CHAPTER III
MATERIALS AND METHODS
3.0 MATERIALS AND METHODS

The experimental design is divided into 4 parts

3.1 Clinical study Methodology.
3.2 Safety Measurements
3.3 Pharmacodynamic Measurements.
3.4 Statistical Analysis.

3.1 CLINICAL STUDY METHODOLOGY

3.1.1 OBJECTIVE

1. To study and compare the lipid lowering effects of three different marketed formulations of atorvastatin in normal human subjects.
2. To determine if these brands of atorvastatin can be safely substituted in general practice.

3.1.2 PRODUCT EVALUATED

*Product A*
Zivast 20 mg tablets each containing 20 mg of atorvastatin manufactured by FDC, India. Batch No. AYT 6061 & Expiry May 2008.

*Product B*
Lipicor 20 mg tablets each containing 20 mg of atorvastatin manufactured by Intas, India. Batch No. DG0571 & Expiry August 2009.

*Product C*
Atocor 20 mg tablets each containing 20 mg of atorvastatin manufactured by Dr. Reddy's Laboratories Limited, India. Batch No. ATH 6007 & Expiry June 2008.

The above mentioned treatments were administered within thirty minutes after breakfast in morning.

3.1.3 STUDY DESIGN

The study was conducted as randomized, open label, parallel in normal human
3.1.4 STUDY SITE

The study was conducted at Ranbaxy Clinical Pharmacology Unit, 2nd Floor, Majeedia Hospital, New Delhi.

3.1.5 SELECTION OF SUBJECTS

Adequate number of subjects were selected randomly from the Volunteer Bank of Clinical Pharmacology Unit and the subjects underwent a standardized screening procedure.

Screening Assessments

Medical histories and demographic data, including name, sex, age, body weight (kg), height (cm) and tobacco use (including number of cigarettes smoked per day) were recorded. Each subject had undergone physical examination and the laboratory tests of hematologic, hepatic and renal functions as listed below.

Laboratory Tests

HEMATOLOGY
- Hemoglobin
- Total leukocyte count
- Differential leukocyte count
- Platelet count

BIO-CHEMISTRY
- BUN
- Creatinine
- Total bilirubin
- Alkaline phosphatase
- AST

URINALYSIS
- PHYSICAL EXAMINATION
  - Colour
  - Appearance
  - pH
  - Specific gravity
  - Protein
  - Glucose

MICROSCOPIC EXAMINATION
- RBC
- WBC

ADDITIONAL TESTS
- HIV I & II
- HBsAg
- HCV
- VDRL
- Urine drug screen (-cannabinoids
- opioids)
Thirty six, male healthy subjects with clinically normal laboratory profiles were selected based on the following inclusion and exclusion criteria.

**Inclusion Criteria**

- Age range of 18-45 years.

- Neither overweight nor underweight for his/her height as per the Life Insurance Corporation of India height/weight chart for non-medical cases.

- Have voluntarily given written informed consent to participate in this study.

- Subjects having normal health as determined by medical history and physical examination of the subjects performed within 28 days prior to the commencement of the study.

**Exclusion Criteria**

The subjects were excluded if they had any of the following exclusion criteria

- History of allergy to atorvastatin and other related drugs.

- Any evidence of organ dysfunction or any clinically significant deviation from the normal, in physical or clinical determinations.

- History of myopathy, rhabdomyolysis, myalgia, pancreatitis and myositis.

- History of recurrent skin rashes, urticaria, angioedema and pruritis.

- History of epistaxis and dizziness
- Presence of disease markers of HIV 1 and 2, Hepatitis B and C viruses and syphilis infection.

- Presence of values which are clinically significantly different from normal reference ranges for haemoglobin, total white blood cells count, differential WBC count and platelet count.

- Positive for urinary screen testing of drugs of abuse (opiates and cannabinoids)

- Presence of values which are significantly different from normal reference ranges for serum creatinine, blood urea nitrogen, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum alkaline phosphatase, serum bilirubin, plasma glucose and serum cholesterol.

- Clinically abnormal chemical and microscopic examination of urine defined as presence of RBC, WBC (>4/HPF), epithelial cells (>4/HPF), glucose (positive) and protein (positive).

- Clinically abnormal ECG or Chest X-ray.

- History of serious gastrointestinal, hepatic, renal, cardiovascular, pulmonary, neurological or haematological disease, diabetes or glaucoma.

- History of any psychiatric illness, which may impair the ability to provide, written informed consent.

- Use of any metabolic enzyme modifying drugs within 30 days prior to Day 1 of this study.

- Regular smokers who smoke more than 20 cigarettes daily or have difficulty abstaining from smoking for the duration of each study period.
- History of drug dependence or excessive alcohol intake on a habitual basis of more than 2 units of alcoholic beverages per day (1 unit equivalent to half pint of beer or 1 glass of wine or 1 measure of spirit) or have difficulty in abstaining for the duration of each study period.

- Subjects who, through completion of this study, would have donated and/or lost more than 350 mL of blood in the past 3 months.

- Participation in any clinical trial within 12 weeks preceding Day 1 of this study.

3.1.6. NUMBER OF SUBJECTS
Enough healthy adult male human subjects were enrolled to allow dosing of 36 subjects so that maximum number of subject/group was 12.

3.1.7 ADMISSION AND STAY
The study subjects were admitted and housed in the Clinical Pharmacology Unit from at least 10 hours before dose administration and were discharged 1 hour after administration of the study drug, if the subjects do not suffer from any adverse drug reaction. In case of an adverse event the subject were monitored until the event subsides.

3.1.8 HANDLING, STORAGE AND ACCOUNTABILITY PROCEDURES
The drug products were procured by the investigator in an appropriate package deemed to maintain the integrity of the products. The drug products were stored under prescribed storage conditions. The investigator was accountable for the study drug products. The study drugs were dispensed according to the randomization schedule.

3.1.9 ASSIGNMENT TO TREATMENT SEQUENCES
The order of receiving study treatments for each subject was determined according to a SAS-generated balanced randomization schedule.
3.1.10 TREATMENTS

Treatment A
A single oral dose of a Zivast 20 mg tablet was administered with 240 mL of drinking water daily for 4 weeks.

Treatment B
A single oral dose of a Lipicor 20 mg tablet was administered with 240 mL of drinking water daily for 4 weeks.

Treatment C
A single oral dose of a Atocor 20 mg tablet was administered with 240 mL of drinking water daily for 4 weeks.

3.1.11 ASSESSMENT OF COMPLIANCE

Compliance was assessed by conducting a thorough examination of the oral cavity by trained study personnel after dosing. At least 80% consumption of medication was the criteria for inclusion of the subjects in the analysis.

3.1.12 FASTING/MEALS

Subjects started the recommended meal 30 minutes prior to administration of the drug products. Study subjects were supposed to eat these meal in 30 minutes or less; however, the drug products were administered within 30 minutes after start of the meal. The drug products were administered with 240 mL (8 fluid ounces) of water. Water was allowed as desired. During housing i.e. day 1 (admission), all meal plans were identical. Subjects received standardized meals – lunch, snacks and dinner at 4, 10 and 13 hours, respectively. All volunteers were advised to follow normal routine diet.

3.1.13 SAMPLING SCHEDULE

A total of five 4-mL blood samples were collected from each subject by a fresh clean venipuncture using a disposable sterilized syringe during the course of the study. The blood samples were collected pre-dose on day 1 and at end of week 1, 2, 3 and 4 for lipid profile estimation. For each subject, the total number of blood draws during the study was five and the total volume of
blood drawn including 16 mL for screening and a 4 mL blood sample collected for safety estimation at the end of the study, did not exceed 40 mL.

3.1.14 RESTRICTIONS

Medications
The study subjects did not take any prescription and/or OTC medications for at least two weeks prior to the study and during the study.

Diet
All subjects were instructed to abstain from alcoholic products during the study period.

Activity
All subjects were dosed while seated and were asked to remain seated or ambulatory following drug administration. Thereafter, subjects were allowed to engage in normal activities.

3.1.15 ETHICAL CONSIDERATIONS

Basic Principles
This research was carried out in accordance with the Basic Principles defined in US 21 CFR Part 312.20, the ICH (62FR 25692, 09 May 1997) 'Guidance for Good Clinical Practice' and the principles enunciated in the Declaration of Helsinki (Edinburgh, October 2000) With notes of Clarification on Paragraph 29 and 30 added by the WMA General Assembly, Washington 2002 and by the WMA General Assembly, Tokyo 2004 respectively.

Institutional Review Board
The protocol and the corresponding informed consent form (ICF) used to obtain informed consent of study subjects were reviewed by the Jamia Hamdard Institutional Review Board and the study subjects were not dosed until the Board has approved the protocol and the ICF, as submitted or with modifications. The protocol and the informed consent form for this study were
reviewed and approved by the Jamia Hamdard Institutional Review Board on 4th October 2006.

Informed Consent
The purpose of the study, the procedures to be carried out and the potential hazards that may be encountered during the conduct of the study were described to the subjects in non-technical terms before the subjects were admitted to the Ranbaxy Clinical Pharmacology Unit. All subjects provided formal written informed consent after attending an oral presentation and after thoroughly reading the version 01 of the Informed Consent Form (ICF).

Drop-out/Withdrawal of Subjects from Study
Subjects were informed that they are free to dropout from the study at any time without stating any reason. The investigator could have withdrawn a subject from the study for any of the following reasons:

(i) The subject suffers from significant intercurrent illness or undergoes surgery during the course of the study.

(ii) The subject experiences an adverse event, and when withdrawal would be in the best interest of the subject.

(iii) The subject fails to comply with the requirements of the protocol. This would include pre-study directions regarding alcohol and drug use, fasting or if the subject is uncooperative during the study.

Volunteer Compensation
The subjects were adequately compensated on account of their participation in the study. In case of drop-out/withdrawal of a subject before completion of the study, the guidelines issued by the Jamia Hamdard Institutional Review Board were final and binding on both Ranbaxy Research Laboratories and the study subjects.
3.1.16 STUDY DOCUMENTATION

All data generated during the conduct of the study were directly entered in the raw data recording forms except the analytical data of clinical laboratory of the Clinical Pharmacology Unit, which was transcribed into the study related forms and the raw data retained by the laboratory for records. The instrument-generated laboratory printouts were also treated as raw data. All raw data and transcribed data forms were completed by the study personnel assisting in the study and were checked wherever applicable for completeness and logistics by the Investigator.

3.1.17 PROTOCOL AND/OR SOP DEVIATIONS

There were no significant protocol and/or SOP deviations.

3.1.18 CONFIDENTIALITY OF DATA

The data identifying each study subject by name will be kept confidential and will be accessible to the study personnel, and if necessary, to the Institutional Review Board- Jamia Hamdard.

3.2 SAFETY

Clinical Safety Measurements
Vital signs of oral temperature, sitting blood pressure and radial pulse were measured during subject admission prior to dosing on day 1, end of week 1, 2, 3 and 4. Vital signs to be measured prior to administration of the dose were taken within 1 hour of the scheduled dosing time.

Brief clinical examination of the subject was conducted by a qualified medical designate on duty after subject admission, prior to dosing on day 1, end of week 1, 2, 3 and 4.

Adverse Events
All the subjects were monitored throughout the study period for adverse events. Subjects were specifically asked about any adverse events after admission and before administration of the dose. Volunteers were instructed
to bring to the notice of the nurse or the doctor any adverse event that may occur throughout the study period.

Liver function test were performed at the time of screening and at end of week 4. All the adverse events were recorded in the final report. Adverse event experienced by subject were followed until the events have resolved.

3.3 PHARMACODYNAMIC ANALYSIS

The following pharmacodynamic parameters will be calculated:

1. Serum Total Cholesterol.
2. Serum LDL Cholesterol
3. Serum HDL Cholesterol.
4. Total Cholesterol to HDL ratio
5. LDL to HDL Ratio
6. Serum Triglycerides.

All these parameters were assessed in samples collected prior to dosing on day 1 and at end of week 1, 2, 3 and 4 during the study period.

3.3.1 Instrument: Dimension® RxL clinical chemistry system

The Dimension® RxL clinical chemistry system is a discrete, random-access, microprocessor-controlled, integrated instrument/chemistry system that measures a variety of analytes, including enzyme activities, in body fluids. For in vitro diagnostic use, it uses the Dade Behring Inc. Flex® multiple-test reagent cartridges, disposable reaction cuvettes, integrated multisensor technology with the Dade Behring Inc. QuicLYTE® integrated multisensor to provide rapid, accurate and precise test results.

3.3.2 Estimation of total serum cholesterol

Cholesterol was measured in the serum with the help of cholesterol flex reagent cartridge. It is an in vitro diagnostic test used for quantitative determination of total cholesterol in serum and plasma.
Principle: Cholesterol esterase (CE) catalyses the hydrolysis of the cholesterol esters to produce free cholesterol which, along with preexisting free cholesterol, is oxidized in a reaction catalyzed by cholesterol oxidase (CO) to form cholest-4-ene-3-one and hydrogen peroxide. In the presence of horseradish peroxide (HPO), the hydrogen peroxide thus formed is used to oxidize N, N-diethylaniline–HCl /4-aminoantipyrine (DEA-HCl/AAP) to produce chromophore that absorbs at 540 nm. The absorbance due to oxidized DEA-HCl/AAP is directly proportional to the cholesterol concentration and is measured using a polychromatic (452, 540, 700 nm) end point technique.

Cholesterol esters $\xrightarrow{\text{CE}}$ Cholesterol + Fatty Acids

$\text{Cholesterol} + \text{O}_2 \xrightarrow{\text{CO}} \text{Cholest-4-ene-3-one} + \text{H}_2\text{O}_2$

$2\text{H}_2\text{O}_2 + \text{DEA-HCl/AAP} \xrightarrow{\text{HPO}} 4\text{H}_2\text{O} + \text{Oxidized DEA-HCl/AAP}$

Reagent Kit Used: Cholesterol flex reagent cartridge was used for the quantitative estimation of serum cholesterol level. The reagents/cartridge were stored at 2°C to 8°C before use.

<table>
<thead>
<tr>
<th>Wells</th>
<th>Form</th>
<th>Ingredients</th>
<th>Concentrations</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>Tablet</td>
<td>CE CO HPO</td>
<td>0.7 U/ml 0.1 U/ml 2.4 U/ml</td>
<td>Fungal microbial plant</td>
</tr>
<tr>
<td>1-3</td>
<td>Tablet</td>
<td>AAP Buffer Cholate</td>
<td>4.5 μmol</td>
<td></td>
</tr>
<tr>
<td>4-6</td>
<td>Tablet</td>
<td>DEA Surfactant</td>
<td>5.8 μmol</td>
<td></td>
</tr>
</tbody>
</table>

Procedure: The test was performed on the dimension clinical chemistry system after the method was calibrated. The CHOL flex reagent cartridge was
required to perform the CHOL test. Sampling, reagent delivery, mixing, processing and printing of the results were automatically performed by Dimension system.

Test Conditions:
- Sample Size: 3 µL
- Reagent 1 Volume: 88 µL
- Reagent 2 Volume: 26 µL
- Diluent Volume: 241 µL
- Test Temperature: 37°C
- Wavelength: 452, 540 & 700 nm
- Type of Measurement: Polychromatic endpoint

3.3.3 Estimation of serum triglycerides

Triglycerides were measured in the serum with the help of triglyceride flex reagent cartridge. It is an in vitro diagnostic test used for quantitative determination of triglycerides in serum and plasma.

Principle: Triglycerides are determined quantitatively in the plasma and serum. The method is based on the enzymatic procedure in which the combination of the enzymes are employed. The sample is incubated with lipoprotein lipase (LPL) enzyme reagent that converts triglycerides into free glycerol and fatty acids. Glycerol kinase (GK) catalyzes the phosphorylation of glycerol by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate, which is oxidized to dihydroxyacetone phosphate and hydrogen peroxide by glycerol-3-phosphate oxidase. The catalytic action of the peroxidase (POD) forms quinoneimine from hydrogen peroxide, aminoantipyrine and 4-chlorophenol. The change in absorbance due to the formation of quinoneimine is directly proportional to the total amount of glycerol and its precursors in the sample and is measured using a bichromatic (510, 700 nm) endpoint technique.

\[
\text{Triglycerides} \xrightarrow{\text{LPL}} \text{Glycerol + Fatty Acids}
\]
Glycerol + ATP $\xrightarrow{GK}$ Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O$_2$ $\xrightarrow{GPO}$ Dihydroxyacetone phosphate + H$_2$O$_2$

$2$H$_2$O$_3$ + Aminoantipyrine + $4$-Chlorophenol $\xrightarrow{PQD}$ Quinoneimine + $\text{HCL} + 4$H$_2$O

Reagent Kit Used: Triglycerides flex reagent cartridge was used for the quantitative estimation of serum triglyceride level. The reagents/cartridge were stored at $2^\circ$ C to $8^\circ$ C before use.

Table 3.2: The reagents provided in the triglycerides kit

<table>
<thead>
<tr>
<th>Wells</th>
<th>Form</th>
<th>Ingredients</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-6</td>
<td>Liquid</td>
<td>Lipoprotein lipase</td>
<td>7.5 KU/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP</td>
<td>3 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycerol kinase</td>
<td>0.5 KU/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycerol-3-phosphate oxidase</td>
<td>2.2 KU/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-aminoantipyrine</td>
<td>0.75 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$4$-chlorophenol</td>
<td>6 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peroxidase</td>
<td>5 KU/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mg$^{2+}$</td>
<td>22.5 mmol/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Buffer pH 7.2</td>
<td>50 mmol/L</td>
</tr>
</tbody>
</table>

Procedure: The test was performed on the dimension clinical chemistry system after the method was validated and instrument was calibrated. The TGL flex reagent cartridge was required to perform the TGL test. Sampling, reagent deliver, mixing, processing and printing of the results were automatically performed by Dimension system.

Test Conditions:
Sample Size: 4 $\mu$L
Reagent 1 Volume: 133 $\mu$L
CHAPTER III: MATERIALS AND METHODS

Test Temperature | 37°C ± 0.1°C
Wavelength | 510 & 700 nm
Type of Measurement | bichromatic endpoint
Autodilute Volume | 2 µL

3.3.4 Estimation of serum HDL levels
HDL-cholesterol was measured in the serum with the help of HDL-cholesterol flex reagent cartridge. It is an *in vitro* diagnostic test used for quantitative determination of HDL-cholesterol in pretreated serum and plasma.

*Principle:* The automated HDL Cholesterol assay is a homogenous method for directly measuring HDL levels without the need for off-line pretreatment or centrifugation steps.

The method is in a two reagent format and depends on the properties of a unique detergent. This method is based on accelerating the reaction of cholesterol oxidase (CO) with non-HDL unesterified cholesterol and dissolving HDL selectively using a specific detergent. In the first reagent, non-HDL unesterified cholesterol is subject to an enzyme reaction and the peroxide generated is consumed by a peroxidase reaction with DSBmT yielding airless product. The second reagent consists of a detergent capable of solubilizing HDL specifically, cholesterol esterase (CE) and chromagenic coupler to develop color for the quantitative determination of HDL.

\[
\text{HDL, LDL, } \xrightarrow{\text{Accelerator + CO}} \text{ Non-reactive LDL, VLDL, Chylomicrons}
\]
\[
\text{VLDL, } \xrightarrow{\text{DSBmT + Peroxidase}} \text{ VLDL, chylomicrons}
\]

HDL Specific Detergent, HDL disrupted

\[
\text{HDL Cholesterol } \xrightarrow{\text{Cholesterol esterase}} \Delta^4 \text{ Cholestenone + H}_2\text{O}_2
\]
\[
\text{H}_2\text{O}_2 + \text{DSBmT + 4-AAP } \xrightarrow{\text{Peroxidase}} \text{ Colour Development}
\]
Reagent Kit Used: HDL-cholesterol flex reagent cartridge was used for the quantitative estimation of serum HDL-cholesterol level. The reagents/cartridge were stored at 2°C to 8°C before use.

Table 3.3: The reagents provided in the HDL flex kit.

<table>
<thead>
<tr>
<th>Wells</th>
<th>Form</th>
<th>Ingredients</th>
<th>Concentrations</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2,3 (Reagent 1)</td>
<td>Liquid</td>
<td>MES Buffer</td>
<td></td>
<td>bacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol oxidase</td>
<td>&lt;1000 U/L</td>
<td>horse-radish</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peroxidase</td>
<td>&lt;1300 ppg U/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N,N-bis(4-sulphobutyl)-m-toluidine-disodium</td>
<td>&lt;1 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Accelerator</td>
<td>&lt;1 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preservative</td>
<td>&lt;0.06%</td>
<td></td>
</tr>
<tr>
<td>4 (Reagent 2)</td>
<td>Liquid</td>
<td>MES buffer</td>
<td></td>
<td>bacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cholesterol esterase</td>
<td>&lt;1500 U/L</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-Aminoantipyrine</td>
<td>&lt;1 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Detergent</td>
<td>&lt;2 %</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Restrainer</td>
<td>&lt;0.15%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preservative</td>
<td>&lt;0.06%</td>
<td></td>
</tr>
<tr>
<td>5,6</td>
<td>Liquid</td>
<td>NaOH</td>
<td>1.00 M</td>
<td>bacterial</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascorbic acid oxidase</td>
<td>&lt;3000 U/L</td>
<td></td>
</tr>
</tbody>
</table>

Procedure: The test was performed on the dimension clinical chemistry system after the method was validated and instrument was calibrated. The HDL flex reagent cartridge was required to perform the HDL test. Sampling, reagent deliver, mixing, processing and printing of the results were automatically performed by Dimension system.

Test Conditions:
Sample Size: 3 μL
Reagent 1 Volume 300 μL
Reagent 2 Volume 100 μL
Test Temperature: 37°C ± 0.1°C
Wavelength: 600 & 700 nm
Type of Measurement: bichromatic endpoint

3.3.5 Estimation of LDL cholesterol

LDL cholesterol will be calculated by the Friedewald formula (Friedewald et al., 1972).

Friedewald formula:

\[
[LDL \text{ cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol} - \frac{1}{5} \times \text{triglyceride}]
\]

3.4 STATISTICAL ANALYSIS

Statistical analyses were performed using the SAS system for Windows, release 8.2 (SAS Institute Inc., USA).

3.4.1 Summary Statistics

Results are presented as mean ± standard deviation (SD). The demographic and other baseline characteristics of the subjects enrolled in the study (age, etc) are summarized in the table. Adverse events experienced by the subject during the course of the study were appropriately summarized and tabulated.

Percentage change from baseline was calculated. Whenever changes from baseline were calculated, only those subjects for whom both baseline and a post-dose assessment (at least 2 weeks) are available were included in the analysis. Percentage change from baseline i.e. percentage difference was calculated as \([\text{Post-treatment (week 1, 2, 3 & 4)-week 0)}\)/week 0 X 100).

Student's Paired t-tests was used to compare baseline & post-treatment values for each variable within each treatment group.

The significance of differences between treatment group was assessed by ANOVA. Factors in the model included treatment. Baseline data were treated as covariate.