Influence of intermittent anaerobic exercise on mouse physical endurance and antioxidant components

D Qiao, L Hou, X Liu

Objective: To determine the effect of intermittent anaerobic exercise on physical endurance, antioxidant capacity, and lipid peroxidation of brain, heart, and skeletal muscles in mice.

Methods: Mice were made to perform intermittent (with short or long rest intervals) anaerobic swimming on six consecutive days. Body weight was monitored. Tissue total antioxidant capacity (T-AOC), superoxide dismutase (SOD), and thiobarbituric acid reaction substance (TBARS) were determined on the 2nd, 4th, and 6th day. Physical endurance was determined on day 7 by using an exhaustive swimming test and a static grasping test.

Results: The intermittent anaerobic exercise resulted in decreased growth rate and physical endurance capacity, as indicated by less weight gain and shorter time to exhaustion during the exhaustive swimming and static grasping test (p<0.05). It also led to a higher T-AOC in muscle, heart, and brain, higher SOD activity in muscle and heart, and higher TBARS content in muscle (p<0.05). This type of exercise had no effect on brain SOD and TBARS. The changes in T-AOC in brain, muscle, and heart were all more pronounced the longer the experiment continued (p<0.05).

Conclusion: Intermittent anaerobic exercise reduced growth and physical endurance and increased tissue antioxidant capacity and lipid peroxidation.

MATERIALS AND METHODS

Experimental animals and swimming protocol

Five week male Kunming albino mice were purchased from the Experiment Animal Research Center (Shanghai, China). They were housed in groups of eight in metallic cages under standard conditions (mean (SD) temperature 24 (2)°C and humidity 50 (5)%). Two hours on the first day of the experiment, they were housed in a room with a 12 hour light/12 hour darkness cycle. The animals were fed with laboratory chow (Experiment Animal Research Center) and tap water ad libitum. All animal procedures were approved by the Shanxi University Animal Investigational Committee and performed in accordance with the Guide for the care and use of laboratory animals published by the Ministry of Health of the People's Republic of China. Ninety six mice were acclimatised for two days and then randomly assigned to one of three groups: anaerobic exercise with short (10 second) rest interval (AESI); anaerobic exercise with long (40 second) rest interval (AEILI); and control (CG). These groups were each subdivided into four subgroups: two days (Day2), four days (Day4), six days (Day6), and behaviour observation group (BG). There were 12 groups in total (eight mice in each group). The exercise protocol was implemented as described by Kawanaka et al.11

Swimming was performed in a glass tank (100 × 50 × 80 cm) filled with water to a height of 60 cm and at 30 (1)°C. All mice swam freely for 15 minutes a day (without any load) for two days so as to adapt to the swimming environment. Subsequently they were made to swim with a load of 10%.

Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; T-AOC, total antioxidant capacity; TBARS, thiobarbituric acid reaction substance.
Measurement of antioxidant systems and lipid peroxidation

Tissue antioxidant systems were evaluated by measuring Cu,Zn-SOD activity and total antioxidant capacity (T-AOC). Tissue lipid peroxidation was measured by determining the content of thiobarbituric acid reaction substance (TBARS). Mice in the exercise and respective control groups were killed by cervical dislocation on the 2nd, 4th, and 6th day of the swimming protocol. Brain (diencephalon), skeletal muscle (hind limb), and heart tissue were immediately excised. Size, colour, and texture of the tissues were observed. They were then trimmed in ice cold saline (0.85% (w/v) NaCl) and homogenized (10% (w/v) in 1.17% KCl in 0.10 M phosphate buffer, pH 7.4) using a glass Potter-type homogeniser at 500–800 rpm in ice. The homogenates were filtered through a muslin cloth and centrifuged at 20 000 × g for 30 minutes at 4°C. The supernatants were immediately collected for measurement of T-AOC, SOD activity, and TBARS and protein concentration.

SOD activity was measured using the nitroblue tetrizolium method described by Sun et al. One unit of SOD is defined as the amount of protein that inhibits the rate of nitroblue tetrazolium reduction by 50%. Data were expressed as U/mg protein.

T-AOC was measured using a kit (Nanjing JianchengBioengineering Institute, Nanjing, China) based on the method of Benzie and Strain with a minor modification. This assay measures the ferric reducing ability of the supernatant. The stable colour of the Fe³⁺-o-phenanthroline complex (produced by the reducing agents in plasma reducing Fe³⁺ to Fe²⁺, which reacts with the substrate o-phenanthroline) was measured at 520 nm. T-AOC was expressed in U/ml where 1 unit is defined as an increase in absorbance (A₅₃₀) of 0.01/min at 37°C.

TBARS concentration (an indicator of lipid peroxidation) was measured using the thiobarbituric acid method of Wasowicz et al using 1,1,3,3-tetramethoxypropane as standard. Data were expressed as nmol/mg protein.

Protein concentration in the supernatants was determined by the method of Bradford with bovine serum albumin as standard.

Observation of behaviour

Endurance capacities and static grasping tests of the BG group were assessed on the day after the last bout of swimming. An interval of one hour was left between the behavioural tests.

Static grasping test

The mice were put on a vertical, suspended glass bar (diameter 0.4 cm, length 20 cm). The mice grasped the glass bar and stayed motionless, so that their muscles were loaded with static stress. The grasping time for each mouse was recorded. The test was repeated three times with an interval of 10 minutes between each test.

Exhaustive swimming test

One hour after the static grasping test, each mouse was made to swim to exhaustion by attaching a weight equal to 7% of body mass to its tail. Exhaustion was defined, according to the criterion of Dawson and Horvath, as the point at which the mouse remained below the water surface for 10 seconds. The time to exhaustion (sinking) was recorded for each mouse.

Statistical analysis

Statistical analysis was performed using SPSS version 10.0 for windows. Body weight and data from the exhaustive swimming test and static grasping test were analysed by Student’s t test. SOD activity, T-AOC, and TBARS content were analysed by one way analysis of variance with Duncan’s multiple range tests. Significance was set at p<0.05. All data are presented as mean (SD).

RESULTS

Body weight changes, exercise capacity, and physical endurance

As shown in table 1, body weight was similar in all groups after the first day of swimming. Mice in the control group gained ~26% of body weight during the six day protocol. The body weight gain in mice in the AESI group was slightly lower than in that in the control group, but the difference was not statistically significant. However, mice in the AESI...
Table 3  Effect of intermittent anaerobic swimming on superoxide dismutase activity in various tissues of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain</th>
<th>Muscle</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>27.19 (3.48)</td>
<td>21.56 (2.51)</td>
<td>20.03 (2.90)</td>
</tr>
<tr>
<td>AESI-Day2</td>
<td>27.54 (2.96)</td>
<td>24.12 (3.39)</td>
<td>22.10 (3.49)</td>
</tr>
<tr>
<td>AESI-Day4</td>
<td>27.88 (1.97)</td>
<td>23.84 (3.53)</td>
<td>23.77 (2.59)**</td>
</tr>
<tr>
<td>AESI-Day6</td>
<td>27.68 (3.21)</td>
<td>25.09 (0.79)**</td>
<td>24.75 (2.06)**</td>
</tr>
<tr>
<td>AEI-Day2</td>
<td>27.54 (2.90)</td>
<td>24.88 (2.10)*</td>
<td>21.89 (2.21)</td>
</tr>
<tr>
<td>AEI-Day4</td>
<td>27.27 (1.97)</td>
<td>30.15 (2.51)**</td>
<td>23.37 (2.15)*</td>
</tr>
<tr>
<td>AEI-Day6</td>
<td>27.33 (2.70)</td>
<td>32.22 (3.18)**</td>
<td>23.67 (3.00)*</td>
</tr>
</tbody>
</table>

Values are mean (SD) (n = 8) expressed as U/mg tissue protein.
*p<0.05, **p<0.01, ***p<0.001 compared with control group (analysed by one way analysis of variance with Duncan’s multiple range test).

Group had significantly lower body weight than the control group after the fourth and sixth day, indicating slower body weight gain (~21%) over the six days. From day 3, animals in the AELI group swam for longer than those in the AESI group. By day 6, all the mice in the AELI groups had completed the exhaustive swimming procedures. One of the eight mice in the AEI group failed to complete the swimming procedures.

Table 2 shows the effect of intermittent anaerobic swimming on physical endurance, as evaluated by the exhaustive swimming and static grasping test. After the six days of intermittent anaerobic swimming, static grasping time had declined in both the BAELI group and the BAESI group comparing with the BCG group, although the changes did not reach statistical significance. The time to swim to exhaustion was significantly shorter in both the BAELI group and the BAESI group compared with the BCG group (both p<0.05) and significantly shorter in the BAESI group than in the BAELI group (p<0.05).

Interruption anaerobic swimming and tissue SOD activities
Table 3 summarises the effect of intermittent anaerobic swimming on SOD activity in brain, skeletal muscle, and heart. SOD activity in brain tissue was similar in all the groups. Intermittent anaerobic swimming resulted in higher SOD activity in both muscle and heart. The changes in SOD activity in skeletal muscle and heart were more pronounced after more days of exercise in both the AELI and AESI groups (all p<0.05).

Interruption anaerobic swimming and tissue T-AOC
After the various swim durations and intensities, T-AOC had increased in brain, muscle, and heart (table 4). In brain, the increase in T-AOC was more pronounced in the AESI groups than the AELI groups, but in muscle and heart the increase tended to be greater in the AELI groups. In all the AELI and AESI groups, T-AOC was greater after more days of exercise (all p<0.05, except the AESI group).

Interruption anaerobic swimming and tissue TBARS content
As shown in table 5, there was no change in brain TBARS content in any of the groups, indicating that intermittent anaerobic swimming does not affect brain TBARS. There was an increase in TBARS in both muscle and heart, although the increase in heart did not reach statistical significance. The intermittent anaerobic swimming induced increases in TBARS in muscle were more pronounced than in control groups (all p<0.05).

Discussion
Intermittent anaerobic swimming and weight gain and physical endurance
Mice gain body weight during their growing period. As expected, the 5 week old mice used in this study gained weight during the six days of experimental procedure. However, the weight gain in the swimming groups, especially the AESI groups, was less than in the control groups (p<0.05). The mice in the AESI groups had a shorter rest time and greater exercise intensity than those in the AELI groups. These results show that the intermittent anaerobic exercise decreased the growth rate. Inhibition of weight gain may result from exercise intensity and duration or frequency.

This study evaluated physical endurance after intermittent anaerobic exercise using the static grasping test and exhaustive swimming test. It is obvious that static grasping time and swimming time to exhaustion were reduced after six days of intermittent anaerobic swimming compared with the control group, although changes in the static grasping
Anaerobic exercise mediated changes in antioxidant capacity and lipid peroxidation were quite different in different tissues. Skeletal muscles are directly involved in anaerobic exercise with short rest intervals has a greater effect on tissue lipid peroxidation and the antioxidant defence system. Interestingly, our data also show that physical endurance and weight gain in the AESI groups were less than in the AELI groups. The data on muscle TBARS and physical endurance and weight gain are consistent. Reducing the rest interval or increasing the intensity of the intermittent anaerobic exercise maximises tissue lipid peroxidation, which overwhelms the compensatory antioxidant capacity, leading to lower physical endurance and even tissue damage. Therefore, during anaerobic exercise, the rest intervals need to be long enough to allow adaptation to oxidative stress and physical endurance.26

In this study, intermittent anaerobic swimming resulted in large increases in SOD activity and T-AOC (p<0.05) and modest increases in TBARS in heart. The altered pattern of heart T-AOC was different from that in skeletal muscle—that is, the increases in heart SOD and T-AOC were greater in the swimming groups with higher swimming intensity or longer swimming time. Kanter et al29 reported that lipid peroxidation increased proportionally with workload during treadmill running in humans. An acute bout of exercise is known to increase the activity of antioxidant enzymes, including catalase and glutathione peroxidases, in skeletal muscle and heart.26 Heart is an aerobic organ and has one of the highest rates of oxygen consumption in the body. It has four times less SOD activity than liver, and catalase activity is also extremely low.26 Therefore heart tissue may be more prone to peroxidative damage from oxidative stress. Anaerobic exercise is an effective method of improving anaerobic energy supply and heart function. The rest intervals during exhaustive exercise need to be long enough to gain maximum benefits from the exercise. In addition, supplementation with antioxidants during high intensity exercise may ameliorate

time did not reach statistical significance. Meanwhile, physical endurance in the AESI groups seemed to be inferior to that in the AELI groups. This suggests that anaerobic swimming with shorter rest intervals has a greater negative effect on physical endurance than anaerobic swimming with longer rest intervals. Wu et al20 reported that, after six days of brief, high intensity, intermittent exercise, rat serum pH was reduced, blood lactate was considerably increased, and liver and muscle glycogen were clearly decreased compared with a control group. We hypothesised that short rest interval high intensity exercise would induce lipid peroxidation in muscle tissues. Therefore we determined tissue lipid peroxidation and antioxidant capacity after intermittent anaerobic exercise.

**Intermittent anaerobic exercise and tissue lipid peroxidation and the antioxidant defence system**

Research on lipid peroxidation has been a major aspect of medical studies on free radicals. SOD, T-AOC, and TBARS are the main indices of oxygen free radical metabolism. SOD is the only enzyme of oxygen requiring species that uses oxygen free radicals as its substrate. The function of SOD is to catalyses the dismutation reaction of the toxic superoxide radical to hydrogen peroxide and to prevent production of hydroxyl radicals. SOD is one of the main antioxidant enzymes that degrade free radicals. TBARS reflects lipid peroxidation. Previous studies have shown that acute endurance exercise stimulates oxygen free radical production and increases TBARS content and lipid peroxidation, leading to exercise induced fatigue and a decline in physical capacity.21

Free radical metabolism is very active in brain. Brain has abundant unsaturated fatty acids, which are susceptible to lipid peroxidation. The influence of exercise induced oxidative stress on the central nervous system has been established.20–23 We found that, after intermittent anaerobic swimming, SOD activity and TBARS content of brain were not changed. This is consistent with the study of Cao et al22 who found that 90 minutes of swimming did not noticeably change TBARS generation and SOD activity in brain. However, brain T-AOC was considerably increased in our study, particularly after anaerobic swimming with shorter rest intervals. It is possible that high intensity swimming increases free radical generation, leading to an increase in antioxidant capacity.26 It is known that exercise alters the enzyme activity of the brain and promotes brain function,24 but little is known about how exercise affects brain function. It has been suggested that it increases capillary growth,25 improves cerebral circulation, and increases the antioxidant capacity of the central nervous system.21 However, the mechanisms whereby antioxidant enzyme activities are not known.

Table 5 Effect of intermittent anaerobic swimming on content of thiobarbituric acid reaction substance in various tissues of mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Brain</th>
<th>Muscle</th>
<th>Heart</th>
</tr>
</thead>
<tbody>
<tr>
<td>CG</td>
<td>4.05 (1.12)</td>
<td>2.09 (0.99)</td>
<td>2.91 (1.38)</td>
</tr>
<tr>
<td>AESI-Day2</td>
<td>4.15 (1.44)</td>
<td>3.73 (1.22)*</td>
<td>3.55 (1.33)</td>
</tr>
<tr>
<td>AESI-Day4</td>
<td>4.17 (0.96)</td>
<td>4.45 (1.20)**</td>
<td>4.01 (1.49)</td>
</tr>
<tr>
<td>AESI-Day6</td>
<td>4.23 (1.42)</td>
<td>4.55 (0.89)**</td>
<td>4.26 (1.70)</td>
</tr>
<tr>
<td>AEU-Day2</td>
<td>4.12 (1.39)</td>
<td>3.60 (0.18)***</td>
<td>3.12 (0.67)</td>
</tr>
<tr>
<td>AEU-Day4</td>
<td>4.06 (1.28)</td>
<td>3.47 (0.35)**</td>
<td>3.30 (0.61)</td>
</tr>
<tr>
<td>AEU-Day6</td>
<td>4.10 (0.97)</td>
<td>3.53 (0.45)***</td>
<td>3.23 (0.68)</td>
</tr>
</tbody>
</table>

Values are mean (SD) (n = 8) expressed as nmol/mg tissue protein. p<0.05, *p<0.01, **p<0.001 compared with control group (analysed by one way analysis of variance with Duncan’s multiple range test). CG, control group; AESI-Day2, AESI-Day4, AESI-Day6, two, four, or six days of anaerobic exercise with short interval; AELI-Day2, AELI-Day4, AELI-Day6, two, four, or six days of anaerobic exercise with long interval.
What is already known on this topic

- Increased oxygen consumption during exercise is accompanied by an increase in the production of reactive oxygen species (ROS), and when ROS production overwhelms the protection and repair mechanisms, the net effect is oxidative stress and oxidative damage to DNA, membrane lipids, and proteins.
- Endurance training generally increases both the activities and gene expression of several enzymatic and non-enzymatic antioxidants, but there are few data on the effects of short term maximal intensity anaerobic exercise.

What this study adds

- Intermittent anaerobic swimming in mice reduced weight gain, exercise capacity, and physical endurance and was associated with increases in tissue TBARS, especially in skeletal muscles.
- Long enough intervals are necessary during anaerobic exercise to avoid a reduction in physical endurance.

lipid peroxidative damage, as our study shows evidence of exercise induced oxidative stress.

It has been postulated that tissue lipid peroxidation contributes to reduced physical endurance—that is, static grasping time and swimming time to exhaustion. In this study, whereas grasping time and swimming time to exhaustion in mice had declined after intermittent anaerobic swimming, brain TBARS and SOD activity were not changed. This may indicate that the brain, or the central nervous system, was not involved in the anaerobic exercise mediated decrease in physical endurance. On the other hand, previously reported anaerobic exercise mediated changes in physical endurance and the muscle oxidant/antioxidant system (especially in skeletal muscles) are consistent with our study.

In summary, this study shows that intermittent anaerobic swimming in mice reduced weight gain, exercise capacity, and physical endurance. These changes were more pronounced with increased swimming intensity (or decreased rest interval). The decrease in physical endurance was associated with increases in tissue TBARS, especially in skeletal muscles. This suggests that the reduction in physical endurance induced by intermittent anaerobic swimming can be attributed to lipid peroxidation, especially in skeletal muscle and heart; long enough rest intervals are necessary during anaerobic exercise to avoid this reduction in physical endurance. Intermittent anaerobic swimming increases the antioxidant capacity of the central nervous system. However, the mechanisms by which antioxidant enzyme activity is increased are not known.

ACKNOWLEDGEMENTS

We thank Professors Enqi Weng and Yun Liu for technical assistance. This research was supported by grant no 30470832 from the National Natural Science Foundation of China and grant no 20051116 from the Science and Technology Foundation of Shanxi Province.

REFERENCES