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Abstract

Individuals can be sensitized to the reinforcing effects of exercise, although it is unknown if this process increases habitual exercise behavior. Sedentary men and women (BMI: 25-35 kg/m^2, N=52), participated in a 12-week aerobic exercise intervention. Exercise reinforcement was determined by how much work was performed for exercise relative to a sedentary alternative in a progressive ratio schedule task. Habitual physical activity was assessed via accelerometry. Post-intervention increases in exercise reinforcement predicted increases in physical activity bouts among those who expended over 2,000 kcal per week in exercise and who compensated for less than 50% of their exercise energy expenditure.

Keywords: Exercise Reinforcement, Incentive Sensitization, MVPA bouts, Weight loss

Introduction

The reinforcing value of exercise refers to one's motivational drive to consistently engage in exercise (Flack et al., 2017b; Flack et al., 2017a). Cross-sectional work has demonstrated that adults who find aerobic exercise highly reinforcing are more likely to meet physical activity (PA) guidelines for vigorous physical activity (VPA) while those who find resistance-type exercise more reinforcing are more likely to meet recommendations for muscular-strengthening activities and VPA (Flack et al., 2017a). The reinforcing value of exercise is also a far greater predictor of habitual physical activity than liking (Flack et al., 2017b), operating on different neurobiological pathways with liking determined more by the central opioid system whereas reinforcement is controlled by central dopamine (Berridge and Robinson, 2003; Berridge and Robinson, 1998; Robinson et al., 2015; Ekkekakis et al., 2011).

Increasing the reinforcing value of exercise among sedentary individuals has great potential for promoting the long-term adoption of exercise behaviors and thus the health of many Americans. Recent evidence points to the process of incentive sensitization, originally used to explain drug addiction, also applying to exercise. Incentive sensitization refers to sensitizing an individual to a reinforcing stimulus after repeated exposures, specifically transforming the perception of stimuli, imbuing them with salience and making them attractive, 'wanted', incentive stimuli (Robinson and Berridge, 1993). This is a prime component of the dopamine hypothesis of reward, well known to be implicated in motivating behaviors such as gambling, eating, and drug abuse (Spanagel and Weiss, 1999). Recent work from our lab has demonstrated single nucleotide polymorphisms (SNPs) important for dopamine signaling and transport previously linked to drug abuse, also to be predictors of exercise reinforcement, tolerance for exercise intensity, and habitual physical activity (Flack et al., 2019a; Robinson et al., 2015).

Using genetic knock-out models, others have demonstrated dopamine transporter and receptor expression to influence physical activity behaviors (Bronikowski et al., 2004; Rhodes and Garland, 2003). This offers an explanation as to why exercise dependency has been demonstrated in both humans (Chan and Grossman, 1988; Chapman and De Castro, 1990; Holden, 2001; Belke, 1997) and rodents (Belke, 1997; Belke, 2000; Iversen, 1993; Lett et al., 2000), with the notion that central dopamine is playing a major role in the choice to be physically active, in line with the dopamine hypothesis of reward (Knab and Lightfoot, 2010).

We have previously demonstrated a high-dose exercise intervention to be effective at increasing exercise reinforcement (five days per week, 600 kcal per session) (Flack, 2019b), while low-dose interventions (three days per week at 150 or 300 kcal per session) are effective at decreasing sedentary behavior reinforcement, but not capable of instilling incentive sensitization for exercise reinforcement (Flack et al., 2019b). The development of sensitization of drug abuse can be dose-dependent (Liu et al., 2005), and if drug abuse and exercise follow similar patterns (i.e. dopamine-mediated reinforcement), we would expect greater doses of exercise to be required in order to instill incentive sensitization. There are still questions regarding the best way to modify the dose of exercise (frequency of sessions, energy expended per session, exercise intensity), and we have yet to demonstrate physiological or behavioral benefits to increasing exercise reinforcement. The current study fills some of this void by using pre-post change in exercise reinforcement to predict changes in physical activity behavior post-intervention, which influences energy compensation to an exercise program and thus weight-loss success. The present investigation's hypothesis was that more frequent but shorter exercise sessions would produce greater increases in exercise reinforcement, compared to less frequent but longer sessions that produce greater energy expenditures per session but lower total expenditure over an entire 12-week intervention. This increase in exercise reinforcement was hypothesized to serve as an independent predictor in the increase in physical activity behaviors post-intervention. As a secondary analysis and hypothesis, we assessed the compensatory response to the exercise intervention, that is, the difference in expected weight loss (based on energy expended) and actual fat and lean mass loss converted to kcal equivalents. For instance, if a participant exercised to expend 30,000 kcal during the intervention but only lost 15,000 kcal, they would have compensated 15,000 kcal, or 50% of their energy expended. Although we did not determine the source of this compensatory response, one possibility is individuals become less active when engaging in exercise, reducing their non-exercise physical activity as a compensatory mechanism (King, 2007). We hypothesized individuals who increase their reinforcing value of exercise would compensate less, possibly by increasing habitual physical activity to increase energy expenditure.

Materials and Methods

Participants

A total of 80 participants aged 18 to 49 years volunteered and were enrolled into the study. Of these 52 completed all baseline tests and were randomized into one of three groups during this longitudinal, randomized, controlled trial. Of these 52 randomized participants, 44 completed the study (32 female), with six (four female) withdrawing for personal reasons and two females being excluded for non-compliance (did not complete the required 85% of exercise sessions assigned per month). A consort diagram is depicted in Figure 1. All participants had a body mass index (BMI) ranging from 25-35 kg/m^2 and were inactive (not engaging in any form of exercise), determined during screening where participants were asked of their exercise behaviors and validated by accelerometry (baseline participant characteristics are presented in Table 1). Participants were also non-smoking and free of any health conditions that may preclude them from exercise (metabolic or heart disease, cancer). Recruitment began in the winter of 2018 and continued until recruitment goals were met (spring of 2019) in and around Lexington, Kentucky. Participants were a sample who responded to recruitment media including printed brochures and flyers and online advertisements placed on University of Kentucky's Center for Clinical and Translational Science (CCTS) website. This study was approved by the University of Kentucky Institutional Review Board. The present analysis is a secondary outcome of a trial aimed at assessing mechanisms of energy compensation at different doses of exercise ClinicalTrials.gov identifier: NCT03413826, currently in review.

Procedures

During the initial screening and consenting visit, participants provided their written informed consent and were screened of eligibility criteria, completing a physical activity readiness questionnaire (PARQ), health history questionnaire, and screened on their dieting, weight loss history, and physical activity behaviors. Participants were provided an ActiGraph Accelerometer (Pensacola, Fla) to wear for the following seven days to objectively assess physical activity prior to completing baseline testing. Participants also completed the Preference for and Tolerance of the Intensity of Exercise Questionnaire (PRETIE-Q) (Ekkekakis et al., 2008; Ekkekakis et al., 2005). Subsequent visits included assessments for exercise liking and reinforcement, rate of energy expenditure during exercise, and body composition (all detailed below).

Study Design

The study was a randomized, controlled trial that included a 12-week exercise intervention of either six sessions (days) per week, two sessions per week, or a sedentary control group (no exercise) blocked on gender. The study statistician generated and maintained the concealed allocation sequence. Participants were randomized upon completion of all baseline assessments with no blinding of intervention assignments. Participants were assessed for outcome measures at baseline and immediately after the intervention. Exercise reinforcement, preference and tolerance for exercise intensity, and body composition were assessed 24 to 48 hours after the participant completed their final exercise session of the 12-week intervention. Seven-day physical activity was assessed prior to beginning baseline assessments and after participants completed all other post-testing assessments. Participants were instructed not to begin a new exercise program during baseline assessments. In the 24-48 hours after the exercise intervention was completed and post-testing assessments for exercise reinforcement, preference and tolerance, and body composition were being performed, participants were instructed not to exercise. Participants were allowed, however, to exercise as they wished during the following 7-days while wearing the accelerometer to assess physical activity post intervention as we were primarily interested if they increased their exercise behaviors once the intervention ceased.

Exercise Intervention

Participants were provided a Polar A-300 heart rate monitor (watch and chest strap, Kempele, Finland) for the duration of the 12-week intervention and instructed to perform aerobic exercise (treadmill, bicycle, or elliptical ergometer) either two or six times per week on their own and were provided access to a fitness center. Participants in the control group were instructed to remain sedentary and return for post-testing 12 weeks later, receiving the exercise intervention after post-testing if they desired. Those in the exercise groups returned to the lab weekly to meet a researcher and download their exercise sessions using the PolarFlow software, which allowed research staff to monitor and track compliance. If a participant was not at least 85% compliant (completed 85% of expected exercise sessions per month) they were removed from the study. The downloaded exercise session reports provided the amount of time spent in each heart rate zone, which allowed for the calculation of total energy expended during each exercise session based off individual rates of energy expenditure averaged across each heart rate zone calculated from the graded exercise test with indirect calorimetry performed at baseline and week six. Participants in the two-day per week group were instructed to perform two long exercise sessions per week and encouraged to try to expend 1,000 kcal per session. Participants in the six-day per week group were instructed to keep their sessions to 400 kcal per session and averaged just over 53 minutes per session. Although most participants in the two-day per week group were not able to attain the 1,000 kcal goal, they still expended significantly greater kcal per session compared to the six-day group and spent on average 94.5 minutes per session. Participants received personalized heart-rate based exercise prescriptions that, if followed, would result in them expending the assigned energy per exercise session. Participants were also provided feedback each week on their energy expenditure of each session of the prior week so they could tailor future exercise sessions. All participants were instructed not to purposely change dietary habits during the intervention, i.e., not begin an energy-restricted diet.

Assessments

Physical activity

Habitual, free-living physical activity was measured using an ActiGraph accelerometer (GT3X+ model; Pensacola, Florida). Each participant wore the device for seven days prior to baseline testing and immediately after completing all other post testing assessments. Participants were instructed to wear the monitor at the hip using the provided belt during all hours awake except when bathing or swimming. Data were cleaned of non-wear time, defined as consecutive strings of zeros greater than 20 minutes. An epoch of 10 seconds was used for data collection as a shorter epoch is more suitable to reflect bout duration under free-living conditions of sedentary individuals where many bouts of sporadic activity last 30 seconds or less (Ayabe et al., 2013; Gabriel et al., 2010). These data were used to determine participants' weekly minutes of moderate to vigorous physical activity (MVPA), number of MVPA bouts, vigorous intensity physical activity (VPA) and sedentary activity using the Crouter et.al algorithm (Crouter et al., 2010), and Freedson cut-points (Freedson et al., 1998).

Liking of Exercise

Participants' liking (hedonic value) of the exercise options (treadmill, elliptical, stationary bike) and sedentary alternatives (computer games, reading, puzzles/Sudoku) was assessed using a 100-point scale (1 = "do not like at all" and 100 = "like very much"). The most liked activity was used for the exercise reinforcement testing session.

Exercise Reinforcement

Exercise reinforcement (specifically, aerobic-type exercise, treadmill, elliptical, or bicycle ergometer) was assessed against a sedentary alternative (playing computer games, reading magazines, doing crossword puzzles, Sudoku). Exercise reinforcement is assessed by evaluating the amount of operant responding (mouse button presses) a participant is willing to complete to gain access to exercise (Bickel et al., 2000; Epstein et al., 2011). The testing space includes two workstations. One station is a computer and mouse on which the participant can earn points towards their most liked exercise activity while the other station is a computer that can be used to earn points toward their most liked sedentary alternative. Participants can switch between stations as much as they choose. The program presents a game that mimics a slot machine; a point is earned each time the shapes match. For every five points, a session is completed, and the participant receives five minutes of access to the reinforcer that was earned (either exercise or sedentary activity). The game is performed until the participant no longer wishes to work for access to either the exercise or sedentary activities. At first, points are delivered after every four presses, but then the schedule of reinforcement doubles (4, 8, 16, 32, […] 1024) each time five points are earned. For instance, the participant initially has to click the mouse button four times to earn each point for Schedule 1. After the first five points are earned, Schedule 1 is complete, and the participant earns five minutes for exercise. Then eight clicks are required to earn each of the next five points for Schedule 2 before another five minutes of exercise is earned. Schedule 3 would require 16 clicks to earn one point, Schedule 4 would require 32 clicks to earn one point, and so on (Epstein et al., 2011; Bickel et al., 2000). Participants engage in the activity for the time earned after they complete the game, which ends when the participant no longer wishes to earn points (time) for exercise or the sedentary alternative. In essence, the more reinforcing exercise or the sedentary behavior is, the more operant responding participants will do for access to these behaviors. Similar button pressing tasks are valid predictors of the reinforcing value of physical versus sedentary activity and for determining the reinforcing value of food (Barkley et al., 2009; Epstein et al., 1999; Epstein et al., 2007). Participants self-selected the intensity level when performing any earned exercise time, which was typically a low to moderate steady-state intensity. These assessments took place in a laboratory space adjacent to the Human Performance Laboratory on the University of

Kentucky campus, equipped with exercise equipment available for the participant to engage the exercise they had earned during the task. The reinforcing value of exercise and sedentary activity was conceptualized as the number of clicks required to earn each point of the last schedule completed (i.e., 4, 8, 16, 32…) for exercise and the sedentary alternative, respectively, each assessed separately and often referred to as Pmax (Scheid et al., 2014; Bickel et al., 2000).

Rate of Energy expenditure

A graded exercise treadmill test was used to determine each participant's rate of energy expenditure at five different heart-rate zones. Oxygen consumed and CO_2 produced were analyzed by indirect calorimetry (VMAX Encore Metabolic Cart, Vyaire Medical, Mettawa, IL) which included an integrated 12 lead ECG for monitoring heart rate and used in conjunction with the Trackmaster TMX428 Metabolic cart interfaced treadmill. Upon completion of a five-minute warm-up walking at 0% grade, 3.0 mph, the treadmill grade increased to 2.5% for three minutes. The treadmill grade was then increased every three minutes to produce an approximately 10 beat per minute increase in heart rate from the previous stage with the speed fixed at 3.0 mph. The test continued until a heart rate of 85% HRR was attained or the participant felt they could no longer continue. Energy expenditure (kcal per minute) was determined from the amount of oxygen consumed and CO_2 expired using the Weir equation (Weir, 1949). The average rate of energy expenditure during the last 30 seconds of each stage of the graded exercise test was regressed against the heart rate averaged over the last 30 seconds of the corresponding stage to calculate the rate of energy expenditure at different heart rates. Heart rate zones were calculated using the heart rate reserve (HRR) formula as (220-age)-resting HR * zone % + resting HR (Swain et al., 1998). Heart rate Zone 1 ranged from 0% to 25% HRR, Zone 2 corresponded to 26-40% HRR, Zone 3 was 41-58% HRR, Zone 4 was 59-75% HRR, and Zone 5 was 76-90%.

Energy expenditure in kcal/min was then averaged across each heart rate zone for determination of energy expenditure per minute for each zone. This test was completed at baseline and week 6 to recalculate rates of energy expenditure to account for improvements in fitness.

Body composition

Body composition was measured using a GE Lunar iDXA machine prior to the exercise test. The iDXA technique allows the non-invasive assessment of soft tissue composition by region with a precision of 1-3% (Rothney et al., 2012). A total body scan was conducted with participants lying supine on the table and arms positioned to the side. Most scans were completed using the thick mode suggested by the software as participants were overweight to obese. All scans were analyzed using GE Lunar enCORE Software (13.60.033). Automatic edge detection was used for scan analyses. The machine was calibrated before each scanning session, using the GE Lunar calibration phantom. Outcome measures included total body weight, fat-free mass (FFM), and fat mass (FM).

Energy Compensation

To calculate compensation for the energy expended during the exercise program (ExEE), the accumulated energy balance (AEB) was calculated from changes in FM and FFM upon completion of the study as body composition changes reflect long-term alterations in energy balance (Rosenkilde et al., 2012). Gains of 1kg FM or 1kg FFM were assumed to reflect 12,000 kcal and 1,780 kcal, respectively (Elia et al., 2003). Losses of 1kg FM or 1kg FFM were assumed to equal 9,417 and 884 kcal, respectively (Forbes, 1990). ExEE was calculated from the training-induced energy expenditure in kcal/session with the addition of 15% excess post-exercise energy expenditure (Bahr et al., 1987). The resting energy expenditure (REE) that would have occurred during the exercise sessions (REE x 1.2) was subtracted. Thus, ExEE = (TrEE x 0.15) + (TrEE – training duration x (REE x 1.2)) (Rosenkilde et al., 2012). The overall compensatory response to the increase in ExEE was assessed as described by Rosenkilde (Rosenkilde et al., 2012), with % kcal compensated calculated as (ExEE + AEB)/ExEE x 100%. A 0% kcal compensated occurs when AEB equals -ExEE, or changes in the energy equivalent of fat mass and fat-free mass equal energy expended during exercise. Positive compensation suggests that changes in body composition indicate a less negative energy balance than expected based on ExEE, whereas negative compensation indicates a greater than expected negative energy balance. ExEE, AEB, and % kcal compensated could be calculated only for those participants who completed the study as both a pre- and post-treatment data points were needed to calculate these variables.

Preference and tolerance for exercise intensity

The Preference for and Tolerance of the Intensity of Exercise Questionnaire (PRETIE-Q) (Ekkekakis et al., 2008; Ekkekakis et al., 2005) assesses how much a person tolerates and/or prefers the discomfort associated with intense exercise (Lind et al., 2005; Gulati et al., 2005; Ekkekakis et al., 2005). This was assessed by questionnaire during the initial screening and consenting visit and during the final follow up visit separate from any bout of exercise. Preference and tolerance scores are associated with the frequency of participation in strenuous exercise and total leisure-time exercise (Ekkekakis et al., 2008), a strong predictor of PA behavior (Flack et al., 2017a), and have been implicated in the process of incentive sensitization for exercise reinforcement (Flack et al., 2019a; Flack et al., 2019b).

Analytic Plan

Baseline participant characteristics were assessed via 1-way ANOVA between groups exercising six- and two-days per week and sedentary control. Differences in the pre-post changes in exercise reinforcement, seven-day MVPA bouts, sedentary reinforcement and changes in body fat were tested between groups and if changes were different from zero using analysis of covariance with the corresponding baseline value as the covariate. Between- group analyses were performed on randomized groups (exercise six-days per week, 2-days per week, or control) in addition to retrospectively split groups on exercise energy expenditure (expending greater than 2,000 kcal per week, less than 2,000 kcal per week, or control), and compensation groups (compensating for greater than 50% of their kcal expended during the exercise intervention, less than 50%, or control). Linear regression analyses were used to predict changes in MVPA bouts, as this was the variable we hypothesized to be effected by our exercise intervention, with specific hypotheses on the relationship between changes in exercise reinforcement and changes in MVPA bouts. Therefore, changes in exercise reinforcement was our primary predictor of interest, with other variables that were differently affected by the exercise intervention (energy expended per week through exercise, percent changes in FM, percent kcal compensated for during the exercise intervention, changes in sedentary behavior reinforcement, and liking of exercise and sedentary activities) also entered as independent variables. Additional separate regression analyses were performed on retrospectivity assigned groups. The choice to split groups on exercise energy expenditure above and below 2,000 kcal and compensation groups above and below 50% was based on weekly energy expenditures per week averaging 2,041.7 kcal and % kcal compensated averaging 50.25. All analyses were performed in IBM SPSS Version 26 (IBM corporation, Armonk, New York). ***Power Analysis:*** Our recent study (Flack, 2019b) demonstrated significant increases in exercise reinforcement after 12-weeks of high dose exercise (five sessions per week, 600 kcal expended per session). Using an 80% power and 95% confidence level, 15 participants per group were needed to detect a significant change in exercise reinforcement (Pmax) from baseline to post intervention.

Results

Baseline characteristics are presented in Table 1, with differences in sedentary behavior reinforcement between all groups, body fat percentage between six-day per week and two-day per week groups, and differences in MVPA bouts between control and two-day per week group. Because of these differences, pre to post change scores were calculated and analysis of covariance was used to determine differences between groups while controlling for the corresponding baseline value. Table 1 also indicates participants were meeting MVPA recommendations (150 minutes per week) despite reporting not engaging in any form of exercise (defined as leisure-time physical activity performed with the goal of increasing fitness and/or losing weight). We believe this is due to most participants accumulating shorter, spontaneous bouts of walking through the day traveling across a sprawling university campus and not indicative of actual exercise. This is supported by the finding that all groups were far below the recommendations for VPA (75 minutes per week). We therefore chose to use MVPA bouts as the primary outcome variable, which would include lower-intensity exercise but only if performed for 10 or more minutes at a time, more indicative of purposeful exercise and in line with current recommendations that exercise sessions should last at least 10 minutes (Piercy and Troiano, 2018).

The mean $\pm$ SE kcal/session for participants in the two day per week group was 745.33 $\pm$ 61.04, while the six-day per week group expended 460.37 $\pm$ 26.04 kcal per session, mean $\pm$ SE, which was different ($P<0.01$) between groups as expected. This equates to 2,762.24 $\pm$ 156.23

kcal per week for the six-day group and 1490.66 ± 122.07 kcal per week in the two-day group, means ± SE. Further information on the exercise intervention outcomes have been reported previously (Flack, 2019a). Table 2 presents the change scores between exercise frequency groups (randomized group), and between retroactively assigned groups based on amount of kcal expended per week during the exercise intervention (over 2,000 kcal vs. under 2,000 kcal) and on the percent of kcal compensated for (over 50% vs. under 50%). There were no differences in the change in exercise reinforcement between any groups or across time, although adjusted differences between six- and two-day groups approached significance (P=0.06). Changes in MVPA bouts were greater in both the six-day and two-day groups compared to control (P<0.01), whereas the control group was the only group who observed significant changes over time, decreasing number of MVPA bouts. Adjusted change in MVPA bouts between groups split on energy expenditure per week (above or below 2,000 kcal per week) were also different from control, while groups split on energy compensation (greater or less than 50% of energy expended during the exercise intervention) were different between each other and between control. Adjusted changes in FM percentage were different when comparing the control group to those exercising either six or two days per week, or above or below 2,000 kcal per week. The six-day per week group, those exercising over 2,000 kcal per week, and those compensating less than 50% of their kcal lost significant FM (change different from zero). All compensation groups were different from each other in FM percent change (P<0.05). Neither the preference for or tolerance of the intensity of exercise (assessed by the Preference for and Tolerance of the Intensity of Exercise Questionnaire, PRETIE-Q) were different between groups at baseline, did not change as a result of the exercise intervention, and did not change differently between any groups. Exercise intensity did not differ between groups, with the 2-day per week group and 6 day per week group spending 52.3 and 47.7% of their time in heart rate zones 3-5, respectively.

Linear regression results are presented in Tables 3-5 predicting changes in MVPA bouts. Changes in exercise reinforcement and % kcal compensated were both independent predictors of changes in MVPA bouts, with greater increases in exercise reinforcement and less energy compensation predicting greater increases in MVPA bouts. Table 4 regression analysis only includes participants expending greater than 2,000 kcal per week (n=16) as when analyzing those expending less than 2,000 kcal per week (n=16) there were no significant predictors of changes in MVPA bouts. Table 5 regression analysis includes only those compensating for less than 50% of the kcal expended during exercise (non-compensators, N=13) as no significant relationships were found for those compensating greater than 50% of the kcal expended during the intervention (N=19). These analyses demonstrate that among all participants, changes in exercise reinforcement predict changes in MVPA bouts when controlling for all relevant variables including energy expended during the exercise intervention, changes in FM, % kcal compensated for, sedentary behavior reinforcement and liking of exercise and sedentary behaviors. Percent kcal compensated and changes in exercise reinforcement remained significant independent predictors of changes in MVPA bouts when analyzed separately from non-significant variables. Changes in exercise reinforcement only predicted changes in MVPA bouts among those expending greater than 2,000 kcal per week during exercise during the intervention and among those who compensated for less than 50% of their kcal expended. An additional regression analysis predicting changes in FM is presented in Table 6, indicating change in MVPA bouts is a significant predictor of FM change when controlling for energy expended during exercise.

Mediation analysis were conducted to test if changes in MVPA bouts mediated changes in exercise reinforcement or amount of weekly energy expended per week may have mediated changes in body fat. There were no significant mediation effects (P>0.05).

Sensitivity analysis was conducted removing males from the analysis (n=12). There was no difference in the overall results, indicating gender was not a confounding variable.

Discussion

There has been a wealth of research centered on behavioral reinforcement as an important component in the participation of certain, reinforcing, behaviors, positing the central dopamine system provides the physiological basis for realizing their reinforcing value (Berridge and Robinson, 1998; Robinson and Berridge, 1993). Recent and current research has focused on drug abuse, nicotine use, gambling, and eating energy dense foods as reinforcing behaviors all operating under the dopamine hypothesis of reward (Berridge and Robinson, 2003; Epstein et al., 2011; Epstein et al., 2007; Liu et al., 2005; Rhodes and Garland, 2003; Robinson et al., 2015; Robinson and Berridge, 1993; Spanagel and Weiss, 1999). These behaviors are all common in that their engagement is not advantageous for one's health (mental or physical), with many researching how we can improve these behaviors by understanding the underling physiological process implicated in their development, with one theory being incentive sensitization. One behavior that is starting to receive greater attention in the context of behavioral reinforcement is exercise, with early work investigating the reinforcing value of active play in children (Barkley et al., 2009; Epstein et al., 1999) and more recent cross-sectional analyses pointing to the reinforcing value of exercise being an important predictor of exercise behavior among adults (Flack et al., 2017a; Flack et al., 2017b). In contrast to the other reinforcing behaviors more traditionally researched, engaging in consistent exercise is beneficial for one's health, making incentive sensitization for exercise an advantageous process. Therefore, we and others have taken an interest in trying to understand ways to induce incentive sensitization for exercise with the goal of increasing physical activity behaviors which would, theoretically, improve health. We have recently demonstrated greater doses of exercise are needed to instigate this process, possibly because a high-dose exercise program can increase the tolerance for exercise intensity to allow it to become a reinforcing behavior (Flack et al., 2019a; Flack et al., 2019b). Specifically, expending 3,000 kcal per week (five sessions/week, 600 kcal per session) increased the reinforcing value of exercise, while exercising to expend 1,500 (five sessions/week 300 kcal per session) did not (Flack, 2019b). These results support an earlier investigation where low doses of exercise (450 or 900 kcal per week) were effective at reducing the reinforcing value of sedentary behaviors but did not increase exercise reinforcement (Flack et al., 2019b). Results of the current investigation are parallel with these findings, as among those in the six day per week group (2,762 kcal expended per week) the increase in exercise reinforcement approached significance (P>0.06) with change scores greater than 30-fold of that compared to the control and those exercising twice per week (1,491 kcal expended per week). The lack of statistical significance despite what appears to be clinically significant differences could be due to unexplained variability among participants, potentially related to genetic polymorphisms in the central dopamine system that have been demonstrated to influence exercise reinforcement (Flack et al., 2019a). The current study did not observe any changes in preference or tolerance for exercise intensity. Since tolerance for exercise intensity appears to be an important player in the process of incentive sensitization for exercise (Flack et al., 2017a; Flack et al., 2019b), the lack of improvements in tolerance may be another reason why improvements in exercise reinforcement did not reach significant levels. Although only speculative, this may be related to the intensity of exercise individuals self-selected, with six-day and two-day groups not differing in time spent in HRR zones 3-5 or 1-2. It is possible that greater intensities are needed to produce tolerance for exercise intensity and improve exercise reinforcement. Research is under way to investigate how high-intensity exercise may work to develop tolerance and and how this may influence incentive sensitization for exercise reinforcement.

Despite the lack of significant changes in exercise reinforcement, this investigation, for the first time, uncovered important implications for increasing exercise reinforcement. These findings support our hypothesis that increasing exercise reinforcement increases exercise behaviors and further justifies future research in this area. We chose to assess MVPA bouts (Freedson cutoff, $\geq$ 10 consecutive minutes of moderate to vigorous intensity) instead of total minutes of MVPA as many of the participants in the study were college students or employees who were obligated to walk sporadically between classes on the college campus, therefore accumulating many bouts of walking less than 10 minutes in duration while not engaging in any structured exercise. Increasing MVPA bouts would therefore be more indicative of increasing purposeful exercise, the goal of our intervention. It is important to note that participants' seven-day assessment of MVPA bouts at post testing were performed between one and two weeks after completion of the exercise intervention as other assessments were performed immediately upon completion. This time between the end of the intervention and habitual activity assessment may have provided the needed break from forced exercise and allowed the process of incentive sensitization to take effect, creating a craving/wanting for exercise, which occurred in spite of participants not told to exercise nor given a fitness center pass as their pass was only valid for the 12-week intervention. In this light, it may have been advantageous to wait a week to perform the post-testing exercise reinforcement task. We also do not know how long lasting the exercise intervention effects were, that is, if these exercise behaviors remained increased several months after the intervention ceased, creating permanent behavior change. Future studies may investigate these issues with multiple post-testing assessments of exercise reinforcement and physical activity, including long-term follow-up assessments.

An additional outcome analyzed in the present investigation centered on changes in percent FM (body fat change in kg/baseline body fat kg). Weight loss, specifically body fat loss, is a prime reason individuals partake in exercise and thus a relevant variable to assess in any exercise intervention (Obert et al., 2017). Indeed, we demonstrated significant decreases in body fat in the six-day per week group and those expending greater than 2,000 kcal per week, slight, but not significant, decreases in the two-day per week group and those expending fewer than 2,000 kcal per week, and non-significant increases in body fat in the control group. This indicates the greater energy expenditures of the six-day per week group and the greater than 2,000 kcal group are needed to sustain the negative energy balance needed for weight loss. When energy expenditure is controlled for, however, one's level of energy compensation determines weight loss success with exercise. Individuals compensating for fewer of the kcal they expended during the exercise intervention are, by definition, in a greater energy deficit compared to individuals who have a greater compensatory response. In the present study, the average % of kcal compensated for was 50.25%, in line with our previous work (Flack et al., 2018). Those who compensated greater than 50% of their kcal were deemed "compensators" and did not display the relationship between changes in exercise reinforcement and changes in MVPA bouts. This is in contrast to the "non-compensators" who were more successful at weight loss and whose changes in exercise reinforcement predicted changes in MVPA bouts. Furthermore, changes MVPA bouts predicted changes in percent FM when controlling for energy expended during exercise. It therefore appears individuals who are less prone to compensate for the energy they expend during exercise realize the reinforcing effects of exercise and increase their exercise behavior, aiding in weight loss. Similar findings have been demonstrated previously, where increases in non-exercise physical activity were associated with lower energy compensation during a high intensity exercise intervention (Schubert et al., 2017). Alternatively, increasing exercise reinforcement could be an effect of successful weight loss with exercise, where improvements in health, well-being, and appearance could feedback to increase exercise reinforcement and increase physical activity. Knowing these two features are inter-related (health physiology and behavioral physiology) is an additionally important finding future research may build upon.

This study is not without limitations. A more robust design may have been to match groups (two-day and six-day) on weekly exercise energy expenditure, to control for some of the variability in the session/week group analysis. The average energy expenditure was just over 2,000 kcal per week, with previous research indicating 1,500 kcal per week to be ineffective at inducing incentive sensitization for exercise reinforcement while 3,000 kcal per week to be effective (Flack, 2019b). Thus, it is possible that weekly energy expenditures of the present study were not great enough for incentive sensitization to take place, although levels approached significance with expenditures of 2,762 kcal per week. If participants exercise at a greater energy expenditure per week, it is likely improvements in exercise reinforcement and potentially greater improvements in MVPA bouts would have resulted. It is also possible that when using greater exercise energy expenditures, mediation analysis between group, exercise reinforcement, and MVPA bouts would have been more fruitful. The analysis also included mostly female, all between the ages of 18 and 40. It is not known if older populations would experience a different effect or of any potential gender effects in play. Additionally, stage of menstrual cycle was not accounted for among female participants, which may have influenced the calculated ExEE during the 12-week intervention. The unsupervised nature of the exercise program may also be considered a limitation as participants could have exercised for additional time while not recording it (did not start watch), although we have no reason to believe this occurred. Finally, calculating energy expenditures averaged across heart rate zones based on the HRR formula may not have been as precise as conducing a maximal exercise test and assigning exercise zones based off of VO_2 max.

Conclusions and Future Directions

Research on increasing exercise reinforcement remains in its infancy, with more questions than answers at this point. The present study provides evidence that physical activity behaviors can be increased as a result of increasing exercise reinforcement while further defining parameters that appear necessary for incentive sensitization to take place. It seems that exercising twice weekly, even when energy expenditures average greater than 740 kcal/session, is inadequate to improve exercise reinforcement and thus exercise behaviors. When exercise is performed six times per week (460 kcal per session) improvements in exercise reinforcement approach significance and positively influences habitual physical activity after the intervention has ceased. This 2,762 kcal per week the present six-day group expended is slightly under the 3,000 kcal/week previously used to induce incentive sensitization, indicating that 3,000 kcal per week may be the minimum energy expenditure needed to increase exercise reinforcement. The optimum frequency, dose, and intensity needed to instill incentive sensitization remains an area of future research, with this investigation adding to that research question. We also demonstrate the interplay between behavioral outcomes (exercise reinforcement, changes in physical activity) and physiological outcomes (improvements in body composition and energy compensation). It appears those who limit their energy compensation and are thus more successful at decreasing body fat through exercise are able to realize exercise as a reinforcing behavior and increase habitual exercise after the intervention has ceased. Although it is uncertain if the behavioral outcomes influenced body fat loss or if greater body fat loss caused exercise to be more reinforcing and made physical activity more appealing or possibly more attainable, a potentially new and interesting research question and an area for future work. Additional research is underway to shed light on some of these questions, with the goal of promoting sustained increases in exercise behaviors, resulting in more Americans meeting physical activity guidelines, attaining a healthy body composition, and improving health.

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Declaration of Conflicting Interests

The Authors declare that there are no conflicts of interest.

Data Accessibility

All raw data associated with the present trial (ClinicalTrials.gov identifier: NCT03413826) is included as supplementary data.

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