CHAPTER 3

METHODS AND PROCEDURES

3.1 SUBJECTS

Thirty marathon runners (19 males and 11 females) between the ages of 20-51 years (mean age = 38.4 ± 8.2 years) volunteered to take part in the study. The subjects had a mean body mass of 73 ± 9.5 kg and a stature of 175 ± 7.2 cm. The mean body mass for the males and females were 75.6 ± 8.0 kg and 61.3 ± 8.0 kg, respectively. The mean stature for the males and females were 177.1 ± 5.5 cm and 167.1 ± 7.6 cm, respectively. All of the participants were members of marathon clubs in and around Pretoria. The subjects were all briefed on the nature of the research project and the possible risks and benefits prior to providing their written informed consent (Appendix A) to participate in the study.

The following specific exclusion criteria were applied:

a) haematology results not within the normal physiological limits;
b) not being able to maintain a minimum running speed of 8 km/h on the treadmill;
c) use of any other ergogenic supplement or aid; and
d) prescription medication usage.

3.2 STUDY DESIGN

The primary aim of the study was to determine the efficacy of both Cellfood® and Switch™ as an ergogenic aid for endurance athletes. In order to reach this goal a pre-test – post-test, double-blind cross-over, placebo controlled experimental design, with two levels of the independent variable, was adopted for the study. Accordingly subjects were randomly assigned to either a placebo, Cellfood® or Switch™ group.
Each of the groups underwent a supplementation period comprising three four-week cycles of varying dosages, as recommended by the manufacturer. After each cycle the subjects stopped supplementation during a two-week washout period, prior to crossing-over to an alternative supplementation and dosage cycle.

Accordingly the groups were arranged as follows over the duration of the study:

<table>
<thead>
<tr>
<th></th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Product</td>
<td>Dosage</td>
<td>Product</td>
</tr>
<tr>
<td>A</td>
<td>Placebo</td>
<td>28ml</td>
<td>Cellfood®</td>
</tr>
<tr>
<td>B</td>
<td>Cellfood®</td>
<td>28ml</td>
<td>Switch™</td>
</tr>
<tr>
<td>C</td>
<td>Switch™</td>
<td>53.2ml</td>
<td>Placebo</td>
</tr>
</tbody>
</table>

No statistically significant differences (p>0.05) were found between any of the groups regarding the variables measured during the baseline tests at the onset of the study, thus reflecting the effectiveness of the pre-study random assignment process. At the end of every cycle each group was subjected to the same testing protocol as during the pre-test.

In order to enhance compliance an individualized dosage schedule was given to each subject. The schedule required ingesting one daily dose upon waking first thing in the morning. This schedule indicated the dates when the subject was required to start and stop using each of the products as well as the relevant dosage of the product for the specific cycle. On recommendation of the manufacturer, the Switch™ dosage was roughly double that of both the Cellfood® and the placebo due to a difference in the relative concentrations of the products (Appendix B).
3.3 INDEPENDENT VARIABLES

3.3.1 Cellfood®

Product content and characteristics as described by the manufacturer:
Cellfood® is a proprietary super energized complex concentrate held in colloidal suspension. It is ingested orally in the form of fluid droplets added to water. It contains 78 trace elements and minerals, combined with 34 enzymes, 17 amino acids, dissolved and nascent oxygen, suspended in a solution of deuterium sulphate (D₂SO₄). Cellfood® is unique due to its ability to create nascent oxygen or "newly born" oxygen (Latin- Nascere). In biochemical terms nascent oxygen refers to a newly born single oxygen (elemental oxygen) that has not yet entered into biochemical reaction. Nascent oxygen is negatively charged (O') while free radicals, on the other hand, are positively charged ions of single oxygen (O'). The opposite charge of these ions cause them to attract each other, forming a single pure oxygen molecule (O₂). Nascent oxygen "seeks out" and neutralizes dangerous free radicals, combining to form pure oxygen in the process.

\[ \text{nascent oxygen} - \text{free radical ions} = \text{stable oxygen} \]

Elements, minerals and trace minerals found in Cellfood®

<table>
<thead>
<tr>
<th>Actinium</th>
<th>Cobalt</th>
<th>Indium</th>
<th>Niobium</th>
<th>Tin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antinomy</td>
<td>Copper</td>
<td>Iodine</td>
<td>Nitrogen</td>
<td>Titanium</td>
</tr>
<tr>
<td>Argon</td>
<td>Dysprosium</td>
<td>Iridium</td>
<td>Osium</td>
<td>Tungsten</td>
</tr>
<tr>
<td>Astatine</td>
<td>Erbium</td>
<td>Iron</td>
<td>Oxygen</td>
<td>Vanadium</td>
</tr>
<tr>
<td>Barium</td>
<td>Europium</td>
<td>Krypton</td>
<td>Palladium</td>
<td>Xenon</td>
</tr>
<tr>
<td>Beryllium</td>
<td>Fluorine</td>
<td>Lanthanum</td>
<td>Phosphorus</td>
<td>Ytterbium</td>
</tr>
<tr>
<td>Bismuth</td>
<td>Gadolinium</td>
<td>Lithium</td>
<td>Platinum</td>
<td>Zinc</td>
</tr>
<tr>
<td>Boron</td>
<td>Gallium</td>
<td>Lutetium</td>
<td>Polonium</td>
<td>Zirconium</td>
</tr>
<tr>
<td>Bromine</td>
<td>Germanium</td>
<td>Magnesium</td>
<td>Potassium</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>Gold</td>
<td>Manganese</td>
<td>Praseodymium</td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>Hafnium</td>
<td>Molybdenum</td>
<td>Promethium</td>
<td></td>
</tr>
<tr>
<td>Cerium</td>
<td>Helium</td>
<td>Neodymium</td>
<td>Rhenium</td>
<td></td>
</tr>
<tr>
<td>Cesium</td>
<td>Holmium</td>
<td>Neon</td>
<td>Rhodium</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td>Hydrogen</td>
<td>Nickel</td>
<td>Rubidium</td>
<td></td>
</tr>
</tbody>
</table>
Digestive and Metabolic Enzymes

- Hydrolases and Carbohydrases: Maltase, Sucrase and Emulsin
- Nucleases: Polynucleotidase and Nucleotidase
- Amidase: Urease
- Peptidase: Aminopolypeptidase, Dipeptidase and Prolinase
- Esterase: Lipase, Phophotase and Sulfatase
- Iron Enzymes: Catalase, Cytochrome oxidase and Peroxidase
- Enzyme containing Coenzymes 1 and / or 2: Lactic Dehydrogenase, Robison Ester and Dehydrogenase
- Copper Enzymes: Tyrosinase and Ascorbic acid oxidase
- Enzymes which reduce Cytochrome: Succinic Dehydrogenase
- Hydrases: Fumarase and Enolase
- Yellow Enzymes: Warburg's Old Yellow Enzymes, Diaphorase, Haas Enzyme and Cytochrome C reductase
- Mutases: Aldehyde mutase and Glyoxalase
- Other Enzymes: Phosphorylase, Phosphohexisomerase, Hexokinase and Phosphoglucomutase

Amino Acids

- Alanine
- Aginine
- Asphartic Acid
- Cystine
- Glutamic Acid
- Glycine
- Histidine
- Isoleucine
- Lysine
- Methionine
- Phenylalanine
- Proline
- Serine
- Threone
- Tryptophan
3.3.2 Switch™

**Product content and characteristics as described by manufacturer:**

Switch™ contains all of the afore-mentioned ingredients of Cellfood® with two added substances. The first substance being Citrin K and the second being L-Carnitine. Citrin-K is a herbal extract from the Garcinia Cambogia fruit plant and contributes 25% to the total Switch™ make-up. L-Carnitine is a nutrient produced naturally in the liver and contributes 20% to the total Switch™ make-up.
3.3.3 Placebo

The placebo comprised a solution with a similar appearance, smell and lemon-flavouring corresponding to the organoleptic (taste) characteristics of both Cellfood® and Switch™.

3.4 DEPENDENT VARIABLES

The following dependent variables were measured. The subjects were not permitted strenuous training the day before each test.

1. Anthropometry
   - Stature
   - Body mass

2. Haematology
   - Ferretin values
   - Haemoglobin
   - Red blood cell count
3. Hematocrit
4. Fasting glucose
3. Pulse oximetry
4. Rate of perceived exertion
5. Heart rate
6. Capillary blood lactate concentrations
7. Oxygen utilization and related spirometry

3.4.1 Anthropometric Measurements

Stature
The stature was measured with a calibrated stadiometer. The subject stood barefoot, feet together with the heels, buttocks and upper part of the back touching the gauge, with the head in the Frankfort plane, not necessarily touching the gauge. The Frankfort plane was considered as the orbital (lower edge of the eye socket) being in the same horizontal plane as the tragion (notch superior to the tragus of the ear). When so aligned the vertex was the highest point on the skull. The measurement was taken to the nearest 0.1 cm at the end of a deep inhalation (Lohman et al., 1988).

Body Mass
Body mass was measured using a Detecto beam balance scale. The measurement was taken to the nearest 0.1 kg, with the subject barefoot, clothed only in appropriate running clothes, and taking care that the:

- Scale was reading zero;
- Subject stood on the centre of the scale without support;
- Subject’s weight distribution was even on both feet; and
- Subject’s head was held up and the eyes looked directly ahead (Lohman et al., 1988).
3.4.2 **Haematology**

The blood analyses were performed by a professional pathology laboratory, namely AMPATH (a division of Du Buisson and Partners pathologists).

The following reference ranges were utilized:

- **Ferretin**: 22 - 322 ng/mL
- **Haemoglobin**: 14.0 - 18.0 g/dL
- **Red blood cell count**: 4.60 - 6.00 $\times 10^{12}$/L
- **Hematocrit**: 42 - 52%
- **Fasting glucose**: 3.5 - 6.0 mmol/L

3.4.3 **Oxygen Utilization**

The maximum oxygen uptake ($V_{O_2}$ max) was determined through direct (open) circuit spirometry, using a Schiller CS-100 gas analyser and a Quinton motorized treadmill (model 24-72). The gas analyser was calibrated before each test with the appropriate gas mixtures supplied by Air Products. The tests were conducted within an air-conditioned laboratory at a temperature of 20°C and barometric pressure of approximately 655 mmHg. The treadmill protocol started at a running speed of 8 km/h and the elevation remained constant at 2% throughout the test. The speed was increased every two minutes (with 2km/h)
until a running speed of 16 km/h was reached. After this point, the treadmill speed was increased by 1 km/h every two minutes, until exhaustion. The athletes were verbally encouraged and the tests were terminated when the athletes could not maintain the running speed. The following criteria were utilized to determine whether a subject had reached their VO$_2$ max:

- respiratory exchange ratio of at least 1.1;
- plateau or decrease in maximal oxygen uptake;
- severe exhaustion; and
- not being able to maintain the running speed despite verbal encouragement (MacDougal et al., 1991).

Gas values were sampled every ten seconds. The following gas analysis values were recorded during the VO$_2$ max test, presented in their abbreviated and defined format as defined by the Schiller CS 200 User Manual (CS-200 User guide, 2000):

- METS: Metabolic Equivalents. Oxygen uptake required for a given task expressed as multiples of resting oxygen uptake.
- RR: Respiration rate. Number of breaths per minute
- VT: Tidal volume. The volume of air actually breathed per breath in ml.
- VE: Minute ventilation. The volume of air taken into or exhaled from the body in one minute. This is conventionally expressed at body temperature, saturated with water at atmospheric pressure (BTPS).
- VO$_2$: Oxygen consumption. The amount of oxygen extracted from the inspired gas in a given period of time, expressed in millilitres or litres per minute, standard pressure and temperature, dry (STPD). This can differ from oxygen consumption under conditions in which oxygen is flowing into or being utilized from the body’s stores. In the steady-state, oxygen uptake equals oxygen consumption.
- VO$_2$ relative: VO$_2$ expressed in ml/kg/min.
- VC0$_2$: The amount of carbon dioxide (C0$_2$) exhaled from the body into the atmosphere per unit time, expressed in millilitres or litres per minute, STPD. This differs from C0$_2$ production rate under conditions in which
additional CO₂ may be evolved from the body stores or CO₂ is added to the body stores. In steady state, CO₂ output equals CO₂ production rate. In rare circumstances, appreciable quantities of CO₂ can be eliminated from the body as bicarbonate via the gastro-intestinal tract or by haemodialysis.

- Respiratory Exchange Ratio (RER): The ratio of carbon dioxide production to oxygen consumption. This ratio reflects the metabolic exchange of gasses in the bodies’ tissue and is dictated by substrate utilization.

- VE/V0₂: Respiration equivalent for oxygen. This is the actual ventilation against absolute oxygen uptake. This parameter indicates how much air (l) must be inhaled to obtain a litre of oxygen.

- VE/VC0₂: Respiration equivalent for carbon dioxide. This is the actual ventilation against absolute carbon dioxide exhaled. This parameter indicates how much air (l) must be exhaled for one litre of carbon dioxide to be expelled. The smaller this parameter the better the carbon dioxide exchange efficiency.

- etO₂: End tidal expired oxygen partial pressure (mmHg). This is the partial oxygen pressure (P0₂) determined in the respired gas at the end of an exhalation. This is typically the lowest P0₂ determined during the alveolar portion of the exhalation.

- etCO₂: End tidal expired carbon dioxide partial pressure (mmHg). This is the partial carbon dioxide pressure (PC0₂) of the respired gas determined at the end of an exhalation. This is commonly the highest PC0₂ measured during the alveolar phase of exhalation.
Figure 3.4: Athlete connected to gas analyser and performing a test.

3.4.4 Pulse Oximetry

Incremental haemoglobin oxygen saturation levels were measured using a Datex-Ohmeda TuftSat hand-held pulse oximeter. The measurements were taken using a finger probe (ClipTip sensor). These measurements were taken at the end of each two-minute stage directly after the blood samples were taken, expressed as a percentage.
3.4.5 Capillary Blood Lactate Concentration

Incremental capillary blood lactate measurements were taken during the treadmill test by using an Accurex BM lactate meter (Roche diagnostics). This required a puncture of the fingertip to obtain a peripheral blood sample. These samples were taken at the end of each two-minute stage during the treadmill test. The values were reported in mmol/l.
3.4.6 Rate of Perceived Exertion

The original Borg scale (6-20) was used to determine the rate of perceived exertion (RPE) for each subject (Borg, 1973). Subjects were asked to indicate their perceived level of exertion on the scale at the end of each two-minute stage during the treadmill run.

3.4.7 Heart Rate

Heart rates were recorded using a Polar Accurex Plus heart rate monitor. Heart rates were recorded continuously during the entire test.
3.5 STATISTICAL ANALYSIS

An independent statistician was consulted to perform all the statistical analyses. Alpha was set at a minimum of $p<0.05$ to indicate significant differences between sets of data. The Kruskall-Wallis method of statistical analyses was used for the analysis of the data. This method is the non-parametric equivalent of a one-way analysis of variance (ANOVA) (Howel, 1992).

Computations to determine standard descriptive statistics (mean and standard deviation) and the non-parametric analysis (Kruskall - Wallis test) were performed using the Statistical Package for Social Science (SPSS), Microsoft Windows release 9.0 (1999).