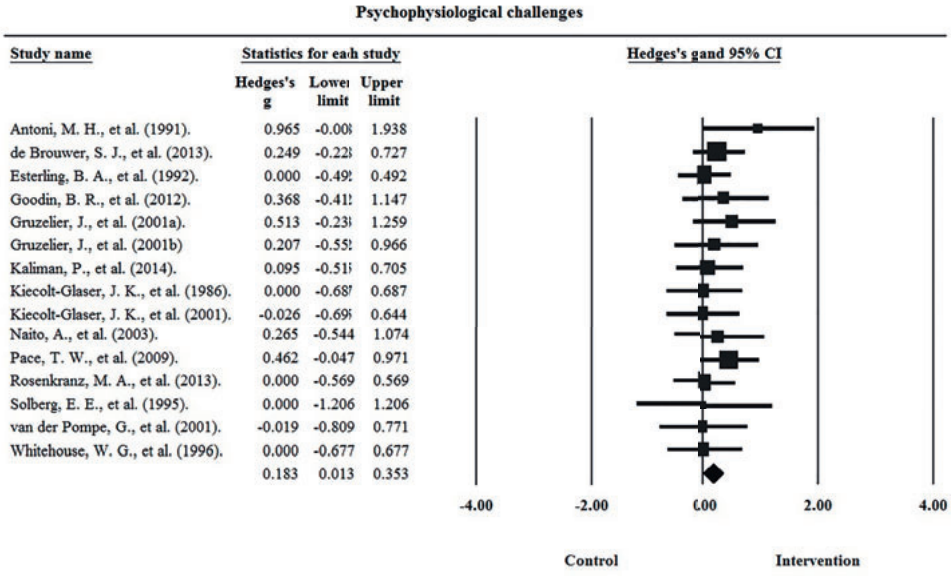

Figure 6. Forest plot of the random-effects meta-analysis on the studies incorporating psychophysiological challenges.

Positive values for g indicate more optimal immune responses in the intervention condition than in the control condition.

Sensitivity analyses

Risk of bias within studies

When studies with a presumed high risk of bias were excluded from the analyses, 23 of 84 outcomes were excluded. However, the overall effect size was not substantially altered (*k* = 61, *g* = .34 [95% CI .20; .48]).

Publication bias

The funnel plot is displayed in Figure 7 and suggests presence of publication bias. The trim and fill method indicates that 12 studies were expected to be missing with below-average effects, as indicated by the black dots. When imputing those studies, the effect size decreased to *g* = .21 [95% CI .09; .32]).

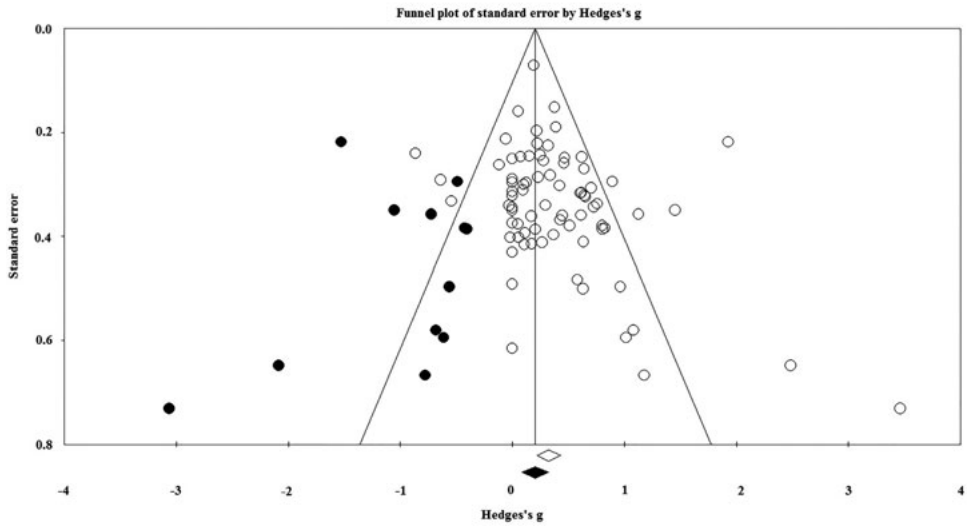

Figure 7. Funnel plot of standardized differences in mean by Hedges g.

Discussion

Over the last few decades, studies have evaluated the effectiveness of stress-reducing psychological interventions on immune function by incorporating chemical, physical, and psychophysiological challenges into the study design. These challenges are thought to present a biologically more valid reflection on the effectiveness of stress-reducing psychological interventions in optimizing immune function as compared to counting cells (170, 252, 253). The present systematic review and meta-analysis, for the first time, summarized immune-related outcomes after a chemical, physical, or psychophysiological

challenge following a stress-reducing psychological intervention in both healthy subjects and patients.

Overall, the findings demonstrated a small to moderate (heterogeneous) positive effect size for optimizing immune function. As a conservative method was applied to handle studies that reported no significant results without further specifying the actual group differences, the overall effect size possibly represents a slightly underestimated effect size. While the largest effects were found for *in vivo* immune-related challenges (especially on studies that incorporated skin tests and wound healing), studies incorporating psychophysiological challenges and *in vitro* immune-related stimulations similarly suggest more optimal immune responses among those receiving stress-reducing interventions.

When focusing on *in vitro* immune-related stimulations, small effect sizes were found. Studies were highly diverse regarding source of material and technical details of the stimulation. For example, studies varied in the target of stimulation (e.g., stimulation of T-cells, NK cells), the types of outcomes (e.g., proliferation, cytokine production, killing monocytes) and the types of concentrations and duration of stimuli. Likewise, a subset of studies stimulated whole blood, hereby performing tests in a biologically normal blood-plasma context, whereas others stimulated peripheral blood mononuclear cells (PBMCs), whereby tests are performed in artificial buffer solutions. Therefore, whole blood stimulations comprise a rather diverse range of cell populations (for example neutrophils, eosinophil's, etc.), whereas the cell populations in PBMCs are more well-defined, resulting in different environments of stimulation. In addition, important details were often lacking from the methods section, such as concentrations used or which type of immune cells were stimulated, while such aspects may substantially influence the results. Future studies are therefore encouraged to report more carefully on the methodological details. This could, for example, be acquired by applying a standard format for reporting the methodology, such as the Minimum Information About a Microarray Experiment (MIAME) guidelines (254) or the Minimal Information About T cell Assays (MIATA) standard (255). In addition, since *in vitro* stimulations are applied outside the body, those challenges may comprise a less biologically relevant valid representation of real-life immune threats as compared to *in vivo* challenges, although *in vitro* immune-related stimulations are easier to implement into the study design.

When focusing on *in vivo* immune-related challenges, studies on skin tests and wound healing found largest effect sizes and were mostly based on evaluating wound size alteration, instead of quantitative immune outcome measures. These outcome

parameters contain a rather unidirectional and straightforward representation of immune function (i.e., faster wound healing represents a more optimal immune response). Thus, of all immune-related challenges examined, most convincing evidence is found for stress-reducing psychological interventions optimizing immune performance in case of wound healing and skin-based tests. Even though these immune-related challenges probably represent a general stimulation of immune performance, this could imply that stress-reducing interventions could be particularly clinically relevant for patients with immune-related skin conditions, such as patients that recover from inflammation-sensitive surgical wounds. Contrary to these findings, only a small to moderate effect size was found for vaccines. Due to the small number of studies that incorporated a vaccine (5 studies), and variation in the type of incorporated vaccines and included time points (influenza vaccines, but also one study with a Hepatitis B vaccine incorporating various measurement points), the present meta-analysis could not provide a conclusive view on this subcategory of *in vivo* immune-related challenges. As few studies incorporated a vaccine, future research would be helpful to further elucidate the effects of psychological interventions on *in vivo* immune-related challenges, particularly in the area of vaccination and related immune outcomes.

For studies incorporating psychophysiological challenges, small effect sizes on immune measures were found when incorporating acute challenges (e.g., exam stress), and small to moderate effect sizes were found when incorporating chronic stressors (e.g., academic stress). Although the data did not seem to display high statistical heterogeneity, the incorporated challenges and immune outcome parameters were highly diverse across studies. More specifically, studies included acute challenges such as exams, speech tasks (some accompanied with or without a mental arithmetic task), a treadmill exercise test, and a cold pressor task, as well as more protracted stress challenges such as serostatus notification for individuals undergoing HIV testing and academic stress experienced by students during an examination period. Since the findings of the present study were based on a small number of studies with mostly limited ecological validity of the stressors, i.e., only some included challenges represented chronic stress as experienced by people in daily life, future work should focus on incorporating stressors with high external validity (e.g., social-evaluative stressors for socially anxious subjects or more daily-life chronic stress such as rumination) in order to evaluate the effects of psychological interventions on immune function (40).

Most the studies that incorporated psychophysiological challenges involved healthy participants (14 out of 17 studies). As healthy participants are supposed to have a well-functioning immune system, they are expected to show responses within the normal range

to standard immune system challenges, also in absence of a stress-reducing psychological intervention (167). The challenging situation to which these healthy participants are exposed therefore must be powerful enough to detect any relevant alterations in immune function in response to a psychological intervention. It is possible that combining a psychophysiological challenge with an *in vivo* immune-related challenge can boost the effects of the separate challenges and possibly provide healthy participants with a more robust immune system challenge. Only one study in the present systematic review and meta-analysis combined an *in vivo* immune-related challenge, i.e., suction blisters on the volar forearm, with a psychophysiological challenge, i.e., a Trier Social Stress Test (TSST), to evaluate the effects of a stress-reducing psychological intervention (200). In this study, participants who received a stress-reducing mindfulness intervention showed a lower post-stress (i.e., post-TSST) inflammatory response to the *in vivo* immune-related and psychophysiological challenges compared to a control group that received a control health enhancement program. The incorporation of both an *in vivo* immune-related challenge and a psychophysiological challenge provides a more elaborate view on the underlying processes of immune function after a psychological intervention, i.e., evaluating immune function after activating the immune system through different challenges that can boost each other's effectiveness. Future studies may consider incorporating multiple challenges in their design when examining immune function in healthy participants in order to hypothetically provide them with a rather robust challenge (256). Due to the heterogeneity that was observed in the included studies, we were not able to analyze the healthy participant studies and somatic patient studies separately. Future studies should systematically incorporate challenges to evaluate the effectiveness of a psychological intervention on immune function and adequately match the incorporated challenge(s) with the included study population, in order to gather a more homogeneous view on this topic.

Regarding the effective components of stress-reducing psychological interventions, no strong conclusions can be drawn at this point due to the substantial heterogeneity in the incorporated intervention elements across studies, including duration and number of sessions, intervention target, and ways of guidance (e.g., self-practice, structured guided sessions, etc.). An exploratory evaluation of the data, however, showed that multiple studies explored the role of self-practice during the intervention (e.g., completing homework assignments) for immune outcomes (181, 183, 186, 191, 192, 197, 200, 204, 213, 220, 223, 251). Most of these studies found a positive association between frequency of self-practice and optimized immune outcomes (181, 191, 192, 197, 200, 213, 223). Although we could not formally test this observation in our meta-analysis due to substantial heterogeneity in study designs (e.g., selection of immune outcomes and

differences in level of details concerning the specification of self-practice frequency), these findings possibly point to the importance of engaging participants with components of the psychological intervention. However, it is important to note that the studies included in the present systematic review and meta-analysis varied widely in the way engagement and the actual effectiveness of the stress-reducing psychological intervention was evaluated. In addition, a substantial number of studies did not report on whether the intervention was actually effective in reducing stress, making it hard to take this factor into account in our analyses. For the same reason, it was not possible to control for confounding factors, including body mass index, recent illness, female menstruation cycle, and so on. As failures to improve immunity can be due to the fact that the stress-reducing psychological interventions were actually not effective in reducing stress, future studies should also carefully evaluate to what extent participants were engaged with the stress-reducing psychological intervention, and whether these interventions were effective in reducing stress. In addition, the present findings were based on the assumption that higher levels of immune activation were associated with a more optimized immune response. However, enhanced immune responses are not necessary beneficial, e.g., in the case of inflammatory and autoimmune disorders (257). In certain cases, optimization is not based on larger immune responses, but on normalization of immune outcomes. Future studies should therefore take the health consequences of the immune response into account when evaluating the effectiveness of a psychological intervention on immune function. Note that as the aim of a stress-reducing psychological intervention is to optimize health outcomes by stress reduction, it would be most relevant to recruit individuals that experience chronic stress with a substantial impact on immune function to evaluate the effectiveness of stress-reducing psychological interventions (169). In addition, future studies should focus on unraveling the effective intervention components in optimizing immune responses by evaluating the effectiveness of intervention components separately, but also in combination with each other.

In conclusion, the present systematic review and meta-analysis provided evidence for the effects of stress-reducing interventions in optimizing immune function when immune outcomes were evaluated by utilizing tests that apply challenges to the immune system. While consistent evidence came from studies that evaluated immune function through an *in vivo* immune-related challenge, specifically studies incorporating skin tests and studies on wound healing, similar but smaller effect sizes were found for *in vitro* immune-related stimulations and immune responses to psychophysiological challenges. Due to the large heterogeneity in study designs, there is a need for future research that incorporates immune- and psychophysiological challenges, as these have a high external validity and are suited for possible clinical applications in immune-related diseases. Studies in healthy

participants have to make sure that the immune challenge is robust enough, for example by combining separate challenges. Finally, future studies should carefully report on the methodological details according to standardized guidelines, including the actual stress-reducing effectiveness of the psychological interventions, and appropriate interpretation of the immune outcomes. This can result in further insights on the immune outcomes that are responsive for change as well as a thorough view on the effective intervention components to optimize immune responses in the short and longer term.

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Supplemental material

Supplemental Table 1. Search terms for Pubmed, EMBASE and PsychInfo.

Pubmed

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EMBASE

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response OR AB stress-evoked cortisol response OR TI combat training OR AB combat training OR TI bungeejump OR AB bungeejump OR TI parachute jump* OR AB parachute jump*) AND ((TI *Hypnosis OR AB *Hypnosis OR DE "Hypnosis" OR DE "Hypnotherapy" OR DE "Self-Disclosure" OR TI Psychotherap* OR AB Psychotherap* OR DE "Psychotherapy" OR TI *Writing OR AB *Writing OR TI *Relaxation therapy OR AB *Relaxation therapy OR DE "Relaxation Therapy" OR DE "Progressive Relaxation Therapy" OR DE "Meditation" OR TI Conditioning OR AB Conditioning OR DE "Conditioning" OR DE "Classical Conditioning" OR DE "Operant Conditioning" OR TI stress management OR AB stress management OR DE "Stress Management" OR TI disclosure OR AB disclosure OR TI mindfulness practice OR AB mindfulness practice OR DE "Mindfulness" OR TI Expressive writing OR AB Expressive writing OR TI Cognitive behavioral therapy OR AB Cognitive behavioral therapy OR TI cognitive-behavi* therap* OR AB cognitive-behavi* therap* OR TI relaxation OR AB relaxation OR TI Guided imagery OR AB Guided imagery OR TI CBT OR AB CBT OR DE "Behavior Therapy" OR DE "Conversion Therapy" OR DE "Dialectical Behavior Therapy" OR DE "Exposure Therapy" OR DE "Implosive Therapy" OR DE "Reciprocal Inhibition Therapy" OR DE "Systematic Desensitization Therapy" OR DE "Cognitive Behavior Therapy" OR TI psychoeducational OR AB psychoeducational OR TI psychoeducation OR AB psychoeducation OR DE "Psychoeducation" OR TI psycho-educational OR AB psycho-educational OR TI psycho-education OR AB psycho-education OR DE "Counseling" OR DE "Educational Counseling" OR DE "Group Counseling" OR DE "Occupational Guidance" OR DE "Psychotherapeutic Counseling" OR DE "Rehabilitation Counseling" OR TI counselling OR AB counselling OR TI counseling OR AB counseling OR ((TI therapy OR AB therapy OR TI therapies OR AB therapies OR TI treatment* OR AB treatment*) AND (TI cognitive OR AB cognitive OR TI behavior OR AB behavior OR TI behavioral OR AB behavioral OR TI behaviour OR AB behaviour OR TI behavioural OR AB behavioural OR TI conditioning OR AB conditioning OR TI cognition OR AB cognition OR Behavior Therapy OR TI behaviour modification OR AB behaviour modification OR TI behavior modification OR AB behavior modification OR TI conditioning therap*)) OR DE "Conditioning" OR AB conditioning therap* OR TI conditioning treatment OR AB conditioning treatment OR TI cognition therap* OR AB cognition therap* OR TI cognitive treatment OR AB cognitive treatment))

Supplemental Table 2. Study characteristics and details concerning the intervention

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Andersen, et al. (2004).	Women surgically treated for regional breast cancer	*N* = 227 *M* age = 50.8, *SD* = 10.8	Multicomponent cognitive behavioral intervention (Control = AO)	4 months	Appointments: 18 group sessions of 1.5 hrs	Psychologist
Andersen, et al. (2007).	See Andersen, B. L., et al. (2004)	See Andersen, B. L., et al. (2004)	Multicomponent cognitive behavioral intervention (Control = AO)	12 months	Appointments: 26 group sessions of 1.5 hrs	Psychologist
Andersen, et al. (2010).	Women with recurrent breast cancer	*N* = 62 *M* age = 53.7, *SD* = 11.3	See Andersen, B. L., et al. (2007	See Andersen, B. L., et al. (2007)	See Andersen, B. L., et al. (2007)	See Andersen, B. L., et al. (2007)
Antoni, et al. (1991).	Healthy gay men	*N* = 47 *M* age = 30.5	Stress management (Control = AO)	2.5 months	Appointments: biweekly group sessions (weekly 1 session of 45 min and 1 session of 1.5 hrs) Self-practice: daily relaxation exercises	Psychologist
Antoni, et al. (2009).	Women who underwent breast cancer treatment	*N* = 128 *M* age = 49.7, *SD* = 7.9	Stress management (Control = Condensed educational version of the intervention during a seminar lasting 5-6 hrs at the midpoint of the 10-week period)	2.5 months	Appointments: weekly group sessions Self-practice: daily relaxation exercises	Guided by intervention facilitators who were supervised by psychologists
Arefnasab, et al. (2016).	Male veterans with pulmonary injury	*N* = 40 *M* age = 49.4, range = 42-59	Mindfulness (Control = WLC)	2 months	Appointments: weekly group sessions of 2 hrs Self-practice: daily home practice	Clinician specialist
Beem, et al. (1999).	Widows	*N* = 18 *M* age = 58.6, *SD* = 4.9	Counseling (Control = AO)	4 months	Appointments: 13 group sessions (2 sessions of 5 hrs and 11 sessions of 2.5 hrs)	Trained counselors who were supervised
Bower, et al. (2003).	Women who lost a close relative to breast cancer	*N* = 43 *M* age = 42.1, *SD* = 8.3	Emotional disclosure (Control = writing about various non-emotional topics)	1 month	Self-practice: weekly 20 min sessions	Unguided appointments; instructions were mailed by a research assistant
Broadbent, et al. (2012).	Patients who underwent surgery (Male: 25%; Female: 75%)	*N* = 60 *M* age = 51.3, *SD* = 16.8	Stress management (Control = CAU)	± 10 days	Appointments: 1 individual session of 45 min Self-practice: daily 20 min with a CD at least 3 days before surgery and 7 days after surgery	Psychologist

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
de Brouwer, et al. (2013).	Patients with RA (Male: 41.9%; Female: 58.1%)	*N* = 74 *M* age = 58.8, *SD* = 10.6	Stress management (Control = CAU)	2 weeks + 7 weeks relapse prevention	Appointments: biweekly individual sessions of 1 hr Self-practice: relapse prevention checklist for 9 weeks	Psychologist
Christensen, et al. (1996).	Male college undergraduates	*N* = 43 Age not specified	Emotional disclosure (Control = Reading about a hypothetical situation that described a student's experience with failing a course)	1 session	Appointment: 1 individual session of ± 35 min	Structured session; way of guidance not further specified
Coates, et al. (1989).	Men with HIV infection	*N* = 64 *M* age = 34.9	Stress management (Control = WLC)	2 months	Appointments: weekly group sessions of 2 hrs and one-day retreat after 1 month	Structured sessions; way of guidance not further specified
Cohen, et al. (2011).	Men with prostate cancer undergoing radical prostatectomy	*N* = 159 *M* age = 60.5, *SD* = 6.7	Stress management (Control = CAU)	2 sessions	Appointments: 2 individual sessions of 1-1.5 hrs and 2 brief booster sessions Self-practice: relaxation by audiotapes	Psychologist
			Counseling (Control = CAU)	2 sessions	Appointments: 2 individual sessions of 1-1.5 hrs and 2 brief booster sessions	Psychologist
Davidson, et al. (2003).	Healthy participants (Male: 29.3%; Female: 70.7%)	*N* = 41 *M* age = 36; Range = 23-56	Mindfulness/meditation (Control = WLC)	2 months	Appointments: weekly group sessions of 2.5-3 hrs and 1 silent retreat of 7 hrs at week 6 Self-practice: 1 hr daily during 6 days per week	Psychologist
Doering, et al. (2007).	Women with a clinical depression after CABG	*N* = 15 *M* age = 59.8, *SD* = 8.6	Multicomponent cognitive behavioral intervention (Control = CAU)	2 months	Appointments: weekly individual sessions of 1 hr Self-practice: homework assignments	Trained nurse therapist
Elsenbruch, et al. (2005).	Patients with UC (Male: 33.3%, Female: 66.7%)	*N* = 30 *M* age = 42.7, *SD* = 10	Multicomponent cognitive behavioral intervention (Control = WLC)	2.5 months	Appointments: weekly sessions of 6 hr	Structured sessions; way of guidance not further specified
Eremin, et al. (2009).	Women with locally advanced breast cancer undergoing multimodality therapy	*N* = 80 *M* age = 49.9, *SD* = 11.5	Relaxation (Control = CAU)	Not specified	Appointments: 5 individual sessions Self-practice: regular home practice through audiotapes	Structured sessions; way of guidance not further specified

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Esterling, et al. (1992).	HIV-1-infected and at risk gay men	*N* = 65 *M* age = 30.5	Stress management (Control = AO)	2.5 months	Appointments: weekly 1 session of 1.5 hrs and 1 session of 45 min Self-practice: take-home imagery tape from week 8 to 10	Psychologist
Fawzy, et al. (1990).	Cancer patients (Male: 45.9%; Female: 54.1%)	*N* = 61 *M* age = 42, range = 19-70	Multicomponent cognitive behavioral intervention (Control = CAU)	1.5 months	Appointments: weekly group sessions of 1.5 hrs	Structured sessions; way of guidance not further specified
Fry, et al. (1964).	Persons with asthma or hay-fever (Gender not specified)	*N* = 47 Age not specified	Hypnosis (Control = AO)	2 weeks	Appointments: 3 group meetings	Structured sessions; way of guidance not further specified
Germond, et al. (1993).	Women with RA	*N* = 14 *M* age = 49, *SD* = 9.4	Multicomponent cognitive behavioral intervention (Control = AO)	2 months	Appointments: biweekly group sessions of 2 hrs	Structured sessions; way of guidance not further specified
Goodin, et al. (2012).	Healthy participants (Male: 50%; Female: 50%)	*N* = 24 *M* age = 19.8, *SD* = 3.0	Hypnosis (Control = AO)	2 weeks	Appointments: weekly sessions	Research assistants who were supervised by a psychologist
Green, et al. (1988).	Students and college employees (Gender not specified)	*N* = 40 Age not specified	Relaxation (Control = WLC)	3 weeks	Appointments: 2 group sessions of 20 min Self-practice: daily 20 min home sessions	Structured sessions; way of guidance not further specified
Gruber, et al. (1993).	Women with breast cancer	*N* = 13 *M* age = 44.6, range = 34-50	Multicomponent cognitive behavioral intervention (Control = WLC)	9 weeks	Appointments: 4 weekly group sessions followed by biweekly biofeedback sessions Self-practice: twice daily relaxation and guided imagery practices	Structured sessions; way of guidance not further specified
Gruzelier, et al. (2001a).	Students (Male: 60.7%; Female: 39.3%)	*N* = 28 *M* age = 20.1	Hypnosis (Control = AO)	3 weeks	Appointments: 1 group session Self-practice: 9 tape recorded hypnosis sessions	Structured sessions; way of guidance not further specified
Gruzelier, et al. (2001b).	Students (Male: 61.3%; Female: 38.7%)	*N* = 31 *M* age = 19.1	Hypnosis (Control = AO)	3 weeks	Appointments: weekly group sessions of 20 min Self-practice: home practice of hypnosis through audio cassettes with a minimum of 3 sessions a week	Researchers
			Relaxation (Control = AO)	3 weeks	Appointments: weekly group sessions of 20 min Self-practice: home practice of hypnosis through audio cassettes with a minimum of 3 sessions a week	Researchers
Hayney, et al. (2014).	Healthy individuals (Male: 18.6%; Female: 81.4%)	*N* = 102 *M* age = 59.4; *SD* = 6.7	Mindfulness/meditation (Control = WLC)	2 months	Appointments: weekly group sessions of 2.5 hrs Self-practice: daily 45 min	Senior exercise physiology staff

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Hosaka, et al. (2002).	Japanese infertile women	*N* = 74 *M* age = 34.8	Multicomponent cognitive behavioral intervention (Control = CAU)	5 weeks	Appointments: weekly group sessions of 1.5 hrs	Nurses / psychiatrists
Irwin, et al. (2015).	Patients with late life insomnia (Male: 24%; Female: 76%)	*N* = 75 *M* age = 65.1, *SD* = 6.6	Multicomponent cognitive behavioral intervention (Control = sleep seminar)	4 months	Appointments: weekly group sessions of 2 hrs	Structured sessions; way of guidance not further specified
Johnson, et al. (1996).	Healthy volunteers (Gender not specified)	*N* = 24 Age not specified	Stress management (Control = AO with hypnotic induction at baseline)	3 weeks	Appointments: 1 session at the start and at the end for 12.5 minutes Self-practice: daily home practice with audiotapes of relaxation	Structured sessions; way of guidance not further specified
Kaliman, et al. (2014).	Expert meditators (Male: 42.5%; Female: 57.5%)	*N* = 40 *M* age = 50.1, *SD* = 10.0	Mindfulness/meditation (Control = intentional activities such as reading, watching documentaries, etc., without unique components of mindfulness)	1 session	Appointments: 1 group session of 8 hrs	Structured session; way of guidance not further specified
Kern-Buell, et al. (2000).	Nonsmoking patients with asthma (Male: 37.5%; Female: 62.5%)	*N* = 16 *M* age = 20.5, *SD* = 5.9	Relaxation (Control = WLC)	2 months	Appointments: 8 sessions of unspecified duration Self-practice: twice a day for 15 min with audiotapes of autogenic relaxation	Structured sessions; way of guidance not further specified
Kiecolt-Glaser, et al. (1985).	Geriatric population (Male: 20%; Female: 80%)	*N* = 45 *M* age = 74, range = 60-88	Relaxation (Control = WLC)	1 month	Appointments: 3 weekly individual sessions of 45 min	Trained psychology students
Kiecolt-Glaser, et al. (1986).	Healthy participants (Male: 64.7%; Female: 35.3%)	*N* = 34 *M* age = 23.5	Stress management (Control = WLC)	2.5 weeks	Appointments: 5-10 possible group sessions in 2.5 week of 35-45 min in length Self-practice: encouraged to practice relaxation outside the group sessions	Psychologist
Kiecolt-Glaser, et al. (2001).	Medical and dental students (Male: 42.4%; Female: 57.6%)	*N* = 33 *M* age = 23.5, *SD* = 2.0	Hypnosis (Control = WLC)	8 days	Appointments: 5 group sessions of 25-40 min Self-practice: daily practice of relaxation-self-hypnosis	Psychologists
Koh, et al. (2008).	Medical students (Male: 66.7%; Female: 33.3%)	*N* = 36 *M* age = 23.7, *SD* = 1.9	Relaxation (Control = WLC)	1 month	Appointments: 2 sessions of 1 hr Self-practice: twice a day for 15 min	Psychiatrist

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Koschwanez, et al. (2013).	Older adults (Male: 42.9%; Female: 57.1%)	*N* = 49 *M* age = 78.8, *SD* = 7.2	Emotional disclosure (Control = writing about their daily activities)	3 days	Appointments: daily individual sessions of 20 min	Researchers
Larson, et al. (2000).	Women with breast cancer	*N* = 41 *M* age = 56, *SD* = 13	Multicomponent cognitive behavioral intervention (Control = CAU)	2 sessions	Appointments: 2 sessions of 1.5 hrs Self-practice: twice daily relaxation practice through audiotapes	Psychologist
Lekander, et al. (1997).	Women with ovarian cancer	*N* = 22 *M* age = 56.8, *SD* = 10.5	Relaxation (Control = CAU)	2 months	Appointments: 3 individual sessions of 20-45 min Self-practice: relaxation practice on a regular basis through audiotapes	Psychologist
Lengacher, et al. (2008).	Women with breast cancer	*N* = 28 *M* age = 52.6, range 25-75	Stress management (Control = CAU)	1 month	Appointments: 1 individual session of 0.5 hrs Self-practice: listening to guided imagery tapes for three times a week	Psychologist
Lengacher, et al. (2013).	Women with breast cancer	*N* = 82 *M* age = 58, *SD* = 9	Mindfulness/meditation (Control = CAU)	1.5 months	Appointments: weekly 2 hrs sessions Self-practice: daily meditation and yoga practice, and other homework assignments	Psychologist
Locke, et al. (1987).	Healthy volunteers (Male: 42.9%; Female: 57.1%)	*N* = 42 *M* age = 26, *SD* = 4	Hypnosis (Control = AO)	3 days	Appointments: 6 sessions of unspecified duration Self-practice: 5 audiotaped reinforcement practice sessions	Structured sessions; way of guidance not further specified
Mawdsley, et al. (2008).	Patients with active UC (Male: 60%; Female: 40%)	*N* = 25 *M* age = 41.0, range = 23-63	Hypnosis (Control = listening to relaxing music of their own choice for 50 min)	1 session	Appointments: 1 session of 50 min	Hypnotherapist
McCain, et al. (2003).	Persons with HIV infection (Male: 80%; Female: 20%)	*N* = 148 *M* age = 39.4, median = 39.0	Stress management (Control = WLC)	2 months	Appointments: weekly group sessions of 1.5 hrs Self-practice: daily practice of the learned skill for 1 week through audiotapes	Structured sessions; way of guidance not further specified Mental health nurse
			Counseling (Control = WLC)	2 months	Appointments: weekly group sessions of 1.5 hrs weekly	

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
McCain, et al. (2008).	Persons with HIV infection (Male: 60.3%; Female: 39.7%)	*N* = 252 *M* age = 42.2	Relaxation (Control = WLC)	2.5 months	Appointments: weekly group sessions of 1.5 hrs Self-practice: routinely practice with relaxation	Experienced and licensed investigator in stress management
McGrady, et al. (1992).	Healthy adults (Male: 56.3%; Female: 43.7%)	*N* = 32 *M* age = 24.9	Relaxation (Control = AO)	1 month	Appointments: weekly group sessions of 0.5 hrs and weekly individual sessions of 0.5 hrs Self-practice: by an autogenic relaxation tape twice a day	Structured sessions; way of guidance not further specified
McGregor, et al. (2004).	Women with early-stage breast cancer	*N* = 29 *M* age = 47.5, *SD* = 6.3	Stress management (Control = AO with 1-day stress management education seminar after 10 weeks)	2.5 months	Appointments: weekly group sessions of 2 hrs Self-practice: weekly homework assignments	Structured sessions; way of guidance not further specified
Moynihan, et al. (2013).	Older adults (Male: 38%; Female: 62%)	*N* = 200 *M* age = 73.5, *SD* = 6.7	Mindfulness/meditation (Control = WLC)	2 months	Appointments: weekly group sessions of 2 hrs and 1 session of 7 hrs	Licensed MBSR trainer
Mulder, et al. (1995).	Men with asymptomatic HIV-infection	*N* = 165 *M* age = 38.7, range = 21-61	Multicomponent cognitive behavioral intervention (Control = WLC)	15 weeks	Appointments: weekly sessions of 2.5 hrs	Structured sessions; way of guidance not further specified
Naito, et al. (2003).	Healthy students (Male: 45.8%; Female: 54.2%)	*N* = 48 Range age = 19-37	Hypnosis (Control = 8 mock neurofeedback sessions over 1 month)	1 month	Appointments: weekly sessions Self-practice: three times a day self-hypnosis for two weeks, thereafter once a day	Hypnotherapist
Nelson, et al. (2008).	Women who survived cervical cancer	*N* = 36 *M* age = 47.9, *SD* = 2.9	Counseling (Control = CAU)	± 3 months	Appointments: 5 weekly individual sessions of 45-50 min and a booster session	Psychologist
Nunes, et al. (2007).	Women with breast cancer undergoing radiotherapy	*N* = 34 *M* age = 52.5, *SD* = 1.8	Relaxation (Control = CAU)	3.5 weeks	Appointments: daily group sessions of 0.5 hrs Self-practice: twice daily at home	Psychologist
O'Leary, et al. (1988).	Women with RA	*N* = 30 *M* age = 49.3, range = 22-75	Multicomponent cognitive behavioral intervention (Control = reading a help book which was also provided to the intervention group)	5 weeks	Appointments: weekly group sessions of 2 hrs	Researchers

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Pace, et al. (2009).	Healthy adults (Male: 47.5%; Female: 52.5%)	*N* = 61 *M* age = 18.5, *SD* = 0.7	Mindfulness/meditation (Control = attending health discussion groups with a requirement of at least 12 hrs participation)	1.5 months	Appointments: biweekly group sessions of 50 min Self-practice: through audiotapes with meditation practice	Researchers
Pennebaker, et al. (1988).	Healthy undergraduates (Male: 28%; Female: 72%)	*N* = 50 Age not specified	Emotional disclosure (Control = writing on an assigned topic without discussing their own thoughts / feelings)	4 days	Appointments: daily individual writing sessions of 20 min	Researchers
Petrie, et al. (1995).	Medical students (Male: 52.5%; Female: 47.5%)	*N* = 40 *M* age = 21.5, *SD* = 2.4	Emotional disclosure (Control = writing on different aspects of their use of time objectively with minimum use of emotions)	4 days	Appointments: daily individual writing sessions	Structured sessions; way of guidance not further specified
van der Pompe, et al. (1997).	Women with breast cancer	*N* = 31 *M* age = 58.8, *SD* = 8.0	Multicomponent cognitive behavioral intervention (Control = WLC)	13 weeks	Appointments: weekly group sessions of 2.5 hrs	Psychologist
van der Pompe, et al. (2001).	See van der Pompe, G., et al. (1997)	See van der Pompe, G., et al. (1997)	See van der Pompe, G., et al. (1997)	See van der Pompe, G., et al. (1997)	See van der Pompe, G., et al. (1997)	See van der Pompe, G., et al. (1997)
Richardson, et al. (1997).	Women with breast cancer	*N* = 47 *M* age = 46, *SD* = 8.7	Counseling (Control = CAU)	1.5 months	Appointments: weekly group sessions	Social workers
			Hypnosis (Control = CAU)	1.5 months	Appointments: weekly group sessions Self-practice: twice daily 20 min relaxation and imagery practice	Hypnotherapist
Robinson, et al. (2003).	Individuals with HIV infection (Male: 94.1%; Female: 5.9%)	*N* = 34 *M* age = 41.0, *SD* = 6.6	Mindfulness/meditation (Control = AO)	2 months	Appointments: weekly group sessions of 2.5 hrs and 1 session of 8 hrs Self-practice: daily practice for at least 45 min	Psychologist
Rosenkranz, et al. (2013).	Healthy volunteers (Male: 20.4%; Female: 79.6%)	*N* = 49 *M* age = 45.9, *SD* = 10.9	Mindfulness/meditation (Control = health enhancement program without the unique components of mindfulness)	2 months	Appointments: weekly sessions of 2.5 hrs and 1 full day session Self-practice: daily at home practice for 45-60 min	Licensed MBSR trainer
Savard, et al. (2005).	Women with breast cancer and chronic insomnia	*N* = 57 *M* age = 54.1, *SD* = 7.4	Multicomponent cognitive behavioral intervention (Control = WLC)	2 months	Appointments: weekly group sessions of 1.5 hrs with 1 optional booster session 1 month after the treatment	Structured sessions; way of guidance not further specified

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Smith, et al. (1992).	Healthy volunteers (Gender not specified)	*N* = 29 *M* age = 45, range = 28-60	Mindfulness/meditation (Control = AO)	1 week	Appointments: daily 2 sessions of 1hr Self-practice: 10 min 6 times daily using the learned techniques and listening to relaxation audiotapes 0.5 hrs upon arising and bedtime for the following week	Researchers
Solberg, et al. (1995).	Male runners	*N* = 12 Median age = 42.5, range = 27-49	Mindfulness/meditation (Control = AO)	7 weeks	Self-practice: regular 30 min sequences at home	Structured sessions; way of guidance not further specified
Stetler, et al. (2006).	Participants from University campus (Male: 10.4%; Female: 89.6%)	*N* = 47 *M* age = 27.5, *SD* = 10.3	Emotional disclosure (Control = writing about their schedule for the upcoming week)	11-15 days	Appointments: 3 individual sessions of 20 min	Researchers
Vedhara, et al. (2003).	Elderly (Male: 44.2%; Female: 55.8%)	*N* = 43 *M* age = 75, *SD* = 7	Stress management (Control = AO)	2 months	Appointments: weekly group sessions of 1 hr	Psychologist
Weinman, et al. (2008).	Male students and university staff	*N* = 36 *M* age = 22.2, *SD* = 4.1	Emotional disclosure (Control = writing about time management)	3 days	Appointments: daily individual writing sessions of 20 min	Structured sessions; way of guidance not further specified
Whitehouse, et al. (1996).	Students (Male: 40%; Female: 60%)	*N* = 35 *M* age = 24.8	Hypnosis (Control = WLC)	3.5 months	Appointments: weekly group sessions of 1.5 hrs Self-practice: daily diaries and 15 min self-hypnosis practice	Psychiatrist
Witek-Janusek, et al. (2008).	Women with breast cancer	*N* = 66 *M* age = 54.6, *SD* = 9.2	Mindfulness/meditation (Control = CAU)	2 months	Appointments: weekly group sessions of 2.5 hrs and 1 full day session Self-practice: homework assignments by a program workbook and audiotapes	Psychologist
Witt (2003).	Humans with birch pollen allergy (Gender not specified)	*N* = 72 *M* age = 42, range = 18-66	Multicomponent cognitive behavioral intervention (Control = AO)	1 month	Appointments: biweekly group sessions of 2.5 hrs	Structured sessions; way of guidance not further specified
Zachariae, et al. (1989).	Highly hypnotic susceptible subjects (Male: 72.2%; Female: 27.8%)	*N* = 18 Age not specified	Hypnosis (Control = AO)	4 days	Appointments: 1 session Self-practice: twice daily 20 min sessions using audiotapes of hypnosis and imagery during 3 days	Structured session; way of guidance not further specified

Study	Sample	Sample size (N) and age (M, SD)	Intervention type	Length	Intensity	Way of guidance
Zachariae, et al. (1990).	Healthy subjects (Gender not specified)	*N* = 14 Age not specified	Stress management (Control = AO)	10 days	Appointments: 1 individual session of 45 min and 1 individual session of 60 min	Structured sessions; way of guidance not further specified
Zachariae, et al. (1994).	Study 1: Healthy subjects (Male: 30%; Female: 70%)	Study 1: *N* = 30 *M* age = 30.5, *SD* = 8.9	Study 1: Stress management (Control = AO)	Study 1: 3 weeks	Study 1: Appointments: weekly individual sessions of 1 hr Self-practice: 5 times a week through an audio cassette tape	Study 1: Structured sessions; way of guidance not further specified
			Relaxation (Control = AO)	3 weeks	Appointments: weekly individual sessions of 1 hr Self-practice: 5 times a week through an audiocassette tape	Study 2: Structured sessions; way of guidance not further specified
	Study 2: Healthy subjects (Male: 30%; Female: 70%)	Study 2: *N* = 30 *M* age = 27.7, *SD* = 6.2	Study 2: Stress management (Control = AO)	Study 2: 3 weeks	Study 2: Appointments: weekly individual sessions of 1 hr Self-practice: 5 times a week through an audio cassette tape	Structured sessions; way of guidance not further specified
			Relaxation (Control = AO)	3 weeks	Appointments: weekly individual sessions of 1 hr Self-practice: 5 times a week through an audiocassette tape	
Zautra, et al. (2008).	Patients with RA (Male: 31.9%; Female: 68.1%)	*N* = 144 *M* age = 54.2, *SD* = 13.6	Mindfulness/meditation (Control = general education concerning RA and other health-related topics)	2 months	Appointments: weekly group sessions Self-practice: weekly homework practices	Psychologist
			Multicomponent cognitive behavioral intervention (Control = general education concerning RA and other health-related topics)	2 months	Appointments: weekly group sessions Self-practice: weekly homework practices	Psychologist

Note. AO = assessment-only; CABG = Coronary Artery Bypass Grafting; CAU = care as usual; CBSM = Cognitive Behavioral Stress Management; CBT = Cognitive Behavioral Therapy; HIV = Human Immunodeficiency Virus; hrs = hours; M = Mean; MBSR = Mindfulness Based Stress Reduction; min = minutes; N = Number; RA = Rheumatoid Arthritis; SD = Standard Deviation; UC = Ulcerative Colitis; WLC = waiting-list control.

Note 2. The reported *N* is based on the total study population for which age and gender were reported. This *N* could deviate from the study population for which the intervention outcomes were measured due to possible drop out during follow up measurements.

Supplemental Table 3. Challenges and outcomes

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
Andersen, et al. (2004).	-	In PBL: PHA → LPR Con A → LPR K562 → NKCC	-	Baseline and after intervention	CD3, CD4, CD8, CD56	↑ LPR to Con A**, ↑ LPR to PHA*	CD3, CD4, CD8, CD56, NKCC
Andersen, et al. (2007).	-	In PBL: PHA → LPR Con A → LPR K562 → NKCC	-	Before intervention, 8 months after intervention	-	↑ LPR to PHA*	LPR to Con A, NKCC
Andersen, et al. (2010).	-	In PBL: PHA → LPR Con A → LPR K562 → NKCC	-	Date of breast cancer recurrence, 4, 8 and 12 months later	-	From 4 to 12 months: ↑ NKCC* At 12 months: ↑ NKCC*, ↑ LPR to PHA*, ↑ LPR to Con A*	At 4 & 8 months: NKCC, LPR to Con A and PHA
Antoni, et al. (1991).	-	In whole blood: PHA → LPR PWM → LPR N/A → NKCC	Serostatus notification	3 days before serostatus notification (after intervention) and one week after serostatus notification	CD4, CD56	In seropositives: ↑ CD4**; ↑ LPR to PHA*; ↑ NKCC**, ↑ CD56*	LPR to PWM
Antoni, et al. (2009).	-	In PBMC: Anti-CD3 → IL-2, IL-4, and IFN- γ production	-	Before intervention, 3 months and 9 months after intervention	CD4, CD8, CD56, $CD56^+CD3^+$, CD19	3 months after intervention: ↑ IL-2 production*, ↑ IFN-γ production**, ↑ IL-2:IL-4 ratio*	At 9 months: IL-2, IFN- γ Overall: IL-4, CD4, CD8, CD56, $CD56^+CD3^+$, CD19
Arefnasab, et al. (2016).	-	In PBMC: PHA → LPR CON A → LPR	-	Before and after intervention	IL-17, $CD4^+$, $CD8^+$, CD56	↑ LPR to Con A**, ↑ LPR to PHA**, ↑ IL-17**	$CD4^+$, $CD8^+$, CD56
Beem, et al. (1999).	-	In PBMC: PHA → LPR anti-CD3 → LPR PWM → LPR K562 → NKCC	-	Before and after intervention	$CD19^+$, $CD20^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD5^+$, $CD16^+$, $CD56^+$, $CD3^-$		LPR to PHA / anti-CD3 / PWM, NKCC, $CD19^+$, $CD20^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD5^+$, $CD16^+$, $CD56^+$, $CD3^-$
Bower, et al. (2003).	-	In PBMC: K562 → NKCC	-	Before and after intervention	$CD3^-$, $CD16^+$, $CD56^+$		NKCC, $CD3^-$, $CD16^+$, $CD56^+$
Broadbent, et al. (2012).	Wound → hydroxyproline deposition	-	-	7 days after intervention	-	↑ Hydroxyproline deposition*	

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
de Brouwer, et al. (2013).	-	-	TSST	1 week after intervention before TSST, and 0, 20, 60 min after TSST and after 7-weeks relapse prevention before TSST, and 0, 20, 60 min after TSST	IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IFN-γ, TNFα	After 7 weeks relapse prevention: ↓ stress-induced IL-8*	1 week after intervention: IL-8. Overall: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-10, IFN-γ, TNFα
Christensen, et al. (1996).	-	In PBL: K562 → NKCC	-	Before and after intervention			Overall: NKCC
Coates, et al. (1989).	-	In N/A: N/A → NKCC Con A → LPR Candida antigen → LPR CMV → LPR	-	Before and after intervention	IgA, CD4, CD8		NKCC, LPR to Con A / candida antigen / CMV, IgA, CD4, CD8
Cohen, L., (2011).	-	In PBMC: K562 → NKCC	-	After intervention and 2 days after surgery	IL-1β, IL-12p70, IFN-γ, IL-6, IL-8, IL-10, TNF-α, CD3, CD19, CD16, CD56, CD4, $CD25^{high}/CD4^{+}$	Stress management group: ↑ NKCC*, ↑ IL-12p70*, ↑ IL-1ß*, ↑ TNF-α*	IL-6, IL-8, IL-10, IFN-γ, CD3, CD19, CD16, CD56, CD4, $CD25^{high}/CD4^{+}$
Davidson, et al. (2003).	Influenza vaccine → Influenza vaccine antibody titers	-	-	3-5 weeks after vaccination, 8-9 weeks after vaccination	-	↑ Antibody titers*	
Doering, et al. (2007).	-	In PBMC: K562 → NKCC	-	Baseline, 3 months and 6 months after surgery	IL-6, CRP		NKCC, IL-6, CRP
Elsenbruch, et al. (2005).	-	In whole blood: LPS → TNF-α production	-	Before and after intervention	CD3, $CD3^{+}CD4^{+}$, $CD3^{+}CD8^{+}$, $CD3^{-}CD16^{+}CD56^{+}$, $CD3^{-}CD20^{+,}$ $CD14^{+}$		TNF-α production, CD3, $CD3^{+}CD4^{+}$, $CD3^{+}CD8^{+}$, $CD3^{-}CD16^{+}CD56^{+}$, $CD3^{-}CD20^{+}$, $CD14^{+}$
Eremin, et al. (2009).	-	In PBMC: K562 → NKCC Daudi → LAK cell activity	-	3 days before start of chemotherapy, during chemotherapy before the 1st, 2nd, 4th and 6th cycle of chemotherapy, the day before surgery, 2 or 3 days after surgery, before radiotherapy, 4 and 12 weeks after radiotherapy	IL-1β, IL-2, IL-4, IL-6, TNF-α, $CD2^{+}$, $CD3^{+}$, $CD4^{+}$, $CD8^{+}$, $CD19^{+}$, $CD25^{+}$, $CD16^{+}$, $CD56^{+}$, $CD14^{+}$	After chemotherapy and 4 weeks after radiotherapy: ↑ $CD3^{+}$* 4 weeks after radiotherapy: ↑ $CD25^{+}$** 12 weeks after radiotherapy: ↑ LAK cell activity*	Other time points: CD3+, CD25+, LAK cell activity Overall: CD2+, CD4+, CD8+, CD19+, CD16+, CD56+, CD14+, IL-1β, IL-2, IL-4, IL-6, TNF-α, NKCC

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
Esterling, et al. (1992).	-	-	Serostatus notification	Before intervention, at week 5, 6 (after serostatus notification), 7, 8, 10 during the intervention	IgG Functional assays: EBV-VCA antibody titers, HHV-6 antibody titers, EBV-EA antibody titers	Week 6, 7, and 10 in HIV-infected participants: ↓ EBV-VCA antibody titers* Week 8 and 10 in HIV-infected participants: ↓ HHV-6 antibody titers**	Overall: IgG. Other time points: EBV-VCA and HHV-6
Fawzy, et al. (1990).	-	In PBL: IFN-augmented NKCC	-	1 week before intervention, immediately before the 5th or 6th intervention meeting, 6 months after intervention	CD4, CD8, CD16, CD56, CD57, CD38	At 6 weeks: ↑ CD57 LGL's*, ↑ CD8 T cell%*, ↑ CD57CD16** At 6 months: ↑ CD57 LGL's*, ↑ CD56**, ↑ CD57CD16*, ↑ CD16*, ↑ CD56**, ↓ CD4*, ↑ IFN-augmented NKCC*	At 6 weeks: CD4, CD56, CD38, IFN-augmented NKCC At 6 months: CD8, CD38
Fry, et al. (1964).	Skin prick test → wheal and flare size	-	-	Before and after hypnosis	-	↑ Decrease in wheal size**	Flare size
Germond, et al. (1993).	-	In N/A: PWM → LPR Con A → LPR PHA → LPR	-	Before intervention, during the 4th week and after the 8th week of intervention	-		LPR to PWM / Con A / PHA
Goodin, et al. (2012).	-	-	CPT	Before intervention, immediately following termination of CPT and at 15, 20, 25, 30, 40 min after CPT	sTNFαRII		sTNFαRII
Green, et al. (1988).	Candida injection → T_{DH} cell activity	-	-	Before and after intervention	sIgA, IgA, IgG and IgM	↓ T_{DH} cell activity*, ↑ sIgA**	IgA, IgG, IgM
Gruber, et al. (1993).	-	In N/A: Con A → LPR N/A → MLR N/A → NKCC	-	Three samples before intervention, weekly during intervention, three monthly after intervention until week 12.	IgG, IgA, IgM, total white cell counts, peripheral blood lymphocytes	Overall: ↓ White blood cell count*, ↑ LPR to Con A**, ↑ MLR**, ↑ peripheral blood lymphocytes**, IgG (direction not specified)**, ↑ NKCC*	IgA, IgM, IL-2 production

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
Gruzelier, et al. (2001a).	-	-	Exams (academic stress)	Before and after intervention	Total white blood count, CD3, CD4, CD8, CD19, CD56	↑ CD56**	Total white blood count, CD3, CD4, CD8, CD19.
Gruzelier, et al. (2001b).	-	-	Exams (academic stress)	Before intervention and after exams	CD3, CD4, CD8, CD19, CD56	↑ CD8*	CD3, CD4, CD19, CD56
Hayney, et al. (2014).	Influenza vaccine → influenza antibody concentrations	-	-	Before immunization, 3 weeks and 3 months after immunization	Nasal IgA Functional assays: IFN- γ, and IL-10 production in PBMC		Influenza antibody concentrations, nasal IgA, production of IFN-γ / IL-10
Hosaka, et al. (2002).	-	In PBMC: K562 → NKCC	-	Before and after intervention	-	After intervention: ↓ NKCC**	
Irwin, et al. (2015).	-	In PBMC: LPS → TNF, IL-6	-	CRP: before intervention, after intervention, 12 months after intervention TNF, IL-6 production: before intervention, 2 months after start of intervention, after intervention, 3 months after intervention, 12 months after intervention	CRP levels	Overall: ↓ CRP*. At 2 months after the start of the intervention: ↓ TNF production*	Overall: IL-6 production Directly after the intervention, at 3 and 12 months after completion of the intervention: TNF production
Johnson, et al. (1996).	-	In N/A: PHA → LPR K562 → NKCC	Doctor-patient role-play	Before intervention, after intervention (directly after the psychological stressor), 1 day or 2 days later	IL-1, IFN-γ, IgA		LPR to PHA, NKCC, IL-1, IFN- γ, IgA
Kaliman, et al. (2014).	-	-	TSST	Before and after intervention	RIPK2, COX2, CCR7, CXCR1, IL-6, TNF-α	↓ RIPK2**, ↓ COX-2*	CCR7, CXCR1, TNF-α, IL-6
Kern-Buell, et al. (2000).	DTH skin test → mumps induration size	-	-	Before and after intervention	White blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils, CD4, CD8, CD56 Functional assays: candida and tetanus antigen response	↓ neutrophils*, ↑ basophils*, ↑ mumps induration size*	Candida and tetanus antigen response, white blood cell counts, lymphocytes, monocytes, eosinophils, CD4, CD8, CD56

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
Kiecolt-Glaser, et al. (1985).	-	In PBMC: PWM → LPR PHA → LPR K562 → NKCC	-	Before intervention, at the end of the intervention, 1 month after the intervention	Functional assays: HSV antibody titers	Overall: ↑ NKCC*, ↓ HSV antibody titers	LPR to PWM / PHA
Kiecolt-Glaser, et al. (1986).	-	In PBMC: K562 → NKCC	Exams (acute stress)	1 month after the first examination series, final day of 3-day examination series during examinations	CD4, CD8		NKCC, CD4, CD8
Kiecolt-Glaser, et al. (2001).	-	In PBL: PHA → LPR Con A → LPR LPS → IL-1ß K562 → NKCC	Exams (academic stress)	Before intervention, 3 days before academic examination	$CD3^+$, $CD4^+$, $CD8^+$, CD14, CD45	↑ LPR to PHA*, ↑ LPR to Con A*, ↑ $CD3^+$*, ↑ $CD4^+$*	IL-1ß production, NKCC, $CD8^+$, CD14, CD45
Koh, et al. (2008).	-	In PBMC: PHA → IL-6, IL-10, and TNF-α production	Exams (academic stress)	Before stress period and after stress period	-	After stress period: ↓ IL-6 production**, ↓ TNF-α production**, ↑ IL-10 production**	
Koschwanez, et al. (2013).	Wound → wound re-epithelialization	In whole blood: LPS → TNF-α, IL-1ß, and IL-6 production	-	Blood assays: Before intervention, immediately before the wound procedure (2 weeks after intervention) Wound assays: 7, 11, 14, 17 and 21 days after punch biopsy	-	At day 11 after punch biopsy: ↑ fully re-epithelialized wound*	At day 7, 14, 17 and 21: wound re-epithelialization Overall: TNF-α, IL-1ß, and IL-6 production
Larson, et al. (2000).	-	In PBMC: Anti-CD3 antibody → IFN-γ production K562 → NKCC	-	Before intervention, after intervention / pre-surgery, post-surgery	-	↑ IFN-γ production**	NKCC
Lekander, et al. (1997).	-	In PBMC: Con A → LPR K562 → NKCC	-	Before and after intervention	Lymphocytes, granulocytes, monocytes, white blood cell count	↑ Lymphocytes**	White blood cell count, monocytes, LPR to Con A, granulocytes, NKCC
Lengacher, et al. (2008).	-	In PBMC: IL-2 → LAK K562 → NKCC	-	Before and after intervention	-	↑ NKCC*, ↑ LAK*	
Lengacher, et al. (2013).	-	In whole blood: PHA → CD3+CD69+, IL-4, IFN- γ	-	Before intervention and after intervention	$CD4^+$, $CD8^+$, $CD19^+$, $CD16^+56^+$, $CD3^+CD69^+$	↑ $CD3^+CD69^+$ stimulation**, ↑ LPR to PHA for Th1/Th2 ratio*	IFN- γ and IL-4 stimulation, $CD4^+$, $CD8^+$, $CD19^+$, $CD16^+56^+$

	Challenges			Timing specifications	Other immune assays	Effects	
Study	*In vivo*	*In vitro* stimulus → target	Psycho-physiological	Measuring time points	Outcome parameters	Present	Absent
Locke, et al. (1987).	Skin testing with antigens → DTH response	-	-	After 24 hrs and after 48 hrs after intervention	-		DTH response
Mawdsley, et al. (2008).	-	In whole blood: LPS → IL-6 and TNF-α production	-	Before intervention, after intervention, 0.5 hrs after intervention	Serum IL-6, serum IL-13, CD16/CD56, platelet activation, leukocyte count, mucosal ROM production, rectal mucosal release of IL-13 and TNF-α	↓ Serum IL-6**, ↓ rectal mucosal fluid concentration of IL-13**, ↓ rectal mucosal blood flow**	Serum IL-13, TNF-α production, IL-6 production, CD16/CD56, platelet activation, leukocyte count, mucosal ROM production
McCain, et al. (2003).	-	In PBMC: K562 → NKCC PHA → cytokine production	-	Before intervention, after intervention, 6 months after intervention	$CD3^+/CD4^+$, $CD3^+/CD8^+/CD57^+$, $CD3^-/CD57^+$ lymphocytes, NKCC, IL-2, IFN-γ, IL-4, IL-10 Functional assay: Host viral load	Social support group immediately post intervention: ↓ IL-4*	Overall: $CD3^+/CD4^+$, $CD3^+/CD8^+$, $CD8^+/CD57^+$, $CD3^-/CD57^+$ lymphocytes, NKCC, IL-2, IFN-γ, IL-10, host viral load After 6 months: IL-4
McCain, et al. (2008).	-	In PBMC: PHA → LPR	-	Before intervention, after intervention, 6 months after intervention	-	Overall: ↑ LPR to PHA*	
McGrady, et al. (1992).	-	In PBMC: PHA → LPR Con A → LPR	-	Before and after intervention	Total white blood cell counts, differential counts	↑ LPR to PHA*, ↓ total white blood cell count*, ↓ neutrophil counts*	LPR to Con A
McGregor, et al. (2004).	-	In PBMC: anti-CD3 → LPR	-	Before intervention, 3 months after intervention	CD3, CD4, CD8, CD19, $CD3^-CD56^+$	↑ LPR to anti-CD3*	CD3, CD4, CD8, CD19, $CD3^-CD56^+$
Moynihan, et al. (2013).	Injection with KLH → anti-KLH antibody levels	-	-	Immediately after intervention (before injection), 3 weeks after intervention, 24 weeks after intervention	-	At 24 weeks follow up after antigen challenge: ↓ anti-KLH antibody levels*	At 3 weeks: anti-KLH antibody levels
Mulder, et al. (1995).	-	In whole blood: anti-CD3 → LPR	-	Before intervention, after every 3 months up to 24 months after intervention	CD4		LPR to anti-CD3, CD4
Naito, et al. (2003).	-	In PBMC: K562 → NKCC	Exams (acute stress)	Before intervention, during exams	CD4+%, CD8+%, CD56+%	↑ CD8+%*	CD56+%, CD4+%, NKCC

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
Nelson, et al. (2008).	-	In PBMC: Anti-CD3 / anti-CD28 → IFN-γ and IL-5	-	Before intervention and 2 weeks after intervention	IL-10, CD3, CD4, CD8, CD14, CD16, CD56		IL-10, IFN-γ production, IL-5 production, CD3, CD4, CD8, CD14, CD16, CD56
Nunes, et al. (2007).	-	In PBMC: PHA with dexamethasone and corticosterone → LPR	-	Before and after intervention	-		LPR to PHA
O'Leary, et al. (1988).	-	In PBMC: PHA → LPR Con A → LPR PWM → LPR	-	Before and after intervention	CD4, CD8		CD4, CD8, LPR to PHA / Con A / PWM
Pace, et al. (2009).	-	-	TSST	After intervention (before the TSST), and 30, 60, 75 and 90 min after TSST	IL-6		IL-6
Pennebaker, et al. (1988).	-	In PBMC: PHA → LPR Con A → LPR	-	LPR to PHA: Before intervention, after intervention, 6 weeks after intervention LPR to Con A: Before intervention, after intervention	-	Overall: ↑ LPR to PHA*	LPR to Con A
Petrie, et al. (1995).	Hepatitis B vaccine → anti-hepatitis B antibody levels	In PBMC: K562 → NKCC	-	After intervention (before the 1st vaccination), 1 month after intervention (before the 1st booster vaccination), 4 months after intervention (before the 2nd booster vaccination), at 6 months after the intervention	CD4, CD8, CD56, basophils	At 4 and 6 months after intervention: ↑ Hepatitis B antibody levels* Directly after intervention: ↓ CD4 counts*, ↓ basophils**	1 month after intervention: Hepatitis B antibody levels. At 4 months and 6 months after intervention: CD4, basophils. Overall: CD8, CD56, NKCC
van der Pompe, et al. (1997).	-	In whole blood: K562 → NKCC PWM → LPR PHA → LPR	-	Before and after intervention	CD4, CD8, CD3, CD16/56	Post treatment: ↓ CD8 cell percentages**, ↓ LPR to PWM**, ↓ CD4 cell percentages**, ↓ CD16/56 cell percentages**	CD3, NKCC, LPR to PHA

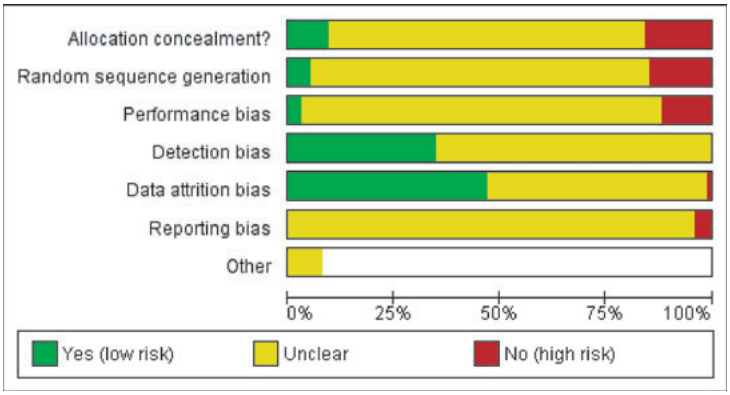
	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
van der Pompe, et al. (2001).	-	In whole blood: K562 → NKCC PWM → LPR PHA → LPR	Speech task	Before intervention (2 times before task onset, 5 min after task onset and 9 min and 37 min after task onset) and after intervention (2 times before task onset, 5 min after task onset and 9 min and 37 min after task onset)	CD3, CD4, CD8, CD16/56, CD19	After intervention (during stress induction): ↓ CD16/56*, ↓ NKCC*	LPR to PHA / PWM, CD3, CD4, CD8, CD19
Richardson, et al. (1997).	-	In PBMC: K562 → NKCC	-	Before and after intervention	IL-1α, IL-1ß, IL-2, IFN-γ, β-endorphins		NKCC, IL-1α, IL-1ß, IL-2, IFN-γ, β-endorphins
Robinson, et al. (2003).	-	In whole blood: K562 → NKCC	-	Before and after intervention	RANTES, SDF-1, CD16/CD56	After intervention: ↑ CD16/CD56**, ↑ NKCC*	SDF-1, RANTES
Rosenkranz, et al. (2013).	Suction blisters on the volar forearm → capsaicin-induced flare size	-	TSST	IL-8 and TNF-α in blister fluid: 4 weeks before intervention, within 4 weeks after the intervention, 4 months after the intervention Capsaicin-induced flare size: 4 weeks before intervention, within 4 weeks after the intervention	Functional assays: IL-8 and TNF-α in blister fluid	↓ capsaicin-induced flare size**	TNF-α and IL-8 in blister fluid
Savard, et al. (2005).	-	In PBL: K562 → NKCC In whole blood: LPS → IL-1ß, IFN- γ	-	Before intervention, after intervention, 3, 6 and 12 months after intervention	Whole blood cell count, $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+/CD56^+$, lymphocyte count	After intervention: ↑ IFN-γ production**, ↓ lymphocyte count*, ↑ IL-1β production* Follow-up: ↑ whole blood cell counts**, ↑ lymphocytes**, ↓↑ IFN-γ production**	Overall: NKCC, $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+/CD56^+$. After intervention: whole blood cell counts Follow-up: IL-1ß production
Smith, et al. (1992).	Varicella Zoster skin test → induration size, varicella zoster antigen response	In PBMC: PHA → LPR	-	Before intervention (before and after skin testing), after intervention at 24 hrs and 48 hrs	-	After 24-hrs: ↑ induration size*	Overall: LPR to PHA, varicella zoster antigen response After 48 hrs: induration size
Solberg, et al. (1995).	-	-	Treadmill exercise test	Before and after physical stress test	$CD2^+$, $CD4^+$, $CD8^+$ cell counts	↓ $CD8^+$ cell count*	$CD2^+$, $CD4^+$ cell counts

	Challenges			Timing specifications	Other immune assays	Effects	
Study	***In vivo***	***In vitro* stimulus → target**	**Psycho-physiological**	**Measuring time points**	**Outcome parameters**	**Present**	**Absent**
Stetler, et al. (2006).	Influenza vaccine → antibody response to vaccine	-	-	Before intervention, 1 and 3 months after vaccination	-	↓ Antibody response to vaccine*	
Vedhara, et al. (2003).	Influenza vaccine → Response to vaccine	-		Before vaccination, after vaccination	-	↑ Clinically appropriate response to vaccination**	
Weinman, et al. (2008).	Skin biopsy at the inner aspect of the upper non-dominant arm → wound diameter	-	-	7, 14, and 21 days after skin biopsy	-	14 and 21 days after skin biopsy: ↓ Wound diameter*	7 days after skin biopsy: wound diameter
Whitehouse, et al. (1996).	-	In PBMC: Con A → LPR PHA → LPR PWM → LPR K562 → NKCC	Exams (acute stress)	Before intervention, after intervention, 3 weeks after intervention (during final exams), 6 weeks after intervention (3 weeks after exams)	Activated T-cells, B-lymphocyte counts, white blood cells, granulocytes, NK cell count	After intervention: ↓ Activated T –cells**	Overall: B-lymphocyte counts, white blood cells, granulocytes, CD56, LPR to Con A / PHA / PWM, NKCC. At follow-up: activated T-cells
Witek-Janusek, et al. (2008).	-	In PBMC: PHA / PMA → IFN- γ, IL-4, IL-6 production K562 → NKCC	-	Before intervention, 1 month after the start of the intervention, after intervention, 1 month after intervention	CD3, CD16, CD19, CD56, CD4, CD8, CD16/CD56	Overall: ↑ NKCC*, ↑ IFN-γ production*, ↓ IL-4 production*, ↓ IL-6 production* After intervention and 1 month after intervention: ↓ IL-10 production*.	Overall: CD3, CD16, CD19, CD56, CD4, CD8, CD16/CD56 1 month after start of the intervention: IL-10 production
Witt (2003).	Histamine provocation, skin prick testing → wheal area	-	-	Before intervention (before start of birch pollen season), after intervention (at start of birch pollen season), 14 weeks after intervention (at end of birch pollen season)	-	Overall: ↓ Wheal area*	
Zachariae, et al. (1989).	Skin prick in the upper dermal layer on the ventral side of both forearms → wheal area, flare size, palpable induration	-	-	Before and after intervention	-	Type I reaction: ↓ flare size* Type IV reaction: ↓ palpable induration**, ↓ flare size*	Type I reaction: wheal area

	Challenges			Timing specifications	Other immune assays	Effects	
Study	*In vivo*	*In vitro* stimulus → target	Psycho-physiological	Measuring time points	Outcome parameters	Present	Absent
Zachariae, et al. (1990).	-	In whole blood: K562 → NKCC	-	Before intervention, 7 days after the 1st intervention session; 10 days after the 1st intervention session	Leukocyte differential count, leukocyte count	↑ NKCC*	Leukocyte differential count, leukocyte count
Zachariae, et al. (1994).	-	Study 1: In PBMC: PHA → LPR Con A → LPR PWM → LPR FMLP → Monocyte chemotaxis	-	Study 1: At the start and end of the 1st, 8th, and 15th day, and 36 days after the start of the intervention	Study 1: Monocyte chemo taxis, lymphocyte production	Study 1: Imagery group: ↑ monocyte chemo taxis at day 8*, ↑ LPR to PHA at day 8*, ↓ LPR to PWM at day 8 and 15** Relaxation group over time: ↑ monocyte chemotaxis at day 8*, ↓ LPR to PWM at day 8*	Study 1: Imagery group: LPR to Con A Relaxation group: LPR to PHA / Con A
	-	Study 2: In PBMC: K562 → NKCC	-		Study 2: -	Study 2: -	Study 2: NKCC
Zautra, et al. (2008).	-	In PBMC: LPS → IL-6	-	Before and after intervention	-	CBT group: ↓ IL-6 production*	Mindfulness meditation and emotion regulation group: IL-6 production

Note. PBL = Peripheral Blood Leukocytes; CBT = Cognitive Behavioral Therapy; CCR = C-C Chemokine Receptor; CD = Classification Determinant; CMV = Cytomegalovirus; Con A = Concanavalin A; COX = Cyclooxygenase; CPT = Cold Pressor Test; CRP = C-reactive Protein; CXCR = CXC Chemokine Receptor; DTH = Delayed Type Hypersensitivity; EBV-VCA = Epstein-Barr Virus Viral Capsid Antigen; EBV-EA = Epstein-Barr Virus Early Antigen; FMLP = Formyl-Methyl-Leucinc Peptide; HHV = Human Herpes Virus; HIV = Human Immunodeficiency Virus; hrs = hours; HSV = Herpes Simplex Virus; Ig = Immunoglobulin; IL = Interleukin; IFN = Interferon; KLH = Keyhole Limpet Hemocyanin; LAK = Lymphokine Activated Killer Cell; LGL = Large Granular Lymphocyte; LPR = Lymphocyte Proliferative Response; LPS = Lipopolysaccharide; min = minutes; MLR = Mixed Lymphocyte Responsiveness; N/A = Not Available; NKCC = Natural Killer Cell Cytotoxicity; PBMC = Peripheral Blood Mononuclear Cells; PHA = Phytohemagglutinin; PMA = Phorbol Myristate Acetate; PWM = Pokeweed Mitogen; RIPK = Receptor-interacting Protein Kinase; RANTES = regulated upon activation, normal T-cell expressed and presumably secreted; ROM = Reactive Oxygen Metabolite; SDF = stromal derived factor; T_{DH} cells = T Delayed Hypersensitivity cells; Th = T helper; TNF = Tumor Necrosis Factor; TSST = Trier Social Stress Test; * = $p \leq .05$; ** = $p \leq .01$.

Note 2. In the last two columns, an effect is specified as present when the intervention condition significantly differed from the control condition after the intervention. When more than two time points (i.e., before and after intervention) were taken into account, the time point on which an effect was found is specified. The direction of the effects is specified by using arrows and represent the outcomes for the intervention condition in perspective to the control condition. When no significant differences between the intervention and control condition were found, the outcome parameters are described in the column "absent".

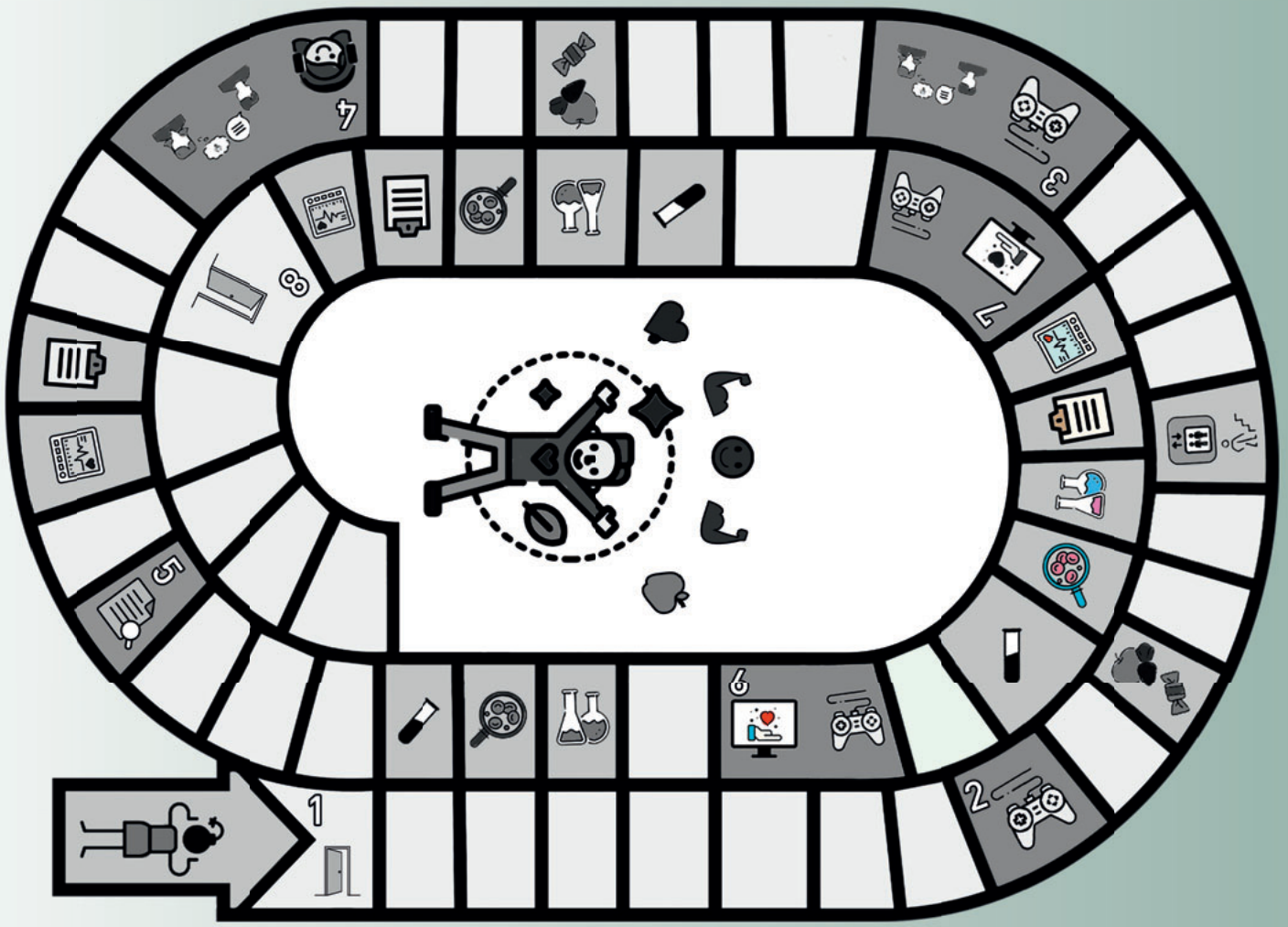


Supplemental Figure 2. Risk of bias graph.

Judgements of the independent review authors about the separate risk of bias items presented as percentages across all included studies.

Supplemental Figure 3. Risk of bias summary.

Judgments of the independent review authors about the separate risk of bias items for each of the included study presented as low, high or unclear.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The effects of a psychological intervention directed at optimizing immune function: Study protocol for a randomized controlled trial

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6

Abstract

Previous research has provided evidence for the link between psychological processes and psychophysiological health outcomes. Psychological interventions such as face-to-face or online cognitive behavioral therapy (CBT) and serious games aimed at improving health have shown promising results in promoting health outcomes. Few studies so far, however, have examined whether internet-based CBT combined with serious gaming elements is effective in modulating health outcomes. Moreover, studies often did not incorporate psychophysiological or immunological challenges in order to gain insight into physiological responses to real-life challenges after psychological interventions. The overall aim of this study is to investigate the effects of a psychological intervention on self-reported and physiological health outcomes in response to immune and psychophysiological challenges. In a randomized controlled trial, 60 healthy males are randomly assigned to either an experimental condition, receiving guided internet-based (e-health) CBT combined with health-related serious gaming elements for 6 weeks, or a control condition receiving no intervention. After the psychological intervention, self-reported vitality is measured, and participants are given an immunological challenge in the form of a *M. bovis* Bacillus Calmette-Guérin (BCG) vaccination. One day after the vaccination, participants are asked to perform several psychophysiological tasks in order to explore the effects of the psychological intervention on participants' stress response following the immune challenge. To assess delayed effects of vaccination on self-reported and physiological health outcomes, a follow-up visit is planned 4 weeks later. Total study duration is approximately 14 weeks. The primary outcome measure is self-reported vitality measured directly after the intervention. Secondary outcome measures include inflammatory and endocrine markers, as well as psychophysiological measures of heart rate and skin conductance in response to the psychophysiological tasks after the BCG vaccination. The innovative design features of this study – e.g., combining guided e-health CBT with health-related serious gaming elements and incorporating immunological and psychophysiological challenges – will provide valuable information on the effects of a psychological intervention on both self-reported and physiological health outcomes. This study will offer further insights into mechanisms underlying the link between psychological factors and health outcomes and is anticipated to contribute to the optimization of healthcare strategies.

Introduction

The conventional way to reduce inflammation is to administer anti-inflammatory pharmaceutical agents such as corticosteroids or non-steroidal anti-inflammatory drugs. These drug treatments, however, often have (severe) side effects or are non-specific (258-261). Previous research has provided evidence for a link between psychological processes and inflammatory responses (41, 262). Psychological interventions aimed at reducing inflammatory processes without the use of pharmaceutical agents could therefore be useful to supplement, or even (partially) replace current drug treatments. For this reason, it is important to increase our understanding of the effectiveness of psychological interventions on health outcomes. However, research on this topic is still in its infancy.
A meta-analysis focusing on the effects of several psychological interventions (e.g. relaxation, conditioning, stress management, hypnosis, and disclosure interventions) on immune-related health outcomes has demonstrated that psychological interventions can modulate certain features of the immune response; these modulations are reflected in lower pro-inflammatory and/or higher anti-inflammatory responses (169). Overall, the meta-analysis provides modest evidence that psychological interventions affect immune function. In light of these findings, it is important to acquire a better understanding of the effects of psychological interventions on self-reported and physiological health outcomes, and of the mechanisms involved.

Internet-based Cognitive Behavioral Therapy (e-health CBT) is an upcoming and innovative tool that may improve the effectiveness of psychological interventions. E-health CBT has been shown to be effective in decreasing psychological stress in various clinical populations, including patients with cancer, chronic pain, and irritable bowel syndrome (263-265). In the area of somatic conditions, a meta-analysis by van Beugen and colleagues (2014) showed that e-health CBT was effective: the effects were comparable with face-to-face CBT (10). Furthermore, some studies have provided evidence that using e-health CBT results in cost savings compared with face-to-face CBT (15, 266, 267). Finally, e-health CBT can be more convenient and flexible than face-to-face therapy, and reduces travelling time (268).

An important part of a CBT intervention is to strengthen the effects of explicit behavior change techniques. To increase the effects, the underlying cognitive processes can be further trained by means of specific cognitive behavioral strategies. Such strategies include principles of reward and evaluative conditioning (269), as can be applied in serious gaming. Recent studies show that the addition of serious gaming elements to e-health interventions can improve knowledge transfer. Moreover, due to the entertainment aspect, adding serious gaming elements can help overcome motivational barriers (28). Serious

games have proved effective in improving knowledge and self-management skills in young people with chronic conditions (29), for example, as well as in increasing knowledge about drug and alcohol use in adolescents (57). Serious games can also have beneficial effects on factors considered important for a healthy lifestyle in general, such as healthy food choices and physical activity (28). So far, serious games have not often been investigated in combination with CBT. A study in bulimia patients and a study in patients with a severe gambling disorder have shown better therapeutic outcomes when CBT interventions are complemented with serious gaming than in the case of a standalone CBT intervention (270, 271). Thus, serious games may be a promising add-on to CBT, as an innovative tool to motivate users to increase their knowledge and skills regarding health-related behavior.

Most studies on the effects of psychological interventions have focused on basal health outcomes (e.g., general levels of psychological and immune functioning). The aim of psychological interventions is to enhance an individual's ability to cope with physical and psychological stressors and daily life hassles, which can presumably best be studied by evoking real-life challenges such as immune challenges (i.e. inflammatory reactions) and psychophysiological challenges (i.e. stress responses). Inflammatory reactions can be elicited experimentally by, for example, *in vivo* or *in vitro* stimulation of the immune system; stress responses can be elicited by having participants perform specific psychophysiological tasks. In a previous study, a relatively short-term inflammatory response was induced by using an *in vivo* stimulation with lipopolysaccharide (LPS). The researchers demonstrated that a physical exercise and breathing intervention directed at optimizing immune functioning showed promising effects when compared to no intervention (272). After the LPS stimulation, the participants who had received the intervention exhibited significantly lower pro-inflammatory cytokine levels and less flu-like symptoms than the control group. Another study evaluated the effects of a stress management training on immune outcomes in patients with rheumatoid arthritis. In response to a potent psychosocial stressor, the group of participants who had received stress management training showed altered cortisol and IL-8 levels compared to the group who had not received training (173, 273).

A relatively safe method to stimulate the immune system *in vivo* is to use a vaccine as an immunological challenge. One live vaccine with a good safety record in children and adults, which is routinely administered to infants in many countries all over the world, is the *M. bovis* Bacillus Calmette-Guérin (BCG) vaccine, the common vaccine against tuberculosis (TB) (274). Furthermore, BCG vaccination induces a pro-inflammatory cytokine response (275). Therefore, BCG vaccination seems an appropriate immunological challenge in order to investigate immune reactivity after a psychological intervention. To obtain more insight

into the effects of a psychological intervention on the responsiveness to stress, participants were additionally exposed to relevant psychophysiological tasks after receiving a BCG vaccination (51, 276, 277).

Based on the theoretical background and empirical findings, a two-armed randomized controlled trial (RCT) has been designed to investigate the effects of a psychological intervention directed at optimizing immune function. We aim to assess the effects of the psychological intervention on self-reported and physiological health outcomes in response to immune and psychophysiological challenges. In this RCT, participants are randomly allocated to an experimental or a control condition. The participants in the experimental condition receive guided e-health CBT in combination with health-related serious gaming elements, whereas the controls receive no interventions. We expect, first, that after the intervention and vaccination participants in the experimental condition will show higher self-reported vitality (as measured by a composite score of vitality and fatigue) compared to the control condition. Second, we expect that participants in the experimental condition will show optimized health outcomes on the psychological and physiological variables assessed after BCG vaccination and the psychophysiological tasks. Below, we describe the study protocol.

Methods/Design

Study Design

In order to investigate whether a psychological intervention, consisting of a guided e-health CBT in combination with health-related serious gaming elements, can modulate self-reported and physiological health outcomes in healthy participants, an RCT will be conducted. The study has been approved by the Medical Ethical Committee of the Leiden University Medical Center (registration number P15.099/NL52434.058.15). The study will be conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation (ICH) Guidelines on Good Clinical Practice (GCP). Figure 1 shows the flowchart of the study design and the Standard Protocol Items: Recommendations for Interventional Trials 2013 (SPIRIT) Checklist is presented as Additional file 1.

Procedures

Participants are recruited from the Leiden University student population via local digital or printed advertisements. Testing takes place at the Leiden University Medical Center in the Netherlands. After providing informed consent to the test leader, participants are first screened for potential physical and psychiatric conditions that might interfere with

the participants' safety or with the study protocol. Participants who meet the inclusion criteria (see below for details) are randomized to the experimental or control condition. Participants in the experimental condition receive a psychological intervention for six weeks; participants in the control condition do not receive any intervention during this period. Subsequently, all participants are given a BCG vaccination to challenge the immune system *in vivo*. One day after vaccination, participants take part in a test day, on which they perform psychophysiological tasks. Four weeks after the test day, finally, a follow-up session is planned in order to evaluate the effects of the psychological intervention on health outcomes in the longer term. The schedule of activities in the study is presented in Table 1.

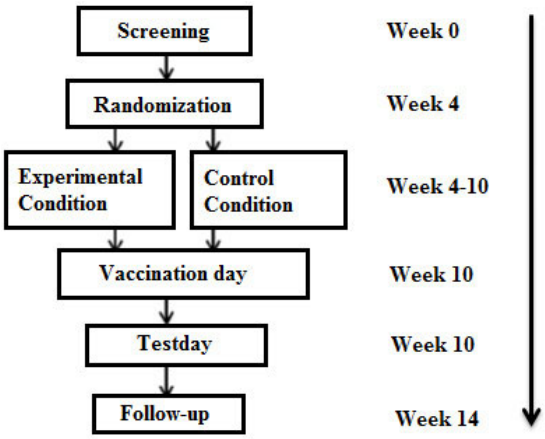

Figure 1. Flowchart of the study design.

Study population, randomization, and blinding

Eligibility is assessed by junior researchers in collaboration with a clinical psychologist and a study nurse in collaboration with a physician; the specific inclusion and exclusion criteria are described below. We aim to include 60 healthy participants between 18 and 35 years of age. Since the menstrual cycle is known to have an effect on immune function (278), only males are included in this study. Further inclusion criteria are having a good understanding of written and spoken Dutch, and being naive for TB. Pre-existing immunity against *Mycobacterium tuberculosis* is actively screened by performing the Quantiferon TB-Gold™ test on all participants at the screening visit; only participants who test negative are included in the study. Moreover, an HIV test is performed for all participants, as infection with HIV may be a contra-indication for vaccination with live vaccines such as BCG. Participants are furthermore excluded from the study if they: a) have a history of inflammatory or cardiovascular diseases; b) are allergic to any of the vaccine components; c) have a history of exposure to open TB, (latent) TB disease, or treatment for TB; d) have

undergone a BCG vaccination at any time prior to entering the trial; e) have received another live vaccination four weeks or less prior to the BCG vaccination; f) have been treated with immune modulating drugs three months or less prior to enrollment; g) have (of have had) a disease affecting the lymphoid organs; h) are known to have congenital or acquired immune deficiencies; i) have psychiatric (DSM-5) or somatic conditions that interfere with their safety and/or the study protocol; j) are professional sports players or perform extreme exercise; k) have a history of excessive drinking or drug use; l) are actively participating in other clinical trials; or m) do not give consent for their General Practitioner to be informed of their BCG vaccination.

Table 1. Scedule of study activities.

			STUDY PERIOD			
Visit	***Screening***	***Allocation***	***Intervention period***	***Vaccination day***	***Testday***	***Follow up***
Time point	***Week 0***	***Week 4***	***Week 4-10***	***Week 10***	***Week 10***	***Week 14***
ENROLMENT:						
Eligibility screen	X					
Informed consent	X					
Allocation		X				
INTERVENTIONS:						
Intervention condition			X			
Control Condition			X			
ASSESSMENTS:						
Heart rate measure	X				X	X
Serum blood sample	X			X	X	X
Heparin blood sample	X			X	X	
Medical screening questionnaire	X					
MINI Psychiatric interview	X					
IGRA test (Interferon Gamma Release Assay	X					
HIV test	X					
Vaccination				X		
Psychophysiological stress tasks					X	
Saliva samples	X			X	X	X
Heart rate, Heart Rate Variability and Skin Conductance Levels	X				X	X
Questionnaires	X			X	X	X

In order to balance the allocation equally during each season, since season is associated with the prevalence rate of influenza (279), a block randomization is performed, with block sizes of four. Block randomization is generated by the first author using an online random number generator (http://www.random.org). In order to avoid possible expectancy effects, the test leader on the test day is blinded to the group allocation of the participants. Participants are aware of allocation, since they have to be informed that they have been randomized to a condition that includes a psychological intervention or a condition that includes no intervention.

Anonymized participant identification codes are used to link data to participants. Study personnel and the person who conducts the data monitoring are the only people who have access to the personalized data forms.

Psychological intervention

Participants allocated to the experimental condition receive guided e-health CBT. Immune function is known to be influenced by behavioral and lifestyle factors such as healthy food and exercise, relaxation and sleep, and cognitions and worldview (273, 280-284). Therefore, these factors are taken into account in this intervention. All modules are based on evidence-based interventions in this area (285, 286). A therapist, referred to as an e-Coach, guides participants through the online environment by giving homework assignments, following the progress of participants, and sending motivational feedback messages. The intervention consists of six modules and starts with a face-to-face intake interview between the e-Coach and the participant. This interview serves as an introduction to the online intervention and to set short-term and long-term goals, based on the various topics of the individual modules of the intervention (Module 1). Modules 2 to 5 focus on lifestyle: healthy food and exercise, relaxation, sleep, and cognitions and worldviews. Each module contains approximately ten online assignments, such as relaxation exercises. At the start of each module, participants make a plan for the week, in which they describe how they aim to reach their goals. Participants fill out a daily diary to keep track of their progress towards their goals and the activities they have undertaken to reach them, and to reflect on wellbeing and sleep quality. At the end of each module, participants receive a summary of what they have learned during the module and are required to reflect on the extent to which they have reached their goals. The intervention ends with a module that focuses on setting long-term goals and preventing relapse.

In addition to the online intervention, participants in the experimental condition play a serious game to optimize immune function called ViaNova©; the game has been developed in a collaboration by Leiden and Delft University. Before starting the game, participants

design an avatar that serves as a representation of their ideal self. Furthermore, the game includes a coach (mirroring the e-Coach of the online intervention), who navigates the avatar through the game. The serious game contains several mini-games based on four themes matching the modules of the online intervention, including lifestyle factors involving healthy food and exercise, relaxation, sleep, and cognitions and worldview; there are four different rooms in the game, with each room reflecting one of these themes. The games are all health-related, based on cognitive behavioral strategies, including principles of reward and evaluative conditioning. An example of a game is the approach avoidance task (72), in which participants have to pull healthy items towards them and push unhealthy items away by clicking on the corresponding arrows on the keyboard. Participants are instructed to play various games five days a week, throughout the six weeks of the intervention.

Vaccination day

After the six-week period, all participants complete questionnaires about the primary and secondary outcomes. Furthermore, a blood sample and a saliva sample are taken, and participants are vaccinated with the live-attenuated BCG by a trained research nurse, through intradermal injection in the upper arm. To monitor for possible side effects, participants fill in a diary for four weeks after the vaccination.

Assessments on the test day

One day after the vaccination, participants complete three different psychophysiological tasks: a modified version of the PASAT (277), the CPT (276), and the TSST (51).

Modified Paced Auditory Serial Addition Task (PASAT)

The PASAT was originally developed as a measure for information processing speed (287). In this task, participants are presented with a series of single-digit numbers, which are delivered through an audio player. The participants' task is to add each number to the number presented previously (287). The task consists of two parts, separated by a 1-minute break, in which four consecutive 2-minute series of digits are presented at different intervals at an increasing pace. To induce psychological stress during this task, we present participants with a modified version, based on a version used in previous studies (277, 288). In this task, participants are exposed to an aversive noise if they give an incorrect response. Furthermore, they are instructed to watch their own face on a computer screen during the task and are informed that these recordings will be analyzed by a body language expert. Previous research has shown that this version of the PASAT can modulate cardiovascular responses, in that stress-induced hemoconcentration appeared

(277). Furthermore, in a previous study investigating the effects of vaccine-induced inflammation on mental stress levels, cardiovascular responses to this version of the PASAT were attenuated by vaccination (289).

Cold Pressor Test (CPT)

In order to induce non-harmful and quickly reversible physical stress, participants are exposed to a CPT (276). Participants are instructed to place their dominant hand in a tank of cold water, at a temperature of about 2 ºC (± 0.1 ºC), until immersion becomes unbearable. The maximum immersion time is four minutes, but the participants are not aware of this time limit. The pain threshold (first moment of pain sensation) and maximum immersion time are recorded.

Trier Social Stress Test (TSST)

Participants are exposed to the TSST (51, 290), a standardized laboratory stress task, consisting of a mock job interview and mental arithmetic task in front of a two-member jury. First, participants are given five minutes to prepare a presentation about their 'dream' job position. Subsequently, participants present in front of a two-member jury while being recorded by a video-camera and voice recorder. During the presentation, the jury members take notes and ask some questions without providing feedback to the participants. After five minutes, participants are instructed to count backwards in steps of 17 from 1965 to 0. When participants make a mistake or do not answer fast enough, they are told to start at 1965 again. The total duration of the TSST is approximately 15 minutes (51). This task has been found to be sensitive to inducing inflammatory responses (154), as well as neuroendocrine and autonomic nervous system responses (51).

Follow-up

Four weeks after the vaccination and test day, a follow-up session is planned in order to evaluate the effects of the psychological intervention on health outcomes in the longer term. During the follow-up session, participants fill in questionnaires. Furthermore, a blood sample and saliva sample are taken, and heart rate and skin conductance are measured at rest.

Self-report outcome measures

Participants fill out several questionnaires at baseline, on the day of vaccination, on the test day, and at the follow-up.

Vitality is measured by a composite of the Subjective Vitality Scale – State version (SVS) (291), and the (reverse-scored) Checklist Individual Strength (CIS-20) (292). The SVS has been validated in a student population as a vitality measure with a good internal consistency and reliability (291, 293). The CIS-20 was developed to measure fatigue severity (294) and has a good internal consistency and reliability (292). A composite score of the SVS and CIS-20 is used as primary outcome measure.

Several other questionnaires are administered exploratively to assess their possible moderating role in the effects of a psychological intervention on self-reported and physiological health outcomes.

Physiological outcome measures

Cardiovascular measures

Heart rate and skin conductance are measured with a BIOPAC MP150 system® at baseline, on the test day, and on the follow-up day.

Inflammatory measures

Serum blood samples are taken to measure inflammatory markers, such as cytokine levels (e.g. IL-6, Il-8), at baseline, on the day of vaccination, at the start and end of the test day, and at follow-up. A heparin blood sample is taken at baseline, on the day of vaccination, and at the start of the test day. This sample is used to stimulate blood cells with LPS *in vitro* in a 37˚C incubator for 6 hours; the stimulated and control plasma samples are then centrifuged and stored at -80°C. All inflammatory parameters are measured in batches, including complete follow-up samples of individual participants.

Endocrine measures

Saliva is taken to assess endocrine responses (e.g. cortisol, alpha-amylase) at the same time points as the serum blood samples and additionally after each stress task on the test day, one day after vaccination.

Statistical analyses

Primary outcome

Effects of the psychological intervention on vitality are assessed in an analysis of variance with inclusion of covariates (ANCOVA) when appropriate. Vitality after the psychological intervention is used as dependent variable, and condition (experimental or control condition) is used as between-subjects factor. Baseline measurement of vitality is used as a covariate.

Secondary outcomes

Inflammatory responses measured in blood at the screening, after the intervention (on the vaccination day), on the test day one day after vaccination, and at the follow-up session are assessed in multilevel models. The inflammatory responses are used as dependent variables, with group allocation, baseline measurements, and time serving as independent variables. Analyses for LPS-stimulated blood (measured at the screening, on the vaccination day, and the start of the test day) are conducted in a similar way.

Endocrine responses measured in saliva at screening, after the intervention (on the vaccination day), after each stress task one day after vaccination, and during follow-up are evaluated as dependent variables in a multilevel model, with group allocation, baseline measurement of the dependent variable, and time as independent variables. Analyses for heart rate and skin conductance are conducted in a similar way.

Demographic variables and self-reported measures are explored as possible predictors of the primary outcomes.

Sample size calculation

The final power calculation was based on a study examining the effects of a stress management intervention on the level of psychological distress, using a comparable design (273). Power analysis of this study indicated that 30 participants per condition would be sufficient to detect an adjusted effect size of f = 0.45 on psychological distress, with a power of 0.80, and an alpha level of 0.05. For the SVS, one representative study have found an effect size of f = 0.44 in change in vitality at post-intervention in an uncontrolled study on a walking intervention including a motivational intervention (295); an effect size of f = 0.40 was found in another representative study for the difference in change in vitality between an endurance intervention group and a control group at post-intervention (296). With regard to the CIS-20 too, similar effect sizes have been reported for cognitive-behavioral interventions in, among other populations, patients with rheumatoid arthritis (297). Therefore, a total sample size of 60 participants was deemed sufficient to identify detectable and clinically relevant differences in the outcome parameters of the current study.

Discussion

The present study evaluates whether self-reported and physiological health outcomes can be modulated by a psychological intervention directed at optimizing immune function. The intervention consists of guided e-health CBT in combination with health-related serious gaming elements and will be tested in healthy males. The study will contribute to the findings on the effects of psychological interventions on psychophysiological stress

reactivity after an immunological challenge and provide further evidence on the link between psychological and immunological mechanisms (169).

A unique feature of this study is that we use a psychological intervention based on multiple strategies (e-health CBT and serious gaming) directed at optimizing immune function. The study is among the first to use a combination of guided e-health CBT and health-related serious gaming elements. Serious gaming can be a promising add-on to e-health CBT, since this highly innovative tool can strengthen skills, attitudes, and knowledge about health in an entertaining manner. The results of the present study will therefore provide more insight into these psychological interventions directed at optimizing immune function and their potential effectiveness on health outcomes, both self-reported and physiological. Furthermore, the advantages of e-health CBT are that both participants and therapists can use and log onto the intervention at the time and place they prefer. Therefore, the intervention developed may be easier to use, more time efficient, and consequently less expensive than traditional face-to-face therapy. Complementing guided e-health CBT with health-related serious gaming elements seems to be a promising approach to optimize health outcomes, since multiple cognitive behavioral strategies are involved. If the combined intervention of guided e-health CBT and health-related serious gaming elements turns out to be effective in modulating self-reported and physiological health outcomes, the individual components of this psychological intervention can be investigated in further studies in order to gain more insight into the effectiveness of the different components.

In addition to an innovative psychological intervention, the study design incorporates validated immunological challenges in the form of *in vivo* and *in vitro* stimulation of immune responses. A previous study incorporated a Hepatitis B vaccination to investigate the effects of an emotional disclosure intervention on immune reactivity (199). The researchers found that participants who received the emotional disclosure intervention showed higher levels of antibodies in response to the Hepatitis B vaccination than the group who did not receive any intervention. Live vaccines, such as the BCG vaccine, come closer than other vaccines to eliciting the same immune response as is observed after natural infection. Consequently, by including BCG vaccination as an immunological challenge, this study will provide more insight into the real-life effects of a psychological intervention on immune function and subsequently on the development of protection against infectious diseases. Besides BCG vaccination, LPS stimulation *in vitro* at baseline, before vaccination, and after vaccination provides more insight into the *in vitro* immune reactivity that occurs in response to a psychological intervention.

The study also incorporates psychophysiological tasks. This allows us to obtain more insight into the psychological intervention's effects on stress reactivity. Previous studies have shown that various stressors can lead to different psychophysiological stress responses. More heightened endocrine responses and anticipatory stress appraisals were found after exposure to the TSST than after exposure to the CPT (165, 298), for example. By combining multiple psychophysiological and physiological stressors, more detailed information can be gathered about the stress response following an immune challenge. In addition, this study is one of few studies to evaluate the effects of a psychological intervention on health outcomes at follow-up (173, 273): the study design incorporates a test session four weeks after the intervention. By implementing *in vivo* and *in vitro* immunological challenges and psychophysiological challenges in combination with a follow-up measurement, this study has the potential to advance scientific knowledge into the mechanisms underlying the relation between psychological and immunological factors.

In conclusion, the present study design is expected to provide valuable information about the role of psychological mechanisms in optimizing health outcomes in healthy males. It will help to unravel the underlying mechanisms of psychophysiological stress reactivity to immunological challenges and may ultimately contribute to the development of new healthcare strategies. If it turns out that this psychological intervention can modulate various health outcomes, it can be implemented in healthcare and may partially or fully replace medication use. This may mean that fewer people suffer from the side effects of medication use and may also lead to reductions in healthcare costs.

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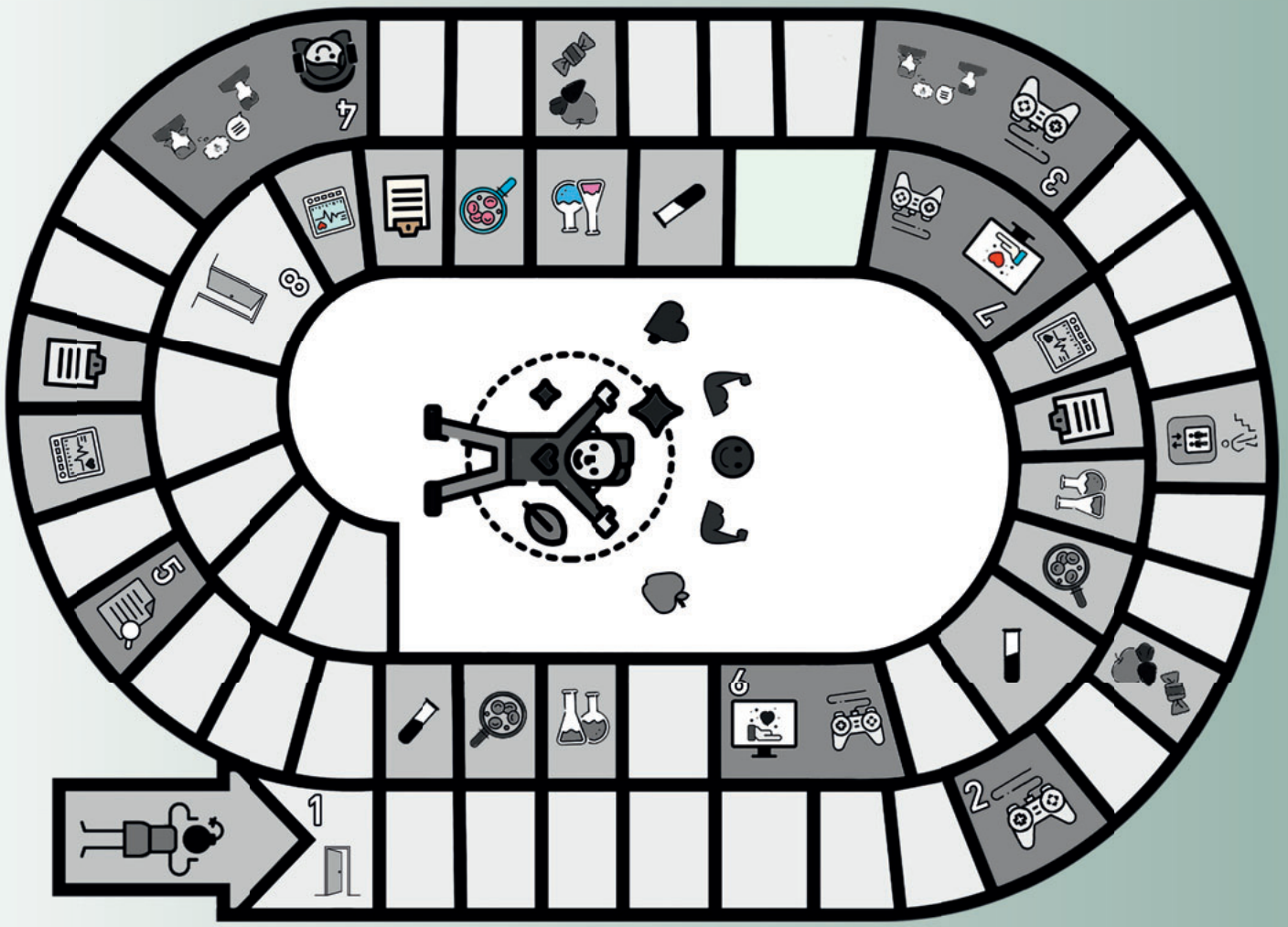
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Optimizing health outcomes in response to immune-related and psychosocial challenges by an e-health psychological intervention: A randomized controlled trial

7

This Chapter is under review for publication as:
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).

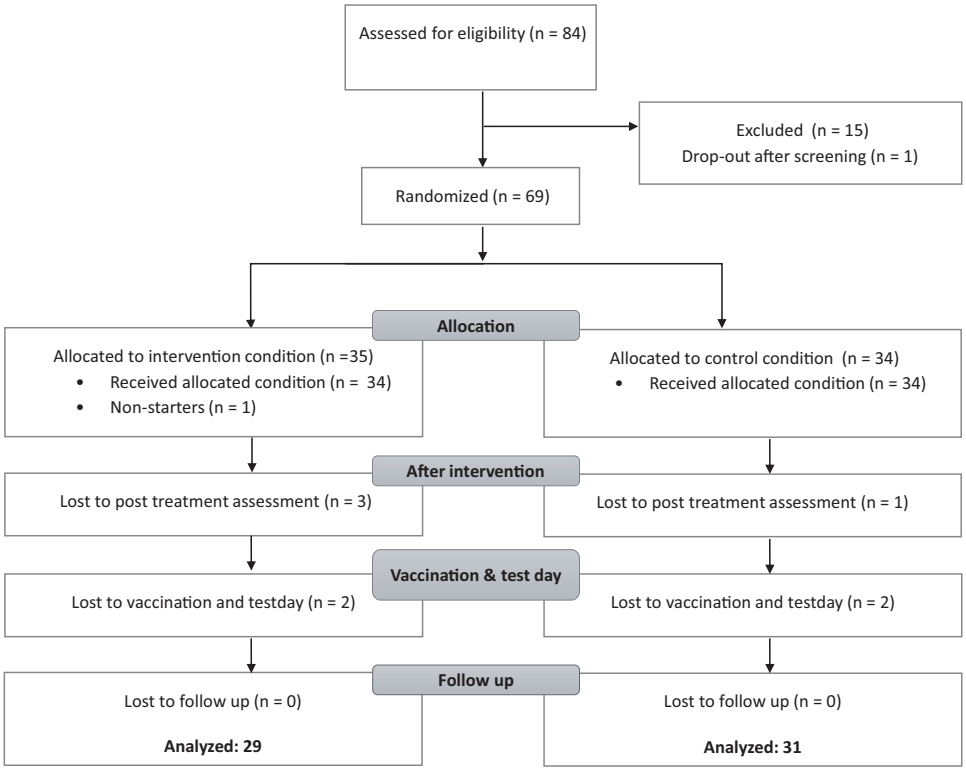


Figure 1. Flow diagram

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.

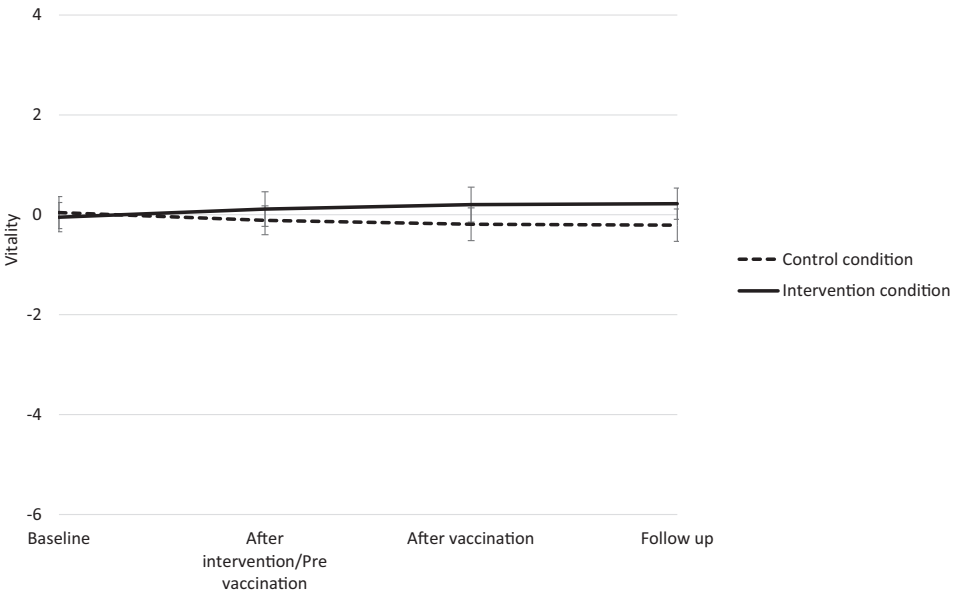


Figure 2. Mean and standard error of self-reported vitality at baseline, after intervention (pre-vaccination), after vaccination, and at follow-up, separately for the control condition and the intervention condition.

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 ($F(1.65, 79.03) = 1.00$, $p = .36$).

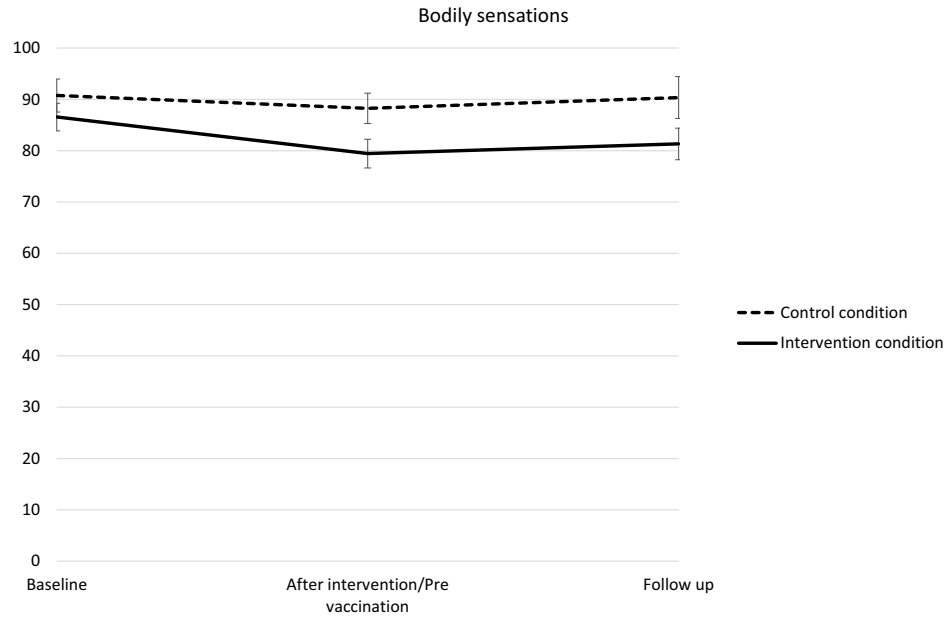


Figure 3. Mean and standard error of the mean of self-reported bodily sensations at baseline, after intervention (pre-vaccination), and at follow-up, separately for the control condition and the intervention condition.

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.

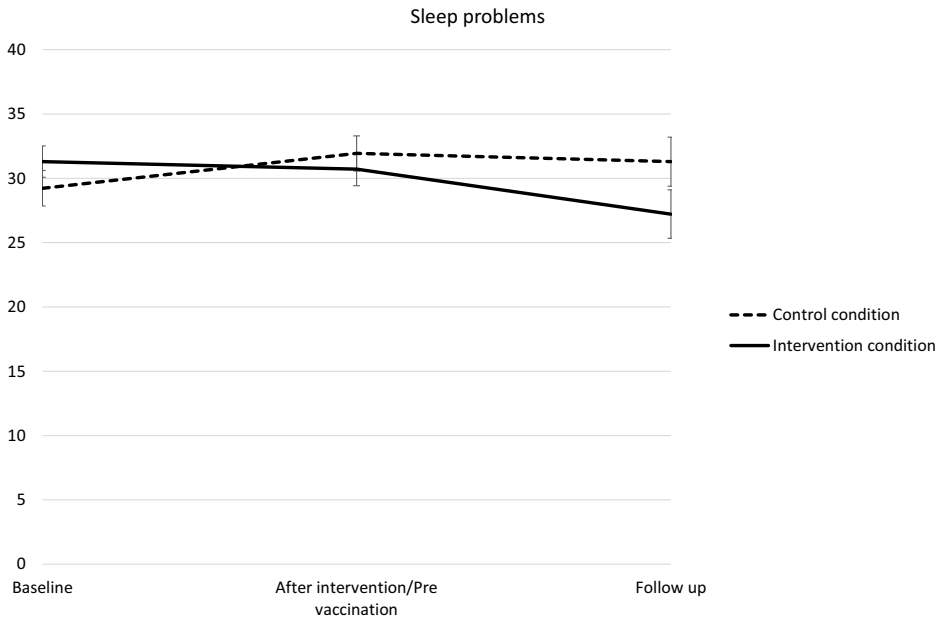


Figure 4. Mean and standard error of the mean of sleep problems at baseline, after intervention (pre-vaccination), and at follow-up, separately for the control condition and the intervention condition.

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.

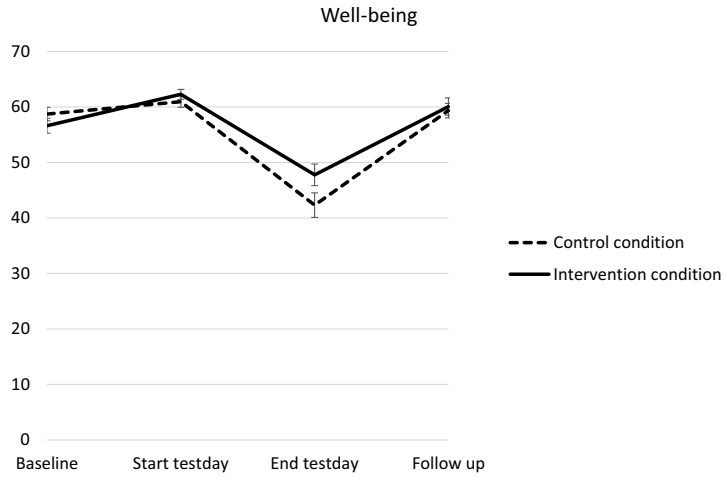


Figure 5. Mean and standard error of the mean of self-reported well-being at baseline, the start of the test day, the end of the test day, and at follow-up, separately for the control condition and the intervention condition.

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).

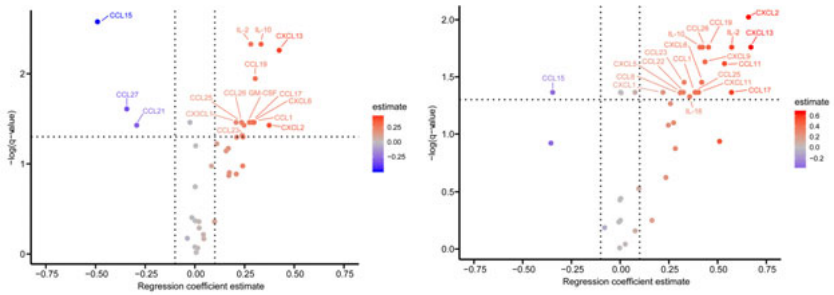


Figure 6. Vulcano plots for the control (upper graph) and intervention condition (lower graph) separately for the comparison pre-vaccination to the end of the test day. Significance is displayed on the y-axis and estimate of variance on the x-axis.

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.

Acknowledgements

The authors acknowledge Chantal Eckhardt, Dion de Hoog, Sander van den Oever, Shirley de Wit and Rafael Bidarra of Delft University of Technology for their help in designing and developing the serious game ViaNova©. Additionally, the authors acknowledge Anne Bruinings, Paige Crompvoets, Annemarie Danton, Lianne Keuning, Jelle van Leusden, Miranda Lutz, Meriem Manai, Rebecca Maurits, Stefanie Meeuwis, Kaya Peerdeman, Maaike de Ronde, Aleksandrina Skvortsova, Ikrame Tajioui and Judith Tekampe for their help with collecting the data.

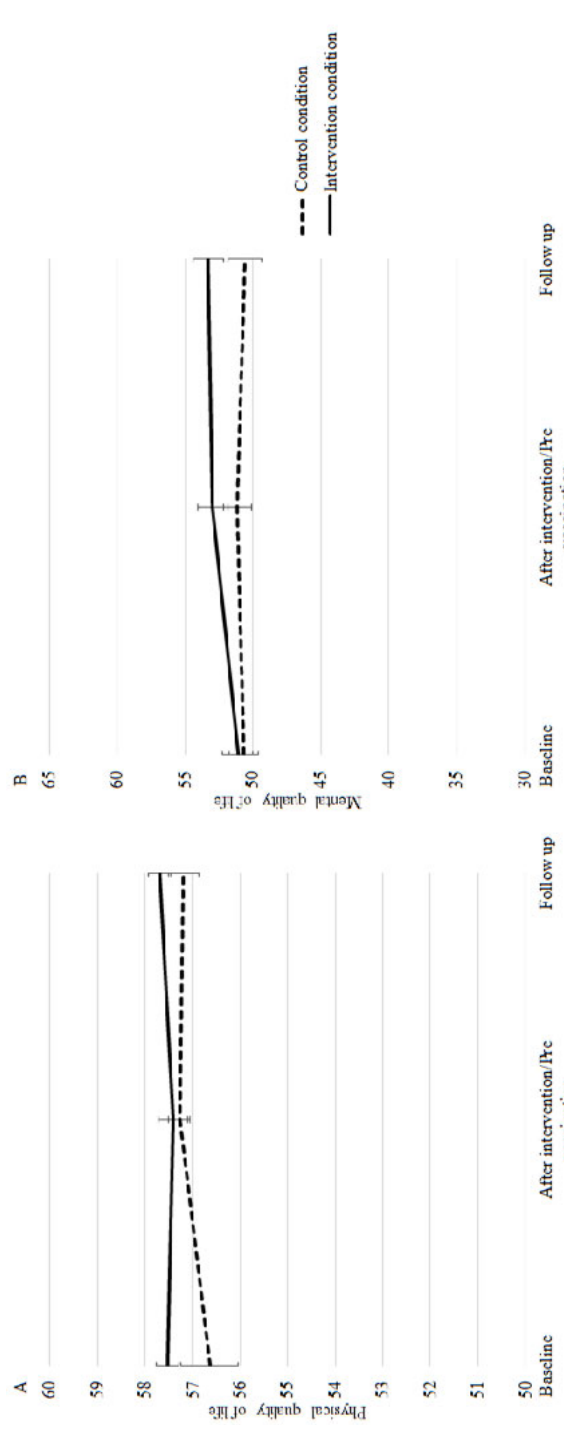
Appendices and supplementary material

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

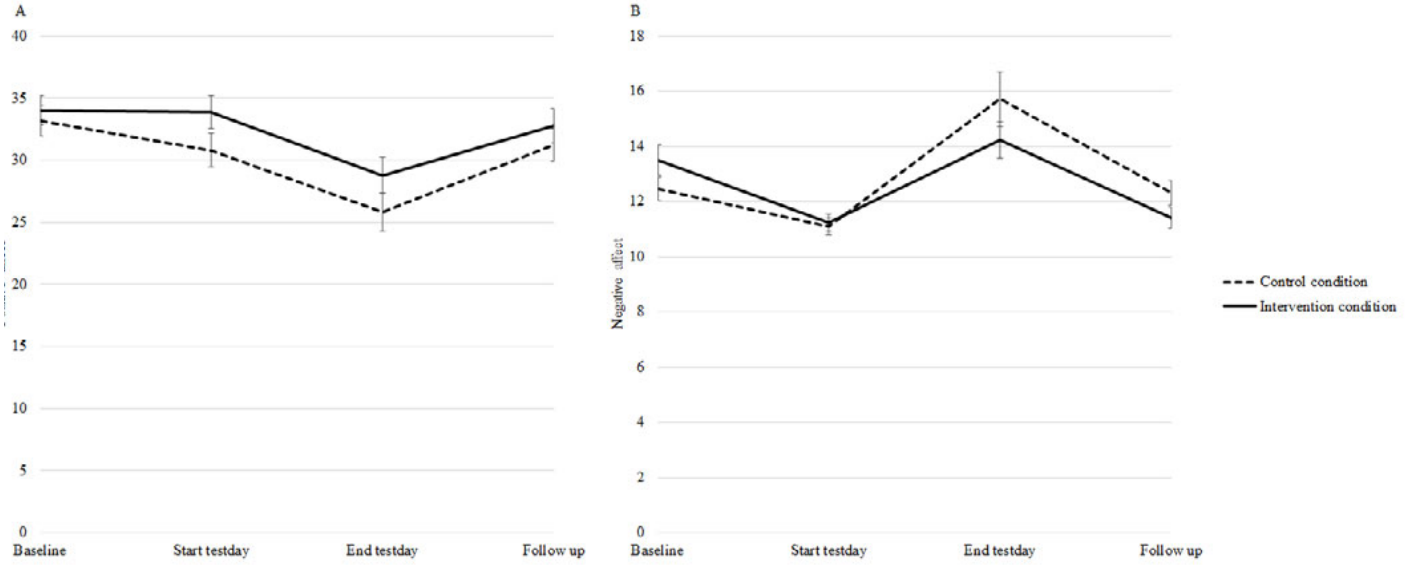
Appendix 2. Details of the self-reported, psychophysiological and immune outcome measures on each measurement point.

	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



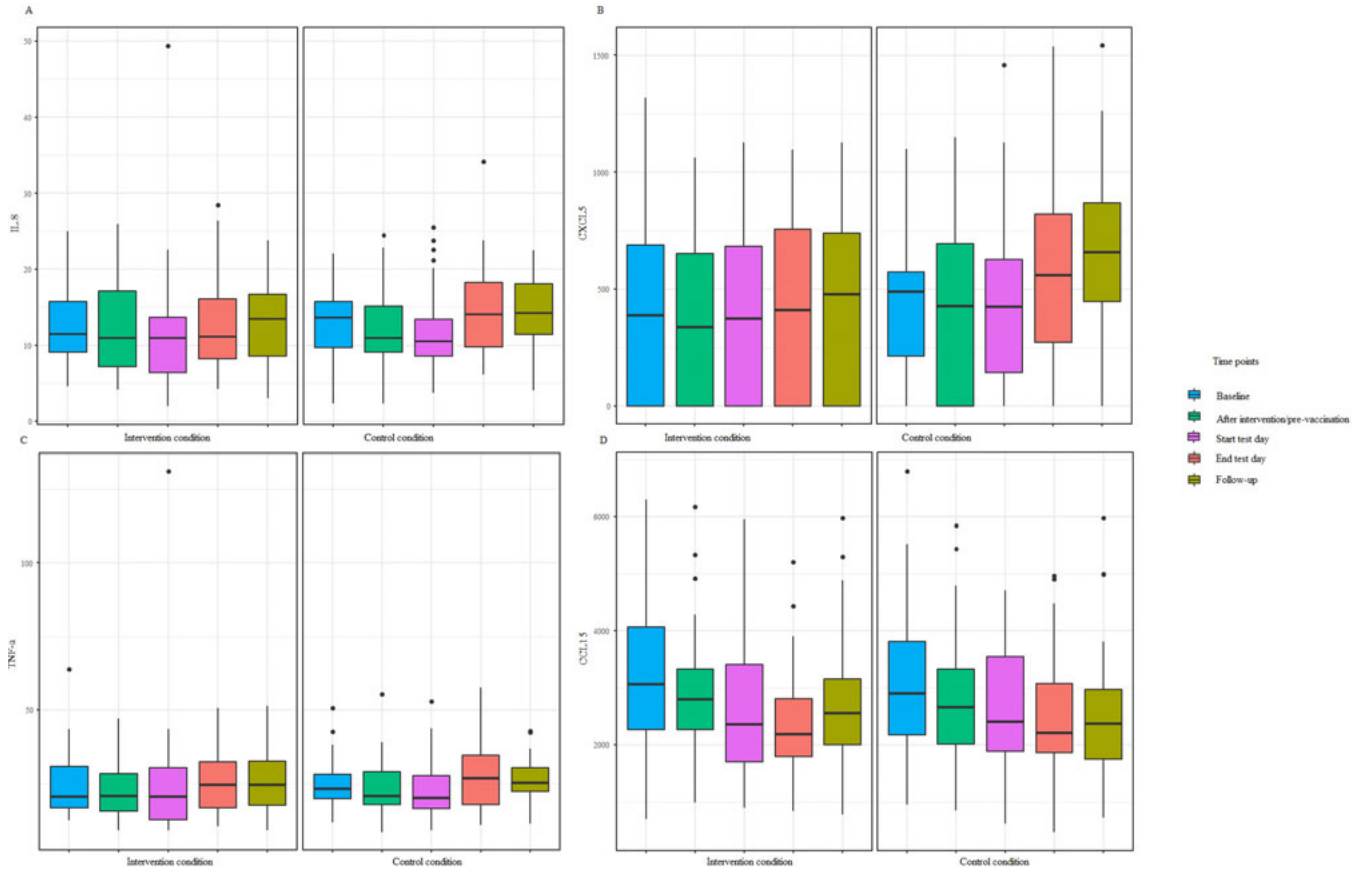
Supplementary Figure 1. Mean and standard error of the mean of self-reported physical quality of life (A) and mental quality of life (B) T-scores at baseline, after intervention (pre-vaccination), and at follow-up, separately for the control condition and the intervention condition.

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



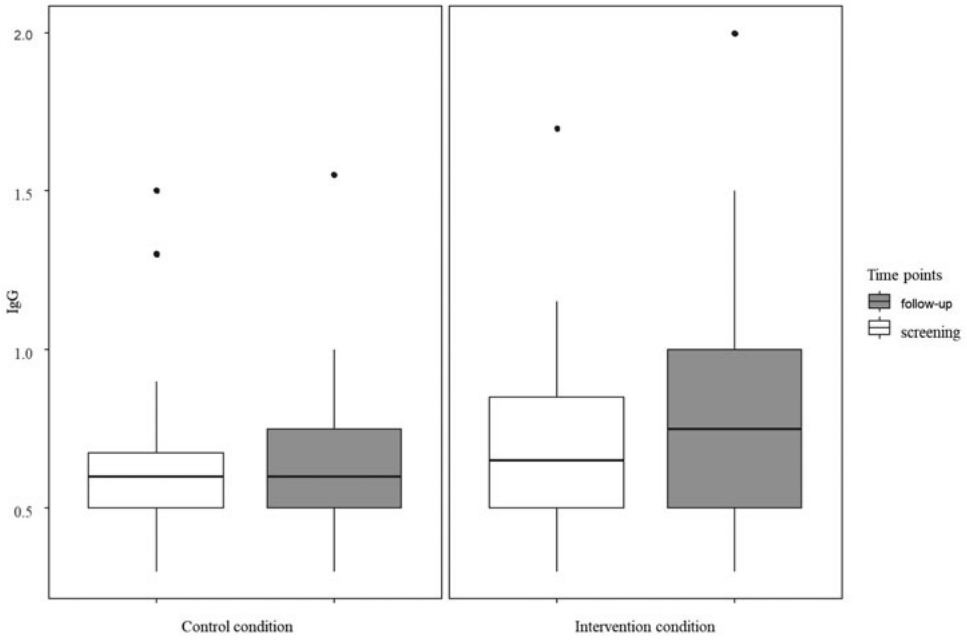
Supplementary Figure 2. Mean and standard error of the mean of the standardized scores for self-reported positive affect (A) and negative affect (B) at baseline, the start of the test day, the end of the test day, and at follow-up, separately for the control condition and the intervention condition.

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



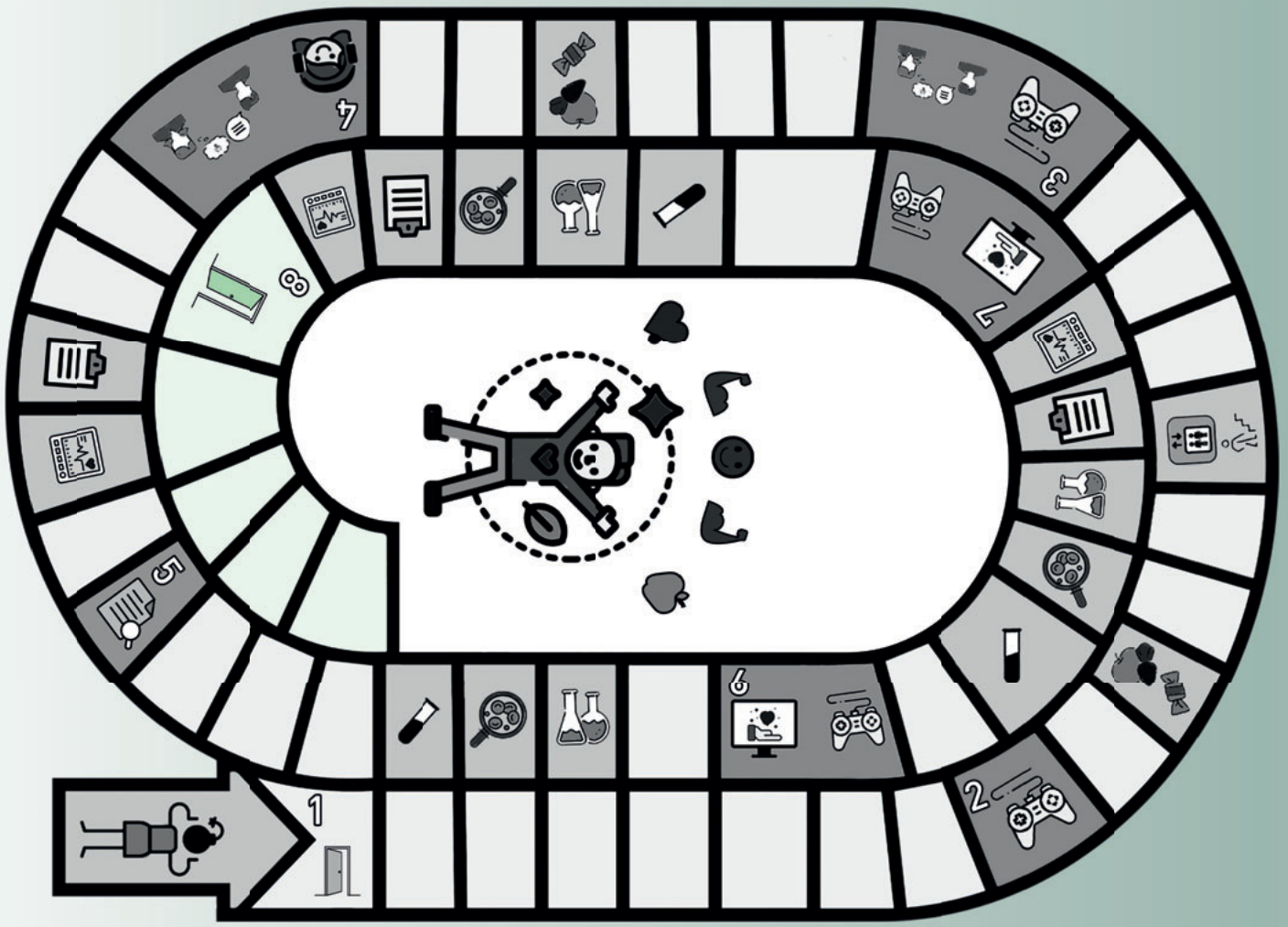
Supplementary Figure 3. Boxplots for the upregulated IL-8 (A), CXCL5 (B) and TNF- α (C), as well as for the downregulated CCL15 (D) for the control condition (left graph) and intervention condition (right graph) separately at baseline, pre-vaccination, start test day, end test day and follow-up.

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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General discussion

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In the present thesis, we aimed to examine the effectiveness of innovative psychological interventions on health outcomes by (1) evaluating the effectiveness of innovative psychological tools, i.e., serious gaming, verbal suggestions, and internet-based interventions, on various health behaviors and psychophysiological outcomes; (2) providing a concise overview of the current existing evidence of psychological interventions in optimizing immune function in response to *in vitro* or *in vivo* immunological as well as psychophysiological challenges; and (3) incorporating various self-reporting, behavioral and psychophysiological outcome measures both at baseline and in response to *in vitro* or *in vivo* immunological as well as psychophysiological challenges, including psychophysiological, physical and/or cognitive stressors, to concisely evaluate the effectiveness of psychological interventions on health outcomes. In this final chapter, we will discuss the findings resulting from the work described in this thesis. We also discuss the findings in light of their possible limitations and highlight potential implications for future research and clinical practice.

Evaluating the effectiveness of innovative psychological tools

Serious gaming to optimize health behaviors

As a first objective, we investigated the effectiveness of innovative psychological tools on health outcomes. A promising innovative psychological tool is serious gaming, as previous literature provided support for the effectiveness of serious gaming in optimizing health outcomes (28, 29). Serious games aim to alter health behaviors by optimizing targets, including self-efficacy, knowledge and skills in an entertaining manner (335). However, a meta-analysis of Desmet and colleagues (2014) showed that the effects of serious gaming varied along the incorporated outcome measures and were most conclusive for the determinants of behavior change, including knowledge and intentions to change behaviors, instead of changing actual health behaviors. The varying effects might be at least partially due to the fact that behavioral evaluations were less often incorporated and high heterogeneity was observed between studies in the incorporated outcome measures. Therefore, in Chapters 2 and 3, we evaluated the effectiveness of serious gaming on self-reported and observed behavioral outcomes in the domains of food and physical activity to receive more insights in the steps underlying health behavior changes. In Chapter 2, we focused on optimizing food outcomes and physical activity by providing participants with several health-related games during one session. These games were based on various serious components, i.e., transferring knowledge, priming and evaluative conditioning, combined with entertaining components, including providing participants with feedback and rewards. In Chapter 3, we specifically focused on optimizing food outcomes by health-related games based on approach and avoidance

training during one session. These games were accompanied or not with verbal suggestions and yielded the same entertaining components as applied in the serious games of Chapter 2. In Chapters 2 and 3, we found comparable results in that the serious gaming session affected predictors of health behaviors but did not affect behavioral outcomes. The results of Chapters 2 and 3 therefore seem restricted to optimized intentions and attitudes rather than actually implemented health behaviors. However, as serious gaming is a rather innovative tool and these studies did mostly incorporate evaluations of predictors of health behaviors rather than evaluations of behavior change (28), there is no conclusive view yet on the components of serious gaming that are effectively resulting in behavioral changes. Serious games can for example vary in the incorporation of serious and entertaining components, as well as in the intensity in which they are provided. Possibly, optimization of these serious gaming components can minimize the gap between intentions and behaviors.

Serious and entertaining components of the serious games

The serious components that were incorporated in the serious games of Chapters 2 and 3 all comprised strategies that were based on behavioral learning principles (68, 105, 336). It can be hypothesized that combining multiple behavioral learning principles would be more beneficial compared to incorporating only a single behavioral learning principle, as it provides the opportunity to tap into multiple learning processes. The meta-analysis of Desmet and colleagues (2014) found that multicomponent games showed higher effect sizes compared to standalone games, but both kind of games showed significant effects on health-related outcomes (28). One recent study also combined multiple behavior change strategies targeting various learning processes to improve snacking behaviors in adolescents using a mobile application, but did not find support for the effectiveness of the intervention (337). Although Chapter 2 was based on multiple behavioral therapeutic learning principles that target various learning processes, including transferring knowledge, priming and approach avoidance training, and Chapter 3 focused specifically on approach avoidance training, both Chapters yielded comparable results. Therefore, no support could be found for the add-on effectiveness of combining multiple strategies. However, as the results so far are heterogeneous and as both Chapters 2 and 3 are proof-of-concept studies, more research is needed on whether combining various evidence-based strategies can strengthen the effectiveness of serious gaming.

Besides the serious components, we incorporated various entertaining components into the serious games. In both Chapters 2 and 3, we included rewards, e.g., by providing participants with trophies and points, and provided the participants with feedback on performance, to enhance engagement with playing the games. Although we did not

incorporate formal evaluations of engagement in our study designs, participants reported that the games were rather enjoyable to play, and we did not find any indications that the games were not engaging. In e-health studies, providing rewards and feedback are most frequently incorporated as incentives to enhance engagement (139). Although literature on the effective entertaining components in serious games is not yet unequivocal, previous studies have pointed out that an optimal blending of the serious components and the entertaining components is essential for the effectiveness of serious games (28). It can be suggested that tailoring the serious games to the specific needs of the individual is needed to provide an optimal personalized blending of the serious gaming components. Previous reviews provided support for optimizing the effectiveness of serious games by tailoring the components to the individual (28, 139).

Intensity of the serious games

It can be suggested that serious gaming during one single session, as in our experiments, can alter precursors of health behaviors, but is not sufficient to alter health behaviors. However, no consensus in the literature exists on the ideal serious gaming duration and frequency. Whereas the average study duration was 4 hours in one meta-analysis (28), and 9 weeks in another (58), both meta-analyses failed to find support for a longer gameplay duration being more effective (28, 58). In contrast to interventions with a longer duration, shorter interventions provide opportunities to reduce time and costs of interventions. Furthermore, no guidelines exist regarding the minimal duration of interventions in order to induce optimized health outcomes. A study analyzing the minimal intervention intensity to optimize health outcomes showed that increasing the intensity of interventions is not always necessary to increase their effectiveness, as interventions with low intensity can also be effective in producing health behavior changes (338). The effects of behavior change interventions, however, were stronger when follow-up guidance was added to the intervention (338). Therefore, future research should investigate whether optimization of health behaviors can be realized when offering personalized guidance according to the specific individual's needs. Guidance can be provided through an online environment in which written guidance feedback is given in response to homework assignments and questions of the participant, as was done in Chapters 6 and 7 in which serious gaming was combined with internet-based cognitive behavioral therapy (CBT). Furthermore, participants can be provided with face-to-face or telephonic follow-up contact focusing on maintaining goals and relapse prevention, as was also done in Chapters 6 and 7.

Alternatively, there exists no consensus for the ideal gameplay absolute intensity and duration, since this may significantly depend on the preexisting knowledge and skills as well as the needs of the individual. It would be interesting to investigate in future research

whether it is effective to tailor the gameplay intensity and duration, as well as the way and intensity in which guidance is provided according to the needs of the individual. This can for example be done by adjusting the guidance according to the individual's goals and needs regarding health behavior optimization.

Optimizing outcome expectancies to enhance the effectiveness of psychological interventions

Another innovative tool that can be implemented into psychological interventions concerns optimizing outcome expectancies, as placebo research on optimizing outcome expectancies showed that verbal suggestions can improve health outcomes (13). There are various ways through which outcome expectancies can be optimized, including instructional learning, conditioning and observational learning (152). In Chapters 3 and 4, we aimed to optimize outcome expectancies through instructional learning by providing participants with verbal suggestions. In Chapter 3, we provided participants with verbal suggestions concerning the effectiveness of serious gaming next to completing the serious games. The findings provided some preliminary support for the add-on effectiveness of verbal suggestions onto serious gaming based on approach-avoidance strategies. As we only found limited support for this add-on effectiveness of the verbal suggestions and not for the verbal suggestion on its own, the results in Chapter 3 are in line with previous research, demonstrating that a combination of techniques, such as for example conditioning and verbal suggestions, is more effective compared to the standalone interventions (339, 340). However, we did not find any support for the add-on effectiveness of verbal suggestions in Chapter 4, in which participants were provided with verbal suggestions concerning the effectiveness of a relaxation practice next to actually performing the relaxation practice. As the results of the proof-of-concept studies described in Chapters 3 and 4 are not conclusive regarding the effectiveness of verbal suggestions in optimizing psychological intervention effects, more research on this topic is needed. It can be presumed that the content of verbal suggestions as well as the frequency in which verbal suggestions are provided to participants are key components that contribute to the effectiveness of verbal suggestions (152, 341).

Content of the verbal suggestions

The verbal suggestions that were applied in Chapter 3 were based on a combination of optimizing outcome expectancies and optimizing contingencies between the stimulus and response, i.e., influencing actions of approaching and avoiding without actually performing these actions (342, 343). In Chapter 4, the verbal suggestions were also based on optimizing outcome expectancies and contained instructions on how to perform a

relaxation practice. Both the verbal suggestions of Chapters 3 and 4 therefore covered the key components of the intervention. Previous studies showed that providing people merely with verbal instructions concerning stimulus-response contingencies without actually performing a training is effective in optimizing approach avoidance effects (342). However, this is not yet confirmed for relaxation instructions and we did not find any support for the effectiveness of the verbal suggestions in Chapter 4. Future studies should therefore further elucidate whether the add-on effectiveness of verbal suggestions can be generalized to various psychological intervention strategies or seems restricted to certain concepts. Although beyond the scope of the proof-of-concept studies of Chapter 3 and 4, it can be presumed that the effective content of verbal suggestions varies along individuals. Prior literature pointed out that there are substantial individual differences in placebo responses (344, 345), for instance due to differences in prior experiences (346). It would therefore be interesting to examine whether providing verbal suggestions that are specifically tailored to the individuals' needs can optimize the effectiveness of the suggestions.

Booster suggestions

As learning processes can be strengthened by repeatedly presenting an individual with the same stimulus (347), increasing the frequency of providing the verbal suggestions could possibly strengthen the effectiveness of the verbal suggestions. In Chapter 3, the verbal suggestions were repeated after each of the outcome measures, whereas the verbal suggestions in Chapter 4 were only presented once. A study of Colloca and colleagues (2010) showed that the number of conditioning trials affected the strength of the analgesic responses, in that a higher number of conditioning trials resulted in increased analgesic effects (348). Although this relation between the frequency of providing verbal suggestions and its subsequent effectiveness is not yet clear yet for verbal suggestions, it could be hypothesized that the differences in the frequency with which the verbal suggestions were presented in Chapters 3 and 4 might have affected the differential findings. It would therefore be interesting to investigate whether increasing the number of (booster) suggestions could strengthen the instructional learning effects in the context of psychological intervention strategies.

Optimizing health outcomes by combining an internet-based intervention with serious gaming

In Chapter 6, we provided the study protocol for evaluating the effectiveness of a six-week guided internet-based CBT intervention accompanied with serious gaming elements in healthy participants. We evaluated the effectiveness of this intervention in Chapter 7 by

incorporating various immunological and psychophysiological challenges, including a BCG-vaccine, and found no significant effects for optimized outcomes on our primary outcome, vitality. However, we found effects on related constructs, in that bodily sensations (including head ache, nausea and other types of sensations that are usually experienced as being annoying) and sleep problems decreased after the intervention. In addition, self-reported well-being decreased less after the test day with psychophysiological challenges, including psychophysiological, physical and/or cognitive stressors, in the intervention condition compared to the control condition. Furthermore, we found some limited support for differential effects on circulating blood chemokines at the end of the test day, as well as for increased IgG antibody levels four weeks after a BCG-vaccination. In addition, it is known that internet-based interventions in general have high drop-out rates, of around 20% (349, 350). However, for our internet-based CBT intervention in Chapter 7, only 3 out of 35 participants (8.6%) discontinued with the intervention, providing support for the effectiveness of our intervention in keeping participants engaged with the intervention. Therefore, a combination of guided internet-based CBT and serious gaming for six weeks provides promising results in optimizing health outcomes.

Possible effective intervention components

Chapter 7 did not only show optimized self-reported outcomes at rest, but also optimized self-reported well-being in response to psychophysiological challenges and limited support for differential chemokine responses and increased IgG antibody levels four weeks after a BCG-vaccination, whereas the results of Chapters 2 – 4 were mainly limited to self-reported outcomes. There are several factors that may have contributed to these findings. First of all, Chapter 7 focused on a combination of internet-based CBT and serious gaming during six weeks, whereas Chapters 2 – 4 were focused on serious gaming or relaxation, respectively, during one single session. It can therefore be hypothesized the combination of multiple innovative intervention tools and the duration have affected the effectiveness of the psychological interventions. Furthermore, the intervention in Chapter 7 was guided by a therapist and tailored to the specific needs of the participant. As the interventions in Chapters 2 – 4 were unguided and not tailored to the specific needs of the individual, this might have led to the less conclusive results compared to Chapter 7. Finally, the results of Chapter 7 were not only measured directly after the intervention as was done in Chapters 2 – 4, but also four weeks afterwards. Moreover, we incorporated a booster session two weeks after the intervention in Chapter 7 to focus on relapse prevention. It can be hypothesized that participants need time to optimize health outcomes and by combining this time with a booster session focusing on relapse prevention contributed to the findings on immune outcomes after a BCG-vaccination.

Although the combination of internet-based CBT and serious gaming already yielded promising results in Chapter 7, it would be interesting to optimize the serious gaming sessions by tailoring the intensity and duration according to the individual needs and by implementing elements of augmented reality. In addition, we did not apply verbal suggestions in Chapter 7. Since Chapter 3 provided some support for the add-on effectiveness of verbal suggestions onto serious gaming, future studies could evaluate whether adding verbal suggestions onto internet-based CBT with serious gaming elements can further optimize these effects. Finally, the usability of the intervention can be further optimized, as the serious games that were provided were only available in a computerized version. It would be interesting to combine the serious game and the internet-based CBT intervention in one mobile application to be able to remind participants with mobile notifications of their homework assignments and to make the intervention even more accessible.

Providing an overview of the evidence of psychological interventions in optimizing immune function

Next to the innovative intervention tools for optimizing health outcomes including immune function, we aimed to provide a concise overview of the currently existing evidence for psychological interventions in optimizing immune function. We therefore investigated the effectiveness of psychological interventions in optimizing immune function by a meta-analytic review in Chapter 5. A previous meta-analytic review did not allow conclusive statements on the effectiveness of psychological interventions on immune function due to substantial heterogeneity in study designs and incorporated outcome measures that were usually assessed during resting states (169). We therefore hypothesized that confronting participants with a challenge, i.e., a stimulus that provokes an immune system response like a vaccine, provides more insights in the actual responsiveness of the immune system to a natural challenge. We performed a meta-analytic review focusing on studies that incorporated an *in vitro, in vivo* or a psychophysiological challenge into the study design. Overall, we found support for a small to moderate effect of psychological interventions on optimizing immune function, although large heterogeneity was observed in the incorporated study population, psychological interventions and immune outcome measures. Moderate effects were found when studies incorporated *in vivo* immunological challenges to evaluate the effectiveness of psychological interventions, while small effect sizes were found for *in vitro* challenges and no significant effects were found for psychophysiological challenges.

In vivo immunological challenges

As highest effect sizes on immune outcomes were found for studies incorporating *in vivo* challenges, such challenges are rather promising to incorporate in future research. Although the results from studies incorporating *in vitro* and psychophysiological challenges provide rather insightful information in the immune response compared to e.g., counting cells in resting states, those challenges presumably yield a more complex interpretation compared to *in vivo* immunological challenges (e.g., faster wound healing). Since *in vitro* immunological challenges are provided outside the body after sample manipulation, they are likely to be more distantly associated with the subtle behavioral responses and thus may confound their detection. Furthermore, it is not known whether psychophysiological challenges yield a direct causal relation with the immune outcome. We therefore hypothesized that incorporating *in vivo* immunological challenges that are carefully matched to the incorporated study population would provide most promising opportunities to evaluate the effectiveness of psychological interventions on immune function directly *ex vivo*. For example, a study of Witt (2003) included patients with birch pollen allergy and challenged them with a histamine provocation test after receiving a psychological intervention or being allocated to the control condition, receiving no intervention (301). They found a differential immune response in participants that received the intervention as compared to the participants that did not receive any intervention. As the results of this study do not only provide insights in that a psychological intervention can alter immune function, but also specifically in how a psychological intervention can result in less symptoms for a certain condition, it provides insights to what extent a psychological intervention can possibly support regular treatments for a specific condition, in this case birch pollen allergy.

In vitro and psychophysiological challenges

As described above, the interpretation of the outcomes derived from *in vitro* and psychophysiological challenges is somewhat more complex compared to the interpretation of the outcomes of *in vivo* immunological challenges. However, *in vitro* and psychophysiological challenges can provide additional insights into immune responsiveness with respect to *in vivo* immunological challenges, as they challenge specific immune cells in a different way. Therefore, *in vitro* and psychophysiological challenges might be suitable add-on challenges onto *in vivo* challenges. Future studies on the effectiveness of psychological interventions on immune outcomes might more consistently incorporate various combinations of challenges in various somatic conditions to gather more insights in the immune response after a psychological intervention. The combination of challenges that should be incorporated in a study depends on the included study population as well as

on the aims of the intervention. For instance, when an intervention is specifically focused on optimizing coping with stress, it would be interesting to incorporate a social evaluative stressor into the study design accompanied with an *in vivo* immunological challenge, such as a Hepatitis B vaccine, as stress can suppress Hepatitis B antibody responses (351). Furthermore, future studies should take the order into account in which they expose participants to specific challenges. It can for instance be presumed that more insights in immune functioning are gathered when the *in vitro* immunological challenge is not only provided prior to the *in vivo* or psychophysiological immunological challenge, but also afterwards. By performing the *in vitro* immunological challenge before and after another challenge, it would be possible to gain more insights in immune function in response to various gradations of challenges.

Incorporating various outcome measures and challenges to evaluate the effectiveness of a psychological intervention on health outcomes

As outlined above, it would be interesting to observe the immune response to a combination of multiple *in vitro* and *in vivo* immunological as well as possibly psychophysiological challenges in order to gather more insights in the underlying mechanisms of the immune response. In Chapters 6 and 7, we therefore explored the effectiveness of a psychological intervention on health outcomes in healthy participants by exposing them to a combination of *in vitro* (i.e., LPS stimulation) and *in vivo* (i.e., BCG-vaccination) immunological as well as psychophysiological challenges (i.e., PASAT, CPT and TSST). When looking at the basal psychophysiological outcomes and the self-reported and psychophysiological outcomes in response to the challenges, some preliminary indications were found for differential outcomes on the physiological outcomes of heart rate. However, no significant effects were found for cortisol and alpha amylase, as measured in saliva. In response to the psychophysiological challenges on the test day, we found higher levels of self-reported well-being after the intervention compared to the control condition. Furthermore, we found some support for increased IgG antibody responses four weeks after the BCG-vaccination in the intervention condition compared to the control condition. In addition, there was some preliminary support for differential effects on immune outcomes after the test day in the intervention condition versus the control condition. These differential immune response patterns were only visible in the context of the challenges, i.e., the *in vitro*, *in vivo* immunological and/or psychophysiological challenges. This is in line with the hypothesis that a combination of challenges is needed to be able to activate a differential immune response pattern. As we incorporated healthy participants, floor effects can be observed likely due to the fact that they already possess an adequate immune system. Therefore, it can be presumed that the challenges are necessary to

uncover potentially differential health outcomes. To provide more insights in the clinical relevance of a psychological intervention on health outcomes, the findings of Chapter 7 should be replicated in persons that are at increased risk of health problems due to immune disorders.

Evaluating health outcomes with various outcome measures

In the studies that were performed in the present thesis, various self-reporting (Chapters 2 – 4, 7), behavioral (Chapters 2 and 3) and psychophysiological (Chapters 4 and 7) outcome measures both at baseline and in response to *in vitro* or *in vivo* immunological (Chapter 7) as well as psychophysiological challenges (Chapters 4 and 7) were included to assess health outcomes. In Chapter 7, we included an *in vivo* challenge of the immune system by providing participants with a live-attenuated BCG-vaccination and found some support for increased IgG antibody levels after the intervention. As antibody responses to the live-attenuated (replicating) BCG vaccine approximate the physiological response to a natural infection, these findings provide limited support for the effectiveness of the psychological intervention of Chapter 7. However, this study was the first incorporating a BCG-vaccination into a study design evaluating the effectiveness of a psychological intervention and as the present findings were not yet conclusive, they should be confirmed and extended by future research. In Chapter 7, the most conclusive findings came from self-reported outcomes, whereas psychophysiological and immune outcomes yielded less conclusive findings. This discrepancy was also observed in Chapters 2 – 4, in which the results were more conclusive for self-reporting outcomes, whereas no significant effects were found for behavioral or psychophysiological outcomes. Studies that evaluate the effectiveness of psychological interventions on health outcomes often rely on self-reported outcomes (28, 352-354), while results on behavioral and psychophysiological outcomes are in general less conclusive. As the primary outcome in Chapters 2, 3, 4, and 7 was based on self-report, the sample size was powered accordingly. Possibly, the studies may therefore not have been sufficiently powered to detect significant differences for the other behavioral and psychophysiological outcomes. Therefore, Chapters 2, 3, 4, and 7 provide a first step in evaluating health outcomes with multiple methods. Future studies should more consistently incorporate behavioral and psychophysiological outcomes next to self-reported outcomes to gather more insights in the underlying mechanisms of health outcome optimization. Moreover, although we observed health behaviors in Chapter 2 and 3, we did not incorporate observations of health behaviors in Chapter 7. Future research should also systematically incorporate observations of health behaviors, including physical activity and eating behaviors. These data can be collected through wearable devices that can detect both energy intake and expenditure (355).

Strengths and limitations

The present thesis has multiple strengths that are worth mentioning. First of all, the present thesis incorporated various innovative psychological intervention tools to optimize health outcomes. By incorporating tools, including serious gaming, verbal suggestions, and internet-based CBT, separately but also combined, more insights were gathered in how to optimize the effectiveness of existing psychological interventions. Second, we incorporated various self-reported, behavioral and psychophysiological outcome measures to evaluate the effectiveness of those tools. In addition, we incorporated various physical and psychophysiological challenges, including a BCG-vaccination to gather more insights in the processes underlying health optimization. As we are aware that multiple testing increases the risk of type 1 errors, we adjusted the relevant p-values accordingly. Therefore, the present thesis was able to gather a multi-perspective view on the effectiveness of innovative psychological interventions on health outcomes.

Next to the above-mentioned strengths of the work presented in this thesis, there are also some limitations that should be mentioned. First of all, the effect sizes that were found in the studies from Chapters 2, 3, 4, and 7 were variable and statistically small. This can at least partially be due to the fact that the experimental studies in these Chapters all entailed proof-of-concept studies in healthy subjects with innovative intervention components and outcome measures. As the included interventions in Chapters 2, 3, 4, and 7 provide a first step towards health optimization, future studies should be carried out to further confirm the present findings. Second, in the experimental studies of Chapters 2, 3, 4, and 7, only healthy participants between 18 and 35 years of age were included. We incorporated a rather homogeneous population in our studies to exclude as many alternative explanations for the conclusions as possible, and to explore whether health outcomes can be further optimized in a healthy population, i.e., primary prevention. The next step would be to investigate whether the interventions can also serve as secondary or tertiary prevention tools. Therefore, the results of our experimental studies should be replicated in populations at risk for health problems or those suffering from chronic diseases. Third, although we do not have indications that the verbal suggestions in Chapters 3 and 4 were not credible to participants, future studies should include manipulation checks to assess the credibility of the verbal suggestions used. Finally, although we aimed to enhance engagement within the studies that included serious gaming elements, i.e., Chapters 2, 3, and 7, we did not evaluate whether engagement with the serious games or with the psychological CBT intervention was actually optimized by playing the games. Therefore, future studies should develop valid assessments of engagement in order to evaluate it more structurally when including serious gaming elements into the study design. Finally, in order to strengthen the theoretical basis of the psychological interventions that we

incorporated in Chapters 2, 3, 4 and 7, we combined multiple techniques and methods into the interventions. However, we were not able to disentangle and examine the effectiveness of the included components in these interventions.

Future research directions

The present thesis extends current knowledge on the effectiveness of innovative psychological intervention tools in optimizing health outcomes by incorporating various evaluation methods, and provides several promising opportunities for future research.
First, the studies performed in Chapters 2 – 4, 6 and 7 are all performed in a rather homogeneous and healthy student sample, which limits the generalizability of the findings. Therefore, the studies should be replicated in patients at increased risk of health problems, including patients with somatic conditions and possibly immune disorders, in order to see whether the interventions can be effective tools for secondary and/or tertiary prevention. More specifically, the serious games that were incorporated in Chapters 2 and 3 should also be provided to populations at risk for health problems due to an unhealthy lifestyle, e.g., people with obesity or metabolic syndrome. In addition, the relaxation practice that was implemented in Chapter 4 should be applied to a population with (sub) clinical levels of experienced stress, to see whether it is actually effective in reducing stress in a population at risk for health problems due to stress. Finally, the internet-based CBT intervention along with the serious game that was incorporated in Chapters 6 and 7 to optimize health outcomes can also be investigated in patients with somatic conditions. As the intervention was effective in optimizing bodily sensations and sleep problems, it would be interesting to apply this intervention to a population where these factors play a major role in the maintenance and/or deterioration of the somatic condition, including for example patients with medically unexplained symptoms such as fibromyalgia (356), but also patients with other somatic and psychological conditions.

Second, future studies concerning serious games should focus on creating an optimal blending of the serious components and the entertaining components in serious games. Therefore, future studies might aim to make rewards in serious games more meaningful for everyday life. This could be realized by adding elements of augmented reality into serious gaming, i.e., applying the serious game in the real world to enhance engagement (357). In the context of optimizing health outcomes, serious games could for example include realistic rewards when people show optimized health behaviors (e.g., discounts on healthy food products and/or subscription to a fitness center) and they could include tailored feedback on how to optimize their health behaviors according to the health goals and values of the individual (358). It can be hypothesized that when features of

augmented reality are added onto the serious games, the gap between intentions and behaviors can be bridged, as users are able to directly apply the acquired knowledge and skills in their daily lives.

Third, we only included verbal suggestions in the context of serious gaming and relaxation interventions. Future studies should also investigate the add-on effectiveness of verbal suggestions to other psychotherapeutic interventions. As described above, it would be interesting to tailor the verbal suggestions onto the specific individual needs, and if booster sessions turn out to strengthen the effectiveness of verbal suggestions, the number of booster sessions could also be individually tailored. Besides, although the present thesis was based on optimizing outcome expectancies through instructional learning, placebo literature supported that outcome expectancies can also be enhanced by observational learning, i.e., observing behavior of another individual (i.e., a demonstrator) to gather more insights in a situation and possible reinforcing consequences of certain behaviors and subsequently to create a change in the behavioral patterns of the observer (152, 339, 359, 360). For example, observational learning has shown to be effective in increasing analgesia (152, 361). A study of Colloca and Benedetti (2009) showed that social observation of a demonstrator who underwent the whole experiment resulted in increased levels of placebo analgesia in the observer when they underwent the experiment themselves (361). Observational learning based on social observation could specifically be interesting in the context of a psychophysiological challenge (e.g., TSST), as was implemented in Chapter 4. Possibly, participants feel more efficacious in coping with the stressor after observing a demonstrator undergoing a social evaluative stressor prior to being exposed to the task by themselves. Finally, the present thesis aimed to optimize psychological intervention tools by combining various intervention components. In Chapters 2 and 3, we included various serious and entertaining components into the serious games. In Chapters 6 and 7, serious gaming components were supplemented to internet-based CBT to optimize the effectiveness of the psychological intervention. As the studies in Chapters 2, 3, 6 and 7 were proof-of-concept studies and performed to evaluate the effectiveness of the psychological intervention as a whole, we were not able to disentangle the effectiveness of the individual techniques and components that were incorporated. Therefore, future research should further investigate the effectiveness of the individual components, as well as the most optimal combination of these components in order to enhance the effectiveness of the psychological intervention. Future studies could for example evaluate whether the combination of multiple components, e.g., various relaxation practices, is more effective than incorporating a single component, e.g., one single relaxation practice, by comparing these interventions on their effectiveness in optimizing health outcomes.

Implications for clinical practice

The results of the present thesis suggest that the incorporated innovative psychological tools can, at least partially, be effective in optimizing health outcomes, which provides promising steps towards clinical practice. Below, we further elaborate on the implications for clinical practice and provide suggestions for future research that can provide further insights to bridge the gap between scientific research and clinical practice.
In Chapters 2 and 3, we investigated the effectiveness of serious gaming in optimizing food outcomes and physical activity. The serious games were effective in optimizing precursors of healthy food outcomes. Therefore, it would be interesting to further optimize the effectiveness of these games, i.e., by providing support through face-to-face contact or by telephone, and take further steps to implement them in primary health prevention programs. In addition, as serious games are known to optimize motivation to complete an intervention (26), serious games presumably are a rather promising add-on tool in existing psychological interventions.

In Chapter 3, we found some preliminary indications that verbal suggestions might be an effective add-on tool onto serious gaming. Although the add-on effectiveness of verbal suggestions was not confirmed by the results in Chapter 4, manipulating outcome expectancies presumably affects the effectiveness of the intervention (152). Future studies should therefore further investigate the effectiveness of verbal suggestions as an add-on tool to psychological interventions. When future studies demonstrate that verbal suggestions are an effective add-on tool to psychological interventions, this can have widespread implications for clinical practice. More specifically, healthcare professionals then should receive standardized education and training regarding the communication with the patient in order to minimize nocebo effects and to optimize placebo effects in clinical practice (344). When applying the verbal suggestions in clinical practice, it would presumably be best from an ethical perspective to yield an open-label approach instead of an hidden approach when providing patients with the verbal suggestions in clinical practice (362).

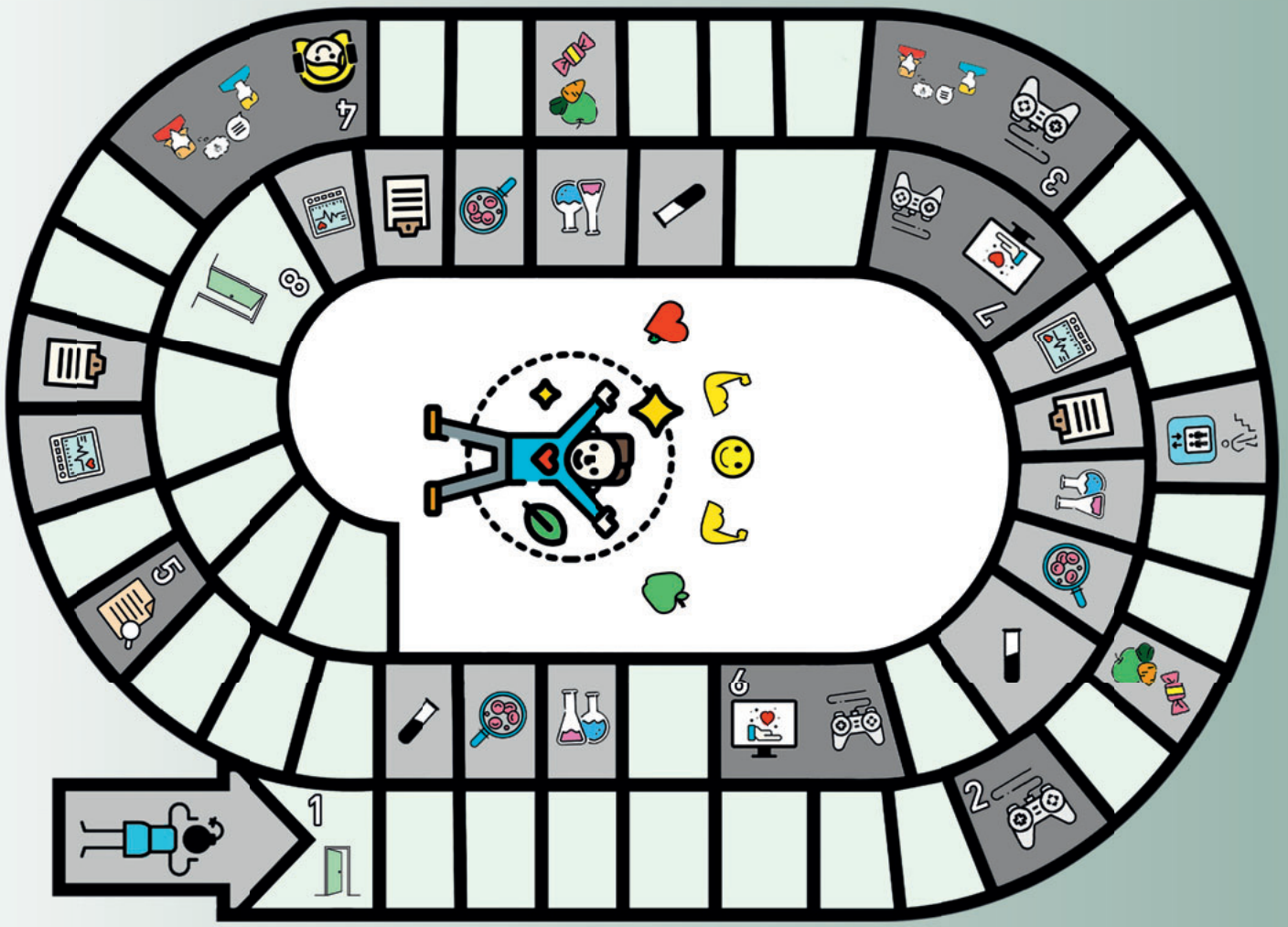
Furthermore, in Chapter 5, we found most conclusive effects for the effectiveness of psychological interventions on immune function when studies incorporated *in vivo* immunological challenges. When evaluating the effectiveness of a psychological intervention by incorporating live BCG-vaccination in Chapter 7, we found differential effects in IgG antibody levels in the intervention condition compared to the control condition, suggesting that the psychological intervention was effective in altering immune function. Previous literature also showed that psychological interventions can be effective in altering immune function when facing an *in vivo* immunological challenge: a

psychological intervention, for instance, showed to be effective in optimizing outcome of the allergic response to birch pollen (301), as well as optimizing wound healing in surgical patients (363). Therefore, it would be interesting for clinical practice to further investigate whether psychological interventions can supplement, or at least partially replace, current drug treatments in various somatic conditions to reduce side effects.
Finally, we found in Chapters 6 and 7 that multiple health outcomes can be optimized by the combination of internet-based CBT and serious gaming. Therefore, it would be interesting to provide patients with the option for internet-based CBT interventions next to regular face-to-face CBT interventions to tailor the way of providing the intervention to the specific preferences of the individual patient. Furthermore, it would be interesting to investigate whether serious gaming is an effective add-on tool towards internet-based CBT interventions. As stated above, the usability of the intervention can be optimized by providing the internet-based CBT intervention along with the serious game in one mobile application.

In summary, participants can benefit from innovative psychological intervention tools, including serious gaming, verbal suggestions, and internet-based interventions. Compared to traditional face-to-face interventions, innovative internet-based interventions can be timesaving, both for the patient but also for the therapist. However, in order to enhance its effectiveness, the effectiveness of tailoring the intervention specifically to the values and goals of the individual should be taken into account.

Conclusion

Taken together, the present thesis provides an overview of innovative psychological intervention tools aimed to optimize health and found preliminary support for the effectiveness of serious gaming, verbal suggestions and internet-based CBT on optimizing various self-reported, observational and psychophysiological measures of health. Evaluating the effectiveness of innovative psychological interventions on health outcomes by incorporating multiple psychophysiological methods and challenges provides more insights in the potential effectiveness of these interventions as well as in the mechanisms underlying health optimization.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Summary

In the last couple of decades, there has been more attention for the relation between health status and psychological processes. The results of studies on the effectiveness of psychological interventions in optimizing health outcomes are promising. However, the underlying processes are not yet fully elucidated. Moreover, it is not often investigated whether adding innovative psychological intervention tools to psychological intervention can optimize the effectiveness of interventions. The aim of the current thesis was to examine the effectiveness of innovative psychological interventions on health optimization by (1) evaluating the effectiveness of innovative psychological tools, i.e., serious gaming (games that contain educative as well as entertaining components), verbal suggestions, and internet-based interventions, to optimize health behavior and psychophysiological outcomes; (2) providing a concise overview of the currently existing evidence of psychological interventions in optimizing immune function in response to *in vitro* or *in vivo* immunological as well as psychophysiological challenges; and (3) incorporating several self-reporting, behavioral and psychophysiological outcome measures with (psycho)physiological stressors to evaluate the effectiveness of psychological interventions on health outcomes.

In **Chapter 2**, we investigated the effectiveness of serious gaming on food-related outcomes and physical activity. We included healthy participants and randomized them to a serious gaming condition, in which subsequently serious games were played for half an hour or a control condition, in which control games were played. The serious games were based on (1) transferring knowledge, (2) priming (i.e., a subconscious process that leads to faster recognizing of and/or reacting on a stimulus that has been observed previously) and (3) evaluative conditioning (i.e., changing the valence of a stimulus by repeatedly providing this stimulus together with other positive or negative stimuli) of health outcomes. The control condition contained games that were not health-related. Afterwards, participants were subjected to self-reported outcome measures, including a food choice task with healthy and unhealthy food products. In this food choice task, participants first had to indicate their preference for each of the healthy and unhealthy food products, and subsequently had to choose between a healthy and unhealthy food product. In addition to this task, observations of actual health behaviors were completed, in that it was observed whether participants took the stairs or the elevator to move from the first to the fifth floor, as well as observations of an actual food choice (healthy or unhealthy) to which people were subjected at the end of the experiment. We found that serious gaming during one session of half an hour could optimize self-reported food preference. However, serious gaming did not affect actual food choice or physical activity. Playing these serious games for half an hour therefore provided a first step towards health optimization, although future studies should further confirm these findings.

To optimize the effectiveness of serious gaming, we investigated whether verbal suggestions can serve as an effective add-on tool in **Chapter 3**, as verbal suggestions directed at manipulating positive outcome expectancies already showed promising results in the placebo literature. In this randomized controlled trial, we specifically focused on optimizing food outcomes. The serious games and verbal suggestions in this study were primarily based on approaching healthy food items and avoiding unhealthy food items. Healthy participants were randomized to a serious gaming condition, accompanied or not with verbal suggestions, a verbal suggestions only condition, or a gaming control condition. Afterwards, participants were subjected to a self-reported food outcome measure (with questions regarding food preference and food choice, see **Chapter 2**), an implicit association task (a test in which participants had to associate food-related words to the label 'positive' or 'negative', as well as the label 'healthy' or 'unhealthy' as fast as possible) and a bogus taste test (a test in which participants were instructed to rate food on specific characteristics, but actually their food consumption was measured). We found that serious gaming, accompanied or not with verbal suggestions, resulted in a healthier implicit food preference. However, no effects were found for actual food consumption. There was some cautious support for the add-on effectiveness of the verbal suggestions, although future research should further elaborate on this topic due to the small effect sizes. Future research should address whether verbal suggestions actually are a significant add-on tool for serious gaming, as the current findings are limited to precursors of health behaviors (e.g., food preference) instead of actual health behaviors.

To further investigate the effectiveness of verbal suggestions in the context of psychological intervention tools, we investigated whether verbal suggestions can strengthen the effects of a brief relaxation intervention in **Chapter 4**. Previous research suggested that verbal suggestions are not only able to optimize health outcomes, but also to facilitate an adaptive stress response. In our randomized controlled study, we allocated healthy participants to a relaxation condition, accompanied or not with verbal suggestions, a verbal suggestions only condition, or a control condition. Afterwards, participants were exposed to a social evaluative stressor and we subjected participants to self-reported outcomes of state anxiety and well-being, as well as psychophysiological outcomes of heart rate, skin conductance, salivary cortisol and alpha amylase. We found that a brief relaxation intervention, accompanied or not with a verbal suggestion, resulted in lower self-reported state anxiety directly after the intervention, but not in response to a social evaluative stressor. Therefore, the brief relaxation intervention, accompanied or not with verbal suggestions, provided a first step towards reducing self-reported anxiety. Future studies should focus on incorporating several kinds of verbal suggestions as well as adjusting the content of the stressor onto the content of the intervention.

Next, we summarized the existing literature concerning the effectiveness of psychological interventions in optimizing immune function. A concise overview of the current existing literature by a systematic review and meta-analysis is provided in **Chapter 5**. In this systematic review and meta-analysis, we included studies that incorporated a chemical, physical or psychophysiological challenge into the study design to evaluate the effectiveness of a stress-reducing psychological intervention. Hereby, more insights in the actual response of the immune system on a natural challenge could be gathered. Overall, we found modest support for the effectiveness of psychological interventions on immune function with most conclusive results for studies that incorporated *in vivo* immune-related challenges. However, the selected studies in this meta-analytic review were rather heterogeneous with respect to the incorporated study populations, psychological interventions, incorporated challenges and immune outcomes. Therefore, future research should carefully consider the incorporated challenge as well as the immune outcome parameters according to the included study population. This will also provide important insights for clinical practice, as it can be investigated whether a psychological intervention can possibly, at least partially, substantiate or reduce medical treatments in patients with chronic somatic conditions.

Finally, we aimed to evaluate the effectiveness of a combination of innovative psychological intervention tools on health outcomes, by including multiple chemical, physical and psychophysiological challenges into the study design, to provide an as robust as possible provocation. In **Chapter 6,** the study design is described, and in **Chapter 7,** the results are described. Participants received a 6-week e-health intervention containing internet-based cognitive behavioral therapy combined with serious gaming. Afterwards, participants were subjected to a BCG-vaccination, as well as a test day including psychophysiological stressors (i.e., a social evaluative stressor, a cognitive stressor, and a physical stressor). We subjected participants to self-reported outcomes of state anxiety and well-being. Furthermore, we incorporated immune outcomes, as well as psychophysiological outcomes of heart rate, skin conductance, and salivary cortisol and alpha amylase at baseline, directly after the intervention, at the start and end of the test day and 4 weeks later. Finally, we determined the IgG antibody responses following the BCG-vaccination at 4 weeks follow-up. Although we did not find evidence for optimized vitality after the intervention, we found that participants in the intervention condition reported lower physical sensations, and fewer sleep problems after the intervention, as compared to the control condition. We also found that self-reported 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 differential outcomes on heart rate

variables as well as increased immunoglobulin G (IgG) antibody responses at 4 weeks follow-up. In addition, differential chemokine outcomes were observed at the end of the test day in the intervention compared to the control condition, when specifically focusing on time effects for both groups separately. The intervention therefore provided a first step towards developing preventive strategies in optimizing health outcomes. Future research should include patients with somatic conditions to investigate whether the intervention can optimize health outcomes in a population at risk for health problems.

Taken together in **Chapter 8,** the findings of the present dissertation underline the potential effectiveness of innovative psychological interventions in optimizing health outcomes. We found preliminary support for the effectiveness of serious gaming, verbal suggestions and internet-based CBT on optimizing various self-reported, observational, immunological, and psychophysiological health outcomes. Incorporating multiple psychophysiological methods and challenges to evaluate the effectiveness of innovative psychological interventions on health outcomes provided more insights in the mechanisms underlying the potential effectiveness of these interventions.

Nederlandse samenvatting

In de afgelopen decennia is er steeds meer aandacht gekomen voor de relatie tussen gezondheidsstatus en psychologische processen. De resultaten van studies naar de effectiviteit van psychologische interventies in het optimaliseren van gezondheidsuitkomsten zijn veelbelovend, maar de processen die hieraan ten grondslag liggen zijn nog niet volledig opgehelderd. Bovendien is niet vaak onderzocht in hoeverre innovatieve interventie componenten toegevoegd kunnen worden aan psychologische interventies om de effectiviteit van interventies te vergroten. Het doel van het huidige proefschrift was om de effectiviteit van innovatieve psychologische interventies op gezondheidsoptimalisatie te onderzoeken door (1) de effectiviteit te evalueren van innovatieve psychologische interventie componenten, d.w.z. 'serious gaming' (spellen die zowel educatieve als entertainment componenten bevatten), verbale suggesties, en internet-gebaseerde interventies, om gezondheidsgedrag en psychofysiologische uitkomsten te optimaliseren; (2) een bondig overzicht te geven van het huidig bestaande bewijs van psychologische interventies in het optimaliseren van immuun functioneren in reactie op *in vivo* of *in vivo* immunologische evenals psychofysiologische provocaties; en (3) het includeren van verscheidene zelf-gerapporteerde, gedragsmatige, en psychofysiologische uitkomstmaten met (psycho)fysiologische provocaties, om de effectiviteit van psychologische interventies op gezondheidsuitkomsten te evalueren.

In **Hoofdstuk 2** onderzochten we de effectiviteit van 'serious gaming' op voedings-gerelateerde uitkomsten en fysieke activiteit. We includeerden gezonde participanten en randomiseerden ze naar een 'serious gaming' conditie, waarin vervolgens gedurende een half uur 'serious games' gespeeld werden of een controle conditie, waarin controle games werden gespeeld. De 'serious games' waren gebaseerd op (1) het overbrengen van kennis, (2) 'priming' (d.w.z. het onbewust sneller herkennen van en/of reageren op een stimulus als men deze al eens heeft waargenomen) en (3) evaluatief conditioneren (d.w.z. het veranderen van een oordeel van een stimulus door deze herhaaldelijk te koppelen aan een andere positieve of negatieve stimulus) van gezondheidsuitkomsten. De controle conditie bevatte spellen die niet gerelateerd waren aan gezondheid. Na afloop werden de deelnemers onderworpen aan zelf-gerapporteerde uitkomstmaten, waaronder een voedingskeuze taak met gezonde en ongezonde voedingsproducten. In de voedingskeuze taak moest men allereerst de voorkeur voor zowel een gezond als ongezond voedingsproduct aangeven. Daaropvolgend moest men kiezen tussen een gezond en ongezond voedingsproduct. Naast deze taak werden observaties van daadwerkelijke gezondheidsgedragingen uitgevoerd. Dit werd gedaan door middel van het observeren of men zich middels de trap of lift van de eerste naar de vijfde verdieping verplaatste, evenals het observeren van een daadwerkelijke voedingskeuze (gezond of

ongezond) waaraan men werd blootgesteld aan het aan het eind van het experiment. We vonden dat 'serious gaming' gedurende één sessie van een half uur zelf-gerapporteerde voedingsvoorkeur kon optimaliseren. Echter, 'serious gaming' oefende geen invloed uit op de daadwerkelijke keuze voor de trap of lift of de daadwerkelijke voedingskeuze. Het spelen van de 'serious games' gedurende een half uur leek hiermee een eerste stap in de richting van gezondheidsoptimalisatie aan te tonen, al moeten toekomstige studies dit verder bevestigen.

Om de effectiviteit van 'serious gaming' verder te optimaliseren hebben we in **Hoofdstuk 3** onderzocht in hoeverre verbale suggesties kunnen dienen als een effectieve toevoeging bovenop 'serious gaming', aangezien verbale suggesties gericht op het manipuleren van positieve uitkomst verwachtingen in de placebo literatuur al veelbelovende effecten op gezondheidsuitkomsten hebben laten zien. In dit gerandomiseerd-gecontroleerde onderzoek hebben we ons specifiek gefocust op het optimaliseren van voedingsuitkomsten. De 'serious games' en verbale suggesties in dit onderzoek waren met name gebaseerd op het benaderen van gezonde voeding en het vermijden van ongezonde voeding. Gezonde participanten werden gerandomiseerd naar een 'serious gaming' conditie, al dan niet gecombineerd met verbale suggesties, een conditie met alleen verbale suggesties, of een 'gaming' controle conditie. Na afloop werden participanten onderworpen aan een zelf-gerapporteerde voedings-uitkomstmaat (met vragen rondom voedingsvoorkeur en voedingskeuze, zie **Hoofdstuk 2**), een impliciete associatie test (een test waarin men zo snel mogelijk voeding gerelateerde woorden moest koppelen aan het label 'positief' of 'negatief', evenals het label 'gezond' of 'ongezond') en een zogenaamde smaaktest (een test waarin men werd geïnstrueerd voeding te beoordelen op bepaalde karakteristieken, maar in werkelijkheid werd gemeten hoeveel men ervan consumeerde). We vonden dat 'serious gaming', al dan niet gecombineerd met verbale suggesties, resulteerde in een gezondere impliciete voedingsvoorkeur. Echter, er werden geen effecten gevonden op daadwerkelijke voedingsconsumptie. Deze bevindingen lieten zien dat verbale suggesties mogelijk de effectiviteit van 'serious gaming' versterken, alhoewel deze eerste resultaten voorzichtig moeten worden geïnterpreteerd vanwege de kleine effecten. Nader onderzoek zal moeten uitwijzen of verbale suggesties inderdaad een significante toevoeging kunnen zijn voor 'serious gaming', aangezien de huidige bevindingen zich voornamelijk beperken tot voorlopers van gezondheidsgedragingen (zoals voedingsvoorkeur) in plaats van daadwerkelijke gedragsveranderingen.

Om de effectiviteit van verbale suggesties in de context van psychologische interventies nader te onderzoeken, hebben we in **Hoofdstuk 4** onderzocht in hoeverre verbale suggesties de effecten van relaxatie interventies kunnen versterken. Voorgaand onderzoek

heeft aangetoond dat verbale suggesties niet alleen gezondheidsuitkomsten kunnen optimaliseren, maar ook een adaptieve stressreactie teweeg kunnen brengen. In dit gerandomiseerd-gecontroleerde onderzoek hebben we gezonde participanten toegewezen aan een relaxatie conditie, al dan niet gecombineerd met verbale suggesties, een conditie met alleen verbale suggesties, of een controle conditie. Na afloop werden participanten onderworpen aan een sociaal evaluatieve stressor, waarin men een presentatie moest geven voor een jury die kritische vragen stelde en geen positieve feedback gaf, evenals een mentale rekentaak waarbij men continu negatieve feedback ontving. We vroegen participanten om op verschillende momenten hun zelf-gerapporteerde angst en welzijn op dat moment aan te geven. Bovendien hebben we de psychofysiologische uitkomsten van hartslag en huidgeleiding meegenomen, evenals speekselmetingen van cortisol en alfa amylase. We vonden dat een korte relaxatie interventie, al dan niet gecombineerd met een verbale suggestie, resulteerde in een lagere zelf-gerapporteerde angst direct na de interventie, maar niet in reactie op een sociaal evaluatieve stressor. De korte relaxatie interventie, al dan niet gecombineerd met een verbale suggestie, is een eerste stap richting het verminderen van zelf-gerapporteerde angst. Vervolgonderzoek zou zich kunnen richten op het onderzoeken van verschillende soorten verbale suggesties evenals het afstemmen van de inhoud van de stressor op de inhoud van de interventie.

Naast het onderzoeken van deze innovatieve psychologische componenten, hebben we de bestaande literatuur betreffende de effectiviteit van psychologische interventies in het optimaliseren van immuun functioneren samengevat. Een overzicht van de huidige bestaande literatuur is middels een systematische review en meta-analyse beschreven in **Hoofdstuk 5**. In deze systematische review en meta-analyse hebben we studies geïncludeerd die een chemische, fysieke en/of psychofysiologische provocatie hadden meegenomen in de studie opzet om de effectiviteit van een stress-verlagende psychologische interventie te evalueren. Dit werd gedaan om meer inzichten te kunnen verkrijgen in de daadwerkelijke reactie van het immuunsysteem op een natuurlijke provocatie. Over het algemeen vonden we matige ondersteuning voor de effectiviteit van psychologische interventies op immuun functioneren. Het meest overtuigende bewijs werd gevonden voor studies die een *in vivo* immuun-gerelateerde provocatie hadden onderzocht. Aangezien de geselecteerde studies in deze systematische review en meta-analyse in sterke mate heterogeen waren betreffende de onderzochte studie populaties, psychologische interventies, geteste provocaties en gemeten immuun uitkomstmaten, wordt aanbevolen dat toekomstig onderzoek nauwkeuriger de geïncludeerde provocatie en immuun uitkomst parameters dient te bepalen en meten. Daarnaast moeten deze worden afgezet tegen de geïncludeerde studie populatie. Dit biedt ook belangrijke inzichten voor de klinische praktijk, aangezien kan worden onderzocht in hoeverre een psychologische interventie mogelijk, tenminste

gedeeltelijk, medicamenteuze behandelingen kan vervangen of verlagen bij patiënten met somatische aandoeningen.

Ten slotte was het doel om de effectiviteit van een combinatie van innovatieve interventie componenten te evalueren op gezondheidsuitkomsten, door het includeren van meerdere chemische, fysieke en psychofysiologische provocaties, om een zo robuust mogelijke provocatie te bieden. De studie opzet van het gerandomiseerd-gecontroleerde onderzoek is in **Hoofdstuk 6** beschreven, en de bevindingen van deze studie zijn in **Hoofdstuk 7** beschreven. Participanten ontvingen een 6-weken durende 'eHealth' interventie gebaseerd op cognitieve gedragstherapie welke via het internet werd aangeboden en welke werd gecombineerd met 'serious gaming' elementen (interventie conditie) of geen interventie gedurende 6 weken (controle conditie). Na afloop werden participanten onderworpen aan een BCG-vaccinatie, evenals een testdag met psychofysiologische stressoren (d.w.z., een sociaal evaluatieve stressor, een cognitieve stressor en een fysieke stressor). We vroegen participanten om op verschillende momenten zelf-gerapporteerde angst en welzijn te beoordelen. Bovendien hebben we immunologische uitkomstmaten meegenomen, evenals psychofysiologische uitkomsten van hartslag, huidgeleiding en speekselmetingen van cortisol en alfa amylase bij aanvang, direct na de interventie, aan het begin en eind van de testdag en 4 weken na de interventie. Ten slotte hebben we 4 weken na de interventie het gehalte aan immunoglobuline G (IgG) antilichaam reacties naar aanleiding van de BCG-vaccinatie bepaald. Alhoewel we geen bewijs vonden voor geoptimaliseerde vitaliteit na de interventie, vonden we wel dat participanten in de interventie conditie minder fysieke sensaties rapporteerden en minder slaapproblemen ondervonden na de interventie, vergeleken met de controle conditie. We vonden tevens dat zelf-gerapporteerde welzijn hoger was in de interventie conditie na de psychofysiologische provocaties. Alhoewel geen significante groepsverschillen gevonden werden voor de psychofysiologische en immunologische uitkomsten boden de data wel voorlopige steun voor differentiële uitkomsten op hartslag variabelen, evenals verhoogde IgG antilichaam reacties na 4 weken. Daarnaast werden, wanneer specifiek werd gekeken naar effecten over de tijd voor beide groepen apart, differentiële chemokine responsen gezien aan het eind van de testdag in de interventie vergeleken met de controle conditie. De interventie had een gunstig effect op verschillende gezondheidsuitkomsten en biedt hiermee een uitgangspunt voor het ontwikkelen van preventieve strategieën gericht op het optimaliseren van gezondheid. Toekomstig onderzoek zou patiënten met somatische aandoeningen moeten includeren om te evalueren in hoeverre de interventie gezondheidsuitkomsten kan optimaliseren in een risicopopulatie met gezondheidsproblemen.

De bevindingen van het huidige proefschrift worden in **Hoofdstuk 8** samengevat en onderstrepen de potentiele effectiviteit van innovatieve psychologische interventies in het optimaliseren van gezondheidsuitkomsten. We vonden beperkte steun voor de effectiviteit van 'serious gaming', verbale suggesties en internet-gebaseerde cognitieve gedragstherapie op het optimaliseren van verscheidene zelf-gerapporteerde, observationele, immunologische en psychofysiologische gezondheidsuitkomsten. Het gebruik van verscheidene psychofysiologische meetmethoden en provocaties om de effectiviteit van innovatieve psychologische interventies op gezondheidsuitkomsten te onderzoeken gaf meer inzichten in de mechanismen onderliggend aan de potentiële effectiviteit van deze interventies.

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Curriculum Vitae

Lemmy Schakel was born on January 31, 1992 in Gorinchem, the Netherlands. In 2010, she completed secondary school education at Lyceum de Oude Hoven in Gorinchem. After graduation, Lemmy started the Bachelor program Psychology at Leiden University and earned her bachelor's degree in 2013. Next, she started the Master program Medical Psychology at Tilburg University and earned her master's degree cum laude in 2015. From September 2015, Lemmy worked as a Ph.D. candidate in the department of Health, Medical and Neuropsychology at Leiden University. During her Ph.D., she supervised a multitude of students working on their bachelor project and master thesis. From December 2018 to February 2019, she also worked as a postdoc researcher in the department of Health, Medical and Neuropsychology at Leiden University in the Health and Well-being track of the Digital Society. From April 2018 to current, Lemmy works as a psychologist in a local care institution (Rivas Zorggroep).

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