Correlation of Haemoglobin Concentration with Maximal Aerobic Capacity - A Prospective Study in First Year MBBS Students of Southern Odisha

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ABSTRACT

BACKGROUND

Decrease in haemoglobin concentration in blood, with normal blood volume results in lower $\dot{V}O_{2max}$ and endurance performance. This may be due to the reduction of the oxygen carrying capacity of blood. Conversely, increase in haemoglobin concentration is associated with increased $\dot{V}O_{2max}$ and endurance capacity. Increase in endurance capacity is also proportional to the increase in the oxygen carrying capacity of blood. Maximal aerobic capacity ($\dot{V}O_{2max}$) is the maximum capacity of individual's body to transport and use oxygen during incremental exercise which reflects physical fitness of that individual. Queen's College Step Test is a standard method to measure one's maximal aerobic capacity using sub maximal exercise in the form of bench stepping suitable for adults. The biological significance of oxygen transport by haemoglobin is well illustrated in anaemia where decreased haemoglobin also decreases exercise performance. The objective of the study is to find out the correlation between haemoglobin concentration and $\dot{V}O_{2max}$ by using Queen's College Step Test.

METHODS

The study was conducted in the Department of Physiology, MKCG Medical College, Berhampur. A total of 150 1st year MBBS students aged between 17 and 24 yrs. were taken as subjects. Out of them 90 were males & 60 were females. Each subject performed the exercise for 3 minutes by Queen's College Step Test. $\dot{V}O_{2max}$ was obtained by calculation. Haemoglobin concentration was measured by Acid Haematin method in Sahli's Haemoglobinometer.

RESULTS

There was a significant positive correlation between haemoglobin concentration with VO_2max in both males ($r = 0.8618$, $P < 0.0001$) and females ($r = 0.5112$, $P < 0.0001$) subjects.

CONCLUSIONS

Increase in Haemoglobin concentration is an indicator of increased $\dot{V}O_{2max}$; hence persons with increased haemoglobin concentration may have increased exercise capability in both male and female subjects.

KEYWORDS

$\dot{V}O_{2max}$, Haemoglobin concentration, Queen's College Step Test

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DOI: 10.18410/jebmh/2021/332

How to Cite This Article:
Patnaik M, Acharya S, Panigrahi AK. Correlation of haemoglobin concentration with maximal aerobic capacity - a prospective study in first year MBBS students of Southern Odisha. J Evid Based Med Healthc 2021;8(22):1758-1763. DOI: 10.18410/jebmh/2021/332

Submission 05-02-2021,
Peer Review 15-02-2021,
Acceptance 16-04-2021,
Published 31-05-2021.

BACKGROUND

Maximal aerobic capacity ($\dot{V}O_{2max}$) is the maximum capacity of individual's body to transport and use oxygen during incremental exercise, which reflects physical fitness of an individual. It is an important determinant of endurance performance which represents a true parametric measure of cardio-respiratory capacity for an individual at a given degree of fitness and oxygen availability.

$\dot{V}O_{2max}$ is considered to be the gold standard for the measurement of cardio-respiratory fitness. It measures the volume of oxygen that human body is capable of consuming and converting it to energy for exercising muscles.

$\dot{V}O_{2max}$ is one of the most ubiquitous measurements in all of exercise science. The concept that there exists a finite rate of maximal oxygen transport from the environment to the mitochondria to support oxidative production of ATP to do physical work began with A.V.

Hill and has been used diversely in clinical science as a measure of exercise performance, a marker of cardio-respiratory function. $\dot{V}O_{2max}$ is basically affected by genetic factors, physical training, gender, age, and body composition. Measuring $\dot{V}O_{2max}$ is not only intended to predict fitness for exercise, it also helps in identifying future health risks associated with low $\dot{V}O_{2max}$.

There are different methods for measuring $\dot{V}O_{2max}$ with maximal and sub-maximal exercise tests like Field test methods, Single stage treadmill test, Bruce sub-maximal treadmill exercise test. Maximal exercise test is not always a feasible or desirable approach; also these are exhaustive and complicated.

Queen's college step test (Mc Ardle et al.) is an easy method to determine $\dot{V}O_{2max,}$ which is recommended as a valid method for evaluation of cardio-respiratory fitness in a young sedentary population, especially in field work where the survey and screening of large number of participants are essential. It is a standard method to measure one's maximal oxygen uptake using sub-maximal exercise in the form of bench stepping suitable for adults.

Haemoglobin (Hb) is responsible for transport and delivery of oxygen to tissues. Oxygen (O_2) must be transported effectively and efficiently from the atmosphere to the tissues in order to maintain essential metabolic pathways. The biological significance of O_2 transport by haemoglobin is well illustrated in anaemia where decreased Hb concentration ([Hb]) also decreases exercise performance despite a compensatory increase in cardiac output, and by improved aerobic performance upon increasing total [Hb].

An acute reduction of blood Hb concentration, even when the circulating blood volume is maintained, results in lower $\dot{V}O_{2max}$ and endurance performance due to reduction of the oxygen carrying capacity of blood.

Objectives

1. To determine $\dot{V}O_{2max}$ in healthy young adults to assess their cardio-respiratory fitness.
2. To correlate Hb concentration with $\dot{V}O_{2max}$ to find out the effect of anaemia on cardio-respiratory functions.

METHODS

A prospective (in relation to time only), cross-sectional study was designed according to the concerned research question. It was carried out in the Postgraduate Research Laboratory of Department of Physiology, MKCG Medical College, Berhampur, Ganjam, Odisha. The laboratory was equipped with the required instruments for the study.

The study period was from 15th October 2017 to 20th June 2019. Period of recruitment of 1st batch of subjects for the study was October 2017 from 2017 - 2018 batch of MBBS students. 2nd batch was recruited from 2018 - 2019 batch of MBBS students in September 2018. Anthropometric measurements and data from Queen's College Step Test were taken throughout the study period. The subjects participated in this study lead a sedentary life style and none of them were exercising.

Sampling Technique

Similar previous published research papers were obtained through searching the journals available in journal section in central library of the institution as well as extensive internet search through Google Scholar and Pub Med. Laxmi et al. conducted a research in India on subjects of 18 - 22 years and used Queen's College Step Test to measure $\dot{V}O_{2max}$. This method uses the basis of linear relation between heart rate and oxygen consumption. We intended to measure $\dot{V}O_{2max}$ with the similar theoretical basis. After consultation with expert statistician, we calculated the minimum sample size by the following formula.[1]

$$\boldsymbol{N = [(Z\alpha + Z\beta)/\ C]^2\ + 3}$$

Where N = Total number of subjects required
Zα / Zβ = The standard normal deviate for α/β
C = 0.5 × ln [(1 + r) / (1 - r)]
r = expected correlation coefficient
For the calculation, we set α (chances of Type I error) = 0 05, β (chances of type II error) = 0.05. Hence, the power of study was (1 - β) = (1 - 0.05) = 0.95 or 95 %.

We made an Excel® Calculator on the basis of the formula and put the required values to get the sample size. Calculated sample size was 51. A dropout rate of 20 % was assumed. Hence, the sample size was multiplied by the factor 1.25.

Final minimum sample size was 64. As there was no major cost involved in the recruitment of the subject and there was minimal risk for subjects, we decided to include all possible interested students. The available time was the only constraining factor.

Sampling Method

Convenience sample was taken from those who were fit (i.e., fulfil inclusion criteria and not barred by exclusion criteria) for the study.

Recruitment Procedure

The students were informed about the study and brief details about the procedure of the study as well as the

parameters to be recorded were declared. Interested students were requested to come to the research laboratory to register their names for the study. After registration, the informed consent form was given to them. Adequate time was allowed to read and understand the consent. The consent form was prepared according to the guidelines by the Institutional Ethics Committee. Participants were requested to fill up the required portion and sign the form if they agreed to provide their voluntary consent for participation in the study.

Participants

Those participants who provided their consent for participation were screened by Physical Activity Readiness Questionnaire (PAR - Q) which was prepared according to the guidelines by American College of Sports Medicine. PAR - Q was designed to identify the participants for whom physical activity might be inappropriate. It was one page questionnaire with 6 questions printed on a white paper with YES / NO response options.[2] The response to the questionnaire was recorded with self-reporting method along with help of the interviewer. Those who qualified after the screening test, were selected for the study. A detailed history was taken from each subject for any other exclusion criteria present.

Inclusion Criteria

- Age between 17 and 24 years
- Consent for participation (i.e., Signed consent form submitted)
- Passed the initial screening by PAR - Q.

Exclusion Criteria

- Any YES answer in the PAR - Q
- Subjects with any history of – Hypertension, Ischemic Heart Disease, Valvular Heart Disease, Chronic Obstructive Pulmonary Disease, Acute Respiratory Tract Infection, Acute asthma, Tuberculosis, Bronchiectasis, Pleural Disease, Diabetes mellitus.
- Subjects with Anaemia.
- Any addiction of Smoking or Alcoholism.

Instruments Used

- Stadiometer.
- Digital weighing scale.
- Sahli's Haemoglobinometer.
- Stepping bench: for Queen's College Step Test.
- Pulse oximeter.
- Stopwatch.
- Metronome.
- Case record form: A case record form was designed to write down particulars including all the anthropometric parameters, heart rate before and after exercise, blood pressure, etc. This form was used as a survey instrument to record all the parameters obtained during the data collection.

Data Collection

The room temperature was set at 23° C and humidity at 50 %. All the measurements were taken between 11:00 am to 1:00 pm. Subjects were informed about the test two days before and were requested to fulfil the pre-test criteria for exercise.

Pre-Test Criteria for Exercise

1) Abstain from eating or drinking 4 hours prior to the test.
2) Abstain from strenuous exercise 24 hours prior to the test.
3) Abstain from caffeine, alcohol, and nicotine etc. 48 hours prior to the test.
4) Void bladder completely within 30 minutes of test.

Anthropometric Measurement

For obtaining the anthropometric measurement of male subjects, measurements were preferably taken in light clothing but subject preference was given priority. Two male students were taken at a time to the laboratory for a mental support as well as to avoid any legal complications. For measurement in female subjects, two female subjects were taken at a time along with one female attendant recruited from the Department of Physiology. Females were instructed to come with light clothing.

Measurement of Weight

Weight was measured by digital weighing scale to nearest 0.1 kg (Precision of instrument 0.1 kg) with minimal clothing.

Measurement of Height

Height was measured by stadiometer to nearest 0.1 cm. Before measurement, the measuring bar and head marker was taken above the estimated height to facilitate subjects step on the base. Subject stood on the base of the stadiometer with bare foot with his / her heels touched together, foot keeping apart at 45 angle between them. Hands were hanging by the side. Subject looked straight forward. The head marker was lowered down to make a touch and the subject was instructed to step down from the base and the reading was recorded.

Calculation of BMI

BMI was calculated according to Quetelet's equation (BMI = Weight in kilogram / square of Height in meter [BMI = kg / m^2]). It was calculated from the measured weight and height in Microsoft Excel spreadsheet equation.

On the same day, Haemoglobin concentration was determined by Sahli's Acid Haematin Method [117 - 119]. With the help of a dropper, N / 10 HCl taken in the graduated haemoglobin tube up to 20 mark on percentage side. Finger prick done taking all aseptic precautions. Blood drawn into the haemoglobin pipette up to 20 µL and immediately transferred into the tube.

The contents were mixed thoroughly with a stirrer and allowed to stand for 10 minutes for conversion of all the haemoglobin into acid haematin (brown colour).

The colour developed due to acid haematin was matched with that of the comparator. The acid haematin was diluted by adding distilled water in drops till its colour matched with that of the standard. Then the reading was taken in gm / dl.

Each subject was briefed about the aim of study and the procedure was demonstrated. A warm up walking was carried out before the test.

A wooden stepping bench of 16.25 inch was used along with android application based metronome and stopwatch. Metronome was used to monitor the stepping cadence, which was set at 96 beats per minute (24 complete steps / minute) for males and 88 beats per minute (22 complete steps / minute) for females. After recording the resting heart rate, each subject was asked to perform each stepping cycle to a four step cadence, up-up down-down continuously for 3 minute. After completion of test, heart rate (beats / minute) was again measured by the pulse oximeter.
$\dot{V}O_{2max}$: determined by the following formula,

For males: $\dot{V}O_{2max}$ (ml / kg/min) = 111.33 - (0.42×HR),
females: $\dot{V}O_{2max}$ (ml / kg / min) = 65.81 - (0.1847×HR)
HR = Heart rate after exercise.

Test termination criteria [174]:

- Onset of angina or angina like symptoms.
- Shortness of breath, wheezing, leg cramps, or claudication.
- Signs of poor perfusion: light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin.
- Failure of heart rate to increase with increased exercise intensity.
- Subject requests to stop.
- Physical or verbal manifestations of severe fatigue.
- Failure of the testing equipment.

Each subject was encouraged to do the exercise and continuous verbal support was given. After 15 minutes of the test, the HR of the subject was measured again and if it was found concordant with the baseline measurement, the test was completed.

Final Sample

Final sample size was 150 subjects with 90 males and 60 females in the age group of 17 - 24 years.

Statistical Methods

All collected data were entered in Microsoft Excel® 2007 spreadsheet. Data of male and females were entered on separate sheet. On a third sheet, combined data of male and females were entered. All the variables were expressed in Mean and Standard Deviation with the help of "AVERAGE" and "STDEV" function of Excel. Anthropometric measurements of male and female subjects were compared statistically by Student's t-test (Unpaired). Male, female and total subjects were categorized according to WHO adult BMI criteria and the proportions were tested statistically by chi-square test.

$\dot{V}O_2$max of two groups (i.e., male and female) were compared by unpaired t-test. For statistical tests, we considered two tail α = 0.05 and β = 0.05 which denotes that two-tail P value < 0.05 was considered statistically significant.

To find the correlation of Hb concentration with $\dot{V}O_{2max}$, Pearson's correlation coefficient (r) and P value obtained by regression and correlation function in GraphPad InStat. Tabulation, pie chart, scattered plot were done in Excel.

All statistical analysis was carried out in Microsoft Excel® 2007 (Microsoft corporation, USA), and GraphPad InStat (version 3.06) for Windows (GraphPad Software, Inc. La Jolla, CA, USA).

RESULTS

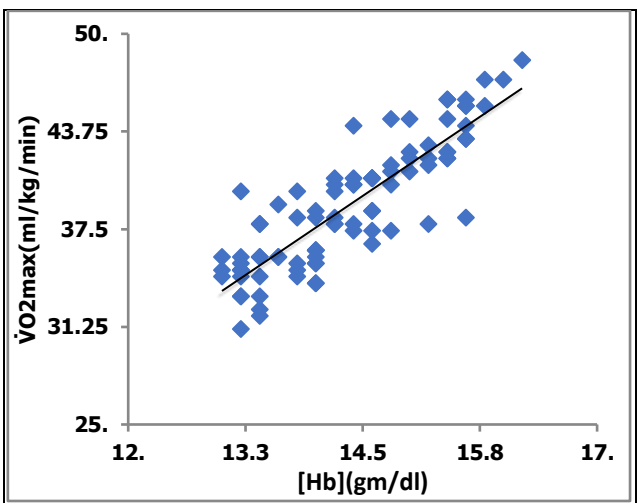

Figure 1. Scatterplot of Correlation between [HB] & $\dot{V}O_{2max}$ in Male

Pearson's correlation coefficient (r) 0.8618,
r^2 0.7426
P - value < 0.0001

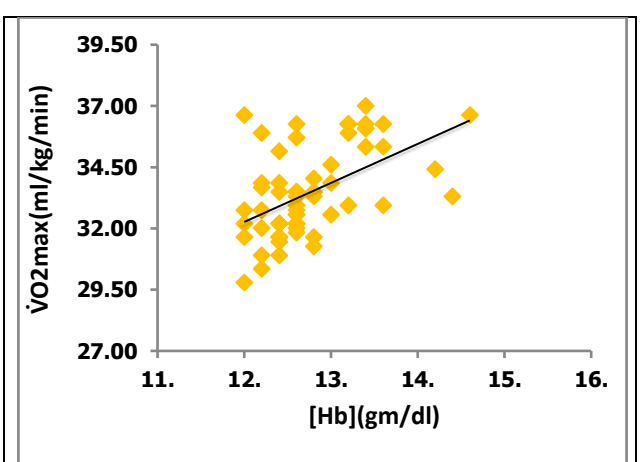

Figure 2. Scatterplot of Correlation between [HB] & $\dot{V}O_{2max}$ in Females

Pearson's correlation coefficient(r) 0.5112,
r^2 0.2613,
P - value < 0.0001

Parameters	Male (MEAN ± SD)	Female (MEAN ± SD)	P – Value
Age (years)	19.27 ± 1.16	19.08 ± 0.92	0.4625
Height (meter)	1.69 ± 0.06	1.57 ± 0.06	< 0.0001*
Weight (kg)	67.86 ± 12.22	57.78 ±8.90	< 0.0001*
BMI (kg / m^2)	23.71 ± 3.75	23.22 ± 3.18	0.5912
Hb (gm / dl)	14.35 ± 0.85	12.74 ± 0.57	< 0.0001*
HR 1 (bpm)	80.83 ± 5.13	79.82 ± 6.54	0.5367
HR 2 (bpm)	172 ± 9.61	175.45 ± 9.65	0.0440*
$\dot{V}O_{2max}$ (ml / kg / min)	39.04 ± 9.04	33.40 ± 1.78	< 0.0001*

Table 1. Measured and Calculated Parameters in Male and Female Subjects, Expressed in Mean ± Standard Deviation

*P value (two-tailed) < 0.05 was considered statistically significant
HR1 = Resting heart rate
HR2 = heart rate after exercise
$\dot{V}O_{2max}$ = Maximal aerobic capacity

BMI (kg/m^2)	Total Subjects (N = 150) Number (%)	Male (N = 90) Number (%)	Female (N = 60) Number (%)	Fisher's Exact Test
Underweight (< 18.50)	4 (2.7)	3 (3.3)	1 (1.7)	P = 0.3608
Normal (18.50 - 22.99)	99 (66.0)	56 (62.2)	43 (71.7)	
Pre obese (23.0 - 24.99)	40 (26.7)	25 (27.8)	15 (25.0)	
Obese (≥ 25.0)	7 (4.7)	6 (6.7)	1 (1.7)	

Table 2. Distribution of Study Groups According to Asia Pacific BMI Category

$\dot{V}O_{2max}$ (ml / kg / min) Category	Male 13 - 19 Years (N = 62)	Male 20 - 24 Years (N = 28)
Poor (< 35.0 - 38.3)	32 (51.61 %)	32 (51.61 %)
Fair (38.4 - 45.1)	25 (40.32 %)	25 (40.32 %)
Good (45.2 - 50.9)	05 (8.06 %)	05 (8.06 %)

Table 3. Distribution of Male Subjects According to $\dot{V}O_{2max}$

VO_{2max} (ml / kg / min) Category	Female 13 - 19 Years (N = 41)	Female 20 - 24 Years (N = 19)
Poor (< 25.0 - 30.9)	1 (2.44 %)	0 (0 %)
Fair (31.0 - 34.9)	30 (73.17 %)	9 (47.36 %)
Good (35.0 - 38.9)	10 (24.39 %)	10 (52.63 %)

Table 4. Distribution of Female Subjects According to $\dot{V}O_{2max}$

DISCUSSION

The present work, "Correlation of Haemoglobin concentration with Maximal Aerobic Capacity: A Prospective study in First Year MBBS Students of Southern Odisha" was conducted and the study population was comprised of 90 male and 60 female subjects. The mean age in males was 19.27 ± 1.16 years and females was 19.08 ± 0.92 years [Table - 1]. The anthropometric variables were measured [Table - 2]. The mean height and weight in males (1.69 ± 0.06 m and 67.86 ± 12.22 kg respectively) were more than that of females (1.57 ± 0.06 m and 57.78 ± 8.90 kg respectively) and the difference was statistically significant (P < 0.0001).

The mean BMI showed no difference in males (23.71 ±3 .75 kg / m^2) and females (23.22 ± 3.18 kg / m^2) (P = 0.591). This may be due to the fact that BMI is calculated by dividing the weight by square of height. A proportional increase in height along with weight causes a little difference in calculated BMI.

There was no significant difference in distribution of male and female (P = 0.3608) in different categories of BMI [Table - 2]. According to Asia Pacific adult BMI cut-off values, 56 % of males and 43 % of females were in 'normal range' of BMI. 25 % of males and 15 % of female were in 'pre-obese' group. Only 6.7 % of males and 1.7 % of females were in 'obese' category. Hence, we had a narrow range of BMI and this was favourable for the study as we got little variation in BMI. However, controlling this narrow range was beyond the scope of the sampling technique we used.

There was no difference between mean resting heart rate (HR1) in males (80.83 ± 5.13 bpm) and females (79.82 ± 6.54 bpm), P = 0.5367.

The mean heart rate just after exercise (HR2) was higher in females (175.45 ± 9.65 bpm) than males (172 ± 9.61 bpm) and the two tailed P value was 0.0440. It is corroborated well with that of Chatterjee et al.[3] They reported significantly higher value of peak heart rate during Queen's College Step Test (QCT) in obese group which indicated greater cardiac load among them.

$\dot{V}O_{2max}$ obtained by using separate formulae for both males and females.[4] The mean $\dot{V}O_{2max}$ was higher in males (39.04 ± 9.04 ml / kg / min) than in females (33.40 ± 1.78 ml / kg / min), P < 0.0001. This gender difference was concordant with the findings of Sharma et al. Żebrowska et al. and Loe et al.[5-7] According to aerobic fitness classification by Katch et al.[8] out of 90 males 62 were in 13 - 19 years age group, out of which $\dot{V}O_{2max}$ of 51.61 % was poor, 40.32 % fair and only 8.06 % was in good category [Table - 3]. Rest 28 males were in 20 - 24 years' age group. Out of which $\dot{V}O_{2max}$ of 28.57 % was poor, 46.42 % fair and 25 % in good category. But in case of females, out of 60, 41 were in the age group of 13 - 19 years. $\dot{V}O_{2max}$ of 2.44 % from this age group was poor, 73.17 % fair and 24.39 % in good category [Table - 4]. Rest of the female subjects (N = 19) in the age group of 20 - 24 years showed fair $\dot{V}O_{2max}$ in 47.36 % and good in 52.63 %.

The mean Hb concentration was higher in males (14.35 ± 0.85 gm / dl) than females (12.74 ± 0.57 gm / dl) and the difference was statistically significant (P < 0.0001).[9-11]

An extremely significant positive correlation was found between Hb concentration and $\dot{V}O_{2max}$ in both males (r = 0.8618, P value < 0.0001) [Figure - 1] and females (r = 0.5112, P value < 0.0001) [Figure - 2]. Philo U Saunders et al. in 2013 found similar correlation but the study was done between haemoglobin mass and maximal oxygen uptake after hypoxic exposure.[12] Other cross-sectional studies indicated a similar strong relationship between Hb_{mass} and $\dot{V}O_{2max}$, which was independent of sex or age.[13,14] Celsing F et al. in 1987 concluded that $\dot{V}O_{2max}$ rises when systemic [Hb] is increased by RBC infusion.[15] Magazanik A et al. in 1991 also found a positive correlation but the study was between $\dot{V}O_{2max}$ and Hb concentration following administration of iron supplements to young training women.[16]

CONCLUSIONS

Haemoglobin concentration shows extremely significant correlation with $\dot{V}O_{2max}$ in both males and females (male: r = 0.8618, P value < 0.0001) and (female: r = 0.5112, P value < 0.0001). Correlation between $\dot{V}O_2$max & Hb conc. was established & found to be positive. Hence persons with

increased haemoglobin concentration may have increased exercise capacity in both males and females. Queen's college step test is recommended as a valid method for evaluation of cardio-respiratory fitness in young population in field work. Haemoglobin influences exercise capacity.

Future Directives

This study was conducted in accordance with calculated sample size; however, future studies with larger sample size would reflect more generalized result. We took a sample from young adult age group only.

Further studies with diverse age range would provide status of aerobic capacity in general population. Other parameters of body composition like body fat % and fat free mass along with BMI can be included as variables. A prospective study with anaemic cases before and after treatment would be better to substantiate Haemoglobin concentration as a predictive variable for $\dot{V}O_{2max}$.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
Disclosure forms provided by the authors are available with the full text of this article at jebmh.com.

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