CHAPTER 3
MATERIAL
AND
METHOD
Study Design:
A randomized non-experimental cross-sectional study was conducted on 488 Gujarati Indian adolescent boys and girls of age group 16 to 19 years studying at the schools and colleges in the Anand district.

Ethical Clearance and Informed Consent:
The study was conducted after the approval of the Human Research Ethics Committee (HREC) of H.M. Patel Centre for Medical Care and Education and after obtaining the informed consent from the participants (Age = 18 years) or the guardian (Age <18 years). (Annexure I & II)

Recruitment of Participants: The inclusive and exclusive criteria mentioned below were used for enrolling the participant in the study:

Inclusion Criteria
1. Gujarati Ethnicity (Based on mother tongue of parents)
2. All socio-economic classes
3. Tanner Stage 5

Exclusion Criteria
1. History of any acute or chronic disease state that would affect the study variables.
2. Pregnancy

Age: Age was self-reported by the participants as age in years to the nearest year.

Sexual Maturation: The participants self reported their Tanner stage after identifying their pubic hair growth with the corresponding Tanner stage using the photographs of the Tanner Stages.
ASSESSMENT OF LIFESTYLE

The lifestyle was assessed for meal frequency; sleep hours at night and physical activity status.

**Meal Frequency:** The participants were asked to report the number of times they eat in a day (inclusive of breakfast, lunch, dinner, snacks) over the last one-year.\(^{(129)}\)

**Sleep Duration at Night:**
The participants were asked to self-report the number of hours for which they slept during most of the nights in a week for the last one-year. The subjects reported the sleeping hours from the time of going to bed to the time they woke up in the morning. Sleep duration of more than or equal to 7 hours per night was considered as Adequate Sleep Duration at Night (ASDN) and sleep duration of less than seven hours was considered as Inadequate Sleep Duration at Night (ISDN).\(^{(30,130)}\)

**Physical Activity Status:**
The participants were judged for their physical activity status for last one year using NASA/Johnson Space Center Physical Activity Rating scale and categorized into Mildly (PA-R: 1), Moderately (PA-R: 2 to3) and Heavily (PA-R: 4 to5) active individuals.\(^{(131)}\)
NASA/Johnson Space Center Physical Activity Rating scale

I. I don’t participate regularly in programmed recreation sport or physical activity:

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Avoid walking or exertion (e.g. always use elevator, drive whenever possible instead of walking)</td>
</tr>
<tr>
<td>1</td>
<td>Walk for pleasure, routinely use stairs, occasionally exercise sufficiently to cause heavy breathing or perspiration.</td>
</tr>
</tbody>
</table>

II. I participate regularly in recreation or work requiring modest physical activity, such as golf, horseback riding, calisthenics, gymnastics, table tennis, bowling weight lifting, or yard work:

<table>
<thead>
<tr>
<th></th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>10 to 60 minutes per week</td>
</tr>
<tr>
<td>3</td>
<td>Over one hour per week</td>
</tr>
</tbody>
</table>

III. I Participate regularly in heavy physical exercise (such as running or jogging, swimming, cycling, rowing, skipping rope, running in place) or engage in vigorous aerobic type activity (such as tennis, basketball, or handball):

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
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<tbody>
<tr>
<td>4</td>
<td>Run less than one mile per week or spends less than 30 min per week in comparable physical activity.</td>
</tr>
<tr>
<td>5</td>
<td>Run 1 to 5 miles per week or spends 30 to 60 min per week in comparable physical activity.</td>
</tr>
<tr>
<td>6</td>
<td>Run 5 to 10 miles per week or spends 1 to 3 hours per week in comparable physical activity.</td>
</tr>
<tr>
<td>7</td>
<td>Run over 10 miles per week or spends over 3 hours per week in comparable physical activity.</td>
</tr>
</tbody>
</table>

Physical Fitness (Predicted VO2 max):
The physical fitness was measured by the formula shown below after measurement of body fat %.

Female: VO2 max = 50.513 + 1.589 (PA-R) - 0.289 (age) - 0.552 (% body fat)

Male: VO2 max = 56.376 + 1.589 (PA-R) - 0.289 (age) - 0.552 (% body fat)
Measurement of Body Composition

Subject Preparation: The body composition was assessed in light clothing with empty bladder.\(^{(132)}\) The participants were asked not to engage in any strenuous/heavy physical exercise for at least 48 hours prior to assessment of body composition.

Total Body Weight (Wt): The body weight (Wt) was recorded barefooted to the nearest 0.5 kg using a calibrated weighing machine.\(^{(132,133)}\)

Height (Ht): The height was measured on the wall using meter scale without footwear to the nearest 1 cm.\(^{(132,133)}\)

Body Mass Index (BMI): BMI was calculated as the weight (kg) divided by the square of height in meters (m\(^2\)).\(^{(132,133)}\)

Waist circumference (WC): WC was measured at the mid point between the lower costal margin and the iliac crest to the nearest 0.5 cm at the end of normal expiration.\(^{(132,133)}\)

Body Fat Percentage (BF %) and Total Body Fat Mass (FM): BF% and FM were assessed by bioelectrical impedance technique using Omron Body Fat Monitor HBF -302 (Figure 3.1).\(^{(134,135)}\) Bioelectrical impedance technique is based on the principle that resistance offered to the passage of current is directly proportional to the amount of fat content in the body, as fat acts as insulator. The Omron Body Fat Monitor sends a weak electrical current of 0.5 mV through the body and receives the resistance encountered by the current in the body using plate electrodes placed in the handles for holding. The inbuilt software then calculates total body fat % (BF %) and total fat mass (FM) based
on the individual's age, gender, height, body weight and the impedance. Fat Free Mass (FFM) was calculated by subtracting FM from Wt.

**Technique of Measurement:** Age, gender, height and total body weight of the participant was registered into the monitor. The participant was then asked to hold the handles (plate electrodes) of the monitor with outstretched hands parallel to the ground and tighten the fist after pressing of the start button.

**Fat Mass Index (FMI) and Fat Free Mass Index (FFMI):**

FMI was calculated as the FM (kg) divided by the square of height (m²). FFMI was calculated as the FFM (kg) divided by the square of height (m²). \(^\text{[136]}\)

![Figure 3.1: Omron Body Fat Monitor HBF-302](image)
ASSESSMENT OF CARDIOVASCULAR PROFILE

Resting Pulse Rate (PR), Arterial Systolic Blood Pressure (SBP), Arterial Diastolic Blood Pressure (DBP), Pulse Pressure (PP), Mean Arterial Blood Pressure (MAP), Cornell Voltage-Duration Product (CVDP) and cardiovascular reactivity to acute sympathetic stress were measured as cardiovascular determinants of blood pressure.

PR was used for assessing cardiac sympathetic activity. Pulse Pressure (PP) and % Rise in Systolic Blood Pressure in response to sustained handgrip (%RSBP) were used as indices of vascular distensibility while MAP was used as indicator of sympathetic vascular tone along with DBP. CVDP was used as an index of left ventricle mass. Percentage rise in PR (%RPR) and Percentage Rise in DBP (%RDBP) in response to sustained handgrip were used as indices of cardiovascular reactivity to sympathetic stress.

Participant Condition: The participants were asked to avoid the intake of any stimulant (drugs, coffee etc) for a period of at least 30 minutes before the measurement. The participants were also asked to empty the bladder before the measurement and relax quietly in sitting position for a period of at least 5 minutes. The pulse rate and blood pressure were measured in the left upper extremity in sitting position with arm and back support, uncrossed legs and feet on the floor.
Pulse Rate and Blood Pressure Recording

The Pulse rate (PR), Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) were measured at the brachial artery from the left arm using the Omron T8 with Intellisense (HEM757A4-C1) Automatic Blood Pressure Monitor (Accuracy, BP: ± 4mmHg, Pulse:±5) which has been validated by the Association for the Advancement of Medical Instrumentation and British Hypertension Society (Figure 3.2). Pulse rate and Blood pressure were recorded at intervals of 1 minute till the difference between two consecutive BP readings was less than 5 mm Hg. The average of the two consecutive readings was used for statistical analysis. 

Pulse pressure (PP) and Mean Arterial Pressure (MAP) were calculated from the average values of SBP and DBP using the formula shown below:

\[
PP = SBP - DBP \\
MAP = DBP + \frac{1}{3}(PP)
\]

Figure 3.2: Omron Intellisense Blood Pressure Monitor T8 (HEM 757)
Cardiovascular Reactivity to Acute Sympathetic Stress:

The cardiovascular response to acute sympathetic stress was assessed by Isometric Hand Grip Test (Sustained Hand Grip Test). Isometric handgrip test evaluates the cardiovascular adrenergic function and it is recommended to be used as an investigational autonomic function test by the American Academy of Neurology. The participants were asked to do maximum voluntary contraction using the Handgrip Dynamometer (INCO, Ambala Figure 3.3) with the dominant hand. Three attempts were made at intervals of 1 minute and the highest reading amongst the attempts was recorded as the maximum voluntary contraction (MVC) for the participant. Pre-exercise Pulse rate and blood pressure were measured prior to exercise at the brachial artery from the arm not involved in contraction (non-dominant arm) using the Omron T8 Automatic Blood Pressure instrument. The participants were then asked to perform isometric handgrip exercise at an intensity of 30% MVC for one minute in the sitting position. Pulse rate and Blood Pressure were measured at one minute of exercise at the brachial artery from the non-dominant arm. The percentage rise in pulse rate (%RPR), percentage rise in SBP (%RSBP) and percentage rise in DBP (%RDBP) were calculated from the pre-exercise and one minute exercise values of PR, SBP, and DBP.

Cornell Voltage Duration Product

The Cornell Voltage Duration Product is a valid and sensitive measure for evaluation of Left ventricle mass (LVM) in normal weight as well as obese individual. A 12 lead electrocardiogram was recorded using CARDIART 108T/MK-VII instrument in supine position. The electrocardiogram

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was recorded at a speed of 25mm/sec after standardization of voltage at 10mm = 1mV.

The Cornell Voltage Duration Product was calculated as the product of Cornell Voltage (RaVL+SV3, with 8 mm added in women in millimeters) and maximum QRS duration recorded to the nearest 40msec based on assessment of all 12 the leads. (142,143, 144)

Figure 3.3: Handgrip Dynamometer

Statistical Analysis:

Statistical analysis was done using Microsoft excel and PEPI statistical package (Version 4.0, <c> PM Gahlinger, JH Abramson, 1993). (145) Unpaired t-test was used for finding significant differences between groups and Pearson's correlation coefficient was used to determine correlationship between dependent and independent variables. P-values <0.05 was considered as significant.