International Journal for Multidisciplinary Research (IJFMR)
E-ISSN : 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Effect of Nitrates on Endurance Athletes - A Case Control Study

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Abstract

Beetroot itself is a precursor of Nitric Oxide (NO), it is used as a supplement. Increased NO availability will enhance oxygen and nutrient delivery to the working muscle by lowering the ATP cost of muscle contractile force production and the oxygen cost of aerobic exercise. The mechanisms contributed to these effects are linked to mitochondrial respiration and biogenesis which shows that enhancing NO bioavailability positively influences exercise performance in endurance athletes.VO2 max (maximal oxygen intake) is a familiar measurement related to aerobic endurance that numerous athletes use to determine their overall fitness as it measures the maximum amount of oxygen that a person can utilize throughout the intense exercise.

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Keywords: Nitric Oxide (NO), VO2 max (maximal oxygen intake), Endurance athletes, Exercise performance, Beetroot, Supplement, Nutrient.

1. Introduction

Beetroot itself is a precursor of Nitric Oxide (NO), it is used as a supplement (Jones A. , 2014) . The process of NO synthesis is thought to be through catabolism of arginine by the enzyme NO Synthase (Stamler & Meissner, 2009). Successfully, arginine supplementation has shown to increase the NO levels (Weitzberg & Lundberg , 2010). Another mechanism of NO synthesis is mediated by inorganic nitrates (NO_3^-) which implies that the high amounts of NO_3^- present in beetroot juice are able to increase NO levels in the body. Around 25% of dietary NO_3^- in the mouth, is reduced by NO_3^- reductase generated by micro-organisms (Potter, Angove, Richardson, & Cole, 2001) to nitrate (NO_2^-) (Gladwin, Lundberg, & Weitzberg, 2008). This NO_2^- is then partially converted to NO through the action of stomach acids which is later absorbed in the gut (Raat , Shiva , & Gladwin , 2009). A part of this NO_2^- enters the blood stream, and, in low oxygen conditions, will be converted into NO (Govoni & Lundberg, 2004).

The absolute bioavailability for dietary nitrate is 100 percent, and the peak plasma levels after dietary consumption are typically reached 1.5-1.8 hours post consumption. The European Food Safety Authority established an Acceptable Daily Intake for nitrate of 3.7 mg/kg daily (0.06 nmol/kg). For a 70kg adult this equates to about 260 mg/d (around 4.2 nmol). In the nitrate loading study, athletes had nearly double the plasma nitrate amount (8.2 nmol), equating to 1.95 times more or 7.2 mg/kg daily. For the same 70kg adult this would mean 505 mg daily.

Endurance fitness is the ability to sustain the necessary activity level for a specific competitive sport. It includes both cardiovascular and muscular endurance required for the sport. Endurance means a general ability to do any kind of physical activity that increases your heart rate above 50% of your maximum. On the higher level it can be divided into general endurance and specific endurance.

Specific endurance is the ability to stand against fatigue in sport specific conditions. The better your sport specific endurance, the better you perform at this specific sport. It can be characterised as a combination of various types of endurance you need to maximize your ability to succeed in your discipline. General endurance characterizes the ability of your whole body to tolerate endurance exercises and diminish fatigue. The better your general endurance the better you can stand longer efforts at various sports disciplines.

VO2 max, or maximal oxygen uptake, is a common measurement linked to aerobic endurance that many athletes use to determine their overall fitness. VO2 max is the measurement of the maximum amount of oxygen that an individual can utilize during intense, or maximal exercise. It is measured as milliliters of oxygen used in one minute per kilogram of body weight (ml/kg/min). It is one factor that may help determine an athlete's capacity to perform sustained exercise.

An athlete's VO2 max score is generally considered by exercise physiologists as one of the best indicators of the athlete's cardiovascular fitness and aerobic endurance. Theoretically, the more oxygen you can use during high-level exercise, the more adenosine triphosphate (ATP) energy you can produce in your cells. This is often the case with elite endurance athletes, who typically have very high VO2 max values.

An athlete's oxygen consumption rises in a linear relationship with exercise intensity—up to a point. There is a specific point at which oxygen consumption plateaus even if the exercise intensity increases. This plateau marks the VO2 max. It's a painful point in VO2 max testing where the athlete moves from aerobic metabolism to anaerobic metabolism. From there, it's not long before muscle fatigue forces the athlete to stop exercising. VO2 max results vary greatly. The average for a sedentary individual is close to 35 ml/kg/min. Elite endurance athletes often average 70 ml/kg/min.

Sabeet is a standardized (2%) powdered extract from the tubers (roots) of Beta vulagris. An athlete needs 5gms of this extract a day to improve his endurance (VO2 max). Some of the common food sources of nitrates are lettuce, beets, carrots, green beans, spinach, parsley, cabbage, radishes, celery and collard greens.

2. METHODOLOGY AND MATERIALS USED

2.1- Aims & objectives: To study the effects of pre-workout supplementation of nitrates (Sabeet) on the performance of endurance athletes.

2.2- Research problem: This study is mainly done to assess the effect of nitrates present in beetroot supplements for elite-level and recreational endurance athletes as there are no previous studies done on endurance athletes in India.

2.3- Research Hypothesis: Consumption of beetroot extract rich in nitrates, 90 minutes prior to a workout session will not increase the VO2 max in endurance athletes.

2.4- Study Design: Interventional Study - It is a type of clinical study in which participants are assigned to groups that receive one or more intervention/treatments (or no intervention) so that researchers can evaluate the effects of the interventions on biomedical or health-related outcomes. The assignments are determined by the study's protocol. Participants may receive diagnostic, therapeutic, or other types of interventions.

-Sample Size: 30 (15 case and 15 control)
-Study Area: Manipal, Karnataka
-Study Period: May to October 2018 (5 months)
-Target Group: Elite and recreational endurance athletes

2.5- Inclusion criteria:

- Elite level and recreational endurance athletes.
- Healthy male without any comorbidities.
- Age between 18-35 years.
- People who can speak in English.
- People who perform exercise daily.

2.6- Exclusion criteria:

- People with significant past medical history such as hypotension, hypertension, cardiovascular disease or any other heart-related disease.
- People who have donated blood anytime within the past 30 days.
- People who smoke and consume alcohol.
- Age below 18 and above 35 years.

2.7-Material used:

2.7.1- Anthropometrical measurements such as weight, height and skin fold thickness at 3 sites namely thighs, abdomen and chest using **Harpenden's caliper** at the beginning of the study.

2.7.2- Blood Pressure using Blood Pressure monitor at the beginning and end of the study.

2.7.3- VO2 Max using **Queen's College Step Test** is a test in which the athlete steps up and down on the platform at a rate of 22 steps (16.25 inches / 41.3 cm step) per minute for females and at 24 steps per minute for males. The subjects are to step using a four-step cadence, 'up-up-down-down' for 3 minutes. The athlete stops immediately on completion of the test, and the heart beats are counted for 15 seconds from 5-20 seconds of recovery. This will be done using **'Digital blood pressure monitor'** which measures the heart rate.

2.7.4- Supplement given: Sabeet (2% standardized nitrate extract from the tubers of *Beta vulgaris*)

2.7.5- Questionnaire will be given to obtain their anthropometry, socio-demographic, physical activity record, food preferences, 3 days diet recall and 6 days dietary recall (in a **diary**) information with respect to the study.

2.7.6- DietCal is a software developed by Ms. Gurdeep Kaur, Senior Dietitian from AIIMS, New Delhi. This software is useful for calculating nutrient content of any food item. It contains all the nutrients given in the book "Nutritive Value Of Indian Foods" by ICMR.

2.8- Statistical methods: T-test and repeated measures in SPSS 16 version.

2.9- Pretesting of questionnaire: Not required

2.10- Data collection methodology:

-Collecting their personal details and topic-related relevant information by providing them questionnaire to fill.
-Divide the population into 2 groups; one case group which is willing to take the supplement and the other control group.
-Assessing their anthropometry values using stadiometer (for measuring height) and weighing scale (for measuring weight) and skinfold calliper (for measuring body percentage).
-Measuring the **VO_2** max (also known as maximal oxygen consumption) for both the groups by subjecting them to Queen's College Step Test.
-Before starting the supplementation, 3 days diet recall for both the case and control groups are taken and will be given a diary that would serve the purpose of a 24 hours dietary recall along with their meal timings and quantity of each meal in which they are supposed to note everything down that they will have for the next 6 days.
-The case group is supplemented with Sabeet (5 grams which contains beetroot extract standardized to 2% nitrate), 90 minutes prior to usual exercise routine for the next 6 days while the control group is allowed to continue their exercise and diet regimen.
-Measuring their **VO_2** max by 'Queen's College Step Test' every day for the case and control groups which will also show their heart rate by using 'heart sensor' and 'Polar Beat App.'
-Measuring the final **VO_2** max after Day 6 for both the groups by subjecting them to Queen's College Step Test again.

3.11- Data Analysis Method:

-Getting approval from IEC and Manipal Sports Council.
-Selection of 30 elite level and recreational endurance athletes.
-Collecting their personal details and topic related relevant information by providing them questionnaire to fill.
-Divide the population into 2 groups; one case group which is willing to take the supplement and the other control group.

-Assessing their anthropometry values using stadiometer (for measuring height) and weighing scale (for measuring weight) and skinfold calliper (for measuring body percentage).
-Measuring the VO_2 max (also known as maximal oxygen consumption) for both the groups by subjecting them to Beep test.
-Before starting the supplementation, 3 days diet recall for both the case and control groups are taken and will be given a diary that would serve the purpose of a 24 hours dietary recall along with their meal timings and quantity of each meal in which they are supposed to note everything down that they will have for the next 6 days.
-The case group is supplemented with Sabeet (5 grams which contains beetroot extract standardized to 2% nitrate), 90 minutes prior to usual exercise routine for the next 6 days while the control group is allowed to continue their exercise and diet regimen.
-Measuring their VO_2 max by 'Queen's College Step Test' every day for the case and control groups which will also show their heart rate by using 'Digital blood pressure monitor.'
-Measuring the final VO_2 max after Day 6 for both the groups by subjecting them to Beep Test again.
-Compare and analyse the performance between both the groups using SPSS 16 version.

3. RESULTS

3.1 : GENERAL INFORMATION

Group	Age in Years	Frequency	Percent
Case	18	3	21.4
	19	2	14.3
	20	3	21.4
	21	3	21.4
	22	1	7.1
	23	1	7.1
	25	1	7.1
	Total	14	100.0
Control	18	4	28.6
	19	2	14.3
	20	5	35.7
	22	2	14.3
	23	1	7.1
	Total	14	100.0

3.1.2- Place of Stay

Group	Place of Stay	Frequency	Percent
Case	Hostel	6	42.9
	Apartment (without parents)	8	57.1
	Total	14	100.0
Control	Hostel	7	50.0
	Apartment (with parents)	2	14.3
	Apartment (without parents)	5	35.7
	Total	14	100.0

3.2- MEDICAL HISTORY

3.2.1- Disease(s) For First Degree Relations

Group	Disease	Frequency	Percent
Case	Diabetes	2	14.3
	Thyroid	1	7.1
	Diabetes and Hypertension	3	21.4
	None	8	57.1
	Total	14	100.0
Control	Diabetes	2	14.3
	Asthma	1	7.1
	Thyroid	1	7.1
	Diabetes and Hypertension	1	7.1
	Cancer	1	7.1
	None	6	42.9
	Diabetes & CVD & Kidney issues & Others	1	7.1
	Asthma & Back Pain	1	7.1
	Total	14	100.0

3.2.2- Disease(s) for Second Degree Relations

Group	Disease	Frequency	Percent
Case	Diabetes	2	14.3
	None	12	85.7
	Total	14	100.0
Control	Diabetes	2	14.3
	Asthma	1	7.1
	None	10	71.4
	Diabetes and CVD	1	7.1
	Total	14	100.0

3.3- ANTHROPOMETRIC AND LIFESTYLE DETAILS

3.3.1- Body Fat Percentage

Group Class		Frequency	Percent
Case	Athletes	11	78.6
	Fitness	3	21.4
	Total	14	100.0
Control	Athletes	10	71.4
	Fitness	4	28.6
	Total	14	100.0

3.3.2- Physical Activity Level

Group	Class	Frequency	Percent
Case	Moderate Activity	13	92.9
	Heavy Activity	1	7.1
	Total	14	100.0
Control	Moderate Activity	13	92.9
	Heavy Activity	1	7.1
	Total	14	100.0

3.3.3- Food Preference

Group Preference	Food	Frequency	Percent
Case	Vegetarian	2	14.3
	Non-Vegetarian	10	71.4
	Vegan	1	7.1
	Ovo lacto	1	7.1
	Total	14	100.0
Control	Vegetarian	3	21.4
	Non-Vegetarian	11	78.6
	Total	14	100.0

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3.3.4- Water Consumption

Group Consumption		Frequency	Percent
Case	2 litres	6	42.9
	3 Litres	6	42.9
	4 litres	1	7.1
	>4 litres	1	7.1
	Total	14	100.0
Control	2 litres	4	28.6
	3 Litres	7	50.0
	4 litres	2	14.3
	>4 litres	1	7.1
	Total	14	100.0

3.3.5- Skipping Breakfast

Group Pattern	Skipping	Frequency	Percent
Case	Daily	3	21.4
	Alternate	1	7.1
	Twice in a week	3	21.4
	Once in a week	2	14.3
	No	5	35.7
	Total	14	100.0
Control	Alternate	2	14.3
	Twice in a week	4	28.6
	Once in a week	1	7.1
	No	7	50.0
	Total	14	100.0

3.3.6- Skipping Lunch

Group Pattern	Skipping	Frequency	Percent
Case	No	14	100.0
Control	Twice a week	2	14.3
	No	12	85.7
	Total	14	100.0

3.3.7- Skipping Dinner

Group Pattern	Skipping	Frequency	Percent
Case	Alternate	1	7.1
	Twice a week	1	7.1
	No	12	85.7
	Total	14	100.0
Control	Twice a week	1	7.1
	No	13	92.9
	Total	14	100.0

3.3.8- Supplement Consumption

Group Consumption		Frequency	Percent
Case	Yes	5	35.7
	No	9	64.3
	Total	14	100.0
Control	Yes	3	21.4
	No	11	78.6
	Total	14	100.0

3.4 – DIET

3.4.1 – Table: Depiction of adequacy of nutrient intake in both case and control groups.

Nutrients	Group	Substantially inadequate	Marginally inadequate	Marginally adequate	Adequate	Excess
Energy(Kcal/d)	Case	2(14.3%)	9(64.3%)	3(21.4%)	-	-
	Control	3(21.4%)	7(50%)	4(28.6%)	-	-
Carbohydrate(g/d)	Case	7(50%)	6(42.9%)	1(7.1%)	-	-
	Control	4(28.6%)	8(57.1%)	2(14.3%)	-	-
Protein(g/d)	Case	2(14.3%)	4(28.6%)	7(50%)	-	1(7.1%)
	Control	3(21.4%)	6(42.9%)	3(21.4%)	-	2(14.3%)
Fat(g/d)	Case	-	3(21.4%)	5(35.7%)	2(14.3%)	4(28.6%)
	Control	1(7.1%)	1(7.1%)	4(28.6%)	4(28.6%)	4(28.6%)
Calcium (mg/d)	Case	9(64.3%)	4(28.6%)	1(7.1%)	-	-
	Control	10(71.4%)	3(21.4%)	1(7.1%)	-	-
Vitamin E (mg/d)	Case	14 (50%)	-	-	-	-
	Control	14 (50%)	-	-	-	-
Vitamin C (mg/d)	Case	2 (14.3%)	5 (35.7%)	5 (35.7%)	-	2(14.3%)
	Control	-	7 (50%)	4 (28.6%)	-	3(21.4%)
Potassium (mg/d)	Case	12 (85.7%)	1 (7.1%)	1 (7.1%)	-	-
	Control	11 (78.6%)	3 (21.4%)	-	-	-
Sodium (mg/d)	Case	-	3 (21.4%)	2 (14.3%)	-	9(64.3%)
	Control	-	1 (7.1%)	2 (14.3%)	1 (7.1%)	10(71.4%)
Iron (mg/d)	Case	3 (21.4%)	6 (42.9%)	3 (21.4%)	-	2 (14.3%)
	Control	4 (28.6%)	6 (42.9%)	2 (14.3%)	2(14.3%)	-

3.4.2- Table: Comparison of nutrient intake (micros) of the participants with the RDA.

Group Classi-fication	Nutrients	Intake Mean±SD	RDA	t-value	p-value	95% Confidence Interval of the Difference	
						Lower	Upper
CASE	Calcium(mg)	428.51±172.97	1000	9.269	<0.001*	328.64	528.39
	Vitamin E(mg)	2.94±1.68	15	6.543	<0.001*	1.96	3.91
	Vitamin C(mg)	46.05±17.63	60	9.774	<0.001*	35.87	56.23
	Potassium(mg)	1787.21±539.34	4700	12.399	<0.001*	1475.80	2098.61
	Sodium(mg)	2712.41±805.59	2300	12.598	<0.001*	2247.27	3177.55
	Iron(mg)	11.55±3.40	18	12.698	<0.001*	9.58	13.51
CON-TROL	Calcium(mg)	467.76±256.21	1000	6.831	<0.001*	319.82	615.69
	Vitamin E(mg)	3.06±2.01	15	5.699	<0.001*	1.90	4.22
	Vitamin C(mg)	51.91±16.57	60	11.720	<0.001*	42.33	61.47
	Potassium(mg)	2037.59±788.29	4700	9.671	<0.001*	1582.44	2492.74
	Sodium(mg)	2886.82±682.35	2300	15.830	<0.001*	2492.84	3280.79
	Iron(mg)	12.16±5.22	18	8.707	<0.001*	9.14	15.17

****Significant at 1% level (p=0.01)**

3.4.3- Table: Comparison of nutrient intake (macros) of the participants with the RDA.

Group Class	Nutrients	Intake Mean±SD	RDA±SD	t-value	p-value	95% Confidence Interval of the Difference	
						Lower	Upper
CASE	Energy (Kcal)	1858.30±420.82	3032.57±533.97	-7.218	<0.001*	-1525.73	-822.81
	Carbohydrates (gms)	267.17±73.29	530.31±68.31	-8.389	<0.001*	-330.90	-195.38
	Protein (gms)	69.61±20.42	97.98±14.65	-4.951	<0.001*	-40.67	-15.96
	Fat (gms)	53.13±9.76	57.64±12.83	-1.328	<0.001*	-11.89	2.83

CON-TROL	Energy (Kcal)	2090.88±398.55	3351.36±668.61	-5.293	<0.001*	-1774.94	-746.01
	Carbohydrates (gms)	301.76±92.80	568.99±141.41	-6.196	<0.001*	-360.41	-174.05
	Protein (gms)	76.33±22.47	108.48±16.89	-3.578	0.207^{NS}	-51.56	-12.74
	Fat (gms)	60.08±12.63	63.06±12.63	-0.545	0.595 NS	-14.78	8.83

****significant at 1% level (p=0.01) , NS-Not Significant**

3.4.4- Table: Comparison of case and control group with respect to their nutrient intake.

Nutrient Intake	Group	Mean	Standard Deviation	t-value	p- value	95% Confidence Interval	
						Lower	Upper
Energy (Kcal)	Case	1858.30	420.822	-1.501	0.145 NS	1615.32	2101.27
	Control	2090.88	398.558	-1.501	0.145 NS	1860.76	2321.00
Carbohydrates (gms)	Case	267.17	73.290	-1.292	0.208 NS	224.854	309.487
	Control	301.75	68.310	-1.292	0.208 NS	262.316	341.199
Protein (gms)	Case	69.612	20.423	-0.828	0.415 NS	57.819	81.404
	Control	76.333	22.475	-0.828	0.415 NS	63.356	89.310
Fat (gms)	Case	53.131	9.757	-1.629	0.115 NS	47.497	58.765
	Control	60.082	12.635	-1.629	0.115 NS	52.787	67.377
Calcium (mg)	Case	428.51	172.975	-0.475	0.639 NS	328.643	528.390
	Control	467.76	256.212	-0.475	0.639 NS	319.829	615.695
Vitamin E	Case	2.940	1.687	-0.173	0.864 NS	1.969	3.911
	Control	3.061	2.010	-0.173	0.864 NS	1.901	4.222
Vitamin C	Case	46.05	17.630	-0.905	0.374 NS	35.874	56.233
	Control	51.90	16.572	-0.905	0.374 NS	42.339	61.476
Potassium	Case	1787.21	539.33	-0.981	0.336 NS	1475.806	2098.616
	Control	2037.59	788.29	-0.981	0.337 NS	1582.446	2492.746
Sodium	Case	2712.41	805.59	-0.618	0.542 NS	2247.279	3177.554
	Control	2886.84	682.35	-0.618	0.542 NS	2492.844	3280.799
Iron	Case	11.55	3.40	-0.366	0.717 NS	9.585	13.516
	Control	12.16	5.22	-0.366	0.717 NS	9.143	15.178

NS- Not Significant

3.5- EXERCISE

3.5.1- Pre VO2 Max Classification

Group	Class	Frequency	Percent
Case	Excellent	11	78.6
	Good	3	21.4
	Total	14	100.0
Control	Excellent	7	50.0
	Good	5	35.7
	Above average	2	14.3
	Total	14	100.0

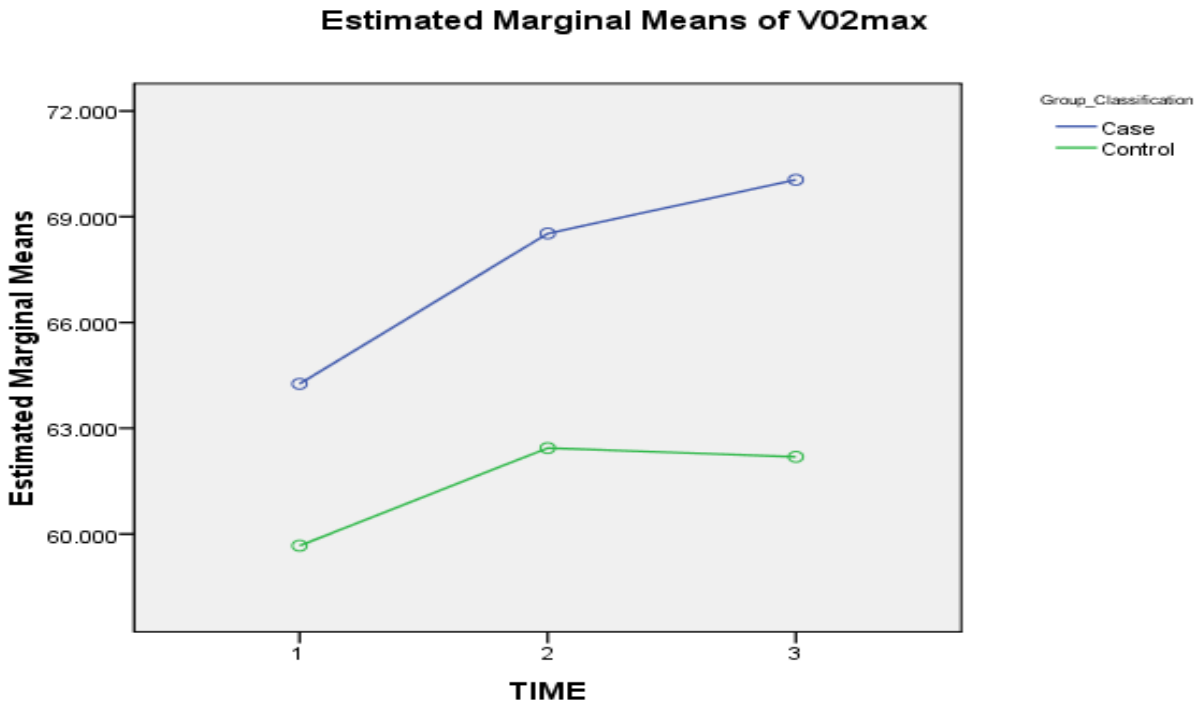
3.5.2. Comparison of vo2max pre, during and post interventions of both the groups using repeated measures

	Group Classification	Mean$\pm SD$	df	F value	p value
Pre_vo2max	Case	64.26±7.74	1.802	19.03	<0.001**
	Control	59.67±7.59			
During_V02max	Case	68.52±5.93			
	Control	62.44±7.19			
Post_vo2max	Case	70.05±7.03			
	Control	62.18±9.56			

Measure: V02max

Group Classification	(I) TIME	(J) TIME	Mean Difference (I-J)	p value	95% Confidence Interval for Difference	
					Lower	Upper
Case	Pre	During	-4.265	.002*	-6.862	-1.668
		Post	-5.790	.000*	-8.592	-2.988
	During	Pre	4.265	.002*	1.668	6.862
		Post	-1.525	.192^{NS}	-3.595	.545
	Post	Pre	5.790	.000*	2.988	8.592
		During	1.525	.192 ^{NS}	-.545	3.595
Control	Pre	During	-2.771	.068 ^{NS}	-5.715	.173
		Post	-2.521	.225 ^{NS}	-6.098	1.055
	During	Pre	2.771	.068 ^{NS}	-.173	5.715
		Post	.249	1.000 ^{NS}	-2.450	2.948
	Post	Pre	2.521	.225 ^{NS}	-1.055	6.098
		During	-.249	1.000 ^{NS}	-2.948	2.450

*** Significant at 5% level (p=0.05) , NS- Not Significant**



The graph shows tremendous increase in the performance of athletes in case group by increasing their vo2max from pre supplementation (day 0) to supplementation (day 1 to day 6) to post supplementation (day 7).
By comparing case and control group, the graph shows the performance of case group are better than the control group. In the control group, initially, it shows an increase in their vo2max but later it shows the decline of their performance due to the decrease in their vo2max.

4. SUMMARY

The study consisted of 28 male athletes of which 14 were in case group and another 14 in control group. The participants were aged between 18-25 years old. In the case group, majority of them, 8 participants stayed in apartments (without parents) while 6 of them stayed in hostels. In the control group, 7 participants stayed in hostels followed by 5 of them staying in apartments (without parents) and 2 stayed in apartments (with parents).

Anthropometric measurements showed that in the case group, majority of the subjects, 11 fell into the category of 'Athletes', while 3 of the subjects fell into the category of 'Fitness'. In the control group, majority of the subjects, 10 fell into the category of 'Athletes', while 4 of the subjects fell into the category of 'Fitness'.

With respect to physical activity level, in the case group, majority of the subjects, 13 fell into the category of 'Moderate Activity' while 1 of them fell into the category of 'Heavy Activity'. In the control group, majority of the subjects, 13 fell into the category of 'Moderate Activity' while 1 of them fell into the category of 'Heavy Activity'.

The food preference of the participants showed that in the case group, majority of the subjects, 10 were Non Vegetarians, while, 2 of them were Vegetarians. 1 of the subjects was Vegan and Ovo- Lacto Vegetarians each respectively. In the control group, majority of the subjects, 11 were Non Vegetarians, while, 3 of them were Vegetarians.

The water consumption of the participants showed that in the case group, 6 of the subjects consumed 2 and 3 litres of water per day each respectively, while 1 of the subjects had 4 and more than 4 litres per day each respectively. In the control group, 7 of the subjects consumed 3 litres of water per day, while 4 of the subjects had 2 litres per day and 2 of the subjects had 4 litres and 1 of the subjects had more than 4 litres per day.

The number of participants who skipped meals in the case group were 9 while rest of the 5 had no habits of skipping meals. In the case of control group 8 of the participants had the habit of skipping meals while 6 had no habits of skipping meals.

In the case group, majority of the subjects, 9 were not taking any supplements, while 5 of them were taking some supplements. In the control group, majority of the subjects, 11 were not taking any supplements, while 3 of them were taking some supplements. The supplements taken were whey protein, creatine, cod liver oil, vitamin D and electrolytes.

The mean intake of the macro nutrients like energy, protein, carbohydrate were found to be less in both the groups compared to the RDA and it showed statistically significant difference at 1% level (p=<0.001).
The mean intake of fat in both the group showed that there was no significant difference (p=>0.001).
The mean intake of micro nutrients like sodium, potassium, calcium, vitamin E, vitamin C and iron were found to be less in both the groups compared to the RDA and it showed statistically significant difference at 1% level (p=<0.001).

From the repeated measures test, it is stated that there is a significant in the consumption of sabeet (nitrate supplement extracted from beetroot) in case group. There is significant difference between pre supplementation to supplementation period (p= <0.05), pre supplementation to post supplementation (p= <0.05), supplementation period to pre supplementation (p= <0.05) and post supplementation to pre supplementation (p= <0.05) in case group. There is no statistically significant difference in control group ie. p=>0.05.

5. CONCLUSION

Intake of 5gms of Sabeet (Nitrate Supplements) for 6 days significantly reduces their heart rate and increases the VO2 max among the athletes.
Nitrate supplement (Sabeet) can be considered as an ergogenic aid, along with dietary counselling to increase their performance by increasing their VO2 max.

6. LIMITATIONS

- Accessibility to Marena Sports Complex's futsal ground inorder to conduct Beep Test or 20 metres shuttle run test.
- Better instruments to measure heart rate like 'Polar H7 Heart Rate Monitor Device'.
- Decreased number of sample size due to non-compliance of the participants.

7. Acknowledgement

I would like to thank Manipal Academy of Higher Education (MAHE) for providing me the resources and facilities to conduct the study and my sponsors, Sami Labs Pvt. Ltd. (Bengaluru) for being generous enough to provide me the Sabeet samples. This study has been completed under the guidance of Mrs. Swathi Acharya, Assistant Professor, Dietetics and Applied Nutrition, Department of Allied Hospitality Studies, MAHE. Her advice, support, constructive ideas and constant encouragement at all stages of my research and the master's program has been an invaluable asset, both in academic and personal levels, for which, I am extremely grateful. I render special thanks to Mr. Baskaran Chandrasekharan, Assistant Professor, Department of Exercise and Sports Science, MAHE for his guidance and support. A sincere thanks to my parents and siblings who provided me strength and emotional support.

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