Plasma Corticosterone Response to Chronic Ethanol Consumption and Exercise Stress

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Abstract. **Acute exposure to ethanol induces a stress response in mice that is manifested by increased plasma corticosterone (PC) concentration. However, during chronic intake of 7.5% w/v ethanol, diurnal fluctuation of PC is dampened. Whether chronic consumption of 20% w/v ethanol alters normal diurnal fluctuation of plasma glucocorticoids is not known. Investigating the PC response in 20% w/v ethanol-consuming mice is of interest because glucocorticoids are known suppressants of natural killer (NK) cell activity and increased concentration or altered diurnal fluctuation of PC may have a modulatory role on NK cells in these mice. Mice given 20% w/v ethanol for at least 7 days and for as long as 10 weeks have suppressed splenic NK cell cytolytic activity. Thus, the purpose of this study was to examine whether mice given 20% w/v ethanol exhibited normal concentrations and diurnal variation of PC. To further define the glucocorticoid response in chronic ethanol-consuming mice, PC concentration was evaluated in response to a secondary stress of physical exercise. After 1 week, ethanol-consuming mice exhibited abnormal diurnal PC periodicity that was progressively dampened during the remaining 9 weeks. Acute physical exercise during Week 1 induced a 2-fold increase in PC concentration compared with pre-exercise values, a response that was independent of ethanol intake. After 6 and 10 weeks, the postexercise PC concentration was attenuated in ethanol-consuming compared with water-drinking mice. It was concluded that suppressed NK cell activity typically observed with this model of chronic ethanol intake is not directly associated with dampened diurnal fluctuation in PC.** [P.S.E.B.M. 1993, Vol 204]

Ethanol given in the drinking water as the sole fluid source is considered stressful to mice (1). Increases in plasma corticosterone (PC) concentration with acute ethanol exposure are strain, time, and dose dependent (2–4). However, mice are capable of developing ethanol tolerance (5, 6) and exhibiting a dampening of PC periodicity with prolonged consumption of 7.5% w/v ethanol (7).

Altered PC concentration in ethanol-consuming mice may be immunomodulatory and this response may be related to the suppression of natural killer (NK) cell activity in mice that chronically consume 20% w/v ethanol (8–11). Therefore, the present study was undertaken to investigate the corticosterone response in mice consuming 20% w/v ethanol for 10 weeks.

To further characterize the glucocorticoid response in these mice, PC concentration was evaluated in response to a secondary stress of physical exercise. Plasma corticosterone concentration increases with physical stress in human subjects (12, 13) and in rodents (14). Treadmill exercise may also elicit exaggerated increases in PC due to psychologic responses to forced exercise stress (15, 16). Chronic exercise stress is associated with adaptation of the hypothalamus-pituitary axis and is reflected by diminished PC concentration after exercise (17, 18). However, it is not known whether ethanol-tolerant mice exhibit an attenuated PC response to an acute exercise stress or whether the glucocorticoid response in ethanol-consuming mice is capable of adapting to chronic exercise stress.

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Materials and Methods

Animals and Housing. Specific pathogen-free mice (C57BL/6J, female, 7–8 weeks old; Jackson Laboratories, Bar Harbor, ME) were group housed (n = 10/cage) upon arrival to the vivarium. Mice were kept in separate quarters maintained at a constant temperature of 23–24°C, 20% humidity, and a 12:12-hr photoperiod with the light cycle beginning at 1200 hr. Pelleted rodent chow (Purina 5001) and water were given *ad libitum*. After 1 week, mice were individually housed and given an additional week to acclimate before assignment to groups. Mice were matched by body weight and assigned to one of four groups: sedentary, water-drinking (SED-H_2O, n = 40); sedentary, ethanol-consuming (SED-ETOH, n = 40); trained, water-drinking (TR-H_2O, n = 20); and trained, ethanol-consuming (TR-ETOH, n = 20). Unequal-sized groups were established to accommodate the differences in blood sampling between SED and TR groups as described below. An additional five mice were group housed and used as controls for anesthesia exposure. Sedentary groups were exposed to normal handling associated with weekly cage cleaning and body weight measures. Ethanol-consuming groups were fed 20% w/v ethanol in the drinking water as the sole fluid source. Body weight and food intake were measured at least biweekly.

Treadmill Exercise. Trained mice exercised on a standard rodent treadmill without electrical stimulation as described previously (11). Exercise occurred at 1350 hr in the same quarters where the mice were housed. Nearly all of the mice were awake before the initiation of exercise. Training was not conducted during the dark cycle of the photoperiod because in the initial weeks of treatment, ETOH mice may be inebriated during the dark cycle and incapable of exercising (11). Treadmill speed and duration of exercise were progressively increased over the first 2 weeks (5 days$\cdot$wk^{-1}). Thereafter, mice ran at a speed of 12 m$\cdot$min^{-1} on an 8° incline for 60 min$\cdot$day^{-1}. During the first 2 weeks of training, some of the TR-ETOH mice (n = 2–3) were inebriated and unable to complete the exercise sessions. This occurrence was infrequent (1–2 times/week) and, subsequently, all of the mice were capable of completing exercise.

Collection of Blood Samples. Blood samples were collected over a 24-hr period at the end of Weeks 1, 2, 6, and 10. Physiologic variables were assessed after 1 week (SED only), after 2 weeks when TR mice achieved the desired training speed and duration, and at monthly intervals. Samples were drawn from five mice per group at 100 hr, 700 hr, 1300 hr (pre-exercise), 1450 hr (postexercise, TR only), and 1900 hr (SED only). Blood samples were collected every 6 hr from SED mice beginning 1 hr into the dark cycle of the photoperiod. Because TR mice were bled immediately after exercise, the 1900-hr measure was omitted from this group. An individual mouse was never bled more than once per month. Because blood samples were drawn from SED mice at the end of Weeks 1 and 2, an additional 20 mice were assigned to these groups. This could not be done in TR groups as the capacity of the treadmill was limited to 40 mice. Inclusion of extra mice in these groups would necessitate an additional exercise session each day, which was not desirable. Blood samples were drawn from the retro-orbital venous plexus into heparinized microcapillary tubes and collected into microcentrifuge tubes 15 sec after each mouse was exposed to methoxyflurane anesthesia and within 1 min of cage disturbance. Blood was kept on ice until centrifuged at 350*g* at 4°C. The plasma fraction was removed and stored for 7 to 14 days at −15°C until analyzed for PC and blood ethanol concentration (BEC).

Hematological Assays. Plasma corticosterone concentration was determined using a standardized ^{125}I corticosterone radioimmunoassay (ICN Biomedical, Inc., Costa Mesa, CA) specific for laboratory mouse and rat serum or plasma total corticosterone. BEC was determined spectrophotometrically (alcohol analytical kit 322-UV; Sigma, St. Louis, MO). To account for potential changes in plasma volume with exercise and/or ethanol intake, packed cell volume (hematocrit) was determined from a 15-μl aliquot from each whole blood sample using a Clay Adams 1000 hematocrit capillary tube reader.

Control Groups for Anesthesia and Treadmill Exposure. During Week 10 (1300 hr), five mice were sacrificed by cervical dislocation and blood was collected via cardiac puncture. The procedure was accomplished in less than 90 sec after removing the mouse from its cage.

To examine the glucocorticoid response in mice exposed to treadmill noise and vibration, an additional experiment was conducted. Mice (SED, n = 10; treadmill control [TR-CON], n = 20) were housed as described above. Treadmill exposure occurred during the same period of the photocycle as exercise for TR mice. TR-CON mice were placed on the treadmill for 60 min$\cdot$day^{-1}, 5 days$\cdot$wk^{-1}, for 6 weeks. Treadmill belt speed was minimal, 1–2 m$\cdot$min^{-1}. At the end of Weeks 2 and 6, blood samples were collected as described above, from SED mice and from TR-CON mice (n = 10) at 1300 hr (pretreadmill exposure for TR-CON group). The remaining TR-CON mice were bled immediately after treadmill exposure.

Statistical Analyses. For the diurnal study, comparisons among values for PC, hematocrit, and BEC were determined by general linear model multivariate analyses, repeated-measures design. Descriptive variables (average daily food intake, ethanol intake, caloric intake, and body weights) were compared by general linear model multivariate analyses of variance, 2 × 2 factorial design. The influence of body weight on postexercise PC was determined by two-factor, analysis of covariance design using SuperAnova Statistical Package for personal computers (Abacus Concepts, Inc., Berkeley, CA). Differences between groups (sedentary versus trained) and treatments (water versus ethanol) were determined posthoc using Fisher's (protected) least significant difference test. The relationship between selected variables was determined from Pearson product moment correlation coefficients. Data from the treadmill exposure experiment were analyzed by two-factor analysis of variance. Average values were considered significantly different at $P < 0.05$.

Results

Nutritional Indices. Body weight and caloric intake are often used as gross indicators of nutritional status in laboratory mice. Body weight in SED-H_2O mice was relatively stable during the initial 2 weeks of treatment (Table I) and increased thereafter. This trend was not substantially changed by ethanol intake in SED mice or by training in H_2O mice. Body weight for TR-ETOH mice was significantly lower than that for the other groups after Weeks 1 and 2. Body weight gains in TR-ETOH mice during the remaining 8 weeks were similar to SED-H_2O mice. TR-ETOH mice were not able to recover initial body weight losses by Week 10.

Food intake for SED-H_2O mice was 14.6 ± 0.3 Cal·day^{-1} (mean ± SE, Table II), which was within the normal range reported by our laboratory (8–11, 19, 20). Physical training by H_2O mice did not effect caloric intake (Table II), which supports previous work (11). Food intake by ETOH mice was approximately 70% of the food calories consumed by SED-H_2O mice, a result that is observed with long-term administration of this model (11). Supplemental calories provided by ethanol contribute to increased body weight and body lipid content compared with SED-H_2O mice, and in this model, ETOH mice are not malnourished (11). Ethanol intake was significantly lower (approximately 13%) in TR-ETOH compared with SED-ETOH mice. This difference was negated when ethanol intake was expressed as grams of ethanol per kilogram of body weight (Table II). Food intake and body weight gains (8.6 ± 3.6 g/6 weeks) were not different between SED and TR-CON groups.

Blood Ethanol Concentration and Hematocrit. Blood ethanol concentration was highly variable, fluctuating within groups and across sampling times. Training had no effect on BEC throughout the experimental treatment. After the second week of ethanol intake, peak concentrations were observed during the dark cycle of the photoperiod (700 hr, 17.6 ± 3.7 m*M*) and the lowest concentrations occurred during the light cycle (1300 and 1450 hr, 5.4 ± 2.2 m*M*). Blood ethanol concentrations were progressively reduced with continued ethanol intake. At the end of Week 10, BEC values ranged from undetectable to 7.5 ± 1.3 m*M*. Hematocrit (52.3 ± 0.1%) was not significantly altered by training, treadmill exposure, or by ethanol intake.

Effect of Anesthesia and Treadmill Exposure on PC. Plasma corticosterone concentration did not differ between samples obtained by cardiac puncture (379.56 ± 144.21 n*M*, SED group) and retro-orbital puncture (341.83 ± 36.35 n*M*, SED group). Measures of PC were not affected by the time required to obtain blood samples ($F_{1,265} = 2.28$, $P = 0.13$). Rather, PC values were significantly affected by the hour at which sampling occurred ($F_{4,265} = 46.34$, $P = 0.0001$).

In the treadmill control experiment, plasma corticosterone concentration, independent of group, was significantly lower at Week 6 compared with Week 2 ($F_{1,54} = 17.14$, $P = 0.0001$), which may indicate accommodation to handling. There were no significant differences in PC values for group ($F_{2,54} = 2.80$, $P = 0.07$) or group × time interaction ($F_{2,54} = 0.62$, $P = 0.54$). At Weeks 2 and 6, PC concentration at 1300 hr was 262.2 ± 81.3 n*M* and 70.7 ± 31.0 n*M* for SED mice and 179.8 ± 52.2 n*M* and 41.1 ± 28.4 n*M* for TR-CON mice, respectively. Treadmill exposure increased PC concentration in TR-CON mice compared with the other groups at Week 2 (384.2 ± 96.2 n*M*) and Week 6 (114.7 ± 24.9 n*M*); however, these differences were not significant.

Diurnal Variation of Plasma Corticosterone Concentration. In laboratory rodents, diurnal variation of PC is primarily synchronized by the photocycle (21, 22). The highest concentrations of PC typically occur

Table I. Body Weight (g) Changes During 10 Weeks of Chronic Ethanol Intake and Exercise Stress^{a}

Group	Initial	Week 1	% Change (0 to Wk 1)	Week 2	% Change (1 to Wk 2)	Week 6	% Change (2 to Wk 6)	Week 10	% Change (6 to 10 Wk)
SED-H_2O	17.3 ± 0.2	17.4 ± 0.2	0.6	17.1 ± 0.2	−1.7	19.8 ± 0.3	15.8	20.6 ± 0.2	4.0
SED-ETOH	17.4 ± 0.1	17.0 ± 0.2	−2.3	16.9 ± 0.2	−0.6	20.4 ± 0.2	20.7	20.6 ± 0.2	1.0
TR-H_2O	17.4 ± 0.2	17.2 ± 0.2	−1.1	17.5 ± 0.3	1.7	19.5 ± 0.3	11.4	20.6 ± 0.3	5.6
TR-ETOH	17.3 ± 0.2	16.0 ± 0.2^{b,c}	−7.5	16.2 ± 0.3^{b,c}	1.3	18.6 ± 0.3^{b,c}	14.8	19.6 ± 0.4^{b,c}	5.4

[a] Values are means ± SE.

[b] Significantly different from H_2O counterpart ($P < 0.05$) within-week comparison.

[c] Significantly different from SED counterparts ($P < 0.05$) within-week comparison.

Table II. Average Daily Food and Ethanol Intake^{a}

Group	Week 1	Week 2	Week 6	Week 10
Food (cal · day^{-1})				
SED-H_2O	14.6 ± 0.2	14.8 ± 0.3	14.0 ± 0.1	15.0 ± 0.3
SED-ETOH	10.2 ± 0.2^{b}	10.8 ± 0.3^{b}	10.8 ± 0.1^{b}	10.6 ± 0.1^{b}
TR-H_2O	15.7 ± 0.2	15.4 ± 0.1	14.9 ± 0.1	15.0 ± 0.1
TR-ETOH	9.3 ± 0.3^{b,d}	10.4 ± 0.3^{b,d}	10.3 ± 0.1$^{b\text{–}d}$	10.2 ± 0.1^{b,c}
Total (cal · day^{-1})				
SED-H_2O	14.6 ± 0.2	14.8 ± 0.3	14.0 ± 0.1	15.0 ± 0.3
SED-ETOH	14.5 ± 0.3	15.2 ± 0.4	16.1 ± 0.3^{b}	15.9 ± 0.3^{b}
TR-H_2O	15.7 ± 0.2	15.4 ± 0.1	14.9 ± 0.1	15.0 ± 0.1
TR-ETOH	13.3 ± 0.4$^{b\text{–}d}$	14.4 ± 0.3^{b,c}	14.7 ± 0.3^{b,c}	14.9 ± 0.3^{c}
Ethanol (cal · day^{-1})				
SED-ETOH	4.3 ± 0.1	4.8 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
TR-ETOH	4.0 ± 0.2	4.2 ± 0.1^{c}	4.6 ± 0.1^{c}	4.7 ± 0.1^{c}
Ethanol (g · kg^{-1} · day^{-1})				
SED-ETOH	36.2 ± 1.0	40.2 ± 1.2	36.7 ± 0.6	36.2 ± 0.7
TR-ETOH	35.8 ± 1.6	37.0 ± 1.3	35.1 ± 1.1	34.0 ± 0.8

[a] Values are means ± SE.
[b] Significantly different from H_2O counterpart ($P < 0.05$) within-week comparison.
[c] Significantly different from SED counterparts ($P < 0.05$) within-week comparison.
[d] Significantly different from SED-H_2O control ($P < 0.05$).

just before waking (dark cycle) and the lowest PC concentrations are observed during the early hours of sleep (light cycle). These changes, reflect the average of a series of intermittent pulsital changes in PC concentrations during the photocycle (23, 24). In this experiment, diurnal variation of PC was considered normal if average group values were significantly different between 100 hr and 1300 hr. After 1 week, SED-H_2O mice exhibited normal PC periodicity (Fig. 1). Peak values occurred during the first hour of the dark cycle (100 hr) and significantly lower PC concentrations were observed during the first hour of the light cycle (1300 hr). Ethanol consumption disrupted this pattern. The lowest PC concentration was observed in SED-ETOH mice at the beginning of the dark cycle (100 hr). During the remaining hours of the photoperiod, PC concentration was highly variable.

Normal PC diurnal variation was maintained in SED-H_2O mice at Week 2 (Fig. 2) and for the remainder of the study (Fig. 3). Diurnal variation in PC concentration appeared normal in the SED-ETOH group at Week 2 (Fig. 2). However, values were not significantly different among time points due to the large within-group variability. At Weeks 6 and 10, SED-ETOH mice exhibited a dampening in PC diurnal variation (Fig. 3, 6-week data not shown).

Training, independent of ethanol intake, altered PC periodicity at Week 2 (Fig. 2). The lowest PC

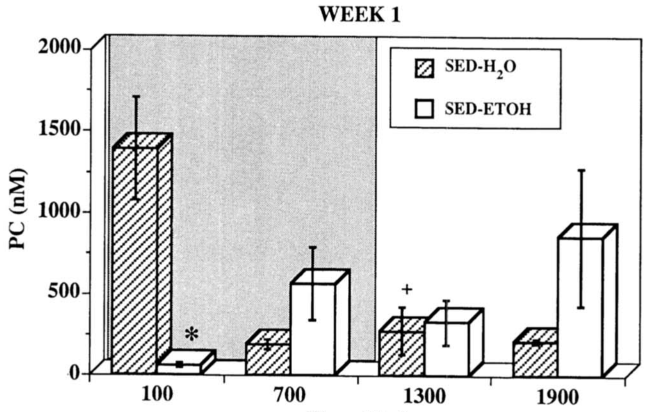

Figure 1. Diurnal variation of PC concentration at Week 1 in sedentary, water-drinking, and ethanol-consuming mice. Values are means ± SE. The shaded and light backgrounds represent the dark and light cycles of the photoperiod, respectively. *Significantly different from SED-H_2O ($P < 0.05$). †Significantly different from 100-hr value within group ($P < 0.05$).

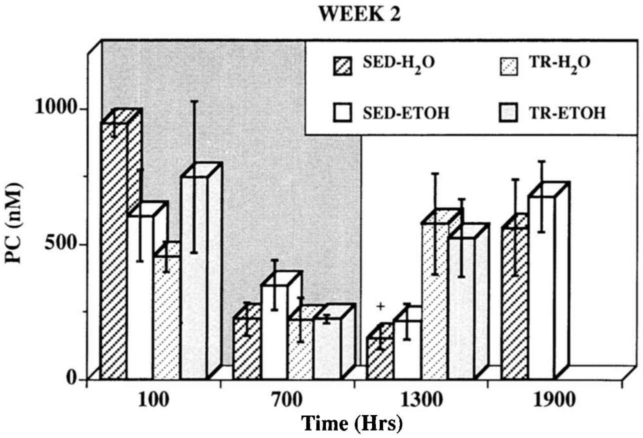

Figure 2. Diurnal variation of PC concentration at Week 2 in sedentary and endurance-trained, water-drinking, and ethanol-consuming mice. The shaded and light backgrounds represent the dark and light cycles of the photoperiod, respectively. Values are means ± SE. †Significantly different from 100-hr value within group ($P < 0.05$).

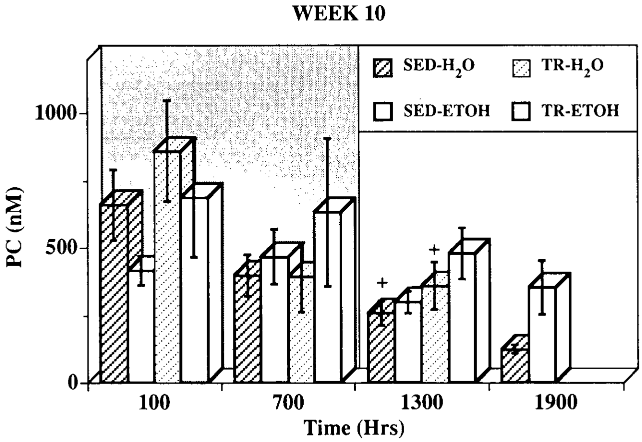

Figure 3. Diurnal variation of PC concentration at Week 10 in sedentary and endurance-trained, water-drinking, and ethanol-consuming mice. The shaded and light backgrounds represent the dark and light cycles of the photoperiod, respectively. Values are means ± SE. †Significantly different from 100-hr value within group ($P < 0.05$).

concentrations were observed in TR mice during the dark cycle (700 hr) rather than at 1300 hr, as occurred in SED mice. Peak PC concentrations were expected at 100 hr; however, in TR mice, PC values at 100 hr and 1300 hr were not significantly different. Immediately before the training session (pre-exercise, 1300 hr), there was a trend for higher PC concentrations in TR (546.15 ± 112.40 n*M*) compared with SED mice (182.53 ± 37.78 n*M*; $F_{3,15} = 2.75$, $P = 0.08$).

Normal diurnal variation of PC was established in TR-H_2O mice by the end of the sixth week of training (data not shown). This is in agreement with the work of Kreiger indicating that diurnal variation of animals is not altered if the stressor is imposed at the same time of day, and the duration of the stress period remains constant (24). At 1300 hr, plasma corticosterone concentration was significantly lower than values obtained at 100 hr, and periodicity was maintained through the 10th week of training in TR-H_2O mice (Fig. 3). Prolonged training (Weeks 6–10) by TR-ETOH mice also appeared to attenuate the dampening effect of ethanol intake on PC diurnal variation. A consistent pattern of diurnal variation was not attained in TR-ETOH mice. Moreover, by Week 10, these mice displayed the largest within-group variability (Fig. 3).

Plasma Corticosterone Response to Acute and Chronic Exercise. After 2 weeks of training, acute physical exercise elicited a 2-fold increase in PC concentration compared with pre-exercise values and this response was independent of ethanol intake (Table III). Although postexercise PC values tended to increase over the course of training, these changes were not statistically significant. Body weight was identified as a significant covariate with treatment (H_2O versus ETOH) and time (2 vs 6 vs 10 weeks) effects for postexercise PC values ($F_{1,16} = 15.3$, $P = 0.001$). Body weight of TR mice was weakly correlated with postexercise PC concentration ($r = 0.82$, $P = 0.04$). Postexercise PC values were adjusted for the effects of the covariate ($F_{11,16} = 12.53$, $P = 0.0001$, $r = 0.95$ for the model). Significant treatment ($F_{1,22} = 13.16$, $P = 0.0015$) and time effects ($F_{2,22} = 48.82$, $P = 0.0001$) were observed for the adjusted postexercise PC values. Postexercise PC values were significantly higher in H_2O compared with ETOH mice. Adjusted values at Week 10 were also significantly greater than 2- and 6-week values. Comparing postexercise to pre-exercise values (% change) indicated that at Weeks 6 and 10, respectively, increases in PC concentration were approximately 3.6 and 1.5 times greater in TR-H_2O compared with TR-ETOH mice (Table III). Pre-exercise (100 hr) PC values tended to decrease over the 10 weeks of training. Although not statistically significant, these changes may reflect an accommodation to handling stresses.

Discussion

The present data indicate that chronic intake of 20% w/v ethanol results in a flattening of the diurnal variation of PC in mice. Defining PC diurnal fluctuation in this model is of interest because of the modulatory role that glucocorticoids have on NK cells. Although transient increases in PC concentration do not necessarily impair NK cell function (25), cytolytic activity is inhibited during continuous exposure to both subphysiologic and physiologic concentrations of glucocorticoids *in vitro* (26–28). It is unknown whether abnormal diurnal PC variation is associated with suppressed NK cell activity. Splenic NK cell cytolytic activity is not suppressed in mice fed 10% w/v ethanol for 4 weeks (29). This concentration of ethanol lies between those (7.5–20% w/v) that induce dampening of PC periodicity. Thus, it is unlikely that dampened PC periodicity in mice consuming 20% w/v ethanol is directly associated with suppressed NK cell cytolytic activity (8–11).

Forced exercise was chosen as a secondary stressor because treadmill exposure in the absence of exercise, elicits a nonsignificant increase in PC concentration (1.5- to 2-fold) in mice that do not have suppressed splenic NK cell activity (11). The glucocorticoid response to physical exercise in rodents and human subjects is related to the intensity and duration of the exercise stress (12, 13, 30–33). Plasma concentrations of glucocorticoids are incrementally increased with graded physical exercise (12, 13, 33). Prolonged submaximal exercise can also elicit significant increases in PC concentration which is proportional to the duration and intensity of exercise (31, 32). Exponential increases in plasma glucocorticoid concentration are observed in response to exercise intensities that exceed 50% of the subject's maximal aerobic power (VO_2max) (31). Ad-

Table III. Changes in Plasma Corticosterone Concentration in Response to Treadmill Exercise[a]

Week	TR-H_2O			TR-ETOH		
	Pre-exercise (nM)	Postexercise (nM)	Change (%)	Pre-exercise (nM)	Postexercise (nM)	Change (%)
2	572.7 ± 188.8	1149.0 ± 200.2[b]	100.6	519.6 ± 144.4	1126.9 ± 120.6[b]	116.9
6	149.1 ± 25.7	1422.9 ± 107.7[b]	854.4	343.4 ± 69.0	1138.4 ± 58.7[b]	231.4
10	358.1 ± 87.9	1912.0 ± 354.7[b]	433.9	480.6 ± 94.6	1884.4 ± 102.4[b]	292.1

[a] Pre- and postexercise values are means ± SE for trained (TR), water-drinking (H_2O, $n = 5$/group) and ethanol-consuming (ETOH, $n = 5$/group) mice. Within weeks, pre-exercise and postexercise values were not significantly different between groups.

[b] Significantly different from pre-exercise values ($P < 0.05$).

aptation to exercise stress is normally observed with endurance training and glucocorticoid concentrations are reduced at absolute and relative power outputs compared with pretraining levels (32, 34, 35).

Mice in this study did not exhibit adaptation of the glucocorticoid response to physical exercise. Body weight of TR mice increased approximately 20% during 10 weeks of training. Therefore, the absolute work load and the physiologic stress of exercise increased with body weight gain. This training model is associated with a 35–45% increase in maximal citrate synthase activity of the quadriceps femoris muscle group, which is indicative of significantly enhanced aerobic capacity of trained skeletal muscle (11). Peripheral adaptations in skeletal muscle, such as these, are typically observed with 10 weeks of endurance training and are in excess of changes observed in whole body maximal oxygen consumption (36). Therefore, it is doubtful that the mice achieved a 20% increase in VO_2max with training, and relative work load (% VO_2max) was also increased with body weight gains. The adjusted scores verify that TR mice did not exhibit adaptation in the glucocorticoid response to exercise stress. However, TR-ETOH mice did exhibit an attenuated postexercise glucocorticoid response. Attenuated PC response in chronic ethanol-consuming rodents is commonly manifested with acute test doses of ethanol (3, 4, 6) and in ethanol pretreated animals exposed to secondary stressors (37, 38). Overall, these findings indicate that after 10 weeks of training, female C57BL/6J mice do not demonstrate an adaptation of the glucocorticoid response to 60 min of forced treadmill exercise. Significant increases in postexercise PC concentration were perhaps associated with psychological stress.

In conclusion, 10 weeks of chronic 20% w/v ethanol did not produce sustained increases in PC in mice. Rather, ethanol-consuming mice exhibited a dampened diurnal fluctuation in PC and an attenuated PC response to a second stressor. These findings extend the understanding of the biologic response to chronic ethanol intake in our model. Furthermore, it appears that suppressed NK cell activity typically observed in this model, is not directly associated with abnormal diurnal fluctuation in PC concentration.

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