EXAMINING THE EFFECTS OF EXERCISE-INDUCED, PHYSICAL OVERSTRESS ON STRESS BIOMARKERS IN ADOLESCENT, C57BL/6 MICE

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Abstract

BACKGROUND: Exercise is known to improve fitness, sports performance, and injury/ health risk reduction. Conversely, exercise overstress (i.e. overtraining syndrome) has shown inverse outcomes in both athletes and general populations. Youth and adolescent training programs have increased exponentially prompting the NSCA to develop the Long-Term Athletic Development Model. Still, there remains a lack of research investigating the negative effects of exercise overstress in adolescents. PURPOSE: The objective of this study was to examine hormone biomarkers, anthropometrics, and behavioral traits associated with exercise overstress, in adolescent C57BL/6 mice. METHODS: Twenty-four adolescent mice, aged 4 to 5 weeks, were randomly assigned to one of three groups: Sedentary Group (SG), Moderate-trained Exercise Group (MEG), and Overtrained Exercise Group (OEG) during an 8-week intervention. An incremental load test was performed at baseline to determine exhaustion velocity (EV) for the MEG and OEG. MEG performed exercise at 60% EV with progressive duration between 15-60 minutes over 8 weeks. The OEG performed downhill running progressing from 60-90% EV and 15-90 minutes over 8 weeks. The SG did not participate in any exercise outside of normal cage activity. Corticosterone, IGF-1, and body composition were conducted at baseline, mid, and post-intervention along with behavioral measures of food consumption, edge and center time. Measures of central tendencies and percentages were used for demographics within and between groups. ANOVA and MANOVA with repeated measures were used to determine if interactions occurred within and between groups for all pre, mid and post intervention data. RESULTS: There were no significant differences in baseline hormone concentrations, food consumption, edge or center time between groups. There was a significant difference between OEG and SG for lean mass (p=0.034059). Following intervention, significant differences were found in plasma corticosterone concentrations across time between OEG and SG (p=0.0022) and OEG and MEG (p=0.0098), respectively with the OEG displaying the highest levels. No significant differences were found in IGF-1 results among groups across time (p=0.9754); however, all groups decreased in IGF-1 levels across time (p=0.0168). All groups significantly increased body weight across time (p<0.0001) with the OEG gaining the least (p=.73). Also, all groups decreased percent fat mass over time (p<0.0001). Behavioral results showed food consumption was significantly greater in the OEG group compared to MEG and SG (p<0.0001) between weeks 4-8. A significant difference was found in edge time between OEG and SG across time (p= 0.0223) but not between OEG and MEG (p=.76). There was no significant difference among groups for center time (p=0.0651). CONCLUSION: Results indicate that exercise overstress in adolescent mice demonstrate negative physiological and behavioral outcomes. Further research is needed to determine which biomarkers can be practically assessed to recognize and reduce potential overtraining and their relevant implications to physicians, coaches, parents, and exercise specialists. PRACTICAL APPLICATIONS: While biomarkers can confirm maladaptation to training stress, feeding and behavior modifications may be practical indicators to observe in adolescent exercise programs to avoid chronic exercise overstress and potential risk of growth rate consequences and injury.

Background

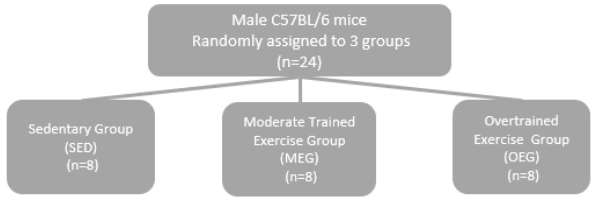
- Overtraining syndrome (OTS) can be defined as a physiological state in which an individual is unable to adapt/recover from training loads that result in decreases in performance as well as negative effects on health.
- Symptoms of OTS include fatigue, depression, bradycardia, “loss of motivation”, insomnia, irritability, agitation, tachycardia, hypertension, restlessness, anorexia, weight loss, “lack of mental concentration”, “heavy, sore/ stiff muscles”, anxiety, and awakening “unrefreshed”.
- Greater than 30% of youth and high school aged athletes have been determined as overtrained with more than 60% of OTS reported in elite runners.
- Youth and adolescent training programs have increased exponentially leading to increased awareness of avoiding exercise over-stressors.
- The NSCA developed the Long-Term Athletic Development Model to combat such exercise overstress behaviors to sustain fun and quality function in youth and adolescents.
- Still, there remains a lack of research investigating the negative effects of exercise overstress in adolescents.

Objective

- The objective of this study was to examine hormone biomarkers, anthropometrics, and behavioral traits associated with exercise overstress, in adolescent C57BL/6 mice.

Methods

- This study utilized a three-group design consisting of an animal-based model for exercise testing and implementation.
- The Study was approved by the Institutional Animal Care and Use Committee (IACUC) of Marshall University
- ***Inclusion Criteria***: Adolescent C57BL/6 male wild type mice aged 4-5 weeks old at the start of the study and appearing physically healthy.
- ***Exclusion Criteria***: Mice that have any noticeable physical deformities or physical signs of disease or illness, mice that refuse to exercise or are unable to perform experimental procedures or mice that develop diseases, health concerns or signs of distress.



Methods

Exercise Assessment & Protocol

- All mice were acclimatized to the Exer-3/6 animal treadmill for 3 days.
- The MEG and OEG underwent an incremental load and exhaustion velocity (EV) test protocol (Pereira et al., (2012)).
- EV = started at 6 m/min with 0% grade increasing speed by 3 m/min every 3 minutes until mice stopped at the end plate five consecutive times in one minute.
- Manual prodding with the use of dull forceps was utilized to encourage the mice to run.

	MEG (n=8)	OEG (n=8)	SED (n=8)
Intensity	60% EV at 0% incline	60% EV at 0% incline (weeks 1-5) 75% EV at -14% incline (week 6) 90% EV at -14% incline (week 7-8)	No exercise outside of their normal physically active behaviors
Frequency	1 session per day 5 days per week	1 session per day (week 1-7) *2 sessions per day (week 8) 5 days per week	
Duration	Week 1: 15 minutes Week 2: 30 minutes Week 3: 45 minutes Week 4-8: 60 minutes	Week 1: 15 minutes Week 2: 30 minutes Week 3: 45 minutes Week 4-5: 60 minutes Week 6: 75 minutes *Week 7-8: 90 minutes	

Data Collection

- Blood collection for Corticosterone & IGF-1 (ELISA), body composition (EchoMRI™-100H), and behavior testing (open field motion capture (ANY- maze software and clear open-field ANY-maze container)) were performed at pre (baseline), mid (4-weeks), and post (8-weeks) study for all groups.
- Body weight (weight scale) and food consumption (weight scale) were measure daily.

Results

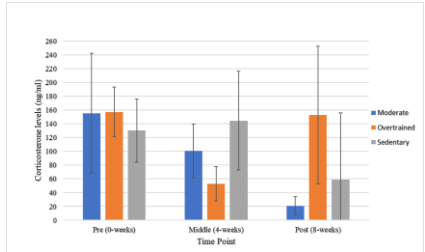
Table 1. One-way ANOVA Results for Comparison of Group Baseline (0-weeks) Assessment Variables

Baseline (0-weeks) Assessment variable	F-value	P-value	Significance Not significant= (NS) Significant = *
Corticosterone (ng/ml)	0.96138	0.398567	NS
IGF-1 (ng/ml)	0.49600	0.61592	NS
Body weight (g)	1.59738	0.226062	NS
Lean Mass (g)	1.07062	0.360781	NS
Fat Mass (g)	2.06111	0.152298	NS
Percent Lean Mass (%)	3.98695	0.034059	*
Percent Fat Mass (%)	0.297696	0.07274	NS
Edge Time (sec)	0.52837	0.597201	NS
Center Time (Sec)	3.31329	0.056152	NS

Note: Significant difference was only observed in % Lean mass, Post Hoc Tukey’s Method results found the difference to be significant between Overtrained and Sedentary groups.

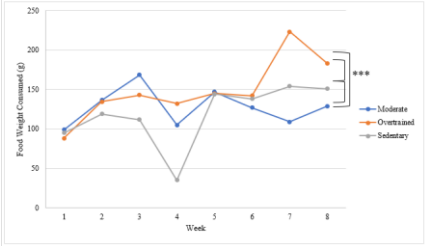
Results

Figure 1. **Corticosterone Levels Among Groups Over Time**



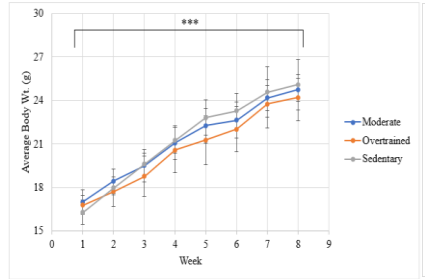
Note: Data includes mean ± S.D. Interaction between Group and time (p=0.0050). Overtrained vs Sedentary significance (p=0.0022). Moderate vs sedentary No significance (p<0.3670). Overtrained vs moderate trained significance (p=0.0098).

Figure 2. **Food Consumption Among Groups Over Time**



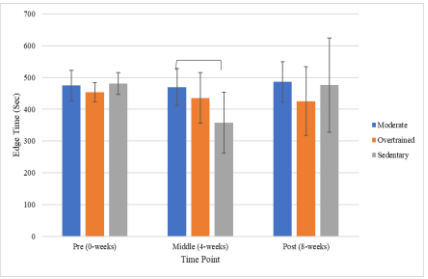
Note: Data includes a single measurement of food weight per group and does not include any mean or Standard deviations. There was an interaction in group by time (p<0.0001). Overtrained vs sedentary was significantly different across time (p<0.0001). Moderate trained vs sedentary was significantly different across time (p<0.0001). Overtrained vs moderate trained was significantly different across time (p<0.0001).

Figure 3. **Bodyweight Among Groups Over Time**



Note: Data includes mean ± SD. There was no interaction in group by time (p=0.1541). There was an interaction in time (weeks), (p<0.0001), and all groups increased body weight (g) over time. There was no significant difference among groups (p=0.389)

Figure 4. **Edge Time Among Groups Over Time**



Note: Data includes mean ± SD. There was a group-by-time interaction (p=0.0280). Overtrained vs Sedentary was significantly different (p=0.0223). Overtrained vs moderate trained was not significantly different (p=0.7607). Moderate trained vs sedentary was significantly different (p=0.0137).

Conclusions

- Corticosterone levels increased significantly from 4-8 weeks in the OEG compared to the MEG and SED. These last 4 weeks were the Overtraining protocol periods.
- On average, SED had the highest body weight throughout the study, and OEG had the lowest body weight however, lean mass was not significantly different across groups.
- Similar increases in food consumption between OEG and MEG from weeks 1-6, but drastically increased in OEG weeks 6-8 and decreased in MEG weeks 6-8 (contrasting with the literature).
- Non- significant differences were observed between OEG and MEG for both edge time and center time displaying similar stress related behaviors.

Future Considerations and Practical Applications

- Determining OTS markers and their roles in OTS remain complex and warrants further investigation.
- Longer term, reliable overstress protocols are needed to determine actual OTS and its associated changes in biomarkers and behaviors in the adolescent population.
- Physicians, Coaches and Parents should assess the symptomatology of OTS with behavior, body composition and eating changes in adolescents to help identify and reduce the risk of OTS .