Pharmacology Biochemistry and Behavior, Vol. 46, pp. 209–213, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Effects of Nicotine Cycling on Weight Loss and Regain in Male Rats

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WINDERS, S. E., D. R. WILKINS II, P. A. RUSHING AND J. E. DEAN. *Effects of nicotine cycling on weight loss and regain in male rats.* PHARMACOL BIOCHEM BEHAV 46(1) 209–213, 1993. – Over successive periods of weight loss and regain caused by deprivation and refeeding, weight loss becomes slower during deprivation and weight is regained quicker during refeeding. One period of nicotine administration and termination results in changes in intake and weight gain comparable to that caused by one period of food deprivation and refeeding. However, no study has examined the effect of multiple periods of nicotine administration and cessation on weight loss and regain. The present study examined the effect of repeated cycles of nicotine administration and cessation on growth rate. Thirty-two male Sprague–Dawley rats underwent three nicotine administration cycles. Cycles consisted of 2 weeks of nicotine or saline followed by 2 weeks of no drug. Nicotine administration decreased growth and food consumption, and cessation resulted in a resumption of normal growth and intake. However, changes in food consumption and body weight were similar across cycles. Thus, although nicotine administration and cessation produced reliable changes in food consumption and body weight similar to those caused by diet cycling, there were no comparable cumulative effects of nicotine cycling on growth rate.

Nicotine Weight cycling Body weight Food consumption Water consumption Growth rate Rats

OVER 50,000 studies have linked cigarette smoking to increased morbidity and mortality from cardiovascular diseases, cancer, and chronic obstructive lung diseases (23). Despite the fact that most Americans are well aware of the health risks associated with smoking, the most recent report of the Surgeon General on smoking estimates that approximately one-fourth of adults in the United States smoke (24). This suggests that there must be some compelling reasons for people to smoke.

One frequently cited reason for continued smoking is the belief that cigarette smoking can control body weight. Wack and Rodin (25) reported that both health researchers and the general public believe that smoking controls body weight and that smoking cessation will produce weight gain. Given the premium placed on thinness in our culture (2,6), it appears that among weight-conscious individuals, the perceived weight control advantages of continued smoking take precedence over the health risks. Surveys of both smokers and nonsmokers suggest that body weight concerns are related to starting and continuing to smoke and may be related to relapse among those who quit [for reviews see (8,9)].

The findings of a large number of cross-sectional and longitudinal studies confirm what the public has long believed. Smokers weigh less than nonsmokers, and smokers who quit smoking gain weight [for a review see (13)]. Although these studies clearly suggest that many smokers will gain *some* weight when they quit smoking, accurate estimates of *how much* ex-smokers might gain were not available until recently. Williamson and colleagues (26) followed body weight and smoking status in a nationally representative cohort of 9004 smokers and nonsmokers from 1971 to 1984. The average weight gain among people who quit smoking was small (approximately 3 kg). However, a sizeable proportion of quitters (9.8% of men and 13.4% of women) experienced major weight gains of 13 kg (28.6 lb) or more. The finding that some people are at a greater risk for major weight gain following cessation than others suggests the presence of moderating variables.

One variable that might distinguish those who will gain a lot from those who will gain less is the number of times they have quit smoking in the past. Regular smokers often quit smoking for extended periods of time, whether for temporary reasons (15) or in an attempt at permanent cessation (18). Indeed, it has been estimated that the typical smoker will start and stop smoking an average of five times (24). Given the weight-altering effects, it is likely that the average smoker would also experience several weight fluctuations over his or her lifetime.

Studies of human dieters have shown that successive periods of weight loss and regain caused by on/off dieting result in a resistance to weight loss (3,19). That is, with each weight loss and regain, the energy reduction necessary to lose one pound becomes greater and the energy increase necessary to gain one pound becomes less. Similar results have been found in studies using food-deprived animals with access to high-fat diets during refeeding (1,4,7,16,20).

Studies using rats receiving nicotine have produced similar changes in body weight to those observed in human smokers.

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Specifically, nicotine administration reduces growth rate, while termination of nicotine results in either a restoration of growth rates to control levels or an increase in growth rate lasting approximately 2 weeks (27). The results of the animal studies suggest that nicotine, the pharmacologically active component of tobacco smoke, is responsible for changes in body weight produced by smoking and smoking cessation. Given the consistent effects of nicotine administration on body weight, it seems reasonable to postulate that on/off nicotine administration might cause a resistance to weight loss similar to that caused by on/off dieting. Despite the obvious parallels between on/off dieting and on/off nicotine administration, there are no available studies (animal or human) that have examined the effects of repeated on/off nicotine on rate of weight loss and regain.

The present study was designed to examine the effect of weight cycling induced by successive periods of nicotine administration and cessation on growth rate and total food and water consumption in 30- and 90-day-old rats with continuous access to a high-fat laboratory chow. Animals of two ages were used to control for changes in the rate of growth that occur as a function of aging in rats (20). A high-fat chow was used because close examination of the weight-cycling literature reveals that studies that have used low-fat chows have failed to find a cycling effect on growth rates (12,22), suggesting that a high-fat diet may be necessary for it to occur. Male animals were used because the weight-cycling effect has been previously demonstrated in males with access to high-fat diets (4).

Based on the data from the nicotine and body weight literature, we hypothesized that during any given cycle, nicotine administration would be accompanied by decreased body weight growth and nicotine cessation would result in a resumption of growth rates to normal or slightly accelerated levels. Based on the weight-cycling literature, we predicted that nicotine-induced weight loss would become less with each nicotine administration and that weight gain would become more rapid with each drug withdrawal.

METHOD

Subjects

Subjects were 32 male Sprague–Dawley rats that were 30 and 90 days old at the beginning of the study (Charles River, Scottdale, PA). Animals were housed individually in stainless steel cages (18.0 × 24.5 × 18.0 cm) in a temperature-controlled room (21–27°C and 45–55% relative humidity) on a 12L : 12D cycle (lights on at 0600 h CST). A high-fat, bland-tasting rat chow (described below) and tap water containing tetracycline (300 mg/l) were available ad lib. Water was delivered via hanging 250-ml water bottles fitted with stainless steel stoppers. Food was supplied in porcelain bowls (5.5 × 11.5 cm) with saucers (30 cm in diameter) attached to the bottom to collect spillage. The cups and saucers were wired securely to the front of the cage to further reduce spillage. Food containers and water bottles were changed once weekly.

Group Assignments

Subjects were assigned to one of two experimental conditions (nicotine cycled and saline/control). Each group contained eight 30-day and eight 90-day animals. Condition assignments were made randomly with the stipulation that with respect to body weight, each group have similar means and standard deviations (computed separately for each age within each group). Animals in the nicotine-cycled condition underwent three 1-month cycles, each consisting of 2 weeks of nicotine followed by 2 weeks of no drug. Animals in the saline/control condition underwent three equivalent cycles consisting of 2 weeks of saline followed by 2 weeks of no drug.

Daily Measurements

Body weight, food, and water were measured daily using a Sartorius IP65 programmable electronic scale. Food cups and water bottles were weighed once upon removal from each cage and once upon refilling before returning them to the cages. To ensure accurate body weight measurements, the mean of 10 weighings, taken once per second over a 10-s period, was recorded for each animal.

Drug Administration

Nicotine and saline were administered SC using Alzet mini-osmotic pumps (model 2002, Alza Corp., Palo Alto, CA). Each minipump released its contents at a rate of approximately 0.5 μl/h for 14 days. Physiological saline was used to make the nicotine solutions and served as the control solution. Animals in the nicotine-cycled condition received 12 mg nicotine (values computed as base) per kg body weight per day during each of the three drug administration periods. Drug dosages and method of administration were chosen because previous research using these procedures has produced results in rats comparable to results of studies using human smokers (10,11,18).

Minipump Implant and Explant Surgeries

To begin each period of nicotine administration, miniosmotic pumps containing the appropriate solution were implanted SC. Prior to pump implantation, animals received an injection of atropine (0.3 mg/kg) followed by IP injection of xylazine and ketamine (50 to 80 mg/kg ketamine, 3 to 6 mg/kg xylazine). Fourteen days after implantation, pumps were removed using a similar surgical procedure. To avoid excess irritation due to implantation of three pumps in a relatively short period of time, the location of pump implantation was rotated in a randomly counterbalanced order from sites in the upper, middle, and lower back.

Lab Diet

All animals had ad lib access to a semisolid, high-fat laboratory rat chow throughout the study. The high-fat chow was made by adding vegetable shortening to a standard bland-tasting laboratory rat chow (Purina Rodent Laboratory Chow 5001). In addition, protein (Purina Assay Protein RP-101) and a vitamin/mineral supplement (TEKLAD AIN-76; TEKLAD 40060) were added to avoid protein deprivation (14). The caloric composition of the diet was approximately 70% fat, 17% protein, and 13% carbohydrate.

Procedure

After a 5-day gentling period, daily measurements of body weight, food, and water were made for 7 days to establish baseline levels. On day 8, each animal received a minipump containing the appropriate solution and the first "on" drug period began. Fourteen days later, minipumps were removed using a similar surgical procedure and the first 14-day "off" drug period began. Animals underwent three consecutive on/off cycles, each lasting 28 days (14 days on, 14 days off).

Body weight, food, and water were measured daily during each of the three cycles.

Statistical Analyses

For the purposes of data analyses, the means of the last 6 days of each experimental period (baseline and each of the on and off periods) were calculated for body weight, food, and water consumption. Six-day means were chosen to ensure that the values being reported were stable and comparable to analyses performed in previous studies using this paradigm (10, 11). Growth rate was computed by subtracting the 6-day body weight mean for each period from the 6-day body weight mean from the preceding period. To determine the effect of multiple periods on nicotine-induced weight loss on growth rate, food consumption, and water consumption, the mean from each of the three on periods were compared for each variable using separate 2 × 2 × 3 repeated measures analysis of covariance (RMANCOVA). To determine the effect of multiple nicotine cessation periods on the dependent variables, an identical set of analyses was performed on the means for each variable obtained during each of the off periods. The two between-subjects factors for these analyses were drug group (saline and 12 mg nicotine/kg/day) and age (30-day vs. 90-day) and the within-subjects factor was time (on 1, on 2, on 3 or off 1, off 2, off 3). Baseline means were used as covariates in all analyses. To control for alpha inflation, a Bonferroni correction was performed at the hypothesis level by dividing the number of univariate tests performed (6) by 0.05. Thus, the alpha level for the overall analyses was set at $p = 0.008$. Follow-up analyses were performed using the Tukey HSD procedure. The alpha level was set at $p = 0.05$ for all follow-up tests. All analyses were done using the SPSS PC+ statistical software package.

RESULTS

Four animals, two from the 30-day-old nicotine condition and one each from the 90-day-old nicotine and saline conditions, died as a result of complications arising from the surgical procedure. Data obtained from these animals were excluded from all analyses. Since none of the dependent variables met univariate sphericity assumptions, all within-subjects effects were adjusted using the Greenhouse/Geisser correction. Thus, the previously described follow-up analyses were performed on adjusted means.

Although the expected main effects of age were obtained for each variable, there were no significant interactions involving age. Therefore, data from both age groups were combined to form one nicotine and one saline/control group for all subsequent analyses and discussion. Figures 1 and 2 present the mean body weight and food consumption, respectively, for the two drug groups during each phase of each cycle of the study.

On Drug

Growth rate. There were significant main effects of both drug condition, $F(1, 23) = 72.21$, $p < 0.001$, and time, $F(2, 48) = 44.58$, $p < 0.001$, on body weight growth, but there were no significant drug by time interactions. Follow-up analyses to determine the effect of drug condition revealed that although both groups gained weight during the three on periods, animals in the nicotine group gained significantly less weight during nicotine administration compared to controls. Follow-up analyses performed to investigate the effects of time revealed that rate of weight gain decreased as a function of time for animals in both experimental conditions.

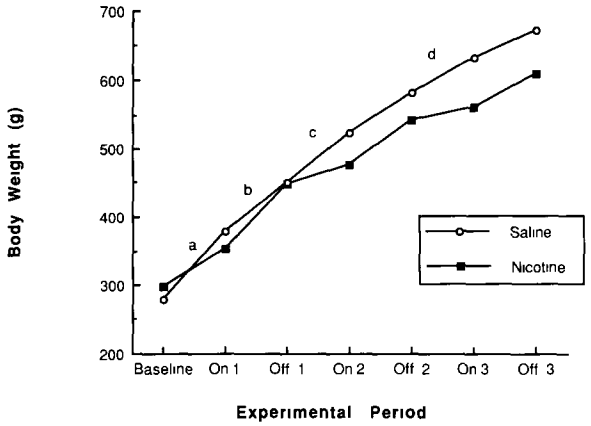

FIG. 1. Body weights averaged over 6-day periods during each phase of each cycle (Baseline, On 1, Off 1, On 2, Off 2, On 3, Off 3). Letters indicate significant between-group differences for each period.

Food consumption. There was a significant main effect of drug treatment on food consumption, $F(1, 23) = 25.90$, $p < 0.001$, but no main effects of time or significant drug by time interactions. Follow-up analyses to investigate the effects of drug condition on food consumption revealed that during each period of nicotine administration animals in the nicotine groups ate significantly less than controls.

Water consumption. Results of the analyses performed on the water data indicated that there were no significant differences between groups or across time in the amount of water consumed on drug.

Off Drug

Growth rate. There were significant main effects of both drug condition, $F(1, 23) = 14.64$, $p < 0.001$, and time, $F(2, 48) = 64.86$, $p < 0.001$, on body weight growth, but there were no significant drug by time interactions. Simple main effects analyses performed to investigate the effect of drug condition indicated that animals in the nicotine group gained

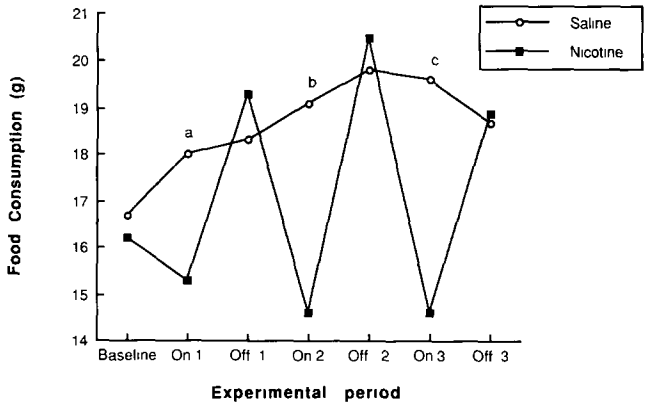

FIG. 2. Food consumption averaged over 6-day periods during each phase of each cycle (Baseline, On 1, Off 1, On 2, Off 2, On 3, Off 3). Letters indicate significant between-group differences for each period.

weight at a significantly faster rate compared to controls during the first off period only. During the second and third off periods, nicotine and saline animals gained weight at a statistically similar rate. Within-group follow-ups designed to investigate the effect of time revealed that growth rates slowed among animals in both conditions as a function of time.

Food consumption. There was a significant main effect of time on food consumption, $F(2, 48) = 9.53$, $p < 0.001$, but no significant effect of drug treatment or drug by time interactions. Follow-up analyses performed to investigate the effects of time revealed that food consumption increased steadily during the first and second off cycles and asymptoted during the third off cycle for all animals.

Water consumption. Results of the analyses performed on the water consumption data indicated that there were no significant differences in the amount of water consumed for any drug group during any of the off periods.

DISCUSSION

The results of the present study provide clear evidence in support of our first hypothesis. Specifically, nicotine administration decreased body weight growth, while nicotine termination was associated with a restoration of growth rates to control levels. In addition, the present study found that changes in body weight were paralleled by changes in food consumption. Thus, results of the present study were consistent with previous single-administration studies (27). Further, the effects of nicotine and body weight and food consumption occurred reliably across three consecutive cycles of administration and termination, and for animals of two different ages. This suggests that the relationship between nicotine administration and body weight is fairly robust and will occur despite the number of previous exposures to nicotine or the age of the animal.

With regard to our second hypothesis, the results were also clear. There was no effect of repeated nicotine administration and cessation on weight loss and regain. More specifically, although both passage of time and drug condition affected growth rate, there were no effects of nicotine cycling on weight gain above (or in addition to) those resulting from aging or nicotine administration and cessation. These results suggest that although nicotine administration and cessation produce changes in food consumption and growth similar to those induced by diet cycling, nicotine cycling does not result in a resistance to weight loss comparable to that observed in food-deprived animals.

It should be noted that although nicotine administration decreased growth rates in the present study, growth rates were always in the positive direction (e.g., animals gained weight continuously throughout the study). In contrast, the majority of the weight-cycling studies using caloric deprivation or fasting have typically induced weight loss well below baseline levels (5). The present study used male animals and bland-tasting, high-fat food. Previous studies using nicotine and body weight have produced more dramatic body weight fluctuations when using female animals and/or high-sweet or high-carbohydrate foods. One possible explanation for the failure of the present study to obtain changes in growth rate as a function of nicotine cycling is that the weight changes produced by nicotine cycling in the present study were not large enough to activate homeostatic mechanisms necessary for the metabolic effects of weight cycling to occur (17). Therefore, it is possible that nicotine cycling might alter weight loss and regain if tested under these conditions.

In summary, the present study found that in male rats with unlimited access to a high-fat laboratory chow, nicotine administration resulted in decreased body weight gain and food consumption, and that cessation from nicotine was associated with a return of these variables to control levels. Further, when the effects of time were accounted for, the magnitude of the effect was similar across three cycles of administration and cessation comparing animals of two different ages. To the extent that these results generalize to humans, they suggest that the typical smoker could start and stop smoking several times without experiencing any long-term physiological changes that would make future weight loss more difficult. Further, given the remarkable stability of the amount lost and regained across cycles, these results suggest that for any given individual, the best predictor of future weight gain upon quitting smoking is the amount gained during the last quit attempt.

ACKNOWLEDGEMENTS

Support for this study was received from a Centers of Excellence grant awarded to the Department of Psychology, Memphis State University, by the state of Tennessee. This study also received support from National Heart, Lung, and Blood Institute grant No. HL-39332. The authors would like to acknowledge Juan Henderson and Racheal Qualls for aid in conduct of the study. In addition, the authors thank Linda Eck, Jean Edgar, Bob Klesges, and Guy Mittleman for their editorial comments.

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