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EFFECT OF SIX WEEK AEROBIC TRAINING, CALCIUM AND IRON SUPPLEMENTATION ON RESTING BIOCHEMICAL PARAMETER CALCIUM LEVEL AMONG SELECTED SCHOOL LEVEL ATHLETES

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ABSTRACT

The intent of this study was to analyze the effect of six-week aerobic training and four treatments (Calcium, Iron, Combo and Placebo supplementation) on the resting biochemical parameter i.e. calcium level among selected school level athletes. To conduct the investigation, 60 male athletes from state sports hostel, Una, Himachal Pradesh ranging from 12 to 19 years age group were selected as subjects on the basis of random sampling. They were further divided into four groups with15 subjects in each group and the groups were named as Calcium, Iron, Combo (Calcium-Iron) and Placebo treatment groups. The four groups undertook a planned aerobic training of six weeks duration and each group was regularly administered one of the Calcium, Iron, Combo (Calcium-Iron) and Placebo supplementations respectively. It was hypothesized that there would be no significant effect of aerobic training and four treatments (Calcium, Iron, Combo and Placebo supplementation) onthe resting biochemical parameter calcium level among selected school level athletes. To cater to the need of the study resting blood samples were obtained from the subjects in the morning i.e. on day-0, day-21 and day-42 and these blood samples were tested in the biochemistry laboratory at IGMC, Shimla. The data obtained was analyzed with the application of one-way repeated measures ANOVA and post hoc test. The result of study exhibited that the four treatments along with aerobic training had a significant effect on the resting calcium level of athletes in the form of increased levels of calcium from one point of time to another with minor exceptions.

KEYWORDS: Aerobic Training, | Biochemical Parameter, Calcium and Iron Supplementation.

INTRODUCTION

Aerobic training refers to certain muscular movements of the parts of the body in the presence of oxygen for a particular period of time by following a similar pattern to enhance the overall well-being and sports performance of athletes. These activities which are referred to as aerobic training or exercise are helpful in increasing heart rate when they are practiced at a balanced rate of anxiety and they are able to produce energy for the cells(Radhakrishnan, 2019). Aerobic exercises result in the form of a parallel positive outcome along with proper nutrition or supplementation including all the constituents known as macro and micronutrients or chemical substances. The body requires them in an appropriate manner or balanced amount to improve results (Clarkson, 1991). They can be obtained from food items and direct supplementation (Heffernan et al., 2019). Calcium, a mineral is essentially required for the body of athletes in a higher amount than the general population, especially in athletes to maintain the health of bones and the regulation of coordination between the brain and other parts of the body. Calcium also plays an important part in cardiovascular and muscle movement. Itis often found in combination with other substances including carbonate and citrate. The supplements help to increase the level of athletic performance as the sports nutritionists most of the time suggest taking calcium supplements along with intense exercise activities (Newman, 2023).

Iron is a vital mineral essential for transporting oxygen to working tissuesall over the body and in addition serves significant roles in metabolism, respiration, and immunologic responses.The human body upholds stores of iron and judiciouslymaintains anequilibrium between iron lost, iron gained, and iron deposited (DellaValle, 2013).

OBJECTIVE OF THE STUDY

The specific objective of the study was to find out the effect of aerobic training along with calcium and iron supplementation on the Biochemical Parameter i.e.resting Calcium level of selected school level athletes.

HYPOTHESIS

It washypothesized that there would be no significant effect of aerobic training and four treatments (Calcium, Iron, Combo and Placebo supplementation) on the resting biochemical parameter calcium level among selected school level athletes.

MATERIALS AND METHODS

The researchers to execute this investigation took 60 male athletes from state sports hostel, Una, Himachal Pradesh. They were ranged between the 12 to 19 years of age and selected on the basis of random sampling. Then, they were divided into four groups and each group consisted of 15 subjects with the names Group-I (Calcium), Group-II (Iron), Group-III (Calcium-Iron or Combo) and Group-IV (Placebo) correspondingly. The subjects were provided with a relevant information regarding the procedure to be adopted for the undertaken research and its possible outcomes as well as its advantages in the field of sports. Moreover, the subjects participating in the study were asked to submit a consent letter to assure their wishful willingness to participate in this study without any enforcement.

The four groups went through a planned aerobic training for a duration of six weeks and each group was regularly provided with one of the Calcium, Iron, Combo (Calcium-Iron) and Placebo supplementation respectively right from day-1. Group-I (Calcium) received 1000mg Calcium gluconate per day, Group-II (Iron) received 10 mg Ferrous Sulphate + 1.5 mg Folic Acid per day; Group-III (Calcium-Iron or Combo) received 1000mg Calcium gluconate per day in the morning and 10 mg Ferrous Sulphate + 1.5 mg Folic Acid per day in the evening; and Group-IV (Placebo) received 100 mg Sugar Coated

Ascorbate per day respectively. Further, resting blood samples were collected from the subjects in the morning i.e. on day-0, day-21 and day-42 with the help of an experienced technician and the blood serum obtained from these samples was analysed in the biochemistry laboratory at IGMC, Shimla by means of fully automated biochemistry analyzer (XL-1000). The outcomes of collecteddatawere statistically analyzed applying statistical procedure one-way repeated measures ANOVA and post hoc test using the SPSS software version 23. The level of significance for testing the hypothesis was set at .01 level.

RESULTS AND DISCUSSION

The results are presented intables 1-10 and they includedescriptive statistics, one-way repeated measures ANOVAand post hoc test values w.r.t. restingbiochemical parameter i.e. calcium level.

Table-1: Descriptive Statistics of the Biochemical Parameter i.e. Calcium Level of School Level Athletes at Rest to Observe the Effect of Aerobic Training and Four Treatments i.e. Calcium, Iron, Combo and Placebo Supplementation at Various Points of Time, i.e. on Day-0, Day-21 and Day-42

	Supplementation Group	**Mean**	**Std. Deviation**	**N**
Pre-Test (Day-0)	Group-I Calcium	8.997	.234	15
	Group-II Iron	9.850	.541	15
	Group-III Combo	9.130	.348	15
	Group-IV Placebo	9.837	.434	15
Mid-Test (Day-21)	Group-I Calcium	9.467	.310	15
	Group-II Iron	10.127	.557	15
	Group-III Combo	9.537	.340	15
	Group-IV Placebo	10.150	.372	15
Post-Test (Day-42)	Group-I Calcium	10.447	.195	15
	Group-II Iron	10.073	.388	15
	Group-III Combo	10.457	.189	15
	Group-IV Placebo	10.113	.440	15

Table-1 reveals the descriptive statistics of the data on calcium level at rest, i.e.,the mean and standard deviation of athletes of different treatment groups at different points of time, i.e., before the training on day-0 (Pre-test), in the middle of training or after 3 weeks of training on day-21 (Mid-test) and after 6 weeks of training on day-42 (Post-test). The mean and standard deviation of the resting calcium level on day-0 (Pre-test) for group-I (Calcium) were respectively 8.997 & .234; group-II (Iron) were 9.850 & .541; group-III (Combo) were 9.130 & .348 and group-IV (Placebo) were 9.837 & .434.

On day-21 (Mid-test), the mean and standard deviation of the resting calcium level for group-I (Calcium) were respectively 9.467 & .310; group-II (Iron) were 10.127 & .557; group-III (Combo) were 9.537 & .340; and group-IV (Placebo) were 10.150 & .372.

The mean and standard deviation on day-42 (Post-test) of the resting calcium level for group-I (Calcium) were respectively 10.447 & .195; group-II (Iron) were 10.073 & 388; group-III (Combo) were 10.457 & .189; and group-IV (Placebo) were 10.113 & .440.

A. Multivariate Test

The results for analysing the above objectives have been reported from both the angles i.e., the multivariate tests and the univariate tests as generated in the output by the software. Firstly, for the interpretation of multivariate tests, the box's test of equality of variance-covariance matrices was checked. To test the assumption of equality of variance-covariance matrices of different scores between four diverse groups i.e., group-I (Calcium), group-II (Iron), group-III (Combo) and group-IV (Placebo) over time for groups, Box's test has been applied and presented below in the table-2:

Table-2: Summary of Box's Test of Equality of Variance-Covariance Matrics w.r.t. Aerobic Training and Four Treatments i.e. Calcium, Iron, Combo and Placebo Supplementation at Rest

Box's M	106.189
F	5.319
Df1	18
Df2	11081.816
Sig	0.000

It is clear from table-2 that the value for Box's matrices is 106.189, F (18, 11081.816) 5.319, $p<.01$, which is found significant. Thus, indicating that the equality of variance and co-variance cannot be assumed. As a result, the assumption is violated and hence, ignored. Furthermore, Pillai's Trace has been taken into consideration to interpret the outcomes for multivariate tests.

Table-3: Summary of Multivariate Test (Pillai's Trace) for Calcium Level of Athletes in Relation to Aerobic Training Programme and Four Treatments i.e. Calcium, Iron, Combo and Placebo Supplementation at Rest

Effect	Value	F	Hypothesis Df	Error Df	Sig.	Partial Eta Squared
Aerobic Training and Four Treatments	.566	7.366	6.00	112.00	.000	.283

The figures in table-3 show that the main effect of repeated measurement over time as a result of six-week aerobic training and four treatments i.e. calcium, iron, combo and placebo supplementation is statistically significant, Pillai's Trace .566, F (6, 112) =7.366, $p<0.01$. Hence, the hypothesis entitled as, *"There would be no significant effect of six weeks aerobic training and four treatments i.e. calcium, iron, combo and placebo supplementation on calcium level of school level athletes at rest",* is **rejected**. In order to see the effect of time, a pairwise comparison of combined resting calcium level at different points of time is also made, and the results are presented in table below:

Table-4: Pairwise Comparison of Combined Resting Calcium Level Scores of Four Treatments Groups over Time

Combined Resting Calcium Level Scores	Mean Difference	Std. Error	Sig.

(Pre-Test) Day-0	**(Mid-Test) Day-21**	.367	.043	.000
(Pre-Test) Day-0	**(Post-Test) Day-42**	.819	.069	.000
(Mid-Test) Day-21	**(Post-Test) Day-42**	.452	.065	.000

Table-4 reveals that there is a significant improvement in the combined resting calcium level scores of four treatment groups from pre-test (day-0) to mid-test (day-21) (mean difference = .367), which is evident from the p-value (.000) for the pairwise difference for pre-test to mid-test. It means that the treatment given to the athletes has resulted in increasing calcium level. Similarly, the p-value for pairwise comparison for pre-test (day-0) to post-test (day-42) (mean difference = .819) and mid-test (day-21) to post-test (day-42) (mean difference = .452) have also been found to be significant at .01 level of confidence which is evident from the p-values for the above-mentioned pairs. The treatment of aerobic training and four supplementations i.e. calcium, iron, combo and placebo given for the first three weeks have resulted in an increase in the calcium level of the athletes. Moreover, the next three weeks of treatment (aerobic training and four supplementations i.e. calcium, iron, combo and placebo) have also resulted in an improvement in calcium level of athletes.

B. Univariate Test (Within-Subjects)

Secondly, after the multivariate test, the other way to interpret the above hypothesis is through the univariate results revealed in table-5.

For the univariate tests, the Sphericity assumption needs to be checked and hence, reported below to verify the results for the hypothesis. The results checked the sphericity assumption through Mauchly's Test of Sphericity and the outcome is accessible below:

Table-5: Mauchly's Test of Sphericity

Within Subjects Effect	**Mauchly's W**	**Approx. Chi-Square**	**Df**	**Sig.**	**Epsilon**		
					Greenhouse Geisser	**Huynh-Feldt**	**Lower Bound**
Calcium Level	.752	15.641	2	.000	.802	.866	.500

Table-5 clarifies that the outcome of Mauchly's test is being found significant which indicates that the variances of the differences between all combinations of related groups due to time (six week aerobic training programme) and four treatments i.e. calcium, iron, combo and placebo supplementation are equal. Therefore, it needs to report then corrections to Sphericity (=1). Herein, the Huynh-Feldt correction (Girden, 1992) will be used since the Greenhouse Geisser Epsilon (E) value (0.802) is greater than 0.75 for the univariate test of mean differences for calcium level scores. This may be due to the equal time span between different measurement's occasions. In order to see the results of the main and interaction effects of time (six weeks aerobic training) and four treatments i.e. calcium, iron, combo and placebo supplementation on the calcium level scores at rest, the proportion of levels amongst the subjects is reported here under:

Table-6: Summary of Univariate Test of Within-Subjects Effects on the Scores of Calcium Level at Rest in Relation to Six-Week Aerobic Training and Four Treatments i.e. Calcium, Iron, Combo and Placebo Supplementation

Source		**Type-III Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**	**Partial Eta Squared**
Aerobic Training and Four	Huynh-Feldt	11.600	5.195	2.233	17.856	.000	.489

Treatments							

From table-6, test of within-subjects effects, it is clear that the main effect of repeated measurements over time as a result of aerobic training and four treatments i.e. calcium, iron, combo and placebo supplementation is statistically significant using Huynh-Feldt Factor, HF (5.195, 2.233) =17.856, $p<0.01$. The Partial Eta Squared indicates a large effect size = .489 (Cohen, 1988; Pituch& Stevens, 2016; Field, 2018).

C. Within-Subjects Contrasts (Trending over Time)

Further to see the type of trending overtime for the implementation of the modules of six-week aerobic training and four treatments i.e. calcium, iron, combo and placebo supplementation on the improvement of resting calcium level, the test of within-subjects contrasts is being stated underneath. The linear trend means that there is a straight line of the trend either going upwards or downwards and a quadratic trend means that there is a consistent curving pattern, upward or downward, marked by a dramatic increase or decrease of means over time or repeated measurements.

Table-7: Test of Within-Subjects Contrasts for the Combined Resting Calcium Level Scores of Four Treatments Groups

Source	Combined Resting Calcium Level	Type-III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Aerobic Training and Four Treatments	Linear	9.786	3	3.262	23.003	.000	.552
	Quadratic	1.814	3	.605	8.090	.000	.302

From table-7, it is seen that the trend for combined resting calcium level due to aerobic training and four treatments i.e. calcium, iron, combo and placebo supplementation has been found significant for linear [$F(3, 56) = 23.003$, $p<.01$] and for quadratic it has again been found significant [$F(3, 56) = 8.090$, $p<.01$], it means that the data is following both of the trends i.e. linear and quadratic for the mean values of the combined resting calcium level (as result of aerobic training and four treatments). It also indicates that due to the implementation of six weeks aerobic training module and four treatments, the resting calcium level increased for all treatment groups from day-1 to the day-21 of training whereas from day-22 to the day-42 of training, the resting calcium level increased for the group-I (Calcium) and group-III (Combo) but in case of group-II (Iron) and group-IV (Placebo) a minor decline is exhibited in the resting calcium level instead of increasing. The same can be realized through the following:

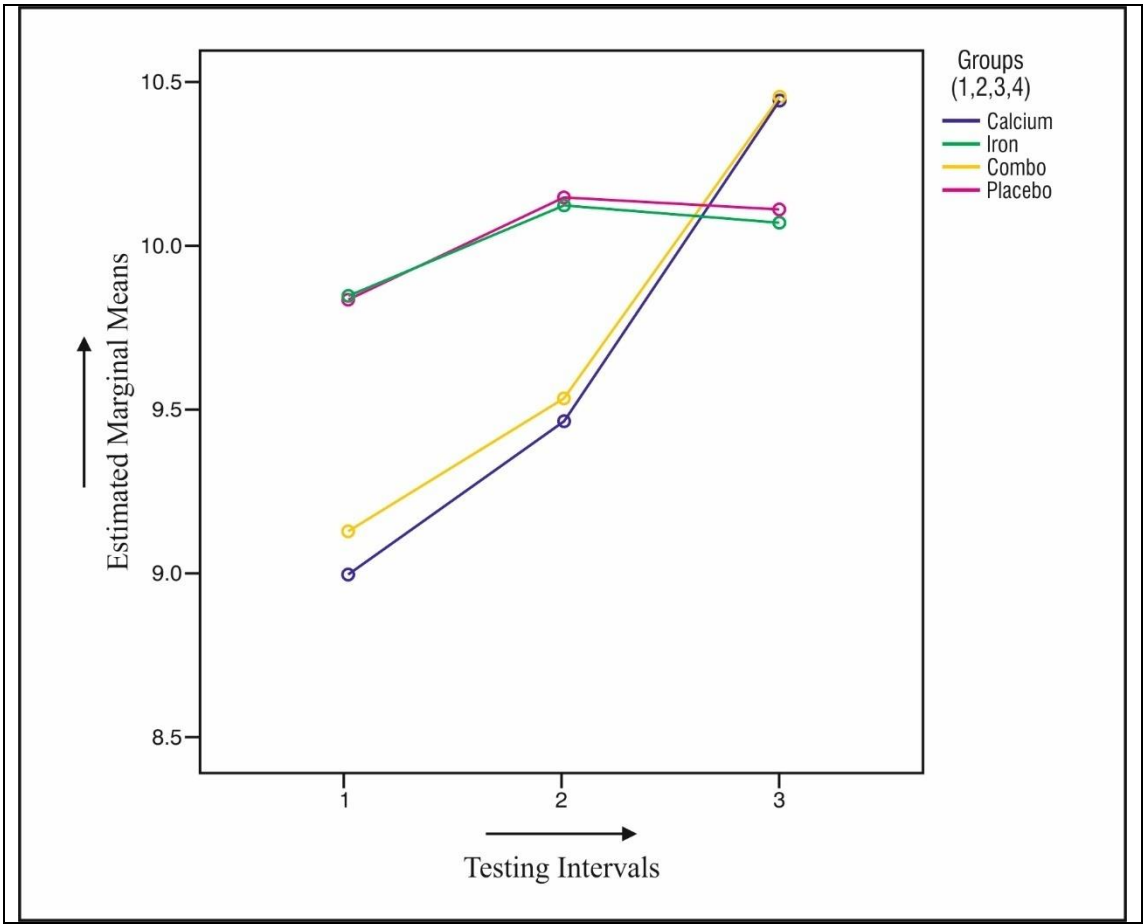

Fig.1: Graphical Representation of the Resting Calcium Levels of the School Level Athletes Undergoing Aerobic Training and Four Treatments (Calcium, Iron, Combo and Placebo Supplementation) at Various Points of Time, i.e. on Day-0, Day-21 and Day-42.

D. Univariate Test (Between-Subjects Effects)

Further, the researcher calculated the univariate ANOVA or between subjects effects to study the main and interaction effects of grouping variables aerobic training and four treatment groups (calcium, iron, combo and placebo supplementation) at each point of measurements i.e. day-0 (pre-test), day-21 (mid-test) and day-42 (post-test) and the results are being presented in the table-8 through Levene's test of equality of error variances.

Table-8: Levene's Test of Equality of Error Variances

	F	Df1	Df2	Sig.
Pre-Test (Day-0) Resting Calcium Level Score	.947	3	56	.424
Mid-Test (Day-21) Resting Calcium Level Score	1.077	3	56	.366
Post-Test (Day-42) Resting Calcium Level Score	5.830	3	56	.002

Above table-8 Levene's Test of equality of error variances shows the intercept of aerobic training and four treatments (calcium, iron, combo and placebo supplementation). The p-value for resting calcium level scores at pre-test and mid-test is greater than .01; hence, the assumption has been met. But at post-test, the p-value for resting calcium level scores is less than .01, hence, the assumption has not been met. Though, the violation of this

assumption is not an issue since the ratio of N's of the largest and the smallest group size is less than 1.5 (15/15 =1), the results of the univariate ANOVA can be considered fairly robust (Petuch and Stevens, 2016).

Table-9: Summary of Univariate ANOVA on the Resting Scores of Calcium Level due to Aerobic Training and Four Treatments i.e. Calcium, Iron, Combo and Placebo Supplementation (Between-Subjects Effects)

Source	Type-III Sum of Squares	Df	Mean Square	F	Sig.	Partial Eta Squared
Intercept	17459.125	1	17459.125	80041.107	.000	.999
Groups (1,2,3,4)	5.720	3	1.907	8.742	.000	.319
Error	12.215	56	.218			

The table-9 reveals that the F-ratio for the averaged resting calcium level scores across time for group-I (Calcium), group-II (Iron), group-III (Combo) and group-IV (Placebo) have been found 8.742. The value is found to be significant at .01 level of confidence. It indicates that the 4 groups differ significantly on the resting calcium level scores averaged for different measurement points of the study. Further, to see the differences averaged across time for resting calcium level scores between groups, researcher used the post hoc test and the output it is being presented through the following table:

Table-10: Post Hoc Values for Calcium Level at Rest Across Time w.r.t. Aerobic Training and Four Treatments i.e. Calcium, Iron, Combo and Placebo Supplementation among 4 Groups

(I) Groups (1,2,3,4)	(J) Groups (1,2,3,4)	Mean Difference (I-J)	Std. Error	Sig.
Calcium	Iron	.380	.098	.004
	Combo	.071	.098	.914
	Placebo	.397	.098	.002
Iron	Combo	.309	.098	.822
	Placebo	.017	.098	.048
Combo	Placebo	.326	.098	.018

The table-10 exhibits that there is a significant difference in the resting calcium levels between calcium group and iron group; calcium group and placebo group at 0.01 level of confidence as the p-values are less than 0.01. However, there is non-significant difference in the resting calcium level between calcium group and combo group at 0.01 level of confidence as the p-value is greater than 0.01. Similarly, there is non-significant difference in the resting calcium levels between iron group and combo group; iron group and placebo group at 0.01 level of confidence as the p-values are greater than 0.01. In the same way, combo group and placebo group has found non-significant difference amongst themselves on the resting calcium level at 0.01 level of confidence as the p-value is greater than 0.01.

Discussion

The findings of the study revealed that the level of calcium in candidates undergoing aerobic training got improved on getting calcium supplementation. This improvement in calcium level was however independent of the fact that as whether the candidate got calcium supplementation in combination with iron or it was given singularly. Hence, it was established that the calcium supplementation enhanced the performance level of sportsmen as minerals like calcium are necessary to maintain the health of bones and to improve the level of performance and it is supported by Clarkson, 1991. The sportsmen are always indulged in strenuous exercise activities which increase the mineral losses from human

body. It must be followed by increased food intakes or oral supplements to fulfill the requirements of energy and minerals. Thus, the athletes should particularly keep in mind the supplementation as previously reported by Maughan, 1999. In short calcium and iron supplementation boosts overall athletic performance (Cinar, Baltaci & Mogulkoc, 2009).

Conclusion

The research investigation lead to the conclusion that calcium and iron supplementation along with aerobic training exhibited a positive impact on calcium level in the form of its increased level from one point of time to another. It may be lostfrom the body due to extensive exercise activities and lead to a level of deficiency which can be overcome by dietary intake or direct supplementation.

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