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Incentive Sensitization for Exercise Reinforcement to Increase Exercise Behaviors

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Abstract

1
2 Individuals can be sensitized to the reinforcing effects of exercise, although it is unknown if this
3 process increases habitual exercise behavior. Sedentary men and women (BMI: 25-35 kg/m²,
4 N=52), participated in a 12-week aerobic exercise intervention. Exercise reinforcement was
5 determined by how much work was performed for exercise relative to a sedentary alternative in a
6 progressive ratio schedule task. Habitual physical activity was assessed via accelerometry. Post-
7 intervention increases in exercise reinforcement predicted increases in physical activity bouts
8 among those who expended over 2,000 kcal per week in exercise and who compensated for less
9 than 50% of their exercise energy expenditure.

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

11 **Introduction**

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

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

34 Using genetic knock-out models, others have demonstrated dopamine transporter and receptor
35 expression to influence physical activity behaviors (Bronikowski et al., 2004; Rhodes and
36 Garland, 2003). This offers an explanation as to why exercise dependency has been
37 demonstrated in both humans (Chan and Grossman, 1988; Chapman and De Castro, 1990;
38 Holden, 2001; Belke, 1997) and rodents (Belke, 1997; Belke, 2000; Iversen, 1993; Lett et al.,
39 2000), with the notion that central dopamine is playing a major role in the choice to be physically
40 active, in line with the dopamine hypothesis of reward (Knab and Lightfoot, 2010).

41 We have previously demonstrated a high-dose exercise intervention to be effective at
42 increasing exercise reinforcement (five days per week, 600 kcal per session) (Flack, 2019b),
43 while low-dose interventions (three days per week at 150 or 300 kcal per session) are effective at
44 decreasing sedentary behavior reinforcement, but not capable of instilling incentive sensitization
45 for exercise reinforcement (Flack et al., 2019b). The development of sensitization of drug abuse
46 can be dose-dependent (Liu et al., 2005), and if drug abuse and exercise follow similar patterns
47 (i.e. dopamine-mediated reinforcement), we would expect greater doses of exercise to be
48 required in order to instill incentive sensitization. There are still questions regarding the best way
49 to modify the dose of exercise (frequency of sessions, energy expended per session, exercise
50 intensity), and we have yet to demonstrate physiological or behavioral benefits to increasing
51 exercise reinforcement. The current study fills some of this void by using pre-post change in
52 exercise reinforcement to predict changes in physical activity behavior post-intervention, which
53 influences energy compensation to an exercise program and thus weight-loss success. The
54 present investigation's hypothesis was that more frequent but shorter exercise sessions would
55 produce greater increases in exercise reinforcement, compared to less frequent but longer
56 sessions that produce greater energy expenditures per session but lower total expenditure over an

57 entire 12-week intervention. This increase in exercise reinforcement was hypothesized to serve
58 as an independent predictor in the increase in physical activity behaviors post-intervention. As a
59 secondary analysis and hypothesis, we assessed the compensatory response to the exercise
60 intervention, that is, the difference in expected weight loss (based on energy expended) and
61 actual fat and lean mass loss converted to kcal equivalents. For instance, if a participant
62 exercised to expend 30,000 kcal during the intervention but only lost 15,000 kcal, they would
63 have compensated 15,000 kcal, or 50% of their energy expended. Although we did not determine
64 the source of this compensatory response, one possibility is individuals become less active when
65 engaging in exercise, reducing their non-exercise physical activity as a compensatory mechanism
66 (King, 2007). We hypothesized individuals who increase their reinforcing value of exercise
67 would compensate less, possibly by increasing habitual physical activity to increase energy
68 expenditure.

69 **Materials and Methods**

70 *Participants*

71 A total of 80 participants aged 18 to 49 years volunteered and were enrolled into the
72 study. Of these 52 completed all baseline tests and were randomized into one of three groups
73 during this longitudinal, randomized, controlled trial. Of these 52 randomized participants, 44
74 completed the study (32 female), with six (four female) withdrawing for personal reasons and
75 two females being excluded for non-compliance (did not complete the required 85% of exercise
76 sessions assigned per month). A consort diagram is depicted in Figure 1. All participants had a
77 body mass index (BMI) ranging from 25-35 kg/m² and were inactive (not engaging in any form
78 of exercise), determined during screening where participants were asked of their exercise

79 behaviors and validated by accelerometry (baseline participant characteristics are presented in
80 Table 1). Participants were also non-smoking and free of any health conditions that may preclude
81 them from exercise (metabolic or heart disease, cancer). Recruitment began in the winter of 2018
82 and continued until recruitment goals were met (spring of 2019) in and around Lexington,
83 Kentucky. Participants were a sample who responded to recruitment media including printed
84 brochures and flyers and online advertisements placed on University of Kentucky's Center for
85 Clinical and Translational Science (CCTS) website. This study was approved by the University
86 of Kentucky Institutional Review Board. The present analysis is a secondary outcome of a trial
87 aimed at assessing mechanisms of energy compensation at different doses of exercise
88 ClinicalTrials.gov identifier: NCT03413826, currently in review.

89 *Procedures*

90 During the initial screening and consenting visit, participants provided their written
91 informed consent and were screened of eligibility criteria, completing a physical activity
92 readiness questionnaire (PARQ), health history questionnaire, and screened on their dieting,
93 weight loss history, and physical activity behaviors. Participants were provided an ActiGraph
94 Accelerometer (Pensacola, Fla) to wear for the following seven days to objectively assess
95 physical activity prior to completing baseline testing. Participants also completed the Preference
96 for and Tolerance of the Intensity of Exercise Questionnaire (PRETIE-Q) (Ekkekakis et al.,
97 2008; Ekkekakis et al., 2005). Subsequent visits included assessments for exercise liking and
98 reinforcement, rate of energy expenditure during exercise, and body composition (all detailed
99 below).

100 *Study Design*

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

117 *Exercise Intervention*

118 Participants were provided a Polar A-300 heart rate monitor (watch and chest strap,
119 Kempele, Finland) for the duration of the 12-week intervention and instructed to perform aerobic
120 exercise (treadmill, bicycle, or elliptical ergometer) either two or six times per week on their own
121 and were provided access to a fitness center. Participants in the control group were instructed to
122 remain sedentary and return for post-testing 12 weeks later, receiving the exercise intervention
123 after post-testing if they desired. Those in the exercise groups returned to the lab weekly to meet

124 a researcher and download their exercise sessions using the PolarFlow software, which allowed
125 research staff to monitor and track compliance. If a participant was not at least 85% compliant
126 (completed 85% of expected exercise sessions per month) they were removed from the study.
127 The downloaded exercise session reports provided the amount of time spent in each heart rate
128 zone, which allowed for the calculation of total energy expended during each exercise session
129 based off individual rates of energy expenditure averaged across each heart rate zone calculated
130 from the graded exercise test with indirect calorimetry performed at baseline and week six.
131 Participants in the two-day per week group were instructed to perform two long exercise sessions
132 per week and encouraged to try to expend 1,000 kcal per session. Participants in the six-day per
133 week group were instructed to keep their sessions to 400 kcal per session and averaged just over
134 53 minutes per session. Although most participants in the two-day per week group were not able
135 to attain the 1,000 kcal goal, they still expended significantly greater kcal per session compared
136 to the six-day group and spent on average 94.5 minutes per session. Participants received
137 personalized heart-rate based exercise prescriptions that, if followed, would result in them
138 expending the assigned energy per exercise session. Participants were also provided feedback
139 each week on their energy expenditure of each session of the prior week so they could tailor
140 future exercise sessions. All participants were instructed not to purposely change dietary habits
141 during the intervention, i.e., not begin an energy-restricted diet.

142 **Assessments**

143 *Physical activity*

144 Habitual, free-living physical activity was measured using an ActiGraph accelerometer
145 (GT3X+ model; Pensacola, Florida). Each participant wore the device for seven days prior to

146 baseline testing and immediately after completing all other post testing assessments. Participants
147 were instructed to wear the monitor at the hip using the provided belt during all hours awake
148 except when bathing or swimming. Data were cleaned of non-wear time, defined as consecutive
149 strings of zeros greater than 20 minutes. An epoch of 10 seconds was used for data collection as
150 a shorter epoch is more suitable to reflect bout duration under free-living conditions of sedentary
151 individuals where many bouts of sporadic activity last 30 seconds or less (Ayabe et al., 2013;
152 Gabriel et al., 2010). These data were used to determine participants' weekly minutes of
153 moderate to vigorous physical activity (MVPA), number of MVPA bouts, vigorous intensity
154 physical activity (VPA) and sedentary activity using the Crouter et.al algorithm (Crouter et al.,
155 2010), and Freedson cut-points (Freedson et al., 1998).

156 *Liking of Exercise*

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

161 *Exercise Reinforcement*

162 Exercise reinforcement (specifically, aerobic-type exercise, treadmill, elliptical, or
163 bicycle ergometer) was assessed against a sedentary alternative (playing computer games,
164 reading magazines, doing crossword puzzles, Sudoku). Exercise reinforcement is assessed by
165 evaluating the amount of operant responding (mouse button presses) a participant is willing to
166 complete to gain access to exercise (Bickel et al., 2000; Epstein et al., 2011). The testing space
167 includes two workstations. One station is a computer and mouse on which the participant can

168 earn points towards their most liked exercise activity while the other station is a computer that
169 can be used to earn points toward their most liked sedentary alternative. Participants can switch
170 between stations as much as they choose. The program presents a game that mimics a slot
171 machine; a point is earned each time the shapes match. For every five points, a session is
172 completed, and the participant receives five minutes of access to the reinforcer that was earned
173 (either exercise or sedentary activity). The game is performed until the participant no longer
174 wishes to work for access to either the exercise or sedentary activities. At first, points are
175 delivered after every four presses, but then the schedule of reinforcement doubles (4, 8, 16, 32,
176 [...] 1024) each time five points are earned. For instance, the participant initially has to click the
177 mouse button four times to earn each point for Schedule 1. After the first five points are earned,
178 Schedule 1 is complete, and the participant earns five minutes for exercise. Then eight clicks are
179 required to earn each of the next five points for Schedule 2 before another five minutes of
180 exercise is earned. Schedule 3 would require 16 clicks to earn one point, Schedule 4 would
181 require 32 clicks to earn one point, and so on (Epstein et al., 2011; Bickel et al., 2000).

182 Participants engage in the activity for the time earned after they complete the game, which ends
183 when the participant no longer wishes to earn points (time) for exercise or the sedentary
184 alternative. In essence, the more reinforcing exercise or the sedentary behavior is, the more
185 operant responding participants will do for access to these behaviors. Similar button pressing
186 tasks are valid predictors of the reinforcing value of physical versus sedentary activity and for
187 determining the reinforcing value of food (Barkley et al., 2009; Epstein et al., 1999; Epstein et
188 al., 2007). Participants self-selected the intensity level when performing any earned exercise
189 time, which was typically a low to moderate steady-state intensity. These assessments took place
190 in a laboratory space adjacent to the Human Performance Laboratory on the University of

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

196 *Rate of Energy expenditure*

197 A graded exercise treadmill test was used to determine each participant's rate of energy
198 expenditure at five different heart-rate zones. Oxygen consumed and CO₂ produced were
199 analyzed by indirect calorimetry (VMAX Encore Metabolic Cart, Vyair Medical, Mettawa, IL)
200 which included an integrated 12 lead ECG for monitoring heart rate and used in conjunction with
201 the Trackmaster TMX428 Metabolic cart interfaced treadmill. Upon completion of a five-minute
202 warm-up walking at 0% grade, 3.0 mph, the treadmill grade increased to 2.5% for three minutes.
203 The treadmill grade was then increased every three minutes to produce an approximately 10 beat
204 per minute increase in heart rate from the previous stage with the speed fixed at 3.0 mph. The
205 test continued until a heart rate of 85% HRR was attained or the participant felt they could no
206 longer continue. Energy expenditure (kcal per minute) was determined from the amount of
207 oxygen consumed and CO₂ expired using the Weir equation (Weir, 1949). The average rate of
208 energy expenditure during the last 30 seconds of each stage of the graded exercise test was
209 regressed against the heart rate averaged over the last 30 seconds of the corresponding stage to
210 calculate the rate of energy expenditure at different heart rates. Heart rate zones were calculated
211 using the heart rate reserve (HRR) formula as $(220 - \text{age}) - \text{resting HR} * \text{zone \%} + \text{resting HR}$
212 (Swain et al., 1998). Heart rate Zone 1 ranged from 0% to 25% HRR, Zone 2 corresponded to
213 26-40% HRR, Zone 3 was 41-58% HRR, Zone 4 was 59-75% HRR, and Zone 5 was 76-90%.

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

217 *Body composition*

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

227 *Energy Compensation*

228 To calculate compensation for the energy expended during the exercise program (ExEE),
229 the accumulated energy balance (AEB) was calculated from changes in FM and FFM upon
230 completion of the study as body composition changes reflect long-term alterations in energy
231 balance (Rosenkilde et al., 2012). Gains of 1kg FM or 1kg FFM were assumed to reflect 12,000
232 kcal and 1,780 kcal, respectively (Elia et al., 2003). Losses of 1kg FM or 1kg FFM were
233 assumed to equal 9,417 and 884 kcal, respectively (Forbes, 1990). ExEE was calculated from the
234 training-induced energy expenditure in kcal/session with the addition of 15% excess post-
235 exercise energy expenditure (Bahr et al., 1987). The resting energy expenditure (REE) that

236 would have occurred during the exercise sessions ($REE \times 1.2$) was subtracted. Thus, $ExEE =$
237 $(TrEE \times 0.15) + (TrEE - \text{training duration} \times (REE \times 1.2))$ (Rosenkilde et al., 2012). The overall
238 compensatory response to the increase in $ExEE$ was assessed as described by Rosenkilde
239 (Rosenkilde et al., 2012), with % kcal compensated calculated as $(ExEE + AEB)/ExEE \times 100\%$.
240 A 0% kcal compensated occurs when AEB equals $-ExEE$, or changes in the energy equivalent of
241 fat mass and fat-free mass equal energy expended during exercise. Positive compensation
242 suggests that changes in body composition indicate a less negative energy balance than expected
243 based on $ExEE$, whereas negative compensation indicates a greater than expected negative
244 energy balance. $ExEE$, AEB , and % kcal compensated could be calculated only for those
245 participants who completed the study as both a pre- and post-treatment data points were needed
246 to calculate these variables.

247 *Preference and tolerance for exercise intensity*

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

257 **Analytic Plan**

258 Baseline participant characteristics were assessed via 1-way ANOVA between groups
259 exercising six- and two-days per week and sedentary control. Differences in the pre-post changes
260 in exercise reinforcement, seven-day MVPA bouts, sedentary reinforcement and changes in body
261 fat were tested between groups and if changes were different from zero using analysis of
262 covariance with the corresponding baseline value as the covariate. Between- group analyses were
263 performed on randomized groups (exercise six-days per week, 2-days per week, or control) in
264 addition to retrospectively split groups on exercise energy expenditure (expending greater than
265 2,000 kcal per week, less than 2,000 kcal per week, or control), and compensation groups
266 (compensating for greater than 50% of their kcal expended during the exercise intervention, less
267 than 50%, or control). Linear regression analyses were used to predict changes in MVPA bouts,
268 as this was the variable we hypothesized to be effected by our exercise intervention, with specific
269 hypotheses on the relationship between changes in exercise reinforcement and changes in MVPA
270 bouts. Therefore, changes in exercise reinforcement was our primary predictor of interest, with
271 other variables that were differently affected by the exercise intervention (energy expended per
272 week through exercise, percent changes in FM, percent kcal compensated for during the exercise
273 intervention, changes in sedentary behavior reinforcement, and liking of exercise and sedentary
274 activities) also entered as independent variables. Additional separate regression analyses were
275 performed on retrospectivity assigned groups. The choice to split groups on exercise energy
276 expenditure above and below 2,000 kcal and compensation groups above and below 50% was
277 based on weekly energy expenditures per week averaging 2,041.7 kcal and % kcal compensated
278 averaging 50.25. All analyses were performed in IBM SPSS Version 26 (IBM corporation,
279 Armonk, New York). **Power Analysis:** Our recent study (Flack, 2019b) demonstrated significant
280 increases in exercise reinforcement after 12-weeks of high dose exercise (five sessions per week,

281 600 kcal expended per session). Using an 80% power and 95% confidence level, 15 participants
282 per group were needed to detect a significant change in exercise reinforcement (P_{max}) from
283 baseline to post intervention.

284 **Results**

285 Baseline characteristics are presented in Table 1, with differences in sedentary behavior
286 reinforcement between all groups, body fat percentage between six-day per week and two-day
287 per week groups, and differences in MVPA bouts between control and two-day per week group.
288 Because of these differences, pre to post change scores were calculated and analysis of
289 covariance was used to determine differences between groups while controlling for the
290 corresponding baseline value. Table 1 also indicates participants were meeting MVPA
291 recommendations (150 minutes per week) despite reporting not engaging in any form of exercise
292 (defined as leisure-time physical activity performed with the goal of increasing fitness and/or
293 losing weight). We believe this is due to most participants accumulating shorter, spontaneous
294 bouts of walking through the day traveling across a sprawling university campus and not
295 indicative of actual exercise. This is supported by the finding that all groups were far below the
296 recommendations for VPA (75 minutes per week). We therefore chose to use MVPA bouts as the
297 primary outcome variable, which would include lower-intensity exercise but only if performed
298 for 10 or more minutes at a time, more indicative of purposeful exercise and in line with current
299 recommendations that exercise sessions should last at least 10 minutes (Piercy and Troiano,
300 2018).

301 The mean \pm SE kcal/session for participants in the two day per week group was $745.33 \pm$
302 61.04 , while the six-day per week group expended 460.37 ± 26.04 kcal per session, mean \pm SE,
303 which was different ($P < 0.01$) between groups as expected. This equates to $2,762.24 \pm 156.23$

304 kcal per week for the six-day group and 1490.66 ± 122.07 kcal per week in the two-day group,
305 means \pm SE. Further information on the exercise intervention outcomes have been reported
306 previously (Flack, 2019a). Table 2 presents the change scores between exercise frequency groups
307 (randomized group), and between retroactively assigned groups based on amount of kcal
308 expended per week during the exercise intervention (over 2,000 kcal vs. under 2,000 kcal) and
309 on the percent of kcal compensated for (over 50% vs. under 50%). There were no differences in
310 the change in exercise reinforcement between any groups or across time, although adjusted
311 differences between six- and two-day groups approached significance ($P=0.06$). Changes in
312 MVPA bouts were greater in both the six-day and two-day groups compared to control ($P<0.01$),
313 whereas the control group was the only group who observed significant changes over time,
314 decreasing number of MVPA bouts. Adjusted change in MVPA bouts between groups split on
315 energy expenditure per week (above or below 2,000 kcal per week) were also different from
316 control, while groups split on energy compensation (greater or less than 50% of energy expended
317 during the exercise intervention) were different between each other and between control.
318 Adjusted changes in FM percentage were different when comparing the control group to those
319 exercising either six or two days per week, or above or below 2,000 kcal per week. The six-day
320 per week group, those exercising over 2,000 kcal per week, and those compensating less than
321 50% of their kcal lost significant FM (change different from zero). All compensation groups
322 were different from each other in FM percent change ($P<0.05$). Neither the preference for or
323 tolerance of the intensity of exercise (assessed by the Preference for and Tolerance of the
324 Intensity of Exercise Questionnaire, PRETIE-Q) were different between groups at baseline, did
325 not change as a result of the exercise intervention, and did not change differently between any

326 groups. Exercise intensity did not differ between groups, with the 2-day per week group and 6
327 day per week group spending 52.3 and 47.7% of their time in heart rate zones 3-5, respectively.

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

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

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

353 **Discussion**

354 There has been a wealth of research centered on behavioral reinforcement as an important
355 component in the participation of certain, reinforcing, behaviors, positing the central dopamine
356 system provides the physiological basis for realizing their reinforcing value (Berridge and
357 Robinson, 1998; Robinson and Berridge, 1993). Recent and current research has focused on drug
358 abuse, nicotine use, gambling, and eating energy dense foods as reinforcing behaviors all
359 operating under the dopamine hypothesis of reward (Berridge and Robinson, 2003; Epstein et al.,
360 2011; Epstein et al., 2007; Liu et al., 2005; Rhodes and Garland, 2003; Robinson et al., 2015;
361 Robinson and Berridge, 1993; Spanagel and Weiss, 1999). These behaviors are all common in
362 that their engagement is not advantageous for one's health (mental or physical), with many
363 researching how we can improve these behaviors by understanding the underling physiological
364 process implicated in their development, with one theory being incentive sensitization. One
365 behavior that is starting to receive greater attention in the context of behavioral reinforcement is
366 exercise, with early work investigating the reinforcing value of active play in children (Barkley
367 et al., 2009; Epstein et al., 1999) and more recent cross-sectional analyses pointing to the
368 reinforcing value of exercise being an important predictor of exercise behavior among adults
369 (Flack et al., 2017a; Flack et al., 2017b). In contrast to the other reinforcing behaviors more
370 traditionally researched, engaging in consistent exercise is beneficial for one's health, making

371 incentive sensitization for exercise an advantageous process. Therefore, we and others have
372 taken an interest in trying to understand ways to induce incentive sensitization for exercise with
373 the goal of increasing physical activity behaviors which would, theoretically, improve health. We
374 have recently demonstrated greater doses of exercise are needed to instigate this process,
375 possibly because a high-dose exercise program can increase the tolerance for exercise intensity
376 to allow it to become a reinforcing behavior (Flack et al., 2019a; Flack et al., 2019b).
377 Specifically, expending 3,000 kcal per week (five sessions/week, 600 kcal per session) increased
378 the reinforcing value of exercise, while exercising to expend 1,500 (five sessions/week 300 kcal
379 per session) did not (Flack, 2019b). These results support an earlier investigation where low
380 doses of exercise (450 or 900 kcal per week) were effective at reducing the reinforcing value of
381 sedentary behaviors but did not increase exercise reinforcement (Flack et al., 2019b). Results of
382 the current investigation are parallel with these findings, as among those in the six day per week
383 group (2,762 kcal expended per week) the increase in exercise reinforcement approached
384 significance ($P > 0.06$) with change scores greater than 30-fold of that compared to the control and
385 those exercising twice per week (1,491 kcal expended per week). The lack of statistical
386 significance despite what appears to be clinically significant differences could be due to
387 unexplained variability among participants, potentially related to genetic polymorphisms in the
388 central dopamine system that have been demonstrated to influence exercise reinforcement (Flack
389 et al., 2019a). The current study did not observe any changes in preference or tolerance for
390 exercise intensity. Since tolerance for exercise intensity appears to be an important player in the
391 process of incentive sensitization for exercise (Flack et al., 2017a; Flack et al., 2019b), the lack
392 of improvements in tolerance may be another reason why improvements in exercise
393 reinforcement did not reach significant levels. Although only speculative, this may be related to

394 the intensity of exercise individuals self-selected, with six-day and two-day groups not differing
395 in time spent in HRR zones 3-5 or 1-2. It is possible that greater intensities are needed to produce
396 tolerance for exercise intensity and improve exercise reinforcement. Research is under way to
397 investigate how high-intensity exercise may work to develop tolerance and and how this may
398 influence incentive sensitization for exercise reinforcement.

399 Despite the lack of significant changes in exercise reinforcement, this investigation, for
400 the first time, uncovered important implications for increasing exercise reinforcement. These
401 findings support our hypothesis that increasing exercise reinforcement increases exercise
402 behaviors and further justifies future research in this area. We chose to assess MVPA bouts
403 (Freedson cutoff, ≥ 10 consecutive minutes of moderate to vigorous intensity) instead of total
404 minutes of MVPA as many of the participants in the study were college students or employees
405 who were obligated to walk sporadically between classes on the college campus, therefore
406 accumulating many bouts of walking less than 10 minutes in duration while not engaging in any
407 structured exercise. Increasing MVPA bouts would therefore be more indicative of increasing
408 purposeful exercise, the goal of our intervention. It is important to note that participants' seven-
409 day assessment of MVPA bouts at post testing were performed between one and two weeks after
410 completion of the exercise intervention as other assessments were performed immediately upon
411 completion. This time between the end of the intervention and habitual activity assessment may
412 have provided the needed break from forced exercise and allowed the process of incentive
413 sensitization to take effect, creating a craving/wanting for exercise, which occurred in spite of
414 participants not told to exercise nor given a fitness center pass as their pass was only valid for the
415 12-week intervention. In this light, it may have been advantageous to wait a week to perform the
416 post-testing exercise reinforcement task. We also do not know how long lasting the exercise

417 intervention effects were, that is, if these exercise behaviors remained increased several months
418 after the intervention ceased, creating permanent behavior change. Future studies may
419 investigate these issues with multiple post-testing assessments of exercise reinforcement and
420 physical activity, including long-term follow-up assessments.

421 An additional outcome analyzed in the present investigation centered on changes in
422 percent FM (body fat change in kg/baseline body fat kg). Weight loss, specifically body fat loss,
423 is a prime reason individuals partake in exercise and thus a relevant variable to assess in any
424 exercise intervention (Obert et al., 2017). Indeed, we demonstrated significant decreases in body
425 fat in the six-day per week group and those expending greater than 2,000 kcal per week, slight,
426 but not significant, decreases in the two-day per week group and those expending fewer than
427 2,000 kcal per week, and non-significant increases in body fat in the control group. This
428 indicates the greater energy expenditures of the six-day per week group and the greater than
429 2,000 kcal group are needed to sustain the negative energy balance needed for weight loss. When
430 energy expenditure is controlled for, however, one's level of energy compensation determines
431 weight loss success with exercise. Individuals compensating for fewer of the kcal they expended
432 during the exercise intervention are, by definition, in a greater energy deficit compared to
433 individuals who have a greater compensatory response. In the present study, the average % of
434 kcal compensated for was 50.25%, in line with our previous work (Flack et al., 2018). Those
435 who compensated greater than 50% of their kcal were deemed "compensators" and did not
436 display the relationship between changes in exercise reinforcement and changes in MVPA bouts.
437 This is in contrast to the "non-compensators" who were more successful at weight loss and
438 whose changes in exercise reinforcement predicted changes in MVPA bouts. Furthermore,
439 changes MVPA bouts predicted changes in percent FM when controlling for energy expended

440 during exercise. It therefore appears individuals who are less prone to compensate for the energy
441 they expend during exercise realize the reinforcing effects of exercise and increase their exercise
442 behavior, aiding in weight loss. Similar findings have been demonstrated previously, where
443 increases in non-exercise physical activity were associated with lower energy compensation
444 during a high intensity exercise intervention (Schubert et al., 2017). Alternatively, increasing
445 exercise reinforcement could be an effect of successful weight loss with exercise, where
446 improvements in health, well-being, and appearance could feedback to increase exercise
447 reinforcement and increase physical activity. Knowing these two features are inter-related (health
448 physiology and behavioral physiology) is an additionally important finding future research may
449 build upon.

450 This study is not without limitations. A more robust design may have been to match
451 groups (two-day and six-day) on weekly exercise energy expenditure, to control for some of the
452 variability in the session/week group analysis. The average energy expenditure was just over
453 2,000 kcal per week, with previous research indicating 1,500 kcal per week to be ineffective at
454 inducing incentive sensitization for exercise reinforcement while 3,000 kcal per week to be
455 effective (Flack, 2019b). Thus, it is possible that weekly energy expenditures of the present study
456 were not great enough for incentive sensitization to take place, although levels approached
457 significance with expenditures of 2,762 kcal per week. If participants exercise at a greater energy
458 expenditure per week, it is likely improvements in exercise reinforcement and potentially greater
459 improvements in MVPA bouts would have resulted. It is also possible that when using greater
460 exercise energy expenditures, mediation analysis between group, exercise reinforcement, and
461 MVPA bouts would have been more fruitful. The analysis also included mostly female, all
462 between the ages of 18 and 40. It is not known if older populations would experience a different

463 effect or of any potential gender effects in play. Additionally, stage of menstrual cycle was not
464 accounted for among female participants, which may have influenced the calculated ExEE
465 during the 12-week intervention. The unsupervised nature of the exercise program may also be
466 considered a limitation as participants could have exercised for additional time while not
467 recording it (did not start watch), although we have no reason to believe this occurred. Finally,
468 calculating energy expenditures averaged across heart rate zones based on the HRR formula may
469 not have been as precise as conducting a maximal exercise test and assigning exercise zones
470 based off of VO_2 max.

471 **Conclusions and Future Directions**

472 Research on increasing exercise reinforcement remains in its infancy, with more
473 questions than answers at this point. The present study provides evidence that physical activity
474 behaviors can be increased as a result of increasing exercise reinforcement while further defining
475 parameters that appear necessary for incentive sensitization to take place. It seems that exercising
476 twice weekly, even when energy expenditures average greater than 740 kcal/session, is
477 inadequate to improve exercise reinforcement and thus exercise behaviors. When exercise is
478 performed six times per week (460 kcal per session) improvements in exercise reinforcement
479 approach significance and positively influences habitual physical activity after the intervention
480 has ceased. This 2,762 kcal per week the present six-day group expended is slightly under the
481 3,000 kcal/week previously used to induce incentive sensitization, indicating that 3,000 kcal per
482 week may be the minimum energy expenditure needed to increase exercise reinforcement. The
483 optimum frequency, dose, and intensity needed to instill incentive sensitization remains an area
484 of future research, with this investigation adding to that research question. We also demonstrate
485 the interplay between behavioral outcomes (exercise reinforcement, changes in physical activity)

486 and physiological outcomes (improvements in body composition and energy compensation). It
487 appears those who limit their energy compensation and are thus more successful at decreasing
488 body fat through exercise are able to realize exercise as a reinforcing behavior and increase
489 habitual exercise after the intervention has ceased. Although it is uncertain if the behavioral
490 outcomes influenced body fat loss or if greater body fat loss caused exercise to be more
491 reinforcing and made physical activity more appealing or possibly more attainable, a potentially
492 new and interesting research question and an area for future work. Additional research is
493 underway to shed light on some of these questions, with the goal of promoting sustained
494 increases in exercise behaviors, resulting in more Americans meeting physical activity
495 guidelines, attaining a healthy body composition, and improving health.

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502 **Declaration of Conflicting Interests**

503 The Authors declare that there are no conflicts of interest.

504 **Data Accessibility**

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

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