5. SUBJECTS AND METHODS

5.1 SELECTION OF SUBJECTS

5.1.1 GENERAL

Normal healthy subjects were chosen for all the studies presented in this thesis. The basic classification was into Yoga and non-Yoga for the purpose of BMR protocol, and sleep protocol, and only yoga-experienced subjects were taken for the rest of the protocol.

Yoga group were students who were in various courses and were having minimum 6 months experience in integrated approach of yoga which includes practice of Āsanas, Prānāyāmas, and Meditation. They were also practising the Jñāna yoga, Bhakti yoga, Rāja Yoga and Karma yoga along with routines of bhajans, lectures and satsaṅgs. Non-yoga group were also from the same Yoga Research Foundation, but not practicing yoga, but involved in different activities of the center. Both the yoga and non-yoga group were living under the same conditions, including eating the same vegetarian food served by the institution. They were matched for age, sex and body weight. The external environment were very similar to both the groups.

5.1.2. INCLUSION CRITERIA

- Age 20-60 years
- Normal health status as declared by them
- Nonsmokers
- Normal body weight
- Normal BMI
- Those who gave written consent to participate in the study
- Not on any medication for temporary discomfort or pain.
- Those who have a minimum of 6 months of experience in yoga practice for yoga group and those who are staying in the Prashanti Kutiram for minimum of six months for non-yoga group.
Subjects & Methods

- Not having any sleeping disorder.

5.1.3. EXCLUSION CRITERIA

- Below the age of 20 and above the age of 60
- Those who were anaemic
- Those who were under medication
- Those suffering from any psychological illness or breathing disorders
- Those whose BMI was greater than 25
- Obese subjects
- Smokers
- Those who do not reside in the Prashanti Kutiram campus of Vivekananda Yoga Research Foundation

Figure 5.1
Flow-chart of selection of subjects

Selection of subjects
Based on those practising yoga for 6 months and above and those not practising yoga but living under the same conditions, at the Yoga Research Foundation Screened N=140, Selected BMR n=104

Sleep Studies Screened n =120, Selected n=88.

Non-yoga group
BMR=49
Sleep=37

BMR & Sleep Studies

Yoga group
BMR =55
Sleep=51

Asana
n=30-34
Relaxation n=(30-34)

Full CM practice
N=48

55
Table 5.1 (a). Details of type of assessment, groups and number of subjects.

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Assessment</th>
<th>Yoga Group</th>
<th>Non-Yoga Group</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>BMR</td>
<td>31</td>
<td>24</td>
<td>55</td>
</tr>
<tr>
<td>2</td>
<td>Sleep</td>
<td>27</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td>3</td>
<td>AKC WA</td>
<td>22</td>
<td>14</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>AKC WC</td>
<td>22</td>
<td>14</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>PH WA</td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>PH WC</td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>AC WA</td>
<td>21</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>8</td>
<td>AC WC</td>
<td>21</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>9</td>
<td>IRT</td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>10</td>
<td>QRT</td>
<td>21</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>11</td>
<td>DRT</td>
<td>22</td>
<td>12</td>
<td>34</td>
</tr>
<tr>
<td>12</td>
<td>Šavāsana</td>
<td>21</td>
<td>13</td>
<td>34</td>
</tr>
<tr>
<td>13</td>
<td>Full CM</td>
<td>36</td>
<td>12</td>
<td>48</td>
</tr>
</tbody>
</table>

5.1.4. REASONS FOR CHOOSING SUBJECTS FROM THE YOGA RESEARCH INSTITUTE

The environment of the surroundings, the type of food eaten, the activities of the subjects, their stress levels, the pollution levels, staying with the family and the related stress have a definite impact on the sleep levels, BMR, and metabolic rate of the subjects. Since it is impossible to select yoga and non-yoga group subjects with identical conditions, (food, environment, stress, working conditions, work atmosphere) the yoga institute at Prashanti kutiram (a residential Yoga Research Foundation) was chosen where both the yoga group and the control group lived under similar conditions including taking simple vegetarian food, which was supplied by the institute. Also all the yoga subjects were trained in a similar way of the yoga school, and all the subjects were able to do the Āsana and relaxation in identical fashion. Also since the subjects lived in the campus they were under supervision. They were all students and volunteers, the subjects followed daily activity pattern, which suggest a sedentary activity, as assessed by 24 hour recall activity pattern. For this reason, all the subjects were recruited from Vivekananda Yoga Research Foundation, which would eliminate the confounding factors such as food, stress, temperature, environmental conditions etc.
5 1.5. PREPARATION OF THE SUBJECTS

The subjects were given a timetable for each different type of study, and were asked to report at the times given in the timetable. An attendance register (Appendix 2A) was maintained to keep track of the recordings, subjects, and time of assessment, type of assessment, and the person who took the assessments with detail time scale. They were generally instructed to ensure that they reported to the lab after 3 hours of taking food, and were allowed to relax for 30 minutes before any assessments were made. They were also instructed not to change their activity pattern during the study period and also instructed not to indulge in any strenuous physical activity including sports, etc.

5.2 DETAILS OF EQUIPMENT USED AND CALIBRATION OF EQUIPMENTS

5.2.1 THE EQUIPMENT

The assessments were done using an OXYCON PRO from Jaeger, Germany. The specifications are given in Appendix 2.

The assessments are based on indirect Calorimetry method of assessment of energy expenditure. The equipment does breath-by-breath analysis of the inhaled and exhaled air concentrations using triple V sensors based on the paramagnetic principle. The analyzer is designed to analyze gases with a speed of 10 ms basis (100 Hz) with breathing level upto 80 breaths /minute.

The assessments are taken using a facemask which covers the mouth and the nose firmly fixed on the face using belts. The subjects breathe comfortably through the nose without discomfort. The mask is checked for any leakage of air after the mask is fixed on the subjects, by asking them to blow hard closing the outlet of air and checking the mask for any leakage.

The equipment was calibrated daily using the auto-calibrations for the flow volume and gas analysis by using certified gases (mixture of 5.2% of CO₂ in nitrogen,
and atmospheric air, BOC, UK) It was ensured that the values of oxygen was (20.9%) Carbon di-oxide (0.03%).

5.2.2. Barometric pressure, temperature and humidity

Since day-to-day variations in barometric pressure are small in the Bangalore city, the inbuilt reader in the equipment measured the barometric pressure and cross-verified using an external barometer. Ambient conditions were checked once at the start of the assessments daily. The equipment has an inbuilt high precision ambient conditions measurement sensor that accurately measured the ambient conditions and was cross verified using a barometer for temperature and humidity.

5.2.3. FLOW VOLUME, FLOW AND GAS CALIBRATIONS

The flow volume was calibrated everyday in the morning to check the delay time and the volume of gases, which are being analyzed. The Oxycon Pro has an in built volume calibrator which are sensitive to 0.2 ml- 2 litres of gases. The delay time is also calculated and verified for any blocks or accumulation of moisture (built-in
Subjects & Methods

software records the delay time, and will not allow calibration if there is an error. It is calibrated by using atmospheric air.

The gas analysis was done daily for the calibrations by using certified gases (mixture of 5.2% of CO₂ in nitrogen, and atmospheric air, BOC, UK). The fast response analyzer based on differential paramagnetic principle, using a facemask. The analyzer is designed to analyze gases with a speed of 10 m sec basis (100 Hz) with breathing level upto 80 breaths /minute.

5.2.4. OXYGEN ANALYZER

The method of oxygen analysis is by using Oxycon Pro differential paramagnetic oxygen analyzer (Jaeger, Germany, EU). This analyzer utilizes the physical properties of oxygen rather than its chemical absorption with a range of 0-25 vol%, accuracy of
Subjects & Methods

0.05 vol% (14-21 vol% O₂ range). Compared to chemical analyzers, these are fast acting, less demanding to use and more precise. The fast response time allows for online recording of data. These analyzers measure the partial pressure of the gas, and assure, a constant relationship between partial pressure and volume percentage of gas. Thus the scale calibration gives an analog output in terms of volume percentage.

The volume flow was regularly calibrated every morning before starting the assessments. The calibrations allow volume sensitivity up to 0.2 ml-2 liters per sec.

The delay time is calculated based on this and any blocks and accumulations of moisture can be detected by the delay time. The ambient air is taken for the calibrations. The basic factors of the calculations in the analysis of oxygen consumption is based on the flow rate and the gas concentrations, and the basic formula is as follows

Flow of air (in)*concentration of oxygen (in)-flow of air (out)* concentration of air (out)= Volume of Oxygen consumed/unit of time.

This can be arrived at, as we know the concentration of oxygen going in and concentration of oxygen going out, along with the concentration of carbon dioxide in and out (William Mc Ardle 1996). Keeping nitrogen concentration as constant in the ambient air, we can arrive at the volume of oxygen consumed. However to arrive at the energy expenditure we need to know what they subject is burning whether it is carbohydrates, Protein or the Fat, as the oxygen consumption depends on them. So it is necessary to arrive at what is known as the respiratory quotient (RQ) according to standard formulas given in William Mc Ardle (1996), and the equipment was designed to generate such reports.
5.3. DEMOGRAPHIC DATA COLLECTION AND INSTRUCTIONS REGARDING FOOD

The information regarding the subjects namely, name, age, weight, height, health status, smokers/non-smokers, profession, and other personal data were recorded in the personal data sheet form. (Appendix 3)

5.3.1. METHODS USED FOR ANTHROPOMETRIC ASSESSMENTS

A. HEIGHT

Height was measured using standard measuring scale with an accuracy of less than 0.02%. The subjects were measured barefoot and the measurements were rounded to the nearest centimeter.

B. WEIGHT

Weight was measured using a digital weighing scale and was measured to the nearest 100 gm.

C. BODY MASS INDEX

The body mass index was calculated using the standard formula (weight in kg/height (m)^2). All the subjects were matched for BMI. The 24 hours result method was used to arrive at the calorie intake of the subjects.

The subjects were instructed to take their normal daily meals without making any changes in their meal pattern. The assessments were made mostly in the mornings on an empty stomach or after 3-4 hours after the meal. All the subjects took the same kind of simple non-spicy, vegetarian meals.

5.4. DESIGN AND PROCEDURE FOR ASSESSMENTS

5.4.1. BMR STUDIES

Selection of subjects for the BMR study

The BMR study was done to determine the basic differences in the metabolic rates at the basal levels.
Subjects & Methods

Once this difference is established, further study can be conducted using this baseline information, as all the activities are determined in the multiples of BMR. (Energy expended over and above the BMR levels).

Table 6.1.1 gives age & Anthropometric characteristics of the subjects. 140 subjects were identified for the study on the BMR. Those living in the Yoga Research Foundation were selected to ensure that both the groups lived under identical conditions. The weight and height, as described earlier, were measured using digital weighing scale, and height was measured using standard scale. Out of the 140 subjects screened, around 104 were selected who matched the inclusion and exclusion criteria. The control consisted of 49 subjects and the yoga group consisted of 55 subjects, and were matched for age, BMI, body weight, and sex (figure 6.1.1). The yoga group consisted of subjects who were practising yoga for a minimum period of six months and above. The non-yoga group consisted of subjects who were not practising yoga but were living under the same condition as the yoga subjects, eating the same kind of food, the only difference was that they were not practising yoga, instead they were carrying on their day-to-day activities such as working in research foundation or in different departments. The energy expenditure pattern of both the group was of Sattvika nature.

PROCEDURE FOR ASSESSMENTS: BMR

The following conditions for measurement of BMR were followed:

1. BMR was assessed after 11-12 hours after the last meal.
2. It was assessed early in the morning before the subjects started any activity.
3. BMR was measured at around 25-26° C.
4. The measurement was taken under no-noise condition, in stress-free atmosphere.
5. The subjects had early dinner on the day prior to the assessment (6.30 pm)
Subjects & Methods

6. The subjects reported to the metabolic lab at 5.30 a.m. or as soon as they woke up.

7. They were made to relax in supine posture for about 30 minutes before the assessments were taken.

8. The BMR assessment was taken for 15 minutes.

Figure 5.4.2- A subject being assessed for sleep recordings.

5.4.2. SLEEP STUDIES

120 subjects were identified for the study after satisfying the inclusion and exclusion criteria.

Table 6.2.1 gives details of the characteristics of the groups. Out of these 120, 88 subjects were selected who were allotted to yoga group, consisting of 51 subjects, and the non-yoga group consisting of 37 subjects, after matching the groups for age, sex and body weight, and BMI. (Figure 6.2.1) Normal BMI subjects were selected for the study.

The yoga group consisted of subjects who were practising yoga for six months and above, and non-yoga group were subjects who were not practising yoga but living under identical conditions, eating the same kind of simple vegetarian meals.
Subjects & Methods

Both the groups were assessed before sleep and after sleep. The after-sleep recordings were similar to BMR studies.

**BEFORE SLEEP RECORDINGS**

The subjects had light simple vegetarian dinner at around 6-6.30 pm, the assessment for the before sleep values were obtained at 9.30 pm three and half hours after meal to eliminate the effect of food on the metabolic rate (specific dynamic action of food). They were asked to relax in supine posture for 30 minutes before the recordings were taken. The recordings were done at around 25-26°C, under no-noise conditions. It was ensured that the subjects were not on any medication prior to the assessments.

**After-sleep recordings:**

The recordings were taken only after the subjects reported good undisturbed sleep.

The subject reported to the lab at 5.30 am, and rested in supine position for 30 minutes.

The recordings were taken at around 25-26°C, under no-noise conditions. The recordings were taken for 15 minutes.

<table>
<thead>
<tr>
<th>30 minutes</th>
<th>5 minutes pre</th>
<th>3-minutes</th>
<th>5 minutes</th>
<th>5 min post</th>
</tr>
</thead>
<tbody>
<tr>
<td>supine rest</td>
<td>Åsana recovery</td>
<td>5 min post</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**5.4.3. ÅSANA STUDIES**

**DESIGN: GENERAL FOR ALL ÅSANAS**

Thirty four subjects of the Yoga group were selected for the Åsana studies. Three different Åsana—Ardhakāta Cakrāsana, Pādahastāsana, and Ardha Cakrāsana, were assessed on the yoga group. There are two different ways doing the Åsana one with awareness and other with count i.e. with 8 counts, each count to go to one position of the Åsana. Each subject was assessed twice on different days for the
each Āsana done in two different ways.

Table 6.3.1A, 6.3.2A, 6.3.3A, 6.3.4A, 6.3.5A, 6.3.6A gives particulars of the Anthropometric characteristics. Those selected from the yoga group were assessed on different days for assessment of different relaxation techniques.

The recordings for the Āsanas and relaxation techniques were taken either in the morning (before breakfast) or after 3-4 hours of food to eliminate the possibility of increased metabolic rate due to ingestion of food (specific dynamic action of food). The subjects rested for 30 minutes before the recordings were taken; under thermo-neutral condition of 25-26°C. The recordings were under no-noise conditions. The design was Self as control design.

**ARDHAKAṬI CAKRĀSANA (AKC)**

Ardhakati Cakrāsana is a side bending posture as shown in figure 5.4.3. The subjects will get up from the supine posture and start with the AKC. It takes 1.20 minutes to perform the AKC on each side with instructions with 1.20 minutes relaxation in between. For AKC with awareness an audiotape is played with instructions for awareness and positions. But in the AKC done in Āsana way, an audiotape is played but only to give position clues such as suggested (eight-step method) in Yoga Āsanas, Prānāyāma, Mudrās and Kriyās (1999), with eyes open.
**PADAHASTASANA**

Pādahastāsana is a forward bending posture (Figure 5.4.3B). It takes 3 minutes to complete the posture and come back to the Tādāsana (standing initial posture for all standing Āsanas). The eyes are closed while practising with awareness and instructions with an audiotape. While performing the Āsana with counts the eight-step method is adopted and the Āsana is done with eyes open. Here also an audiotape is used for giving clues to come to the particular position in the Āsana as in the eight-step methods.

**ARDHA CAKRĀSANA**

This is a backward bending posture (Figure 5.4.4C). It takes 3 minutes to complete the posture and come back to the Tādāsana (standing initial posture for all standing Āsanas). The eyes are closed while performing the Āsana.

Figure 5.4.3C
Subject performing Ardha Cakrāsana
5.4.4. RELAXATION STUDIES

A. 30-34 subjects of yoga group were selected for further studies on relaxation and Āsana. Table 6.4.3A, 6.4.2A gives the anthropometric characteristics of the group.

The relaxation posture time was fixed and an audiotape containing instructions was played for IRT, DRT, QRT, and for the practice of Āsana with instructions for awareness, the timings were matched for the performance of Āsana in the Āsana way with counts, but instructions for awareness and positions were not given. Śavāsana was recorded for 10 minutes without any instructions. The pre-levels and post-levels were recorded for 5 minutes for all the āsanās and relaxation postures. Five minutes of recovery phase was allowed for all the āsana and relaxation postures before the post-recordings were taken. This was to allow the metabolic rate to recover to the pre-levels so that a realistic post-levels were obtained.

B. IRT

\[
\begin{array}{c|c|c|c|c}
30 \text{ minutes} & 5 \text{ minutes pre} & 1\text{-minute IRT} & 5 \text{ minutes recovery} & 5 \text{ min post} \\
\text{supine rest} & & & & \\
\end{array}
\]

Instant relaxation technique as the name suggests is done for one minute with progressive contraction of muscles from toes to the head and hands done rapidly with instructions for the same and letting go of all the tensions and collapsing the entire body, then relaxing with full awareness on the relaxation of the stretched muscles. This is also done with instructions and with eyes closed.

C. QRT

Quick Relaxation technique is done in three phases. Phase 1 consists of watching the abdominal movements with instructions and with awareness, Phase 2 consists
of synchronization of the abdominal movements with deep breathing, Phase 3 consists of awareness with invoking of positive emotions and feeling of purification and mental sensations and let go of all the negative emotions. Throughout the practice the eyes are closed and instructions are given through an audiotape.

D. DRT

Deep Relaxation technique is done in supine rest for about 7 minutes. It is done 6 phases of step-wise relaxation as described By Nagendra and Nagarathna (1988)

<table>
<thead>
<tr>
<th>Step</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine rest</td>
<td>30 minutes</td>
</tr>
<tr>
<td>Pre-DRT</td>
<td>5 minutes</td>
</tr>
<tr>
<td>DRT</td>
<td>6 minutes</td>
</tr>
<tr>
<td>Recovery</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Post-DRT</td>
<td>5 minutes</td>
</tr>
</tbody>
</table>

Phase 1 involves relaxation of the body from toes to waist mentioning each part to keep an awareness followed by chanting of the AAA syllable. Phase 2 involves relaxation from waist to neck and hands followed by chanting of UUU, Phase 3 involves relaxation from head region followed by chanting of MMM, Phase 4 involves whole body awareness with a chanting on OM, phase 5 involves body apartness with instructions, to be a witness to oneself, and to drop-down the body. Phase 6 involves merging the self into the expansive awareness. The entire practice is done with eyes closed and with instructions.

While performing the Åsana with counts the eight-step method is adopted and the Åsana is done with eyes open. Here also an audiotape is used for giving clues to come to the particular position in the Åsana as in the eight-step methods.

5.4.5. CYCLIC MEDITATION

Table 6.5.1 gives details of anthropometric characteristics of the subjects. The practice of cyclic meditation which consists of both Åsana and relaxation posture interspersed, as a capsule, consisting of 23 minutes of practice were assessed on 48 subjects, matched for age and BMI.

An audiotape was played while the subjects practised with eyes closed throughout the practice. The practice starts with IRT in supine position, which involves
contraction of muscles from toes to head very rapidly, followed by relaxation of the whole body. Then the subjects come to standing position to do Āsanas, which consists of Ardhakaṭi Cakrāsana (side bending posture), Pādahastāsana (forward bending posture), and Ardha Cakrāsana (Back bending posture), which takes about 9 minutes. After the Āsanas the subjects went through the DRT relaxation in supine posture. All the practises are done very slowly with closed eyes with full awareness on the changes of stimulation and relaxation. The total effect of practise on the bio-energetics was studied.

| 30 minutes supine rest | 5 minutes pre | 23-minutes CM | 5 minutes recovery | 5 min post |

5.5 DETAILS OF PARAMETERS MEASURED

The following parameters were measured using Oxycon Pro metabolic analyzer for all the five protocols described above:

1. Volume of oxygen consumed (VO₂)
2. Volume of Carbon di-oxide eliminated (VCO₂)
3. Breath rate
4. Ventilation
5. Energy expenditure in terms of Kcal/day
6. Heart rate

5.6 DATA COLLECTION:

There was provision for online data collection and transfer. The data was collected for each breath and averaged out for the purpose of analysis. Minute by minute analysis of data was also done in a single subject to know the energy expenditure pattern in detail. The online data so collected was transferred to a different excel file for the purpose of further analysis.

Each subject underwent 14 types of assessments under different protocols.
5.7. DATA ANALYSIS: STATISTICAL PROCEDURE

The sample size was determined by using the statistical formula

\[ N = \frac{Z^2 \cdot S^2}{D^2} \]

Where Z is the level of significance, S is the standard deviation, and D is the % of difference expressed. These values were basically arrived at by the values obtained in the pilot studies and that of the literature. Generally more number of subjects was taken wherever possible.

Kolmogorov-Smirnov, Shapiro-Wilk test was done to assess the normality of distribution for all the Asana, relaxation techniques and as well as for the BMR and sleep studies.

Paired t test was done for all the relaxation studies, along with MANOVA, further general linear mode multivariate analysis was done to compare the differences in the relaxation component of all relaxation as well as for the Asana's. Tukeys post-hoc tests were done in most of the comparisons for the pre and post as well as between the yoga and non-yoga groups. ANCOVA was carried out for the differences in the covariate for BMR and the sleep group. The body weight was used as covariate for all the rest of the assessments. Homogeneity test was conducted to assess the equality of variance in the pre-values of all the relaxation as well as asana postures. The groups were matched for age, body weight, height, and BMI using the independent t test. Chi-square test was used to match the gender distribution between yoga and non-yoga groups.

The breath by breath data was averaged as pre, during, recovery, and post data. The averages of the group were statistically analyzed by using appropriate tests, using SPSS version 10 software. It was considered significant when the p value was below 0.05 levels.