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ARTICLE

Efficacy of Spirulina Supplementation on Isometric Strength and Isometric Endurance of Quadriceps in Trained and Untrained Individuals – a comparative study

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Abstract
Objective: To determine the efficacy of spirulina supplementation in enhancing isometric muscle strength and endurance in trained and untrained human beings.
Design: Placebo controlled, different subject experimental.
Setting: University level athletes and college students of Guru Nanak Dev University, Amritsar, India.
Subjects: Forty healthy subjects (20 trained and 20 untrained) participated in the study and were divided into 4 groups, supplemented untrained (n=10), placebo untrained (n=10), supplemented trained (n=10), placebo trained (n=10). Subjects in both supplemented groups volunteered to take spirulina for 8 weeks in addition to their normal diet. Subjects in the placebo groups served as control and were given capsules filled with flour.
Outcome measures: Peak force, average force and fatigue index of dominant quadriceps muscle were measured before and after 8 week of supplementation. Values were compared within and between the groups.
Results: The results show that spirulina supplementation is effective with time in increasing peak force ($p<0.01$), average force ($p<0.01$) and decreasing fatigue index ($p<0.01$) on paired t-test. Significant group and supplementation effects were also found with ANOVA analysis for peak and average force. However no significant effects ($p>0.05$) were found between the groups with ANOVA in decreasing fatigue index.
Conclusion: Spirulina for 8 weeks is effective in increasing the isometric muscle strength and isometric muscle endurance. Spirulina supplementation with training was found to be better than the spirulina only and training only in increasing muscle strength but no group was found to be better in increasing muscular endurance.

Key words: Spirulina – Muscle Strength – Fatigue – Sports Nutrition

Introduction

Spirulina, microscopic blue green algae have been known to be consumed since ancient times as a nutritional supplement as it has very high protein content (70 p.100 of dry matter) (1). In India, athletes have been eating Spirulina while training for track and field events (2). The Chinese and Cuban Olympic teams are also known to eat spirulina daily during their training and competition (3). These suggest some strength or exercise performance related effects of spirulina supplementation in humans.

It has been found to be excellent food for rapid recovery in children from malnutrition related diseases in Mexico, Togo, Romania, China, Rwanda, Zaire, India, Ukraine, and Belarus (4). Many National programs have been undertaken to cure malnourishment with spirulina in Indian and African subcontinent (5).

Spirulina is unicellular, filamentous, helicoidal shaped green algae with length of 0.2-0.3mm. It's special advantage over other natural food is that it contains 65-70% protein on dry weight basis which is higher than any other natural food, 14% carbohydrates (Ramnose, Glucane, Phosphorolated Cyclitols, Glucosamine, Muramic acid, Glycogen), 6% lipids (Palmitic acid, Linoleic acid, Gamma linoleic acid, Alpha linolenic acid, Beta carotene), all eight essential amino acids (Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophane, Valine), several vitamins and trace minerals like Ni, Cu, Fe, Ca, Co etc (4,6).

According to previous evaluations, spirulina seems to be a good alimentary protein source for human subjects. Spirulina supplementation has shown to increase the protein synthesis rates in soleus as well as myosin content in gastrocnemius muscle of rats (7).

Initially the interest in spirulina focused on its nutritive value, more recently, some preclinical testing suggests it has several therapeutic properties such as anti-inflammatory and antioxidant (8). Spirulina supplementation has shown preventive effects on skeletal muscle damage under exercise-induced oxidative stress (9). It is evident that muscular exercise promotes production of free radicals, which are responsible for exercise-induced protein oxidation and contribute to muscle fatigue (10). This oxidative stress has been observed both after exhaustive aerobic as well after isometric exercises (11). However, spirulina's effects in increasing muscular strength and delaying muscular fatigue owing to its high protein content and antioxidant potential in normal healthy subjects have not been documented. Previous clinical studies on spirulina supplementation have used dosage ranging from 0.5gms to 10gms for various therapeutic purposes. We intended to study the effects of spirulina supplementation on muscular strength and fatigue at the minimal therapeutic dose of 2gm/day for 8 weeks (12).

Performance in sports and in any physical task depends upon neuromuscular, psychological, environmental and nutritional factors. Proper nutrition provides fuel for biologic work and essential elements for repairing existing cells and synthesizing new tissues, forming the foundation for physical and muscular performance. Muscle performance refers to the capacity of muscle to do work. Key elements of muscle performance are strength and endurance. Muscle strength refers to the ability of contractile tissue to produce tension and a resultant force based on demands placed upon the muscle. Muscle endurance is the ability of a muscle to contract repeatedly against a load, generate and sustain tension, and resist fatigue over an extended period of time (13).

The use of nutritional supplements in sport is widespread. Nutritional ergogenic aids are aimed primarily at enhancing performance. Athletes regularly consume these supplements to increase resistance to infection and improve general health. This is important in reducing the interruptions to training due to minor illness and infection. Also nutritional supplements aim at increasing lean body mass or muscle mass by stimulation of protein synthesis and at reducing body fat content (14). This provoked us to check whether spirulina due to its high protein content and antioxidant potential can be used as a nutritional supplement to combat the stress of exercise on muscle and enhance the muscular performance by increasing the muscular strength and endurance.

Materials and Methods

A different subject experimental design for inter group and intra group comparison was used. A total of 40 trained and untrained male and female subjects volunteered in our study. The term ' trained' was used to refer to athletes who have been consistently involved in university level competition. This group included subjects participating in boxing and shotput throwing for at least 3 years. The term 'untrained' was used to refer to subjects not involved in any kind of sporting activity during the last 1 year or during the study period.

Subjects were informed of the possible risks and benefits of participation before signing the consent. The study was approved from the Institutional Medical Ethics Committee of Guru Nanak Dev University, Amritsar who further approved the protocols therein. 20 untrained subjects (10 males, 10 females) with mean age (25.2±3.5), mean weight (60±7.8), mean height (165.5±9.8) were selected and randomly divided into 2 groups- Supplemented untrained (group I) and Placebo untrained (group II)

20 trained subjects (12 males, 8 females) with mean age (24.4±3.4), mean weight (68±6.3), mean height (170±8.8) were selected and randomly divided into 2 groups- Supplemented trained (group III) and Placebo trained (group IV).

Table 1: Physical characteristic of the tested individuals

Characteristic	Untrained	Trained
Age (year)	25.2±3.5	24.4±3.4
Weight (kg)	60±7.8	68±6.3
Height (cm)	165.5±9.8	170±8.8

Trained subjects were found to have greater weight and height compared to the untrained may be due to the training effects.

Experimental trained subjects continued with their regular training sessions which involved predominantly strength training (exercise machines and resistance bands) and conditioning for the sport involved for average 5hrs/day for 8 weeks.

Inclusion criteria for the study required untrained subjects not participating in any sporting activity, not having any history of lower limb injury and not taking any dietary supplement for past 6 months. Exclusion criteria included any history of lower limb or back injury or surgery, taking any kind of medication or dietary supplement.

Inclusion criteria for trained subjects included regular participation in university level sporting activity and involved in resistance training for at least 3 years, no history of lower limb injury and not taking any kind of protein or dietary supplement. Exclusion criteria were same as for untrained. All subjects who participated in this study were university hostel residents so had the same type of dietary intake.

Testing Protocol

Instructions to subjects were given prior to the testing protocol. Subjects were asked not to consume coffee, tea, heavy meal or other stimulants within 2-3 hours of testing. All were asked to refrain from strenuous activity or exertional physical work at least 24 hours before testing. The HUR1* 5340 Leg Extension/Curl computer controlled instrument (HUR, Finland) was used for evaluating the isometric angle specific peak torque. Each subject visited the laboratory before the start of the actual study and performed 2 maximum voluntary isometric contraction with 10 second and 60 second hold with a rest period of 2 minute in between the two contractions, to determine peak force (PF), average force (AVF) and fatigue index (FI) of the quadriceps muscle of the dominant leg measured at optimal standardized angle of knee joint (for which the quadriceps muscle applies maximal torque i.e. 60° of knee flexion; 0° means full knee extension) (15).

Isometric Strength Measurement

The torque (Newton–metre) was measured at 10 sec. isometric hold at 60° knee flexion for quadriceps in both groups. It was normalized to force (Newton) by dividing torque by lever arm length.

$$\text{Force (Newton)} = \frac{\text{Torque (Nm)}}{\text{Lever Arm Length (m)}}$$

Peak force (N) and Average force (N) (average force of 4 quarters (1 quarter = 2.5 sec) for 10 sec was calculated) from peak torque (Nm) and Average torque (Nm) respectively.

Isometric endurance measurement

After 2 min rest with no activity, fatigue index (FI) was calculated as a measure of isometric endurance with the same seat position and knee angle as above. Isometric hold of 60 sec was performed, to calculate isometric endurance. Torque in 1st sec (T1) and torque at 60th sec (T60) were observed. Torque T1 and T60 was normalized to force F1 and F60 respectively. Fatigue Index designed by Milner and Brown (16) was calculated using the formula;

$$\text{Fatigue – index} = \frac{F_1 - F_{60}}{F_1} \times 100\ (\%)$$

No visual or verbal feedback was given during the test session to the subject so that no external stimuli were instituted except for the subject's own maximal effort and hold.

Experiment Protocol

The study utilized a placebo controlled different subject experimental design with supplemented and placebo trials completed in a random order in 8 weeks. The pre-protocol measurement of isometric strength and endurance were done. The subjects assigned to supplemented group (both trained and untrained) consumed organic Spirulina (by Parry Nutraceuticals, Tamil Nadu, India, USDA - NOP certified) in capsular form (500mg each) in addition to their normal diet. Each subject was asked to take 2 capsules in the morning and in evening and not to alter their regular eating habits during the study period, thus total dosage of

2gms per day for 8 weeks. The dose given was the minimal therapeutic dose (12). The subjects assigned to placebo group (both trained and untrained) consumed empty capsules filled with flour. At the end of supplementation period all subjects were again tested for any change in peak force, average force and fatigue index.

Statistical Analysis

Data was statistically analyzed using the Statistical Package for Social Sciences (SPSS)/14.0. (Copyright© SPSS Inc) .Results is expressed as mean ± standard deviation (SD). A paired Student's t was used for the statistical analysis to compare the mean difference between pre- and post-tests within group. Intergroup comparisons have been made by ANOVA.

Results

Table 2 shows comparison between the mean values before and after supplementation by paired t-test analysis. Peak force ($t=5.83$, $p<0.01$), average force ($t=6.67$, $p<0.01$) and fatigue index ($t=6.03$, $p<0.01$) showed significant difference in supplemented trained group before and after supplementation. Significant differences were also found in the supplemented untrained subjects for peak force ($t=6.29$, $p<0.01$), average force ($t=5.73$, $p<0.01$) and fatigue index ($t=9.92$, $p<0.01$) before and after supplementation. No significant difference was found for any parameter in the placebo groups before and after supplementation.

Pre supplementation values were compared by ANOVA. The results (table 3) showed that the pre- values of peak force differ significantly ($F=3.599$, $p<0.05$) between the groups. On applying Post Hoc Multiple Scheffe Range Test significant difference was found in trained and untrained groups. No statistically significant difference was found between trained and untrained groups for average force ($F=2.612$, $p>0.05$) and fatigue index ($F=0.587$, $p>0.05$).

There is significant difference seen for parameters peak force and average force when compared between groups after supplementation. ANOVA followed by Post Hoc Multiple Scheffe Range Test after completion of supplementation yielded ($F = 5.622$, $p < 0.01$) group III to be statistically most significant ($p<0.01$) in enhancing peak force followed by group I ($p<0.05$) when compared to placebo groups. This implies spirulina supplementation with training was better than only training or only spirulina group.
Similarly significant difference in average force ($F = 4.815$, $p<0.01$) was found with ANOVA. On applying multiple scheffe, group III was found to be most significant but no significant difference was found in any of the other group. Fatigue index was found to be statistically insignificant ($F= 2.086$, $P>0.05$) in between the groups with ANOVA.

Discussion

The present study was undertaken with the objective of evaluating the effects of Spirulina on exercise performance. Peak force, average force and fatigue index have been

Table 2 - Comparisons of effect of spirulina supplementation on peak force, average force, and fatigue index of dominant quadriceps muscle in supplemented and placebo trained and untrained groups. Values are shown as mean values with standard deviation

		Peak force (in Newton)			Average force (in Newton)			Fatigue index		
		Pre	Post	t	Pre	Post	t	pre	post	t
Supplemented	Trained	326.50 ±72.74	398.40 ±84.77	5.83**	268.80 ±65.53	337.40 ±77.40	6.67**	34.60 ±13.50	25.57 ±12.32	6.03**
	Untrained	231.30 ±41.14	302.30 ±59.15	6.29**	198.40 ±43.68	266.80 ±61.18	5.73**	41.05 ±17.94	31.93 ±17.43	9.92**
Placebo	Trained	295.40 ±69.88	293.10 ±62.20	0.451	252 ±60.19	255.7 ±56.42	0.818	36.34 ±15.19	36.27 ±15.43	0.221
	Untrained	286.50 ±74.86	283.10 ±75.53	0.678	230. ±65.87	229.8 ±68.29	0.047	43.06 ±18.16	42.83 ±17.84	0.641

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measured in trained and untrained individuals as a measure of muscular performance.

Spirulina, blue green algae have been widely used as a nutritional supplement all over the world. It has high protein content (over 60%) and contains all essential amino acids. Besides this it is also a rich source of vitamins and antioxidants.

Many studies have proven the antioxidant and disease modifying potential of spirulina and its effectiveness in various oxidative stress or free radical induced diseases (8,22-24). However its effect in delaying the muscular fatigue which incur due to this free radical production during exercise and in increasing muscular strength due to high protein content have not been studied.

Spirulina has high protein content as well high PER (protein efficiency ratio) of 87% and a NPU (net protein utilization) of 92% when compared to 100% values for casein (17). This implies that about 92% of dietary nitrogen (amino acids) supplied has been converted to proteins. Amino acids play an important role in regulating muscle protein synthesis and this modulation may be affected by dietary protein quality and quantity. Fabricio et al found increased

Table 3: Comparison of peak force, average force and fatigue index in between the groups before and after supplementation by ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Peak Force (pre-supplementation)	Between Groups	47163.275	3	15721.092	3.599	.023*
	Within Groups	157271.500	36	4368.653		
	Total	204434.775	39			
Peak Force (post-supplementation)	Between Groups	85426.675	3	28475.558	5.622	.003**
	Within Groups	182352.300	36	5065.342		
	Total	267778.975	39			
Average Force (pre-supplementation)	Between Groups	27748.400	3	9249.467	2.612	.066
	Within Groups	127492.000	36	3541.444		
	Total	155240.400	39			
Average Force (post-supplementation)	Between Groups	63500.075	3	21166.692	4.815	.006*
	Within Groups	158249.700	36	4395.825		
	Total	221749.775	39			
Fatigue Index (pre-supplementation)	Between Groups	468.961	3	156.320	.587	.628
	Within Groups	9588.333	36	266.343		
	Total	10057.294	39			
Fatigue Index (post-supplementation)	Between Groups	1583.816	3	527.939	2.086	.119
	Within Groups	9111.284	36	253.091		
	Total	10695.100	39			

skeletal muscle protein and increased protein synthesis rate in muscle of growing rats with spirulina ingestion (7). We studied its implication in enhancing the muscle strength due to enhanced muscle protein and found increase in muscular strength after 8 weeks of spirulina supplementation.

Peak force and average force exerted by dominant quadriceps muscle were measured as measures of muscular strength.

Huczel et al (25) compared the strength trained and the untrained subjects and concluded that muscular strength was enhanced in women engaged in a training program designed primarily to increase muscular strength and hypertrophy, but fatigability is not affected. We found similar results in our study. The values of all the parameters before supplementation (pre-values) were analyzed by ANOVA test. The results showed that the pre values of peak force differ significantly between the trained and untrained groups ($F=3.599$, $p<0.05$). This is because the trained groups showed high peak force values than the untrained groups due to the training effect. Similarly the trained group also showed better values before supplementation for the average force than the untrained groups but the result was not statistically significant ($F=2.612$, $p>0.05$). The fatigue index as used in our study involved the use of a sustained isometric contraction for 60 sec, did not show any significant difference ($F=0.587$, $p>0.05$) between the strength trained and untrained.

The pre and post values of peak force exerted showed significant increase ($p<0.01$, table 2) in both the supplemented groups by paired t-test analysis. Mean value increased from 326 to 398 N in supplemented trained and from 231 to 302 N in supplemented untrained. No significant difference was found in any of the placebo group. Significant difference was found in post values of peak force by ANOVA analysis. On applying multiple scheffe, significant difference was found between the supplemented trained and placebo trained groups and between the supplemented trained and supplemented untrained group. This implies spirulina supplementation with training is better than only spirulina ingestion or only training in enhancing peak force.

The pre and post values of average force exerted also showed significant increase ($p<0.01$, table 2) in both the supplemented groups by paired t-test analysis. Mean value increased from 268 to 337 N in supplemented trained and from 198 to 266 N in supplemented untrained. Significant difference was found in post values of average force by ANOVA analysis. On applying multiple scheffe, significant difference was only found in between supplemented trained and placebo trained. This implies that spirulina supplementation is better with training in enhancing average force compared to only training but is equally effective without training.

Huang et al (3) found that spirulina supplementation decreased free radicals and increased soleus superoxide dismutase activities significantly in rats after exhaustive exercise. Hsueh kuan et al (9) also found that 3 week spirulina supplementation decreased activity of creatine kinase and LDH, which are indicators of skeletal muscle damage in response to exercise. It is well documented that muscular exercise promotes the production of free radicals and other reactive oxygen species in the working muscle due to increase in oxygen consumption in tissues and the failure to remove free radicals could lead to oxidative damage of cellular biomolecules (18). These reactive oxygen species are responsible for exercise-induced protein oxidation and contribute to muscle fatigue (9). These muscle-derived ROS primarily act on myofibrillar proteins to inhibit calcium sensitivity and depress force (19). Free radicals formed during chronic exercise may exceed the protective capacity of the antioxidant defense system, thereby making the muscle more immune to fatigue and injury.

In our study, the pre and post values of fatigue index measured as percentage fall in maximal force over a period of 60 sec by dominant quadriceps muscle showed significant change ($p<0.01$, table 2) in both the supplemented groups by paired t-test. Mean value decreased from 34.6 to 25.5 in supplemented trained and from 41 to 31.9 in supplemented untrained. Decrease in fatigue index implies increase in muscular endurance. No significant difference was found on ANOVA analysis in between the groups. This implies human supplementation with spirulina has the effect of increasing muscular endurance, but there is no significant difference as far as training levels are concerned.

Adverse Reaction

No level of any adverse effects has been found with the dosage we used (2gm/day) after 8 weeks of spirulina supplementation in any of the subject. This is in accordance with studies done in animals where no body or organ toxicity has been reported for acute, sub-acute and chronic toxicity (20). Salazar et al (21) also concluded that spirulina up to high feeding levels did not produce adverse effects in mice after subchronic treatment of 13 weeks.

This study shows that spirulina supplementation is effective in enhancing the muscular performance and can be used as a nutritional supplement. Spirulina is a natural food other than artificial concentrated compounds. If taking spirulina has the effect on exercise related advantage, it could probably decrease the side effect and dependence, which usually appear with long-term use of artificial concentrated compounds on human body.

Limitations of study

The sample size was small (n=40) and limited to a narrow range (20-30 years). Other limitations of the study may be that only isometric force could be studied. Psychological and motivational component of the subject during the testing of maximal voluntary isometric contraction might have affected the outcome of the study.

Conclusions

Summarizing the results of our study we conclude significant group (trained vs untrained) and supplementation (spirulina vs placebo) effects for the isometric muscle strength after spirulina supplementation. Spirulina with training is effective in increasing muscle strength than only spirulina or only training. It is also effective with time in enhancing muscular endurance but no significant group effect was found. So it can be used as a nutritional supplement by athletes and non-athletes in enhancing muscle strength and endurance.

Conflict of Interest: None declared

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