



Swimming and running endurance capacity study in mice drinking water treated by ZF-10M Filter

TESTING FACILITY:

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PERFORMED FOR:

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SUMMARY

Forty C56 Black mice were randomly divided into 4 groups.

Group one and two were for running endurance study. In group one (n=10), all mice drank ZF-10M Filtered water. In group two (n=10), all mice drank Evian water. Group three, and four were for swimming endurance study. In group three (n=10), all mice drank ZF-10M Filtered water, In group four (n=10), all mice Evian water. There is a trend that mice drinking ZF-10M Filtered water has better running and swimming endurance than mice drinking Evian water.

Introduction

ZF-10M Filter uses high reactivity carbon mixture (nanocarbon the same as graphene) as a filtering media. This special water filter has been used in Russia for several years. The known function of this filter include: enhancing filtered water with iodine and potassium, removing arsenic and heavy metals etc. Recently, it has been empirically found that people drinking the filter water often experience increased endurance to physical exercise. Evian water has been deemed as the most pure drinking bottle water, hence has been very popular for years all over the world.

Objectives

The objective of this study is to compare the efficacy of water treated by ZF-10M filter and Evian water in swimming and running endurance capacity in mice

Materials and Methods

The testing was performed based on study protocol. 40 animals were used for this study.

1. Testing system

Genus:	<i>Rodentia</i>
Species:	<i>Mouse</i>
Strain:	C57 black/6
Sex:	Male only
Age:	8-10 weeks
Weight:	20-30 gram (+/- 20% of mean)
Source of Animals	Charles River Laboratories, Inc.
Number of Animals	40

2. Acclimation

Mice were acclimated for at least 3 days after clinical health examination and prior to testing to minimize shipping stress and ensure general good health. Upon arrival, all animals were examined for general health and weighed.

3. Housing

40 mice were divided in four groups (n=10 for each group). Mice were housed in plastic, solid bottom cages filled hardwood chip bedding during the study. Each mouse were housed individually in one cage, all cages in each group were kept in one cage rack. Housing was in compliance with the space recommendations of the Guide. Room temperatures were maintained at 70 +/- 2 degrees Fahrenheit. The relative humidity during the study was generally maintained at 50 ± 20%. Lighting was automatically controlled to provide 12 hours of light followed by 12 hours of darkness.

4. Food

Mice diet was obtained from Charles River Laboratories and was accompany shipments of animals to ensure no change in nutrition during the study. Mice food was provided in sanitized feeding devices, *ad libitum*.

5. Water

ZF-10M Filtered water was produced in Brunswick Laboratories. Briefly, fresh tap water from town of Norton Ma was connected with ZF-10M Filter which was from A-Z Comp. The ZF-10M Filtered water of the first 10 litters was discarded. The ZF-10M Filtered water was provided in sanitized water bottles for mice in group one and group three. Evian water was purchased in local department store. Evian water was provided in sanitized water bottles for mice in group two and group four.

7. Experiment design

Group Number	Study	Treatment (30 days)
Group 1 (N=10)	Running endurance	ZF-10M Filtered water
Group 2 (N=10)	Running endurance	Evian water
Group 3 (N=10)	Swimming endurance	ZF-10M Filtered water
Group 4 (N=10)	Swimming endurance	Evian water

After the acclimation period, all mice in each group were given water for 30 days according to experimental design. Water bottles were changed every day.

For running endurance study, individual mouse was trained for running on a Rota-Rod treadmills (ENV-575M Med Associated Inc.) for three days prior to running study. During training, the speed of the Rota-rod was increased incrementally to make animals exercise more intensely and running for at least one minute. Mice were subjected to running test every other day beginning at 11th day after water treatment as follows:

11th	13th	15th	17th	19th	21th	23th	25th	27th	29th
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The Rota-Rod treadmills had five testing zones, each zone had a photo bean that sensed when the animal dropped off the treadmills which was connected with computer to calculate running time on the treadmills automatically. The speed of the Rota-rod was set to 35 RPM. The maximum time running until exhaustion (drops from the Rota-rod) was used as the index of running endurance capacity. The maximum time of running until exhaustion in each mouse was recorded.

For swimming endurance study, prior to experiment, each mouse was tested for feasibility testing. All mice in swimming groups could swim. All mice were subjected to swim in a water pool (48x28x24 inch), filled 19 inch deep of water maintained at 25°C. During swimming testing, each mouse was fixed four paper clips with a plastic string on the tail. The paper clips and plastic string weighted about 1.81 grams. Bearing this weight, all mice were forced to swim very hard. All mice were subjected to swim until exhaustion (cease movement of limbs and float). The maximum time swimming until mice exhausted was used as the index of swimming endurance capacity. The maximum time

swimming until exhaustion in each mouse were recorded. The swimming was performed every other day beginning at 11th day after water treatment as follows:

11th	13th	15th	17th	19th	21th	23th	25th	27th
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At 29th day, the swimming distance in one minute was measured. Briefly, a galvanized tank pool (Diameter: 183 cm) was filled with water (25°C) to a height of about 5 cm. An automatic tracking system (a video camera, a computer with software Smart Junior v 1.0.04, Harvard Apparatus) was set to monitor each mouse's swimming distance for one minute. The distance was automatically calculated by the computer.

10. Data collection

10.1 The swimming and running endurance:

Swimming and running endurance was collected as described above.

10.2 The Oxygen Radical Absorbance Capacity (ORAC) test:

After 30 days of water treatment and the completion of endurance testing, all mice were euthanized with an overdose of pentobarbital sodium (200mg/kg) ip injection. Serum was collected from the heart. Brain, heart, liver kidney were immediately removed, weighed and stored at -80°C.

Free radical damage related oxidative stress have been implicated in a wide range of biological fields, these including pathophysiological process of many diseases, increased aging and reduced exercise endurance etc. Superoxide (O_2^-) scavenging capacity in the human body is the first line of defense against oxidative stress. It is estimated that one percent of the total oxygen consumed by an adult (70 kg body mass) is converted to the superoxide anion. Therefore, the Superoxide scavenging capacity in blood is a very important parameter to gauge an individual's antioxidant status. ORAC assay quantifies the Superoxide (O_2^-) scavenging activity. Mice serum was evaluated for ORAC assay using standard protocol in our lab.

Result

Body weight (Gram, Mean±SD)

	Group 1	Group 2	Group 3	Group 4
before	21±1	22±1	21±1	22±2
Day 2	21±1	22±1	21±1	21±2
Day 3	20±1	22±1	21±1	21±1
Day 4	21±1	22±1	21±1	22±1
Day 5	21±1	22±1	21±1	22±1
Day 6	21±1	22±1	22±1	22±2
Day 7	21±1	23±1	22±1	23±1
Day 8	21±1	23±1	22±1	23±1
Day 9	22±1	23±1	22±1	23±1
Day 10	22±1	23±1	22±1	23±2
Day 11	22±1	23±1	23±1	23±2
Day 12	22±1	23±1	23±1	23±2
Day 13	22±1	23±1	23±1	23±2
Day 14	22±1	24±1	23±1	24±2
Day 15	22±1	24±2	22±1	24±2
Day 16	23±1	24±1	24±1	24±2
Day 17	23±1	24±1	24±2	24±2
Day 18	23±1	24±1	24±1	24±2
Day 19	23±1	24±1	24±1	24±1
Day 20	23±1	24±1	24±2	24±2
Day 21	23±1	24±1	24±1	24±2
Day 22	22±1	25±1	24±1	24±2
Day 23	22±1	25±1	24±2	25±2
Day 24	23±1	25±1	24±1	25±2
Day 25	23±1	25±1	25±1	25±2
Day 26	23±1	25±1	25±1	25±2
Day 27	24±1	25±1	24±2	25±2
Day 28	24±1	25±1	25±1	25±2
Day 29	23±1	25±1	25±1	25±2
Day 30	24±1	25±1	25±1	26±2

Water intake (Gram, Mean±SD)

	Group 1	Group 2	Group 3	Group 4
Day 4	5.1±0.7	5.9±1.1	5.5±1.4	5.1±0.9
Day 5	5.6±0.8	5.4±0.7	5.5±0.8	5.1±1.0
Day 6	5.2±0.6	4.9±1.3	5.8±0.9	5.6±0.8

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Day 7	5.7±0.9	5.2±0.8	6.1±0.7	5.5±0.8
Day 8	5.2±0.8	5.4±0.7	5.5±1.1	5.5±1.1
Day 9	5.5±0.7	5.5±0.5	5.9±0.9	5.5±0.5
Day 10	4.8±0.9	5.6±0.7	6.2±0.6	5.6±0.8
Day 11	5.4±1.0	5.6±0.8	6.2±0.9	5.3±0.7
Day 12	5.5±0.8	5.5±0.5	6.2±1.1	5.7±0.8
Day 13	6.0±0.8	5.8±0.9	5.8±1.1	6.3±0.8
Day 14	5.4±0.5	5.1±1.4	6.1±1.6	5.5±1.1
Day 15	5.8±0.8	6.4±0.7	4.7±3.1	5.8±1.7
Day 16	6.0±0.7	7.1±1.0	6.7±1.4	7.2±0.6
Day 17	5.5±0.8	6.1±0.6	7.0±1.3	7.1±1.9
Day 18	6.5±0.8	7.1±1.0	6.7±0.9	6.4±1.2
Day 19	6.3±0.9	6.0±1.1	6.7±1.1	6.6±0.5
Day 20	6.2±0.8	7.0±0.8	6.2±0.8	6.9±0.6
Day 21	5.8±0.8	6.1±0.7	6.4±0.8	5.9±0.7
Day 22	6.8±0.9	8.1±1.1	6.4±1.1	6.9±0.7
Day 23	5.3±0.9	5.9±0.7	5.8±0.8	5.6±1.0
Day 24	5.1±0.6	6.2±0.8	6.1±1.3	6.3±0.7
Day 25	5.3±1.0	5.7±0.8	5.7±1.9	5.9±0.9
Day 26	5.7±0.9	8.4±1.2	6.1±1.3	8.3±3.0
Day 27	4.7±0.9	5.6±0.8	4.4±3.0	6.0±1.1
Day 28	5.9±0.9	6.5±1.4	4.9±2.0	6.8±1.0
Day 29	5.7±0.7	6.6±0.5	5.5±1.0	6.2±1.3
Day 30	5.5±0.5	7.1±0.7	6.2±1.3	7.3±0.8

Running endurance (Second, Mean±SD)

	Group one (n=10)	Group two (n=10)
Day 17	698±447	505±368
Day 19	745±407	626±315
Day 21	572±280	516±287
Day 23	590±357	442±272
Day 25	428±144	512±273
Day 27	620±333	462±267
Day 29	622±284	403±123

Number of running with endurance of >20 minutes vs endurance of <20 minutes

		Group one	Group two
Day 17	>20 minutes	4	1
	<20 minutes	6	9
Day 19	>20 minutes	4	2
	<20 minutes	6	8
Day 21	>20 minutes	1	1
	<20 minutes	9	9
Day 23	>20 minutes	2	1

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	<20 minutes	8	9
Day 25	>20 minutes	0	1
	<20 minutes	10	9
Day 27	>20 minutes	2	1
	<20 minutes	8	9
Day 29	>20 minutes	1	0
	<20 minutes	9	10
*Total number of running from Day 17 to Day 29	>20 minutes	14	7
	<20 minutes	56	63

Swimming endurance (Second, Mean±SD)

	Group three (n=10)	Group four (n=10)
Day 15	167±42	89±16
Day 17	145±27	124±24
Day 19	151±22	105±20
Day 21	87±18	75±20
Day 23	83±18	76±22
Day 25	82±22	77±13
Day 27	82±15	67±32

Swimming distance in one minute tested at day 29 (Centimeter, Mean±SD)

	Group three (n=10)	Group four (n=10)
First test	1105±139	1106±272
Second test	1075±159	947±261

Serum ORAC

	ORAC value (Mean±SD, μmole TE/mL)
Group one	8.08±2.23
Group two	6.51±0.90
Group three	6.88±0.94
Group four	6.24±0.81

Conclusions

There is a trend that mice drinking ZF-10M Filtered water has better running and swimming endurance than mice drinking Evian water, mice drinking ZF-10M Filtered water also showed more antioxidative capability than mice drinking Evian water.

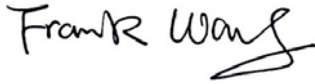
Key personnel responsibilities

Frank Wang: animal testing, data analysis

Dylan McKenna: animal testing and animal care.

Miwako Kondo: ORAC testing

Signature:



08/16/2010

Frank Wang, M.D. Study Director

Date