METHODOLOGY

Research Design - Different subject experimental design

3.1 SUBJECTS

The study was carried out at Guru Nanak Dev University, Amritsar, and nodal centers of Sports Medicine Centre, Roorkee between March 2011 and October 2014. All exercise interventions were carried in evening session with an ambient temperature between 18 to 28 °C.

A total of 241 young adult male subjects volunteered for the study. After obtaining written informed consent to participate in the study, participants were randomly assigned to either high-intensity interval training (HIIT) group or a slow continuous training (SCT) group using block randomization. 02 subjects dropped in the 1st week of the study while 01 changed his residence in the 2nd week and was unable to continue, making the total number of dropouts as 03. These 3 dropouts were not considered for tabulation of the results. The final composition of the groups was

HIIT  n=119
SCT  n=119

3.2 INCLUSION CRITERIA

– Male
– Age > 18 yrs, < 30 yrs
– BMI > 16kg/m², < 25 kg/m²
– Recreationally active individuals with no h/o of competitive sports participation
– Absence of any health related complication in past 01 yrs
– Lack of any disease as elicited during history and routine medical examination

3.3 EXCLUSION CRITERIA

– Physical deformity
– Diagnosed or suspected lifestyle disease
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– known musculoskeletal or cardiorespiratory disease
– Competitive sports participation or professional training aimed at competitive participation

All subjects were instructed to continue normal daily activities and were asked not to undertake any other training until the completion of the study.

3.4 Parameters Studied

The parameters studied, and the equipment and methods used have been explained in detail as below.

3.4.1 Age: Calendar age was calculated based on subject’s declaration of date of birth. It was rounded off to the nearest whole year as on the date of beginning of experimental protocol of the subject.

Height: Height was measured using collapsible board stadiometer. Subjects removed their shoes and standing height was measured barefoot. The subjects were asked to stand with their back against the measuring bar. The subject stood erect with legs placed together and weight evenly distributed on both feet. The subject’s position was verified from both the front and from the left side of the body. Next, the subject’s head was positioned in the Frankfort Horizontal Plane. In this position, an imaginary line can be drawn from the bottom of the eye socket (orbital margin) to the external opening of the ear (external auditory canal). He was asked to inhale deeply and hold his breath while maintaining the head and body in the same position. The moveable headpiece is brought onto the upper most point on the head with sufficient pressure to compress the hair. The measurement was recorded to the nearest 0.1cm.
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Figure 1: correct way to record height

Figure 1 shows the correct posture to record height using a stadiometer. The subject should be standing erect, with the head, shoulder blades, buttock and heels touching the measuring surface.

Figure 2: Frankfort Horizontal plane

Figure 2 depicts use of Frankfort horizontal plane for correct recording of height. It is an imaginary line passing through external ear canal and across the top of lower bone of the eye socket, immediately under the eye.
3.4.2 **Weight**: Weight was measured using Toyo weighing scale which was calibrated by the manufacturer. Before each weight was taken, it was ensured that the scale showed “zero” without weight. Subjects were asked to strip to their briefs and stand on the scale with weight evenly distributed on both feet, arm hanging freely by the side and head facing straight ahead. Weight was read from the digital scale and was recorded to the nearest 0.1 kg.

**Figure 3: Taking weight using Toyo weighing machine**

![Image](image1.jpg)

Figure 3 shows the correct way to use the toyo weighing machine. Weight is taken with subject in a comfortable position, with minimum clothes.

3.4.3 **Body Composition Analysis**: This was done using 4 electrode Bioelectric impedance analysis (BODYSTAT 1500MDD). The Bodystat 1500MDD uses bio – impedance analysis with lock in signal conversion technology. Detailed specifications are mentioned below.
Figure 4 shows detailed specification of Bodystat 1500 MDD. The Bodystat 1500 MDD uses Bio Impedence to assess body composition. It uses currents in frequency of 2 & 50 KHz, and has a wide operating range.

Figure 5: Measurement of body composition using Bodystat 1500 MDD

Figure 5 shows the correct placement of electrodes while measuring body composition using Bodystat 1500 MDD.
Measurement is taken with subject in a relaxed state. He should not be in contact with any metallic object, including any jewellery.

Height and weight were recorded as per procedure described above. The following parameters were recorded:

(a) Height (m)
(b) Weight (kg)
(c) BMI (kg/m\(^2\))
(d) Body Fat (kg)
(e) Body Fat percent (%)
(f) BMR (kcal/day)
(g) BMR per kg body weight (kcal/kg/day)
(h) Lean Mass (kg)

3.4.4 Blood Glucose Analysis: Oral Glucose Tolerance Test (GTT) was performed for assessing the fasting and post prandial glucose levels. Venous blood sample was collected from ante – cubital vein the subject after overnight fasting and subjected to blood glucose analysis. 75 g glucose was administered to the subject orally with plain water and the time of administration of glucose was noted. Another venous blood sample was collected from ante – cubital vein after 02 hrs of administration of glucose. Blood was collected in vacutainer.NaF was used as glycolytic inhibitor. Analysis of blood glucose was done using semi – auto analyser (MISPA PLUS) by Trinder’s method. Details of the method used are mentioned below.

Equation 1: Reactions in Glucose Estimation by Trinder’s Method

\[
\begin{align*}
\text{D-Glucose} + H_2O + O_2 & \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2 \\
H_2O_2 + \text{phenol} + 4\text{AAP} & \xrightarrow{\text{Peroxidase}} \text{Red Dye} + 2H_2O
\end{align*}
\]

Equation 1 depicts reactions that lead to production of colour which can be measured photometrically.
Detailed assay parameter for photometer is given below

**Figure 6: Assay parameters of photometer for glucose estimation**

<table>
<thead>
<tr>
<th>ASSAY PARAMETERS FOR PHOTOMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode</td>
</tr>
<tr>
<td>Wavelength 1 (nm)</td>
</tr>
<tr>
<td>Wavelength 2 (nm)</td>
</tr>
<tr>
<td>Sample Volume (µl)</td>
</tr>
<tr>
<td>Reagent Volume (µl)</td>
</tr>
<tr>
<td>Incubation time (min.)</td>
</tr>
<tr>
<td>Incubation temp. (°C)</td>
</tr>
<tr>
<td>Normal Low (mg/dl)</td>
</tr>
<tr>
<td>Normal High (mg/dl)</td>
</tr>
<tr>
<td>Linearity Low (mg/dl)</td>
</tr>
<tr>
<td>Linearity High (mg/dl)</td>
</tr>
<tr>
<td>Concentration of Standard</td>
</tr>
<tr>
<td>Blank with</td>
</tr>
<tr>
<td>Absorbance limit (max.)</td>
</tr>
<tr>
<td>Units</td>
</tr>
</tbody>
</table>

Figure 6 depicts details of reagents and wavelengths used for checking the concentration of glucose

**3.4.5 Lipid Profile Analysis:** Venous blood sample was collected from ante–cubital vein the subject after 14 hr fasting and subjected to Lipid Profile analysis. Blood was collected in sterile plain vacutainer. The following readings were recorded

a) Triglycerides - by GsPO Trinder method (Abidin et al., 2013), end point using semi – auto analyser (MISPA PLUS)

b) Total Cholesterol- by CHOD – PAP method (Trinder and Webster, 1984) end point using semi – auto analyser (MISPA PLUS)

c) HDL Cholesterol- by Phosphotungstate method (Lopes-Virella et al., 1977) using semi – auto analyser (MISPA PLUS)

d) Total/HDL Ratio- by calculation
Methodology

Cholesterol esters are enzymatically hydrolyzed by cholesterol esterase (CE) to cholesterol and free fatty acids. Free cholesterol, including that originally present, is then oxidized by cholesterol oxidase (CHOD) to cholest-4-en-3-one and H2O2. In presence of peroxidase (POD), the formed hydrogen peroxide formed effects the oxidative coupling of phenol and 4- aminoantipyrine (4-AAP) to form a red-colored quinoneimine dye.

The intensity of the color produced is directly proportional to cholesterol concentration. It is determined by measuring the increase in absorbance at 500 – 550 nm.

3.4.6 **Heart Rate Analysis:** The subjects were demonstrated the method of measurement of radial pulse of left upper limb using three fingers and thumb of their right hand. Once they have attained proficiency in the same, they were asked to measure early morning pulse everyday immediately after waking and record the same in a diary. The same was used for estimation of resting Heart Rate. The record of pre and post exercise Heart Rate was done using Heart Rate monitor (Polar S 410 Heart Rate monitor including Polar T61 transmitter, elastic strap for securing transmitter to chest and Polar S 410 wrist receiver). The following parameters were recorded

a) Resting heart rate

b) Peak heart rate to be recorded immediately after exercise.

c) Recovery heart rate during active recovery at 02, 03 and 05 min post exercise.

3.4.7 **Lactate Analysis:** For calculating the Resting and Peak lactate capillary sample of blood was collected from pulp of finger and analysed immediately using YSI 1500 Sport Lactate Analyser. Sample was collected before the exercise (Resting sample) and 02 min after termination of exercise (peak lactate sample).

The YSI 1500 Sport Lactate Analyser uses principles conceived by Dr. Leland Clark, formerly of Children's Hospital Foundation, Cincinnati, Ohio. The immobilized enzyme membrane has been invented by YSI (U.S. Patent 4,073,713)
Figure 7: Sensor Probe and Enzyme membrane of YSI lactate analyser.

Figure 7 depicts different steps in estimation of lactate using YSI Lactate Analyser. The sensor probe is fitted with three layer membrane. Substrate passes through the three layers. The exact place where the reaction takes place are also shown

The probe is fitted with a three-layer membrane containing immobilized lactate oxidase in the middle layer. The face of the probe, covered by the membrane, is situated in a buffer-filled sample chamber into which a sample is injected. Some of the substrate diffuses through the membrane. When it contacts the immobilized enzyme (lactate oxidase), it is rapidly oxidized, producing hydrogen peroxide (Reaction 1). The hydrogen peroxide (H2O2) is, in turn, oxidized at the platinum anode, producing electrons (Reaction 2). A dynamic equilibrium is achieved when the rate of H2O2 production and the rate at which H2O2 leaves the immobilized enzyme layer become constant. Equilibrium is indicated by a steady state response. The electron flow is linearly proportional to the steady state H2O2 concentration and, therefore, to the concentration of lactate.

Equation 2 : Equation Depicting Steps of Lactate Estimation by YSI Lactate Analyser
Reaction 1 (lactate) : Lactate + O2 LOx Pyruvate + H2O2

Reaction 2 : H2O2 Pt anode 2H+ + O2+ 2e-

Equation 2 gives details of the reactions that take place in probe and membrane of YSI Lactate Analyser

The platinum electrode is held at an anodic potential and is capable of oxidizing many substances other than H2O2. To prevent these reducing agents from contributing to sensor current, the membrane contains an inner layer consisting of a very thin film of cellulose acetate. This film readily passes H2O2 but excludes chemical compounds with molecular weights above approximately 200. The cellulose acetate film also protects the platinum surface from proteins, detergents and other substances that could foul it. However, the cellulose acetate film can be penetrated by such compounds as hydrogen sulfide, low molecular weight mercaptans, hydroxylamines, hydrazines, phenols and anilines.

The YSI Sport Lactate analyser was calibrated as per the protocol given below using lactate standard (YSI 2327 5 mmol/L) in order to compare the relative concentration of the standard with the relative concentration of the unknown sample.

1. Every day at the beginning of testing
2. Whenever the membrane was changed or new reagent fluids were installed
3. Whenever the instrument was powered on
4. After every fifth sample
5. Whenever injection devices were changed

3.4.8 Endurance: Endurance was tested by using 20 m Shuttle test (Beep test). Subjects were asked to run between two lines drawn 20 m apart in a way to time the placement of feet on the line with the “beep” played from the software. The beeps are pre arranged to correspond to an increase in speed by 0.5km/hr, starting at 8.5 km/hr (Leger et al., 1988; Brewer et al., 1988). Test was carried out till volitional exhaustion. Test was terminated when the subject failed to place his leading foot on designated line 02 consecutive times. Constant encouragement was provided during the test. The following was recorded for each subject.
Methodology

a) Stages and levels finally achieved
b) VO₂ max calculated
c) Maximum velocity achieved
d) The test was also be used as maximal exercise stimulus for assessing peak lactate, peak heart rate and recovery heart rates.

3.5 EXPERIMENTAL PROTOCOL

3.5.1 Pre-experimental procedures: Subjects were familiarized with the testing procedures before baseline testing.

3.5.2 Pre-experimental testing: All subjects were administered the following tests prior to beginning of exercise intervention.

3.5.2.1 Body composition analysis: This was done using 4 electrode Bioelectric impedance analysis (BODYSSTAT 1500MDD). Height was recorded using standardized stadiometer and weight was recorded using standardized digital weighing machine. Other measurements were taken using standard measuring tape. The following parameters were recorded

a) Height (m)
b) Weight (kg)
c) BMI (kg/m²)
d) Body Fat (kg)
e) Body Fat percent (%)
f) BMR (kcal/day)
g) BMR per kg body weight (kcal/kg/day)
h) Lean Mass (kg)

3.5.2.2 Oral Glucose Tolerance Test (GTT): Oral GTT was performed using method described by American Diabetes Association (2014). Venous blood sample was collected from ante – cubital vein the subject after overnight fasting and subjected to blood glucose analysis. 75 g glucose was administered to the subject orally with plain
water and the time of administration of glucose was noted. Another venous blood sample was collected from ante – cubital vein after 02 hrs of administration of glucose. Blood was collected in vacutainer containing NaF. Analysis of blood glucose was done using semi – auto analyser (MISPA PLUS)

3.5.2.3 Lipid Profile: Venous blood sample was collected from ante – cubital vein the subject after 14 hr fasting and subjected to Lipid Profile analysis. Blood was collected in sterile plain vacutainer. The following readings were recorded

(a) Triglycerides - by GsPO Trinder method (Abidin et al., 2013), end point using semi – auto analyser (MISPA PLUS)

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(c) HDL Cholesterol- by Phosphotungstate method (Lopes-Virella et al., 1977) using semi – auto analyser (MISPA PLUS)

(d) Total/HDL Ratio- by calculation

3.5.2.4 Heart Rate: The subjects were demonstrated the method of measurement of radial pulse of left upper limb using three fingers and thumb of their right hand. Once they have attained proficiency in the same, they were asked to measure early morning pulse everyday immediately after waking and record the same in a diary. The same was used for estimation of resting Heart Rate. The record of pre and post exercise Heart Rate was done using Heart Rate monitor (Polar S 410 Heart Rate monitor including Polar T61 transmitter, elastic strap for securing transmitter to chest and Polar S 410 wrist receiver). The following parameters were recorded

(a) Resting heart rate

(b) Peak heart rate to be recorded immediately after exercise.

(c) Recovery heart rate during active recovery at 02, 03 and 05 min post exercise.

3.5.2.5 Resting and Peak lactate analysis: Capillary sample was collected from pulp of finger and analysed immediately using YSI 1500 Sport Lactate Analyser. Sample was collected before the exercise (Resting sample) and 02 min after termination of exercise (peak lactate sample).
3.5.2.6 **Endurance**: Endurance was tested by using 20 m Shuttle test (Beep test). Subjects were asked to run between two lines drawn 20 m apart in a way to time the placement of feet on the line with the “beep” played from the software. The beeps are pre arranged to correspond to an increase in speed by 0.5 km/hr, starting at 8.5 km/hr (Leger et al., 1988; Brewer et al., 1988). Test was carried out till volitional exhaustion. Test was terminated when the subject failed to place his leading foot on designated line 02 consecutive times. Constant encouragement was provided during the test. The following was recorded for each subject.

(a) Stages and levels finally achieved
(b) $\text{VO}_2\text{max}$ calculated
(c) Maximum velocity achieved
(d) The test was also be used as maximal exercise stimulus for assessing peak lactate, peak heart rate and recovery heart rates.

3.5.3 **Experimental procedures**: The subjects were randomly divided into High Intensity Interval Training (HIIT) group and Slow Continuous Training (SCT) group. Each group was assigned under a supervisor who ensured that training was carried out as per the required protocol. Training given to each group was as under.

3.5.3.1 **Warm up**: Each group underwent a warm up sessions lasting 15 min of running. During warm up, maximum velocity reached was 50% of that achieved during pre – test Beep Test. Generalised stretching was a part of warm up protocol. The entire session was supervised.

3.5.3.2 **High Intensity Interval Training (HIIT) group** underwent the following training

1. Frequency : 03 sessions per week X 06 weeks
2. Intensity : maximal, all out running
   
   Training velocity not less than maximum velocity achieved in Beep test
3. Type : Interval
   
   Training/Active rest – 30s/30s
   
   05 reps per set
   
   04 sets per session with 03 min rest between sets
4. Duration : Exercise time – 10 min/session

30 min/week

Total time – 26 min/session

3.5.3.3 Slow Continuous Training (SCT) group underwent the following training

1. Frequency : 05 sessions per week X 06 weeks

2. Intensity : sub – maximal

Training at velocity equal to 60 – 70% of maximum velocity achieved in Beep test

3. Type : Continuous

4. Duration : Exercise time – 30 min/session

– 150 min/wk

3.5.3.4 Cool down: All subjects underwent a cooling down sessions lasting 15 min of running. Generalised stretching was a part of cool down protocol. The entire session was supervised.

3.6 EXPERIMENTAL TESTING

All the tests conducted as part of pre – experimental testing were repeated after 03 weeks and 06 weeks of testing. The tests will be so scheduled so as to avoid any interruption of training.

3.7 DIETARY CONSIDERATIONS

Subjects were asked to note down their diet on the day before the initial performance tests and to use similar diet before the mid- and post-training tests. They were also instructed to abstain from caffeine or alcohol for at least 8 hours before testing.