ADAPTATIONS OF SKELETAL MUSCLE IN AGING RATS: COMBINED INTERVENTION OF AN ANTIOXIDANT-BASED FOOD SUPPLEMENT AND PHYSICAL ACTIVITY

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Abstract:

Physical training has always remained a suitable intervention to overcome the muscle degenerative situations such as sarcopenia not only in the old but also in late-adult and middle-age. This study is aimed to investigate the intervention of physical activity and naturally occurring biologically active compounds of medicinal value in addressing age related skeletal muscle changes. Basella albaalso known as Indian spinach has antiulcer potential, anti-inflammatory activity and hypo-glycemic activity, a natural antioxidant can offer better defense against age related changes in the supplemented physical trained rats. Rats were categorized into four sub-groups. Sedentary control, SE-C; sedentary supplemented, SE-C(S); swim trainees at thermo neutral temperature, SW-T(N) and supplemented swim trainees at thermo neutral temperature, SW-T(N+S). The results suggest that swimming is capable of improving skeletal muscle endurance and mass. Basella alba supplementation ameliorates the benefits of swim exercise.Basella alba and swim training showed changes and increase in the endurance capacity of skeletal muscles.

Keywords: basella alba, extensor digitorum longus,gastrocnemius, soleus,physical activity.

Introduction:

The growing problem of frailty and muscle degeneration with aging calls for research to prolong the onset of occurrence of such disabilities especially from late adult to middle-age in order to move towards quality life in old age. However, studies defining the features of exercise training in terms of the type, duration and age of onset of training as strong determining factors for obtaining benefits from such an intervention still remain elusive in terms of the adaptations of different types of skeletal muscles. Unlike what has been known hitherto that regular exercise confers protection with age, how supplementation can impact the adaptations of the muscle for physical training remains elusive that needs further research. The overall aim of this investigation was to study the possible adaptations to exercise training of the skeletal muscles, namely the soleus (SOL), extensor digitorum longus(EDL) and gastrocnemius (GC), to overcome age-related changes. An adjoining interest was to look into the possibilities of combining antioxidant-based food supplement with physical training to boost the adaptations for overcoming declines. Swimming may be beneficial in terms of reducing the deleterious changes, in the aging EDL, SOL and GC muscles of trained rats. Basella alba (B.alba) containing several active components including flavinoids exhibit antioxidative, anti-proliferative and anti-inflammatory properties in biological systems and may provide better adaptations in the skeletal muscles.

Material and Methods:

Swim training of animals

Rats were categorized into four sub-groups. Sedentary control, SE-C; sedentary supplemented, SE-C(S); swim trainees at thermoneutral temperature, SW-T(N) and supplemented swim trainees at thermoneutral temperature, SW-T(N+S). Rats of the trainee groups were swim exercised for 30 min day^{-1} for 5 days week^{-1} and for a total period of 45 days with a load of 3% body weight in the adult (3 mo-), late-adult (12 mo-) and middle-aged (18 mo-) rats. The rats of the supplemented groups received B.alba leaf extract dissolved in distilled water at the dose of 800 mg/Kg body weight orally before swim training.

Preparation of Basella leaf extract

The Basella alba plant was identified and authenticated as **Basella alba Linn.** belonging to the family ‘Basellaceae’ (RRCBI-01023) by the National Ayurveda Dietetics Research Institute, Bangalore, India. The leaves were shade-dried and powdered.

Acute toxicity study

Acute toxicity study was performed. The various dose level of Basella extract was 500, 1000 and 1500 mg/kg body weight for toxicity test. After oral administration of test dose, dissolved in distilled water, the animals were kept under observation for 8 h and at the end of 24 h checked if there were any symptoms of distress.

Tissue Somatic Index

The relation between body weight and muscle weight was calculated for the muscles, which is used as an index for sarcopenia, in order to have a comprehensive overview of the effects of aging on different types of muscles.

Plasma lactate

Plasma lactate was measured by the method of Barker and Summerson (1941). Deproteinised sample was treated with 20% copper sulphate and diluted with distilled water. $Ca(OH)_2$ was added, and incubated at room temperature (RT). The samples were centrifuged. To the supernatant, 4% copper sulphate was added followed by concentrated sulphuric acid. The samples were boiled and cooled. This was followed by the addition of p-hydroxy diphenyl reagent. Samples were placed in a water bath at 30°C, followed by 90 secs in vigorously boiling water. After cooling, the absorbance was read at 560 nm using lithium lactate as the standard. Blood lactic acid was expressed in terms of mmols/L.

Statistical Analyses

Data are shown as mean ± S.E.M of five animals /sub-group. Changes between the sub-groups and age groups of swim trained rats in thermoneutral waters and supplemented trainees were compared with their respective sedentary. Data was further analyzed by two-way ANOVA and tested by Bonferroni post-hoc test using GraphPad Prism 5 and $p < 0.05$ was considered significant.

Results

Acute toxicity study

In acute toxicity study there were no behavioral changes up to 8 hours and no distress was observed even at the maximum tested dose level of 1500 mg/kg BW. An effective dose of 800 mg/Kg body mass was considered for the present study.

Body Mass

Body mass in all the experimental animals of different age groups gradually increased during the experimental study period. However, the mean body mass of the swim trained at thermoneutral temperature and supplemented trainees were significantly less compared to the sedentary during the training period (Table I).

Table I. Changes in the body mass (g)

Sub-groups	Age (months)		
	3^{A}	12^{B}	18^{C}
SE-C	331.6 ± 19.0$^{\mathbf{a}}$	419.2 ± 0.1$^{\mathbf{a}}$	490.66 ± 4.2$^{\mathbf{a}}$
SE-C(S)	290.3 ± 3.91$^{\mathbf{b}}$	436.0 ± 1.5$^{\mathbf{a}}$	496.10 ± 3.5$^{\mathbf{a}}$
SW-T(N)	273.3 ± 9.21$^{\mathbf{b}}$	300.8 ± 13$^{\mathbf{b}}$	406.60 ± 4.0$^{\mathbf{b}}$
SW-T(N+S)	268.3 ± 8.01$^{\mathbf{b}}$	335.6 ± 6.7$^{\mathbf{c}}$	398.66 ± 5.7$^{\mathbf{b}}$

Values are mean ± SEM (n=5 animals/sub-group). Sedentary control, SE-C; sedentary supplemented, SE-C(S); Swim trained at thermoneutral temperature, SW-T(N); supplemented swim trainees at thermoneutral temperature, SW-T (N+S). A two-way ANOVA with Bonferroni's post-hoc test was used to compare the age groups and significance is represented in upper case, and between the sub-groups in lower case. Those having dissimilar upper and lower cases are significantly different at $p < 0.05$.

Muscle mass and Tissue Somatic Index

Table II. Muscle mass and Tissue somatic index

Sub-groups	Age (months)		
	EDL		
	3^{A}	12^{B}	18^{C}
SE-C	0.350 ± 0.01	0.442 ± 0.03	0.574 ± 0.32
SE-C(S)	0.359 ± 0.05	0.451 ± 0.09	0.586 ± 0.02
SW-T(N)	0.401 ± 0.01	0.510 ± 0.03	0.612 ± 0.07
SW-T(N+S)	0.432 ± 0.01	0.542 ± 0.04	0.643 ± 0.06
	TSI		
SE-C	0.106 ± 0.01^{a}	0.105 ± 0.03^{a}	0.117 ± 0.02^{a}
SE-C(S)	0.123 ± 0.05^{b}	0.103 ± 0.06^{a}	0.118 ± 0.07^{a}
SW-T(N)	0.149 ± 0.04^{c}	0.169 ± 0.09^{b}	0.150 ± 0.06^{b}
SW-T(N+S)	0.161 ± 0.01^{c}	0.161 ± 0.01^{b}	0.153 ± 0.01^{b}
	SOL		
	3^{A}	12^{B}	18^{C}
SE-C	0.332 ± 0.13	0.432 ± 0.08	0.564 ± 0.06
SE-C(S)	0.340 ± 0.03	0.440 ± 0.01	0.566 ± 0.01
SW-T(N)	0.392 ± 0.05	0.504 ± 0.04	0.603 ± 0.02

SW-T(N+S)	0.412 ± 0.07	0.532 ± 0.07	0.633 ± 0.06
		TSI	
	3 A	12 B	18 C
SE-C	0.100 ± 0.09 a	0.103 ± 0.04 a	0.115 ± 0.06 a
SE-C(S)	0.117 ± 0.09 b	0.102 ± 0.08 a	0.114 ± 0.02 a
SW-T(N)	0.143 ± 0.04 c	0.168 ± 0.01 b	0.148 ± 0.02 b
SW-T(N+S)	0.153 ± 0.01 d	0.158 ± 0.03 c	0.159 ± 0.05 c
		GC	
	3 A	12 B	18 C
SE-C	2.412 ± 0.22 a	2.912 ± 0.40 a	3.315 ± 0.10 a
SE-C(S)	2.613 ± 0.14ab	3.012 ± 0.08ab	3.811 ± 0.10ab
SW-T(N)	2.918 ± 0.06bc	3.313 ± 0.40bc	4.113 ± 0.50bc
SW-T(N+S)	3.212 ± 0.04c	3.614 ± 0.10 c	4.512 ± 0.20 c
		TSI	
SE-C	0.721 ± 0.20	0.692 ± 0.4	0.673 ± 0.10
SE-C(S)	0.89 0± 0.10	0.690 ± 0.4	0.766 ± 0.10
SW-T(N)	1.061 ± 0.40	1.090 ± 0.4	1.000 ± 0.50
SW-T(N+S)	1.202 ± 0.10	1.070 ± 0.1	1.130 ± 0.20

Training effects are indeed highly tissue-specific. In this study, SOL muscle was studied as a representative slow oxidative muscle; EDL as fast glycolytic muscle and GC consisting of white (rich in type IIb fiber) and red (rich in type IIa) fibers. (Fig.2.).The EDL, SOL and GC muscle mass at the end of the training period showed significant increases in their mass in the swim trainees and supplemented trainees irrespective of age. Increases in the tissue-somatic index (TSI) at the end of the training period was observed in the swim trainees and supplemented trainees of the adult, late-adult and middle-aged rats with respect to their sedentary (Table II). Although swim training resulted in increased TSI in all the age groups, the extent of increase was greater for the SOL than for the EDL which was in turn greater than the GC muscle.Our investigation revealed significant differences in the tissue-somatic index of 3 mo, 12 mo and 18 mo old muscles.A drop in the sarcopeniaindex indicates muscle wasting.A significant increase was seen in the SOL of 3 mo-, 12 mo- and 18 mo- old swim trainees and supplemented trainees shows beneficial effects of training. A considerable increase in the GC was observed between the sedentary and swim trainees and supplemented trainees.

Table III. Results of the weekly endurance test (min)

Weeks of swim	Age (months)					
	3A	3 A	12 B	12 B	18 C	18 C
	SW-T(N) a	SW-T(N+S) b	SW-T(N) a	SW-T(N+S)b	SW-T(N) a	SW-T(N+S) b
1	30±1.2 a	36±2.1 a	30±1.5a	35±1.4a	15±2.1a	17±1.4a
2	40±1.4 b	48±1.4 b	37±2.4a	45±1.3bc	15±2.5a	20±1.9ac
3	45±1.4 bc	51±1.5 bc	45±1.6b	52±1.2c	20±2.6ab	25±1.5ac
4	50±1.8 c	58±1.9 c	50±1.4b	59±1.4c	22±2.4ab	27±1.8bc
5	60±1.9 d	69±2.1 d	80±1.3c	89±1.6d	23±1.4ab	29±1.5cd
6	70±1.3 e	81±1.7 e	95±1.5d	102±1.7e	25±1.9b	32±1.3d
7	100±1.6 f	117±1.0 f	100±2.1de	108±1.8ef	25±2.1b	36±1.7de
8	110±1.5 g	129±2.5 g	105±1.9ef	115±1.5f	30±1.7bc	39±1.9de

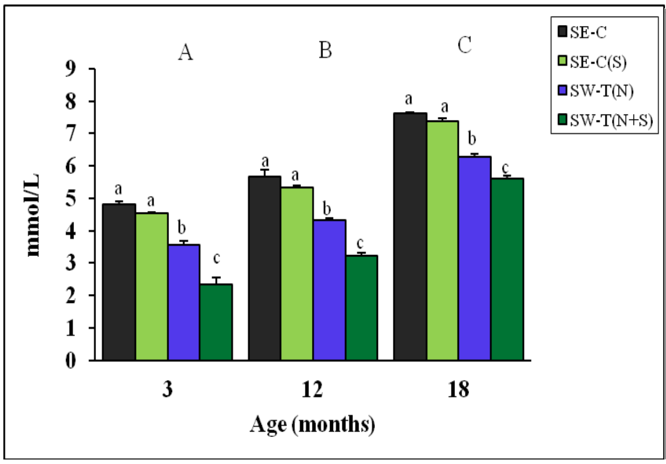
9	125±2.1 h	145±1.8 h	110±1.7f	130±1.2g	35±1.8c	45±1.8e

A three-way ANOVA with Bonferroni's post-hoc test was used to compare between the age groups and significance is represented in upper case, and between the sub-groups and weeks of training in lower case. Those having dissimilar upper and lower cases are significantly different at $p < 0.05$.

Swim endurance and plasma lactate

Swim endurance was measured at the end of the fifth and ninth week of training, and was compared to that measured at the end of the first week of training. Swim endurance capacity of the adult increased by 2-to 4-fold at the end of fifth and ninth week of training and this was regardless of supplementation. However, in the late-adults, endurance increased among the supplemented trainees by 3.6-fold as compared to a 2.6-fold in the unsupplemented one but trained at the end of the fifth and ninth week of training. In the middle-aged rats, the increase in endurance was by 2.64 by the end of ninth week in the supplemented trainees compared to 1.7-fold in the unsupplemented trainees (Table. III).

Plasma lactate was reduced by 33%, 33% and 15% in the 3-, 12- and 18-mo-old swim trainees and by 33%, 35% and 23% in the supplemented trainees as compared to the values with their respective sedentary (Fig.1).



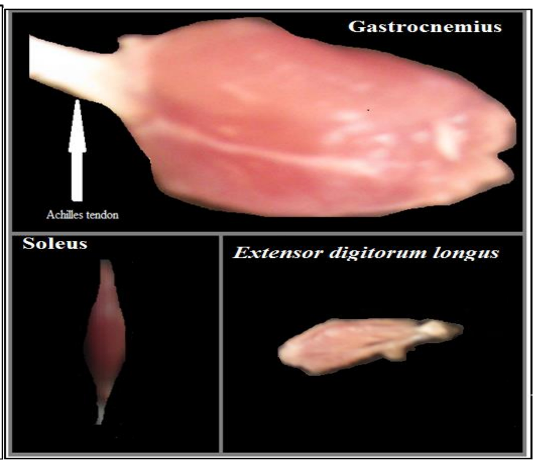


Fig.1.Plasma lactate level Fig.2. GC, SOL and EDL muscles rat hind limb

Discussion

Aging is associated with reduction in muscle function. Exercise is a part of physical therapy and weight loss.Itimproves or maintains physical fitness and strengthens muscles. In this study, results are provided in support of our hypothesis that exercise training and B.alba supplementation can alleviate changes in the aging skeletal muscle than any one of the single interventions.Growth-related gain of body and skeletal muscle weight was observed. Swim exercise significantly reduced the body mass and increased the muscle mass in the adult, late-adult and middle-aged animals. The data on body mass demonstrated a significant increase with age among the inactive rats. Reference**[1]**reported decreased body weight in swim trained male C57BL/6 mice unlike the sedentary, which showed significant increases in body weight during the experimental period. [2] demonstrated a 10% reduction of body weight in healthy swim trained Wistar rats which were fed a chow diet possibly due to the reduction of relative and absolute body fat.

TheEDL, SOL and GC muscle masses were significantly increased in the trained in relation to the sedentary as seen from the muscle mass as a percentage of body mass. Our findings on increased muscle hypertrophy based on the muscle-wet mass in the trained may be explained in terms of a possible increase in contractile proteins and collagen which may also explain, in part, the improved endurance seen in the trained. Significant correlation between antioxidant activity and total phenolic content indicating total phenolic content as the major contributor to the antioxidant activity of the plant has been reported [3].

Swim training the animals had significant effect on the muscle mass of GC in young, late-adult and middle-aged, of the SOL in the adult and of the GC. In the present study, when the percent muscle weight-to-body weight - TSI was considered, there was a significant increase in the TSI of the SOL with age. In contrast to the SOL muscle, EDL and GC muscles showed insignificant increases in TSI irrespective of age, suggesting that the EDL and GC may be the hind-limb muscles which are recruited to a lesser extent during endurance swim exercise as reported in [4], and the soleus muscle is one of the most recruited muscles during the exercise training with a large proportion of type I fibers. Soleus and plantaris muscles are the primary working muscles in prolonged swimming exercise. Swim training altered the endurance capacity.

A higher extent of increase in endurance with a lower plasma lactate level was achieved in the trained late-adult and middle-aged rats compared to the levels seen in their sedentary counterparts, and the responses were further improved in trainees supplemented with B.alba thereby suggesting an anti-fatigue effect of the supplement.Vigorous physical activity resulting in the generation and gradual storage of Lactic acid (LA) in the skeletal muscle is a matter of great concern to exercise physiologists because of its impact on muscle fatigue. Muscles produce LA during swimming, which leads to their accumulation in the blood [5].Endurance exercise training improves lactate clearance capacity [6].In the current study, an increase in the level of plasma lactate was observed with aging. Further, the observed decrease in plasma lactate levels of swimmers could be indicative of an increased aerobic capacity in them. Plasma lactate has been used to assess the possible anaerobic metabolism in energy production during exercise in animal models; some studies have shown that regular endurance exercise increases glycolysis and oxidative metabolism [7]. Although the adult, late-adult and middle-aged rats show a similar trend in blood lactate with training, the extent of lactate reduction was lowest in the middle-aged. The decreases observed in blood lactate suggests an increase in aerobic capacity and increase in blood lactate clearance that have been reported in endurance trained rats.

People exercise less; lose muscle mass and gain weight as they age [8]. Swimming is a natural ability in rats, they work at higher intensities when made to swim to exhaustion, for survival and hence increase the performance level. In addition, swimming-to-exhaustion endurance tests have been extensively used to test performance before and after, long-term training regimens [9]. In the present study, the endurance at the end of the training period increased significantly in the thermoneutral swimmers compared to the endurance in the beginning of the training period, in adult and late-adults, whereas the increase in endurance was significant between the age groups in thermoneutral swimmers. In the supplemented trainees significant increases was seen in the 3mo and 12 mo-old rats in the endurance capacity and considerable increase in the 18 m0-olds. Locally grown plants have been used worldwide as supplements and therapies for several ailments [10]. Phytochemicals are promising therapeutic agents because many phytochemicals have anti-inflammatory, antioxidative as well as anticholinesterase activities. [11]

Collectively, the observed differential responses of the types of muscles with exercise and age explains the interaction of exercise in reducing the age related changes, and thereby improving the endurance of the skeletal muscles, which otherwise leads to increased muscle disuse-related dysfunction in the sedentary and aged. The results on the responses of the three types of aging muscles to exercise and supplementation with Basella suggests that B.alba as a antioxidant rich in polyphenols and flavanoids, can reduce the changes related to aging and improve the swim endurance even in the middle-aged rats. A combination of exercise training and B.alba is beneficial in lowering age-related changes, while improving the adaptive capacity to exercise of aerobic type, improving swim endurance, especially in the middle-aged are definitely age-and fiber-specific.

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