METHODS AND MATERIALS
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Globesity has now become so common that it is replacing the more traditional public health concerns including undernutrition and infectious diseases, as one of the most significant contributor to ill health. Furthermore, obesity can be seen as first wave of a defined cluster of several NCDs now observed in both developed and developing societies. Though it is less common in Asia, there is evidence that its prevalence is increasing at an alarming rate throughout the life cycle. The problem appears to be increasing rapidly in children as obesity in school children is already approaching 10% not only in industrialised countries but also in industrialising countries like India (Yedav 2002). The increasing prevalence of obesity in a population and particularly among children is an early indicator of emerging the global epidemic of health burden due to NCDs in developing countries.

Various reports show an increase in rate of obesity in school children in developed countries whereas the magnitude of the problem in Indian and regional context remains relatively unknown. Therefore the present study was planned to find the magnitude and trends of overweight and obesity amongst school children (12-18 years) of urban Vadodara and to identify the risk factors associated with it.

PLACE OF STUDY

The study was carried out in private schools of Urban Vadodara, in the western part of Gujarat state, India.

SELECTION OF SCHOOLS

An exhaustive zone-wise list of private schools having higher secondary division was obtained from the Office of the District Education Officer, Vadodara. At the time of the survey there were 57 schools in Urban Vadodara. Out of these, 10 schools from different zones were selected randomly such that each of them
was regular and co-educational and imparting education through either English or Gujarati medium or both. The site map of Vadodara indicating zonal distribution of the selected schools is given in Figure 3.1.

**SELECTION OF SUBJECTS**

All the children studying in 8th-12th standards were enrolled for the study. In all, there were 4808 children comprising of 2890 boys & 1918 girls. The school wise strength of the children is given in Table 3.1.

For assessing the prevalence rate of obesity using an estimated prevalence of 10% and a precision of 15% and a confidence interval of 95%, the required sample size was calculated to be 870. However to prepare normal growth percentiles, WHO recommends that at least 200 children be selected for each age and sex. Thus the obtained sample size (4808) was adequate to make a valid estimate of the prevalence of overweight & obesity.

**EXPERIMENTAL PLAN**

The study was divided into three phases. The detailed experimental plan is given in Figure 3.2. Methodology for the study is given in Table 3.2.

**PHASE I: MAGNITUDE OF THE PROBLEM OF CHILDHOOD OBESITY**

**General Information about the Schools**

The detailed information on the selected schools comprising medium of teaching, number of shifts, total class wise strength, various facilities available, place for outdoor physical activities, extra curricular activities and health check-up programme was obtained by using a pretested structured questionnaire (Appendix 1).
FIGURE 3.1
LOCATION OF SELECTED SCHOOLS IN URBAN VADODARA
## TABLE 3.1
SCHOOL WISE STRENGTH OF CHILDREN

<table>
<thead>
<tr>
<th>School</th>
<th>Boys</th>
<th>Girls</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>School 1</td>
<td>561</td>
<td>444</td>
<td>1005</td>
</tr>
<tr>
<td>School 2</td>
<td>267</td>
<td>158</td>
<td>425</td>
</tr>
<tr>
<td>School 3</td>
<td>116</td>
<td>79</td>
<td>195</td>
</tr>
<tr>
<td>School 4</td>
<td>529</td>
<td>172</td>
<td>701</td>
</tr>
<tr>
<td>School 5</td>
<td>143</td>
<td>139</td>
<td>282</td>
</tr>
<tr>
<td>School 6</td>
<td>594</td>
<td>169</td>
<td>763</td>
</tr>
<tr>
<td>School 7</td>
<td>151</td>
<td>53</td>
<td>204</td>
</tr>
<tr>
<td>School 8</td>
<td>112</td>
<td>341</td>
<td>453</td>
</tr>
<tr>
<td>School 9</td>
<td>261</td>
<td>218</td>
<td>479</td>
</tr>
<tr>
<td>School 10</td>
<td>156</td>
<td>145</td>
<td>301</td>
</tr>
<tr>
<td>Total</td>
<td>2890</td>
<td>1918</td>
<td>4808</td>
</tr>
</tbody>
</table>
FIGURE 3.2
EXPERIMENTAL PLAN

Phase I

Setting
Private Schools of Urban Vadodara
(Coeducational schools with higher secondary division)

Sampling
Random selection of schools (N=10) representing different zones of Urban Vadodara

Subjects
All children studying in 8th -12th classes
N=4808 ; boys=2890, girls=1918

Data
Socioeconomic profile
Anthropometric measurements
Height, weight, waist, hip

Main outcome measures
BMI, WHR
Phase II

Risk factor analysis

Non Ow/Ob children
N=65

Overweight children
N=111

Obese children
N=38

Dietary pattern
Activity pattern
Biochemical parameters
(Blood sugar, Lipid profile)
Phase III

10 schools

5 schools selected for longitudinal study

Follow up of overweight and obese children

Overweight N=215
Obese N=33

Height, weight, waist, hip measurements at the interval of 6, 12, and 24 months
### TABLE 3.2
**METHODOLOGY USED FOR THE STUDY**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Socioeconomic status</td>
<td>Structured pretested questionnaire</td>
</tr>
<tr>
<td>Anthropometric measurements</td>
<td>Height, weight, body mass index, waist to hip ratio - standard methods</td>
</tr>
<tr>
<td>Dietary pattern</td>
<td>• 24-hour dietary recall method</td>
</tr>
<tr>
<td></td>
<td>• Food frequency</td>
</tr>
<tr>
<td>Activity pattern</td>
<td>Self administered pretested questionnaire</td>
</tr>
<tr>
<td>Biochemical parameter</td>
<td>• FBS</td>
</tr>
<tr>
<td></td>
<td>• TC</td>
</tr>
<tr>
<td></td>
<td>• TG</td>
</tr>
<tr>
<td></td>
<td>• HDL-C</td>
</tr>
<tr>
<td></td>
<td>• LDL-C</td>
</tr>
<tr>
<td></td>
<td>• VLDL-C</td>
</tr>
</tbody>
</table>

- By calculation
- Enzymatic Kits

110
General Information of the Children

On enrollment the following information was elicited from the children using a pretested structured questionnaire (Appendix 2).

- Date of birth, age, sex, religion, family composition
- Education of parents
- Socio-economic status
- Exercise pattern
- Mode of transport to school
- Date of birth of the child was confirmed from the school records

Details of the Parameters Studied

Assessment of overweight and obesity

The measurements of weight, height, waist and hip of the children were recorded using standard methods and quality control measures. For measuring weight, height, waist and hip a separate room for boys and girls with adequate privacy and proper ventilation and lighting was identified in the school. A good rapport was established (using exercise or game on nutrition related topics) with children. Before taking actual measurements, children were told about measurements of the body to be taken, its objectives and how it will be taken. The children with endocrinai disorders were not enrolled for the study.

Age

The exact age of the children was verified from the school records and their parents (birth certificates wherever available were checked).

Weight

Bathroom scale which was calibrated daily with known weights was used. The scale was adjusted to zero mark regularly before taking the measurements. The investigator did the standardisation on 10 children for measuring weights. The weight was recorded in triplicate to an accuracy of 0.1 kg. The weight was taken in school uniform without shoes, jumpers, sweaters etc. The child was asked to stand on a scale with weight evenly balanced on both the feet and the feet about 25-30 cms apart. The head was held comfortably erect with the lower
border of the orbit of the eye in the same horizontal plane as the external canal of
the ear and the arms hanging loosely at the sides.

**Height**

A flat floor against a perpendicular wall was identified in that room and it
was marked using fibre tape to an accuracy of 0.1 cms. The child was asked to
stand bare feet on a flat floor against a perpendicular wall with feet parallel and
with heels, buttocks, shoulders and back of the head touching the wall. The head
was held comfortably erect and marked for measuring height was made on a wall
with a flat scale touching the top of the head horizontally and its vertical edge flat
against the wall.

**Waist and Hip measurements**

Each child stood with weight evenly balanced on both the feet and the feet
about 25-30 cms apart. The child was asked to breathe normally and at the time
of making measurement was asked to breathe out gently. This prevented them
from contracting their muscles or from holding their breath.

**Waist**

The measurements were done midway between the lower rib margin and
iliac crest. For this the level of the lowest rib margin and iliac crest in the mid
axillary line was palpated and it was marked on the skin. A non-stretchable fibre
glass tape was then passed around this point and the waist circumference was
measured.

**Hip**

The measurement was done at the point yielding the maximum
circumference over the buttocks with the tape held horizontally.

**Body Mass Index (BMI)**

BMI was calculated using following formula.

\[
BMI = \frac{\text{Weight (kg)}}{\text{Height (m)}^2}
\]

**Waist to Hip ratio (WHR)**

WHR was calculated using waist (cms)/Hip (cms) formula.
For all the children BMI and WHR were calculated. Age and sex specific Cole et al standards were used to classify children into non overweight/obese, overweight and obese categories (Appendix 3).

**PHASE II : RISK FACTOR ANALYSIS**

In this phase as per the experimental plan, the children were divided into non overweight/obese, overweight and obese based on the international cutoff points of BMI given by Cole et al (2000). From these, 65 non overweight/obese, 111 overweight and 38 obese children were included in the study. Information was collected regarding their dietary pattern, activity pattern and biochemical parameters. Biochemical estimations were carried out on 197 children.

**Dietary Pattern**

A structured questionnaire was used to obtain the general dietary information and the dietary profile of the children. Dietary intakes were taken using 24-hour dietary recall method. The nutritive values of foods consumed were calculated from the raw ingredients using the values given in 'Nutritive Value of Indian Foods' by Gopalan (1998). The details of dietary information are given in Appendix 4.

**Activity Pattern**

The general activity information was obtained using a structured questionnaire and the seven days activity profile of the children was taken using self-administered pretested questionnaire as given in Appendix 5.

An exhaustive list, which was representative of the physical activities of children of the present study, was made (Appendix 6). These activities were then classified into light, moderate and heavy activities as per the description of FAO/WHO expert committee's report on energy requirements (1985); Light activity which involved 75% sitting and 25% standing and moving, Moderate activity mainly involving standing and walking but no other physical activity and Heavy activity which included heavy physical activity.
The factors of energy expenditure were given to these activities according to the report by FAO/WHO (1985) and total energy expenditure was calculated. The details of energy expenditure calculations are given in Appendix 7.

Biochemical Analysis

Fasting venous blood samples of the children were collected by a trained technician and following estimations were carried out.

- Fasting blood sugar
- Total cholesterol
- High density lipoprotein cholesterol
- Triglyceride

Procedures for biochemical estimations

Estimation of serum sugar

Estimation of serum sugar was done using enzymatic kit (GOD/POD) supplied by Ecoline (Raabo, 1969).

Principle: Glucose is oxidised by the enzyme Glucose Oxidase (GOD) to give D-gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of enzyme Peroxidase (POD) oxidizes phenol, which combines with 4-amino antipyrine to produce a red coloured quinoneimine dye. The intensity of the colour developed is proportional to glucose concentration in the sample.

\[
\text{GOD} \quad \text{D-glucose} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{D-gluconic} + \text{H}_2\text{O}_2
\]

\[
\text{POD} \quad \text{H}_2\text{O}_2 + \text{4-amino antipyrine} + \text{Phenol} \rightarrow \text{Quinoneimine dye} + \text{H}_2\text{O}
\]

Estimation of triglyceride

The triglyceride estimation in the serum was estimated by GPO method using enzymatic kit supplied by Chema (Mc Gowan 1983).

Principle: The triglyceride is hydrolysed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by ATP in the presence of Glycerol Kinase (GK) to
Glycerol 3-Phosphate (G-3-P) which is oxidised by the enzyme Glycerol-3 Phosphatic Oxidase (G-3-P-O) producing hydrogen peroxide which reacts with 4-amino antipyrine and 3-5 Dichloride 2 Hydroxy Benzene Sulfonic acid (DHBS) in the presence of enzyme peroxidase (POD) to produce a red quinoneimine dye. The intensity of the colour developed is proportional to the triglyceride concentration in the sample and is measured at 546 nm.

**Lipoprotein Lipase**

\[
\text{Triglyceride} + \text{H}_2\text{O} \rightarrow \text{Glycerol} + \text{Fatty acid}
\]

**GLC**

\[
\text{Glycerol} + \text{ATP} \rightarrow \text{Glycerol-3-P} + \text{ADP Mg}^{2+}
\]

**GPO**

\[
\text{Glycerol 3-Phosphate} + \text{O}_2 \rightarrow \text{Dihydroxy acetone phosphate} + \text{H}_2\text{O}_2
\]

**POD**

\[
\text{H}_2\text{O}_2 + \text{Aminoantipyrine} + \text{DHBS} \rightarrow \text{Quinoneimine} + \text{H}_2\text{O}
\]

**Estimation of total cholesterol**

Total cholesterol (TC) was estimated using Chema enzymatic kit (Glueck 1989)

**Principle:** Cholesterol esters are hydrolysed by cholesterol esterase (CE) to free cholesterol and fatty acid. Free cholesterol is oxidised by cholesterol oxidase (CO) to cholest 4 en 3 one and hydrogen peroxide. Hydrogen peroxide produced couples with 4 amino antipyrine and phenol in the presence of peroxidase to form a pink coloured quinoneimine dye. The intensity of the colour developed is proportional to the cholesterol concentration and is measured at 505 nm.

**CE**

\[
\text{Cholesterol ester} + \text{H}_2\text{O} \rightarrow \text{Cholesterol} + \text{Fatty acid}
\]

**CO**

\[
\text{Cholesterol} + \text{O}_2 \rightarrow \text{Cholest 4 en 3 one} + \text{H}_2\text{O}_2
\]
POD

\[ 2\text{H}_2\text{O}_2 + 4 \text{ Amino antipyrine } + \text{ Phenol} \rightarrow \text{ quinoneimine dye } + 4 \text{ H}_2\text{O} \]

**Estimation of HDL–C**

The VLD–C and LDL–C fraction of serum sample was precipitated using phosphotungstic acid (Warnick et al 1982). The supernatant was used for HDL–C estimation by the Chema enzymatic kit as described in total cholesterol estimation.

**Estimation of LDL–C**

The serum LDL–C was calculated by Friedwald's formula

\[ \text{LDL in mg\%} = \text{TC–HDL–TG/5} \]

**Estimation of VLDL–C**

VLDL–C was calculated by the formula \[ \text{VLDL} = \text{TG/5} \]

**PHASE III: LONGITUDINAL STUDY**

A mixed longitudinal design was used to study the degree of tracking of overweight status categories over a period of two years. Five schools were selected. A total of 248 children (n=215 overweight and n=33 obese) studying in 8th-12th standard who were identified as overweight/obese at the base line were followed up, at an interval of 6, 12 and 24 months. Height, weight, waist and hip measurements were recorded. Age, BMI and WHR were calculated and children were classified into overweight/obese category based on Cole et al standards at each interval. Each child was examined for 4 times. Those children who for some reasons or the other left their school or completed 12th standard were followed up at their home. At the final follow up 201 children were available for the analysis. The anthropometric measurements were made during the period of June 2001 to August 2003.
DATA PROCESSING AND STATISTICAL ANALYSIS

The data collected was processed on an IBM PC/XT compatible system. The data was entered in the FoxBase package, verified and cleaned. The following statistical analysis was performed using the Statistical Package for Social Sciences (SPSS package).

- Frequency distribution, percentages, percentiles, means and standard deviation were calculated for all parameters expressed numerically.
- Independent Student's 't' test was used to compare differences between the means in different groups.
- Chi-square test was used to test the difference between the frequency distribution of various parameters in different groups.
- Correlation analysis was carried out between energy intake, energy expenditure, per capita income, type of diet, heredity factor, lipid profile of the children.
- Multiple regression analysis was used to investigate the determinants of overweight and obesity in children.
- Kappa index of agreement to measure concordance between different standards to classify overweight and obesity in children was calculated.