APPLICATION AND DEVELOPMENT OF INDIRECT MEASURES OF FREE-LIVING ENERGY EXPENDITURE

by

LARA KEYTEL

This thesis is Presented for the Degree of

DOCTOR OF PHILOSOPHY

In the Department of Human Biology

University of Cape Town

SOUTH AFRICA

MARCH 2004

MRC/UCT Exercise Science and Sports Medicine Research Unit

Sports Science Institute of South Africa

Boundary Road

Newlands

7700

South Africa
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This thesis is dedicated to my grandparents, Smittie, Masha, Vincent and Ruth, my biggest fans.
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ACKNOWLEDGMENTS

I wish to acknowledge and express my sincerest thanks and gratitude to the following people for their support and assistance during the compilation of this thesis:

Vicki Lambert, my supervisor, life-mentor and friend. Your guidance as well as your unfaltering belief in my work and me, has meant the world to me and I look forward to many more successful years together.

Tim Noakes, the “father” of our department. Your excitement, enthusiasm and passion, are inspirational and I feel honoured to be a playing on the same team.

Paul Schoffelen, my “silent” supervisor. Words are not really enough to express my sincerest gratitude and thanks, without your patience, guidance, support and friendship, I would not be in the position I am today and I am eternally grateful.

Jonathan Dugas, my best friend and fiancé, you are my rock and you give me wings to fly. Without you, my world has no balance.

My parents, Ron and Anna, for giving me the belief and confidence to go for what it is I really want and not only because it may please someone else, and my brothers, Bruce and Alistair, we have come a long road together, and each time we are in each others company it only gets more satisfying.

Lize Van Der Merwe, Statistician par excellence. Your statistical assistance was fundamental to the development of much of this thesis, and I am grateful for your
willingness to learn about physiological models and further your acceptance of me and your absolute selflessness in putting together many of the models in this thesis.

**Willem Krige**, for your enthusiasm and boundless energy for going the extra mile, taking the time to get Labview exactly correct and as a result taking both of us where we have never gone before.

**The University of Maastricht** and Department of Human Biology, more specifically **Professor Klaas Westerterp** and **Loek Wouters**, who willingly came aboard a project that was out of this World, and went out of their way to make a success of both the doubly labelled water protocol and isotopic analysis.

**Raija Laukkanen** and **Polar**, Electro, Oy, Kempele Finland, for their sponsorship and support of this thesis.

**Winning Wellness BODYiQ** and **Travis Noakes**, for their vision, support and sponsorship of much this thesis.

**Mark Shuttleworth** and the **First African in Space** project. To live your dream is truly a great thing; to live your dream and give back so much at the same time is even greater.

**The Medical Research Council of South Africa** and **The University of Cape Town**, for their financial assistance during the compilation of this thesis.

**Nellie Atkinson** and **Harry Crossley Staff Research Funds** of the University of Cape Town for their financial assistance during this thesis.
Both the staff, particularly to Mike Lambert and Malcolm Collins, and administrative staff, particularly Lesa Sivewright, who have always made my life a lot easier and always available to bounce ideas around.

The University of Cape Town Department of Nursing, particularly Rosalie Thompson, Pat Mayers, Renee Hill and Minette Coetzee, for their unflagging support, encouragement and belief in me.

Gayle McArthur, and Sherri and Jack Dugas, who are my “family” away from my family. Your friendship, love and on-going support, reminds me what is important in life, keeps me laughing and my head above water.

Lisa, Julia, Jojo, Candice and friends, both at work and at play, who have lent me so much support over the last couple of years and kept me laughing.

All the subjects, who so willingly and graciously donated their precious time.

Finally, the examiners, for whom I am grateful for their time and commitment to furthering our discipline.
DECLARATION

I, Lara Ruth Keytel, do hereby declare that the experiments presented in this thesis were conceived and executed by myself except where otherwise stated.

Neither the substance nor any part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree in the University or any other University.

This thesis is presented in fulfilment of the requirements for the degree of PhD.

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Signed: ____________________________

Date: ______________________________
LIST OF PUBLICATIONS

PEER REVIEWED PUBLICATIONS RESULTING FROM THIS DISSERTATION.


PUBLISHED ABSTRACTS RESULTING FROM THIS DISSERTATION.


PROFESSIONAL PRESENTATIONS RESULTING FROM THIS DISSERTATION.


ABSTRACT

The aims of this thesis were to explore the accuracy in measuring free-living total daily energy expenditure (TDEE), by examining existing indirect measures of energy expenditure (EE) measurement and further, developing new techniques, for improved accuracy and application, in population-based studies. In a number of the studies, the research focus is the heart rate (HR) monitoring technique, for TDEE estimation as a result of its low cost and ease of implementation in large population-based studies.

This thesis represents a progression from the application of the HR monitoring technique for estimating EE in response to training, or as a means to validate a physical activity recall instrument. However, what is highlighted are the limitations of the existing methodology for estimated TDEE in this way. Therefore, this thesis introduces a novel concept in the HR monitoring technique, incorporating group-based EE equations, and further, by including the effects of the previous minutes HR response on the estimation of EE from HR. Finally, this thesis validates these modifications, using a respiration chamber, purpose-built as a part of this dissertation. It should be noted, however, that in some instances, the thesis was constrained by opportunistic sampling, or the fact that in the case of Chapter 4, the study sample was part of a larger study designed for another purpose. Nevertheless, the outcomes of this research, in particular, the group-based HR-EE prediction equations, have important implications for large population-based epidemiological research concerning physical activity dose-response.

In the first part of this thesis, we explore free-living TDEE estimation, using an existing HR monitoring technique, reliant on individual subject calibration, in a sample of 19 sedentary women, before and after an 8-week exercise-training program. This study lays the
foundation for this thesis, as it explores the HR monitoring technique’s ability to track free-living EE measurement in the presence of change, characterised in this study by fitness. Based on previous research, introducing the concept of the individual HR-EE calibration test, subjects were required to undergo an individual HR-EE calibration test, prior to EE estimation. The TDEE, estimated sleep EE, resting metabolic rate (RMR), resting respiratory exchange ratio (RER) and the sum of the 24hr heart beats (HR) was measured during pre and post testing, in the exercise group (EX, N=9) and a control group (CON, N=10).

Mean submaximal HR during steady-state exercise in EX was lower after training compared to CON (P<0.05). However, neither VO2max, or estimated TDEE, RMR, total energy intake nor total HB changed over 8 weeks, in either group. Day-to-day variability in estimated EE in non-exercising CON subjects (N=10) was determined on the two non-exercising days. Total HR over 24hr correlated reasonably well between the two control days (r=0.71, P<0.02) and the coefficient of variation was 6.4%. However, when this was converted into TDEE using the individual regression equations, there was a great deal of variability between the two control days (r=0.31, P<0.383) and the coefficient of variation was 23.1% for the measurements.

It was concluded that in this population of previously sedentary postmenopausal women, exercise training was not associated with any increases in TDEE, however, it could not be excluded that this failure to demonstrate any increase in TDEE may also be due, to the large degree, to the intra-individual variability in the HR monitoring technique for estimating TDEE.

In the second study, we used the HR monitoring technique as a means to validate the 7-day physical activity recall instrument. In this chapter, we employed individual subject
calibration for the HR-EE technique, and compared the results measured over 1 working day, to reported levels of activity, averaged over 5 working days using a modified version of the 7-day Physical Activity Recall questionnaire (Stanford 5-City project questionnaire). Hospital employees (N=59), ranging from unskilled labourers to skilled medical professionals, were recruited. Subjects completed the questionnaire for 5 working days (5-day recall), and then proceeded to have their TDEE measured, using the individual subject HR-EE calibration technique, and 24hr HR monitoring. In this study, it was found that TDEE, estimated using the HR monitoring technique, was positively correlated to TDEE (r=0.36, P<0.0003) and physical activity energy expenditure (PAEE), measured using the 5-day recall (r=0.34, P<0.002). However, in general, EE using the 5-day recall was significantly over-reported (P<0.001). The over-reporting could not be explained by differences in gender, level of education, or occupation. From this study we concluded that the HR monitoring technique provided a reasonable tool to validate a physical activity questionnaire, in a sample of hospital employees, however we were unable to conclude whether differences between the two techniques were as a result of inaccuracies solely attributed to the physical activity recall method and not the HR monitoring method. Finally, the need for group-generated EE equations was identified, as the current technique of individually calibrating subjects was time consuming. Future research should therefore explore group-generated EE equations, for the use during HR monitoring measurement, therefore doing away with the need for individual subject calibration.

In the third chapter of this dissertation, we attempted to develop these group-based HR-EE equations, by incorporating factors that may also modulate this relationship, such as: mode of exercise, body composition and training. We recruited 115 active subjects, who completed three, steady-state exercise stages on either the treadmill (10-min) or the cycle ergometer (15-min) at 35%, 62% and 80% of VO$_2$max, corresponding to 57%, 77% and
90% of maximal HR. HR and respiratory exchange ratio data were collected during each stage. We then used a mixed model analysis to identify the factors that best predicted the relationship between HR and PAEE. Factors that significantly correlated to PAEE were HR, body weight, gender, VO₂max and age. These significant variables were then included in the final mixed model. The correlation coefficient between the measured PAEE and the estimated PAEE was $r=0.913$. The model therefore yielded an $R^2$ of 0.83. Further, in an independent validation sample of subjects, completing a 20-minute gym training session, the mixed model accounted for 60% of the variance in measured EE. Based on these results we concluded that it is possible to estimate PAEE from HR, using group-generated EE equations, with good accuracy after adjusting for gender, body weight and fitness levels, during submaximal exercise.

In the fourth study of this dissertation, we further developed the model, taking into consideration, the likely effects of cardiovascular drift as the duration of exercise increases, as well as the temporal dissociation between heart rate and oxygen consumption during intermittent activity. For this study, we recruited 65 regularly exercising subjects, who underwent individual HR-EE calibration, using an intermittent protocol, which included twelve 4-min workloads, separated by four 1-min rest periods; HR and VO₂ were continuously monitored. Age, gender, VO₂max, current minute’s HR, preceding minute HR and an interaction variable consisting of previous minute HR and VO₂max all contributed significantly to the final mixed model, which yielded an $R^2$ of 0.81. In addition, in a separate sample (N=17), completing a 55-minute gym training session, comprising both cardiovascular and toning activities, the final mixed model accounted for 76% of the variance between measured and estimated EE. We therefore concluded that HR in the preceding minute improves the accuracy of predicting EE from HR and other determinants during intermittent exercise, which is more reflective of daily activity. Including HR during
preceding workload or recovery periods, and an interaction variable consisting of previous minute HR and VO_{2\text{max}}, may provide additional accuracy for EE prediction.

While we are confident that we can accurately account for EE during physical activity, as seen in the previous study, it has been the primary aim of this thesis to develop accurate measures of 24 hr free-living EE. Therefore our fifth study explored the accuracy of the novel intermittent mixed model (developed in Chapter 5) and the conventional continuous mixed model (developed in Chapter 4) EE equations, used in combination with estimated resting metabolic rate, in the estimation of 24 hr free-living EE. The EE estimates were compared to two existing measures of TDEE, the respiration chamber, and the motion sensing accelerometer. One of the main objectives at the onset of this thesis was to put into operation a respiration chamber specifically for the use of TDEE measurement. Following the construction and validation of the respiration chamber, a sample of 5 regularly exercising male subjects were recruited. Prior to the entry into the respiration chamber, subjects were fitted with a telemetric HR monitor and 3-dimensional accelerometer. For the next 23 hours, subjects followed a fixed activity protocol, which included periods of rest, physical activity (one cycling bout and one stepping bout) and participated in the normal activities of daily living. After excluding data during sleeping time and missing data, EE data from between 12-15 hours, for each subject, were obtained and used in the final analysis. EE estimated using the novel intermittent and conventional continuous EE equations yielded \( R^2 \) values of 0.85 and 0.80, for the novel and intermittent equations respectively, while EE estimated from the 3-dimensional accelerometer accounted for only 62% of the variance in measured EE, using the respiration chamber.

EE estimated using the HR-EE equations was not different from measured EE, during physical activity, or sedentary activity. Further the novel intermittent equation provided
increased accuracy during the physical activity bouts, compared to the conventional continuous equation. To our knowledge, these results represent the first validation study of group-based equations for predicting energy expenditure using a respiration chamber. Furthermore, we believe that the novel, intermittent equation may be more suitable for estimating energy expenditure during activities, which are stochastic in nature, and more similar to activities of daily living.

We believe these equations represent an easily administrable method for validating both existing and new methods for EE estimation in the field. While whole room calorimetry is undeniably the gold standard in metabolic measurement, its uses are limited to single subject evaluation, and further remove the subject from their natural environment. EE estimated using HR-EE equations, on the other hand, keeps the subject in their natural environment, while still obtaining accurate estimates of TDEE.

In the addendum to this dissertation, we report on a unique case study, in which heart rate monitoring was used to estimate energy expenditure under quite novel conditions. As part of the First African in Space project, we participated in a case study, of a single cosmonaut, and estimated the EE of this individual completing a 10-day space mission, on board the International Space Station (ISS). This was compared to EE measured using the doubly labelled water technique (DLW). The study was completed in two parts, an Earth-based (Part 1) and In-flight (Part 2) component, both Part 1 and Part 2 were 10 days in length. During Part 1 and 2, the subject consumed a pre-weighted dose of the doubly labelled water, and proceeded to collect saliva samples, on days 0, 1, 4, 7 & 10 the protocol. The subject underwent HR-EE calibration during Part 1, and this individual HR-EE equation estimate of EE, as well as the group-based equation estimate of EE were compared to the doubly labelled water, on land, and in the ISS. In addition, the subject was required to
complete daily food records, to estimate energy intake. During Part 1, TDEE did not differ between the DLW and EE estimated using the novel equation. However, EE estimated using the individual calibration was different to the DLW technique. In Part 2, both the HR monitoring techniques were different to the DLW measurement. We concluded that this was likely the response to the well-documented cardiovascular adaptations that take place in a microgravity environment. This case study therefore serves as evidence for the application of the HR monitoring technique, for EE estimation, in healthy populations only, and not those groups experiencing any form of cardiovascular disease, metabolic abnormalities or currently using medications that either alter HR or have any effects on the autonomic nervous system.

In summary, this thesis has demonstrated that free-living EE estimation may be accurately measured in large groups using modifications of existing HR monitoring techniques. The accurate execution of this technique is reliant on developing EE estimation equations, which incorporate other explanatory variables into regression equations that are likely to impact upon the HR-EE relationship. Further, we have shown that it is important to consider the nature of the day-to-day variability of the activities of daily living, by incorporating an intermittent calibration protocol. Finally, this thesis demonstrates that EE estimation need not be time consuming and laborious, or place a high degree of strain on the investigation team, to obtain accurate estimate for population-based research, in communities with limited resources and funds.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BMI</td>
<td>Body mass index calculated as weight (kg) divided by (height)$^2$</td>
</tr>
<tr>
<td>BMR</td>
<td>Basal metabolic rate</td>
</tr>
<tr>
<td>Bpm</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>Cm</td>
<td>Centimetres</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DLW</td>
<td>Doubly labelled water</td>
</tr>
<tr>
<td>EE</td>
<td>Energy expenditure, measured in kJ.min$^{-1}$</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>G</td>
<td>Grams, unit of weight</td>
</tr>
<tr>
<td>HR</td>
<td>Heart rate, measured in beats per minute</td>
</tr>
<tr>
<td>HR-EE</td>
<td>Heart rate-energy expenditure</td>
</tr>
<tr>
<td>IPAQ</td>
<td>International physical activity questionnaire</td>
</tr>
<tr>
<td>ISS</td>
<td>International Space Station</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilograms</td>
</tr>
<tr>
<td>kJ.min$^{-1}$</td>
<td>Unit of energy expenditure measurement</td>
</tr>
<tr>
<td>Km</td>
<td>Kilometres</td>
</tr>
<tr>
<td>MET</td>
<td>Unit of EE measurement and represents a metabolic equivalent</td>
</tr>
<tr>
<td>Min</td>
<td>Minutes</td>
</tr>
<tr>
<td>Min.day$^{-1}$</td>
<td>Minutes per day</td>
</tr>
<tr>
<td>N$_2$</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>PAQ</td>
<td>Physical activity questionnaire</td>
</tr>
<tr>
<td>PAEE</td>
<td>Physical activity energy expenditure</td>
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<tr>
<td>PAL</td>
<td>Physical activity level</td>
</tr>
<tr>
<td>PAR</td>
<td>Physical activity recall</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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<tr>
<td>PARQ</td>
<td>Physical activity recall questionnaire</td>
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<tr>
<td>PPO</td>
<td>Peak power output</td>
</tr>
<tr>
<td>PTRS</td>
<td>Peak treadmill running speed</td>
</tr>
<tr>
<td>RER</td>
<td>Respiratory exchange ratio</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>RQ</td>
<td>Respiratory quotient</td>
</tr>
<tr>
<td>S</td>
<td>Seconds</td>
</tr>
<tr>
<td>SMR</td>
<td>Sleep metabolic rate</td>
</tr>
<tr>
<td>TDEE</td>
<td>Total daily energy expenditure</td>
</tr>
<tr>
<td>TEE</td>
<td>Thermic effect of exercise</td>
</tr>
<tr>
<td>TEF</td>
<td>Thermic effect of food</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Oxygen consumption (expressed as either l.min&lt;sup&gt;-1&lt;/sup&gt; or ml.kg&lt;sup&gt;-1&lt;/sup&gt;.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>VCO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Carbon dioxide production</td>
</tr>
<tr>
<td>VO&lt;sub&gt;2&lt;/sub&gt;max</td>
<td>Maximal oxygen consumption (expressed as either L.min&lt;sup&gt;-1&lt;/sup&gt; or ml.kg&lt;sup&gt;-1&lt;/sup&gt;.min&lt;sup&gt;-1&lt;/sup&gt;)</td>
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<tr>
<td>W</td>
<td>Watts</td>
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<td>Yrs</td>
<td>Years</td>
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5-day recall  modified Stanford 7 day physical activity recall questionnaire, for 5 days

7-day recall  Stanford seven day physical activity recall questionnaire
CHAPTER ONE

LITERATURE REVIEW
1.1. INTRODUCTION

In recent years there has been much emphasis placed on the accurate measurement of physical activity energy expenditure (PAEE). A large part of this interest is almost certainly as a result of documents such as the US Surgeon General's report on physical activity and health (2), which states that individuals who are moderately active have lower mortality rates than individuals with sedentary habits. In the United States of America, data from the 2000 National Health Interview Survey (3), indicated that over 74% of women and 64% of men are currently inactive. Further, the relationship between physical activity and all-cause mortality is widely recognised (63;66;96;123;161). A review by Samitz (123) observed that individuals who are fit (either moderately or very fit) experience mortality rates that vary from one-third to one-half of those individuals less fit or active. In addition, Samitz concluded that cardiorespiratory fitness offered some form of protection against other predictors of mortality and that the relationship between fitness and mortality could be described by a dose-response curve, i.e. the greatest benefit may be attained by those who are the least active. Morss et al. (96) recently reported on the dose-response to exercise in women (DREW project) and health promotion. It was concluded that total energy expenditure is the most important component of an exercise program. Through the exploration of the dose-response relationship and by identifying the key factors influencing this relationship, including: age, and lifestyle risk factors, as well as, exercise frequency, duration and intensity, health-related outcomes, such as cardiovascular disease, may be improved.

In a recent meta-analysis by Wilmore (161) it was concluded that those most benefiting from physical activity are individuals who appear to be less healthy than their regularly exercising counterparts, with respect to changes in blood pressure, lipids and lipoproteins.
Further, it was concluded that men benefit more from activity, as a result of typically having a higher risk profile, including: higher blood pressures and total triglycerides, compared to women. However, this was not the case with regards to age, where there were no differences in response to training, even in light of unfavourable profiles associated with ageing. Consequently, there is much debate, and more recently investigation, into the issues of “how much exercise is enough” and further, a re-examination of the dose-response relationship with respect to physical activity. Specifically, this debate revolves around identifying how much physical activity is enough for the attainment and maintenance of good health, and to minimise the likelihood of risk for non-communicable diseases, such as diabetes and cardiovascular disease.

For example, Iwasaki et al. (63) completed a dose-response investigation in 11 sedentary subjects, over a one-year period. Initially, subjects began training between 1.5 and 2 hours a week. By the end of the one-year period, the subjects were exercising between 7- and 9-hours a week and all had successfully completed either a 42.2km road race, a triathlon or 100km road cycle race. Results from this study showed that moderate amounts of physical activity training over an initial three-month period resulted in significant changes in blood pressure and heart rate regulation. Furthermore, it was noted that any additional training time did not enhance the benefits already experienced from the initial exercise training, and that the greatest response to training came from those completing moderate intensity exercise as opposed to either very high or low intensity exercise training. It was suggested that the dose-response relationship might therefore follow a bell- or "U"-shaped curve, with the maximum benefit gained from moderate amounts of training, as opposed to prolonged high intensity training bouts.
Westerterp (153) argues this case further, suggesting that those benefiting the most from physical activity are not those engaging in regular physical activity exercise training, moderate or otherwise, but that the benefits result more as a consequence of leading an active lifestyle. It was observed that individuals who included short spurts of vigorous activity, into an otherwise sedentary lifestyle, were worse off their healthier counterparts who appeared to have higher levels of habitual physical activity throughout their daily routines and activities of daily living. In another study, it has been demonstrated that low spontaneous physical activity was a risk factor for weight gain in men (166).

As a result, it becomes increasingly necessary for health professionals to have at their disposal accurate and reliable tools that enable them to complete population-specific testing, in an attempt to accurately quantify physical activity prescription for public health recommendations. For example, a study completed by Jakicic et al. (66) evaluated exercise adherence in a group of previously sedentary, overweight women, over an 18-month period. Participants were randomised into three groups. The first group comprised a long-bout exercise group, who at study completion were exercising 5 times a week for 40 min.day⁻¹. The second group was a short-bout exercise group, who at study completion were exercising 5 times a week, for 40 min.day⁻¹, separated into four 10-min bouts. Finally, the third group was also exercised in short-bouts, but in addition, they received motorized home treadmills. Participants in this study were monitored for changes in body composition (using dual-energy x-ray absorptiometry), cardiorespiratory fitness (using a submaximal graded cycling test), dietary intake (using a food frequency questionnaire), as well as, exercise adherence (using a physical activity questionnaire, a leisure time physical activity questionnaire and accelerometers).
Weight loss in the short-bout, home-based group was significantly greater (P<0.05) than in the short bout activity group, and they maintained a higher level of exercise (P<0.05) compared to those in either the short-bout or long-bout group. All groups showed increases in cardiorespiratory fitness, and there was no difference in weight loss experienced across groups. However, when weight loss was analysed as min.week⁻¹ of exercise, those participating in more than 200 min.week⁻¹, experienced the greatest weight loss. This study suggests that short-bout activity may be as beneficial as long-bout activity (40-min accumulated through 10-min exercise bouts versus 40-min of continuous exercise), but only in individuals completing home-based exercise programs. In addition, this study provides evidence for a dose-response relationship between amount of exercise per week (min) and long-term weight loss. Finally, this study highlights the need for validated tools, which measure accurately, habitual physical activity energy expenditure and its role in the dose-response relationship, so that population specific guidelines for physical activity may be implemented.

1.2. ENERGY EXPENDITURE MEASUREMENT

The measurement of energy expenditure, and specifically physical activity energy expenditure, is achieved through the use of either direct or indirect calorimetry. Calorimetry is essentially the measurement of heat production (90). All life processes use energy, of which our human energy source is food. Food is metabolised in the body into the energy forms from which a portion is required to sustain growth and life. The rest is given off as heat. Direct calorimetry is the process by which heat production is measured to determine energy expenditure, while indirect calorimetry is the measure of the gas exchange associated with the oxidation of energy substrates (90). Energy expenditure can be calculated based on the amount of oxygen consumed and carbon dioxide produced.
Historically, the first measurement of heat production from small laboratory animals was well over 200 hundred years ago. In these experiments, rodents were placed in closed circuit chambers, surrounded by a water jacket and the rise in the water temperature was documented (90). By the start of the twentieth century, good agreement between direct and indirect calorimetry (1) had been demonstrated, and attention was then focused primarily on the indirect method as a result of ease of testing, when compared to using direct methods.

**Figure 1.2.1.** Time line illustrating evolvement of calorimetry systems, data adapted from McLean et al. (90).
With these indirect calorimetry methods available, it has been possible to accurately quantify the different components of total daily energy expenditure (TDEE). Of the four major components comprising TDEE, physical activity energy expenditure (PAEE) is reported as being the most variable (157). The other three components include sleep metabolic rate (SMR), the energy cost of arousal and the thermic effect of food (TEF) (152). Basal metabolic rate (BMR), which incorporates the energy cost of arousal and SMR, is known to contribute to between 60–80 % of TDEE (18;110), while TEF accounts for approximately 10% of TDEE in individuals in energy balance who are ingesting a mixed diet (157). These four components are affected by a number of determinants, which either increase or decrease the expression of TDEE.

**Figure 1.2.2.** Components of 24hr free living total daily energy expenditure, data adapted from Bouten et al. unpublished thesis data (21).
1.3. ESTIMATING 24 HOUR TOTAL DAILY ENERGY EXPENDITURE AND THE DETERMINANTS THEREOF

1.3.1. Measurements using the respiration chamber

Probably the most useful tool for investigating the determinants of 24hr TDEE in man and considered to be the "gold standard" of metabolic measurement is the human respiration chamber, (32;42;95;128). A volunteer enters an airtight room, and typically remains there for a minimum duration of 23 hours, up to a period of several days. A stream of fresh air is directed through the chamber, during which time, using first-order linear differential equations (26;30;128), the net flow and accumulation of gas is calculated to determine metabolic rate (95). Despite the confinement to the single room, subjects are able to carry on with the normal activities of daily living, in addition to completing some form of exercise, usually on a cycle ergometer placed within the chamber. The with-in subject coefficient of variation for 24hr TDEE measurement is approximately 1.5-5% (160), while the inter-subject variation ranges from as low as 2% (98) to approximately 11% (9). In addition, 80-90 % of the differences in TDEE between individuals may be explained by differences in lean body tissue (9;112) and fat mass (51). Further in a study by White et al. (160) 24hr energy expenditure, respiratory quotient and substrate oxidation, whether expressed as group or for individuals, were found to have good test-retest reproducibility. However, factors such as the pre-chamber diet, as well as familiarisation with the chamber, significantly impact on the day-to-day variability of these measures. In a study by De Boer et al. (41) a high reproducibility was also found over a 3-day measurement period, provided that the subjects remained weight-stable and followed a standardised activity protocol. In this study it was also found that reproducibility was improved following a 1-day familiarisation to the chamber.
In one of the earlier studies, characterising determinants of 24hr energy expenditure in man, Ravussin et al. (111) investigated both obese and normal weight individuals, following a fixed-activity physical activity protocol, in a respiration chamber. Overall obese subjects had a significantly higher resting metabolic rate, even after adjusting for fat free mass, however, substrate oxidation was not different between groups, and total energy expenditure, expressed relative to fat-free mass was also not different between groups. The single most important determinant of resting, and 24hr energy expenditure in this study was fat-free mass, accounting for 79 and 67% of the variance in resting metabolic rate and 24hr energy expenditure, respectively.

In a follow-up study of 177 subjects, Ravussin et al. (112) further explored the determinants of the variability of 24hr energy expenditure in man. The subjects were once again restricted to a fixed activity protocol and consumed 4 meals, constituting 80% of their habitual diet. Radar positioned in the chamber recorded any spontaneous physical activity. Initially data showed a coefficient of variation of 2.4% for 24hr energy expenditure, 3.7% for RMR and 4.7% for sleep metabolic rate during the respiration chamber stay. TEF was highly variable with a coefficient of variation of 43% in this study. Further, body weight, height and body surface area all correlated significantly with 24hr energy expenditure and accounted for 73-82% of the variance in 24hr energy expenditure. As previously found (38;40;111), fat free mass correlated significantly with 24hr energy expenditure, $R^2=0.81$. In addition this study confirmed that energy expenditure decreases during sleep, by approximately 10%, when compared to the basal metabolic rate and varied widely between subjects. Basal metabolic rate correlated significantly with fat free mass, $R^2=0.69$, while the TEF did not correlate to any of the subjects' morphological characteristics. Finally, in this study, the energy cost of physical activity energy expenditure was related to body weight
(R^2=0.46) and fat free mass (R^2=0.50) and the variation in physical activity energy expenditure accounted for large differences in 24hr energy expenditure. The variability in physical activity energy expenditure in this study was approximately 9% and ranged from 4-17%.

Astrup et al. (9) further investigated the impact of physical characteristics and body composition on 24hr energy expenditure. During this trial, 10 subjects were investigated during two 24hr chamber stays, separated by 4 weeks. Subjects consumed a standardised diet and completed two 15-minute exercise sessions over the 24hr period. Reproducibility data showed no significant differences between the duplicate measures, although 24hr energy expenditure varied widely from subject to subject. When 24hr energy expenditure was expressed relative to lean body mass, between-subject variation was significantly reduced. As previously found by Ravussin et al. (111), body weight, height and lean body mass all contributed significantly to 24hr energy expenditure. Lean body mass correlated significantly with sleep energy expenditure, R^2=0.92, with basal metabolic rate, R^2=0.91 and with 24hr energy expenditure, R^2=0.93. While this study reports one of the highest correlations for lean body mass and 24hr energy expenditure, it is suggested that this finding is primarily a result of the lean subject sample used. It is reported that in a study by Owen et al. (101), only 55–57% of the variance in TDEE could be accounted for by lean body mass, in a group of non-athletic women. This suggests that in the normal or overweight population, variables, other than lean body weight may become important in the prediction of 24hr TDEE.
1.3.2. Variability in 24hr respiration chamber measurement

Rumpler et al. (120) studied the repeatability of 24hr energy expenditure measurements. This study was separated into two parts, with part one comprised of 4 men, spending 3 overnight stays in a respiration chamber, separated by 1 day. Part two consisted of 5 men from a weight maintenance group, in which energy expenditure was measured following weight loss, 5 times over 12 weeks (0, 1, 4, 8 and 12 weeks). Subjects followed a fixed activity protocol while in the chamber, exercising at self-selected exercise intensities for three 30-minute exercise sessions in part one and two 30-minute exercise sessions in part two. The within-subject coefficient of variability was 2.7% for part one and 4.6% during part two. During part one the BMR accounted for 64% the total daily energy expenditure in subjects and the within subject coefficient of variation was 2.3%. The within subject variability in part two for BMR was 2.9%. Physical activity energy expenditure accounted for 15% of the total daily energy expenditure in part one and 6% during part two as a result of the reduced exercise session. The within-subject coefficient of variation during exercise was 2.3% for part one and 3.3% during part two. In this study, physical activity energy expenditure contributed between 2-19% of the TDEE, and the thermic effect of food and spontaneous physical activity accounted for the remaining 18-29%. Thus, physical activity energy expenditure is a significant source of variation in the measurement of 24hr TDEE.

A study by Anderson et al. (7) was designed to assess the stability of physical activity energy expenditure measurement over an extended period of time. This work investigated the 24hr energy expenditure variation in a group of 4 subjects, over 10 months. This study found that much of the variation in the 24hr energy expenditure measurement over this time was independent of changes in body composition. Further, environmental or seasonal changes had no impact on the variation in metabolic rate. In this study, the subjects...
followed a fixed activity physical activity protocol in a direct respiration chamber and were measured twice (before and after) a ten-month period. Menstrual cycle was controlled for in the women. The 24hr energy expenditure coefficient of variation was between 2.5-3.4% and changes in the fat mass and fat free mass had no influence over the results. The intra-subject variation for sleep energy expenditure, thermic effect of food, and physical activity energy expenditure was 5.3, 7.6 and 8.3% respectively. During this study the largest source of variation came from the two cycling bouts, of 75W for 30-minutes.

This corroborates previous findings concerning physical activity energy expenditure, specifically, that physical activity energy expenditure accounts for a large proportion of the day-to-day variability in TDEE, contributing, on average, between 5 and 15% to the 24hr TDEE. However, in all of these studies, individuals were removed from their free-living environment and placed in a confined room. Therefore, it is not likely that physical activity energy expenditure in a respiration chamber is representative of that experienced day-to-day. Westerterp et al. (154) recently compared physical activity energy expenditure, measured in a respiration chamber, with that measured by using doubly labelled water. Subjects (N=45) completed an overnight sedentary stay in a respiration chamber, and then proceeded to complete a two-week study of free-living energy expenditure, using doubly labelled water. The subjects were restricted to low-intensity activities of daily living, during the two-week investigation period. The first finding was that physical activity energy expenditure measured inside the chamber was only 47% of the value outside of the chamber, however the two were significantly correlated. And secondly, the physical activity energy expenditure measured in the chamber explained only 25% of the variance in free-living physical activity energy expenditure. This study has important implications for the utility of the human respiration chamber in quantifying TDEE, especially for measurement under sedentary conditions.
In a recent study, Ribeyre et al. (115) found that individuals who were athletic had higher physical activity energy expenditure than those who were not athletic. Adolescents (N=49) were separated into one of four groups, according to gender, and athletic status. Subjects spent 36hrs in a respiration chamber, during which time they followed a fixed activity protocol consisting of 2 exercise periods. They were given four meals and required to wear heart rate monitors for continuous heart rate measurement. TDEE was significantly higher in the boys compared to the girls. On further examination it was found the TDEE was significantly greater in the athletic subjects, for both boys and girls, when compared to those who were not athletic. These differences remained, even after adjusting for fat free mass. The energy expenditure related to sedentary activities was increased in the athletic groups. This study is important as it suggests that while lean body mass has an important role to play in TDEE, habitual physical activity or spontaneous physical activity is an important determinant of TDEE and therefore corroborates recent work by Westerterp (154).

One of the challenges in using whole room respirometry is that of achieving energy balance. De Jonge et al. (42) have developed algorithms to predict 24hr TDEE at both high and low levels of physical activity, within 3 and 7 hours of beginning the 24hr trial. The algorithm assumes TDEE in the chamber to be 15% less than that measured during free-living conditions. Further, researchers use either a high (1.8 x RMR) or low (1.4 x RMR) activity protocol. Using the TDEE prediction algorithm, investigators have been able to predict final total 24hr energy expenditure value at 3- and 7-hours into the respiration chamber stay. During both days in the respiration chamber, the final measured 24hr energy expenditure was not significantly different to the predicted 24hr energy expenditure at 3 (96% of final measured TDEE value) and 7 hours (97% of final measured TDEE value). Further, the 24hr
energy expenditure was not significantly different between the two measurement periods. The within-coefficient of variation for the 24hr energy expenditure measurement was 5.2%, while the inter-individual variation was 12.6%. It was however, concluded that while 3 and 7-hour measurements are preferable to 24hr measurement; caution should be taken as a result of the increased inter-individual variation in the measurement technique.

Another area that may provide unwanted variation in 24hr TDEE measurement is the inconsistent practice of familiarisation with the human respiration chamber. Although studies (9;37) have now suggested that a one-day familiarisation reduces the intra-individual differences. Another source of variation in TDEE is the timing of the overnight stay in the respiration chamber with respect to the menstrual cycle. De Boer et al. (41) studied 10 women, each of whom completed a 4-day, 3-night stay in a respiration chamber, while consuming an experimental diet and following a fixed activity protocol that included five 15-minute cycling sessions. The trial was repeated on a second occasion, separated by between 2-24 months. Investigators found that the with-in subject variation in energy expenditure measurement for first chamber stay was 2.6% and for the second stay 1.8%. The coefficient of variation for the trial was 3.1%, however when subjects that were not familiarised with the chamber were excluded from the analysis the coefficient of variation was 1.9%. In another study, Bisdee and James (13) reported a 6% increase in the sleep metabolic rate during the premenstrual stage when compared to the pre-ovulatory stage. Therefore, it may important to control for the phase of the menstrual cycle during metabolic studies.

In human respiration chamber studies, the day-to-day variability with regards to TDEE is actually quite small and the largest factor impacting upon the measurement and quantification of individual TDEE is related to accurately quantifying energy expenditure
associated with physical activity. Therefore, in order to accurately quantify TDEE, it is necessary to understand the nature of free-living physical activity energy expenditure, and how it impacts upon TDEE. As a result, there have been a number of tools developed to take the subject out of the confined spaces of the laboratory or respiration chamber, and allow them to be measured in the true free-living environment. These tools range from the most basic of technologies such as physical activity questionnaires (4-6;36;117;149) to more complex tools such as motion sensors including accelerometers (23;92;151;155), pedometers (124;147) and global positioning systems (131), to heart rate monitors (29;47;62;77;81;114;136) and the doubly labelled water method (125-127;155;158;159). As a result, the individual being monitored is “freed” from the confines of the human respirometry chamber, and measured in their natural environment. Of course it is necessary to validate each of these tools accurately with respect to physical activity measurement and here the respiration chamber has proved invaluable.

1.3.3. Measuring Total daily energy expenditure, using the doubly labelled water method

One of the most accurate tools for determining free-living TDEE is the doubly labelled water method (DLW). This stable non-invasive isotope method, introduced for TDEE measurement over 20 years ago, has been extensively validated in humans using whole room respirometry (34;126;127) and produces results that are within 2% of measured energy expenditure. A dose of the stable isotope $^2\text{H}_2^{18}\text{O}$ is given to a volunteer, and over a period from several days up to several weeks, the $^2\text{H}$ is eliminated from the body as water and the $^{18}\text{O}$ is eliminated as water and CO$_2$. The difference in the rates of $^2\text{H}$ and $^{18}\text{O}$ elimination is proportional to the production of CO$_2$ and consequently allows for the estimation of TDEE. In one study Schoeller et al. (127) compared 5 days of DLW
measurement to respiratory gas exchange measurement, using a portable system of indirect calorimetry. The DLW method had a coefficient of variation of 8%.

Schoeller et al. (125) completed a study specifically looking at the main sources of variation when using the DLW method to measure TDEE. In this study, measurements of the post-absorptive resting metabolic rate and the thermic effect of food were also included. Women (N=6) participated in this trial and were tested before and after a 6-month interval. Schoeller et al. found that the DLW technique did not detect any seasonal variation in energy expenditure in the women. Further, the within-subject variation was 8%, with 6% of this being attributed to physiological variation. Similar to the respiration chamber, the largest source of variation was from the estimation of physical activity energy expenditure during the TDEE measurement.

A study by Westerterp et al. (156) measured energy expenditure using DLW compared to respirometry during both low and high levels of physical activity. Five subjects were tested on 6 days, during which time they were instructed to complete mainly sedentary activities, such as deskwork. The average TDEE was $1.4 \pm 0.1$ times that of their sleeping rate. A further 4 subjects were then measured twice over 3.5 days, during which time they were instructed to cycle on 2 of the days, at a heavy workload. The addition of the cycling workloads to the days activities, elicited TDEE $2.6 \pm 0.3$ times that of sleeping metabolic rate. The DLW energy expenditure measured during the low activity protocol was, on average, $1.4 \pm 3.9\%$ larger than the energy expenditure measured using the whole room respirometry chamber, while the DLW energy expenditure measured during the high activity protocol was on average $1.0 \pm 7.0\%$ lower than that obtained during the whole room respirometry measurements. The differences between the two techniques were relatively
small and provide evidence for the applicability of the technique in accounting for differences in TDEE.

While it is widely accepted that the DLW method presents an accurate method for measurement of free-living TDEE, it is costly. Therefore, the utility of the DLW is that it may be used to validate other “free-living” measurement techniques, aimed at collecting data from larger, population-based samples. The DLW method provides an excellent platform for the validation of other physical activity tools, in the free-living population. Coupled with measurements of resting and sleep metabolic rate, it provides a very accurate assessment of free-living TDEE. For example, Bouten et al. (22) used the DLW to validate a motion sensor for TDEE measurement. In the sample of 30 subjects, resting metabolic rate was measured overnight using a human respiration chamber, and subjects wore a motion sensor during waking hours. The motion sensor explained 64% of the variation in TDEE, after adjusting for sleep metabolic rate, and was able to differentiate between activity levels.

In another study by Conway et al. (33), physical activity questionnaires and physical activity records were assessed for validity and compared to the DLW method. For resting metabolic rate measurements, subjects completed an overnight stay in a human respiration chamber. Physical activity records, on average, overestimated physical activity energy expenditure by 8%, while the physical activity recall methods overestimated physical activity energy expenditure on average by 31%, highlighting the utility of DLW for distinguishing between the different techniques and providing a sound reference point for free living TDEE measurement.

Finally, with respect to DLW, while it may not be an appropriate tool for the estimation of TDEE in large population-based epidemiological research, as a result of cost, it is invaluable.
for research during which subjects are removed from their normal or habitual environments or are unable to maintain contact with investigators. For example, Westerterp et al (158) used the DLW technique to explore energy balance in cyclists, competing in the 3-week Tour De France cycle race (158) and Branth et al. (25) completed investigations in yachtsmen during a 10-day, single leg of the transatlantic yacht race, using the DLW technique.

Another extreme environment, in which the measurement of energy expenditure is important, but difficult, is that of space flight and microgravity. Numerous studies have investigated the effects of microgravity on TDEE (75;76;137-140). Most of these studies have been conducted using the DLW technique (76;140). Lane et al. (76) compared TDEE in 13 male astronauts measured on Earth and in the microgravity environment, during 8- to 14-day Space Shuttle missions, using the DLW technique. TDEE was not statistically different between the Earth-based and microgravity environments. However, the total energy intake was significantly lower than the TDEE during the in-flight versus the ground-based studies, accounting for the significant weight losses experienced by the astronauts. The decrease in energy intake was attributed to the motion sickness, which astronauts typically experience during exposure to the microgravity environment.

This finding was corroborated by research conducted by Stein et al. (140) who compared a 16-day in-flight space mission to a 16-day 6° head-down bed-rest study, using the DLW technique. The microgravity environment resulted in a significant difference in body weight, nitrogen retention and energy intake compared to best rest. Further, in the microgravity environment, there were significant decreases in weight and nitrogen retention, in addition dietary intake was also reduced, while energy expenditure remained unchanged in-flight, resulting in a significant loss in overall body weight.
In the microgravity environment, other techniques such as motion sensing and physical activity questionnaires are impractical. For example, motion sensing would under-report activity, as a result of the weightlessness environment. However, in environments where physiological adaptations are present, such as the microgravity environment or even simply as a result of temperature fluctuations, the DLW technique proves very useful. For example, Hebestreit et al. (58) showed significant heart rate changes in temperatures above 22°C, with heart rate increasing by approximately 1.5 beats°C⁻¹. This environmental effect would have significant consequences for the estimation of energy expenditure, using the heart rate monitoring technique.

1.3.4. The heart rate monitoring method and total daily energy expenditure measurement

As previously mentioned doubly labelled water provides the “platform” against which more cost-effective, widely applicable methods for quantifying physical activity energy expenditure may be compared. One such technique is the heart rate monitoring technique. Heart rate monitoring has been extensively investigated over the last fifteen to eighteen years, for the purpose of TDEE measurement. Heart rate monitoring provides physiological information about the type of activities being performed and describes the nature of day-to-day variability in energy expenditure (58;85). While whole room respirometry and indirect calorimetry provide physiological information into the nature of the activity to be performed, these tools are not only costly to maintain, but often take the subject out of their natural environment for the duration of the measurement period (85). Further, heart rate monitoring provides one of the most efficient and economical means of measuring energy expenditure, in large groups of people (29;88;95;136).
The basis of the heart rate monitoring technique is the underlying assumption that minute heart rate (HR) is closely correlated with oxygen consumption (VO₂) or energy expenditure during periods of physical activity (29;136) or above the so-called "flex heart rate". Spurr et al. (136) introduced the concept of the "flex heart rate", a critical point below which the relationship between heart rate and oxygen consumption is not linear.

**Figure 1.3.4.1.** Example of the heart rate-energy expenditure relationship, during rest and exercise, above flex heart rate. Adapted from Spurr et al. (136).

Flex heart rate was calculated by averaging the highest heart rate achieved during resting activities, and the lowest heart rate achieved during physical activity. As a result of the linear relationship between heart rate and oxygen consumption, above flex heart rate, it is possible to derive heart rate-energy expenditure regression equations for each individual subject. These heart rate-energy expenditure relationships have been subsequently used to determine energy expenditure from the heart rate recordings, by means of predicting the appropriate energy expenditure value for the corresponding heart rate. Individual calibration requires that each subject complete a progressive exercise test, during which
time heart rate is simultaneously measured, along with indirect calorimetry to estimate energy expenditure (29;136). The individual calibration protocol usually consists of at least eight continuous incremental activities, designed to elicit an average 10-bpm increase in heart rate for each successive workload. Typically, each calibration test includes resting measurements, usually obtained during sitting and standing activities.

Results from early studies for predicting energy expenditure from heart rate were not promising. A study conducted by Christensen et al. (31), found poor agreement for the estimation of energy expenditure from heart rate, using individually determined heart rate-oxygen consumption regressions. In this sample, 17 subjects completed individual heart rate-oxygen consumption calibration tests on consecutive days, using a heart rate-energy expenditure calibration protocol consisting of eight workloads. The first 2 workloads constituted resting measurements and comprised 20-minutes of supine lying and 20-minutes of sitting quietly. The next 6 workloads were physical activity workloads and comprised 3 workloads of incremental cycling intensities and 3 workloads of incremental walking intensities. Each of the 6 physical activity workloads were 10-minutes in length and subjects were continuously monitored for heart rate and oxygen consumption. Subjects proceeded to collect 2 days of heart rate monitoring, using a portable ECG monitor.

Results indicated that generally there was good agreement (average $R^2$ for sample not given) between the duplicate measurement days for subjects, however there was a large degree of individual variation, with only 7 of the 17 subjects having differences for duplicate measures of energy expenditure that were less than 20% of the first value. Further, the slopes and intercepts varied greatly between the two measurement days for the subjects. As a result, estimates of energy expenditure were significantly different when calculated using either of the two calibration days. It was concluded that energy expenditure
estimation using individual heart rate-energy expenditure was not suitable for measurement in subjects who participated in activities of daily living that were associated with low energy expenditure.

Further research was, however, conducted by numerous investigators (29;81), all of whom have made significant contributions to the methodology of the technique and thereby improved the accuracy for use in the estimation of free-living TDEE in man. For example, Spurr et al. (136) were able to accurately predict physical activity energy expenditure in 22 men and women (aged 18-47 years old) using a small, portable, lightweight, heart rate recorder and the subjects' own individually-calibrated heart rate and energy expenditure relationship. In this study, the calibration protocol involved a series of incremental cycling workloads that were increased in Watts every 3 minutes until heart rate was approximately 150 beats per minute. Each calibration test included a number of resting measurements, obtained during sitting and standing workloads, prior to the cycling bouts. From the individual calibration test individual heart rate-energy expenditure relationships were calculated. Subjects then proceeded to spend an overnight stay in a respiration chamber, following either 1 of 4 fixed activity protocols. The fixed activity protocols varied in the amount of exercise that the subject performed during the 24hr stay, with the first protocol including the least amount physical activity and the fourth protocol consisting of the greatest amount of activity.

Using heart rate averaged to one minute intervals and the energy expenditure generated in the respiration chamber, it was found that TDEE measured using heart rate monitoring varied by as much as 20 to −15%. Further, it was found that there were significant gender differences in the establishment of the flex heart rate point, with women achieving the critical point approximately 10 bpm lower than the men. In addition, the slope of the heart
rate-energy expenditure relationship for the cycling workloads was significantly higher in the men, compared to the women. This variability in the technique was however, thought to be acceptable for energy expenditure measurement, especially in light of the ease and cost-effectiveness of the technique.

The work by Spurr et al. (136) was novel, as previously heart rate averaged over the entire measurement period were entered into a linear regression equation, as in Christensen et al. (31) and it was assumed that the relationship between heart rate and energy expenditure was linear, even at very low levels of activity or at resting heart rate. However, this study introduced the concept of the critical cut-off point, below which the relationship between heart rate and energy expenditure is no longer linear. As a result, researchers were able to significantly improve the estimation of energy expenditure from heart rate monitoring. In the study by Spurr et al, energy expenditure estimated using the heart rate monitoring technique accounted for 76% of the variance in measured energy expenditure, measured using the respiration chamber.

Ceesay et al. (29) further investigated the heart rate-energy expenditure relationship. A sample of 20 men and women (aged 17-36 years old) completed an individual heart rate-energy expenditure calibration test, before spending 24hrs in a respiration chamber, following a fixed-activity protocol. The individual heart rate-energy expenditure calibration test consisted of approximately 12 workloads, which included 3 sedentary activity workloads, and a number of cycling, stepping and running workloads. Subjects rested between each workload for about 5 minutes. The heart rate-energy expenditure relationship was calculated according to the flex heart rate method and was based either on 8 or 12 activity workloads. Heart rates during the 5 minutes rest periods were not used in the regression equation. The fixed activity protocol in the whole room chamber consisted of three 30-
minute exercise sessions. During the 24hr measurement period, any time the measured
heart rate fell below flex heart rate-energy expenditure values were assigned according to
the Schofield equations (129) for BMR. Otherwise, the individually determined heart rate-
energy expenditure equation was used to calculate minute-by-minute energy expenditure.

TDEE, using the heart rate method, only underestimated energy expenditure measured in
the whole room chamber by an average 1.2%, (range from −11.4 to 10.6%). However, the
energy expenditure estimated during the night was significantly different from that measured by indirect calorimetry, with an average difference of 6.2%, this may be as a consequence of the Schofield equation versus measured BMR. For accurate TDEE estimation, the heart rate monitoring method (as with the doubly labelled water method), requires resting metabolic rate measurements, usually completed with indirect calorimetry or as in the current study, using validated equations for predicting basal metabolic rate, such as the Schofield equations (129). It is perhaps noteworthy that in this study, the magnitude of error when using the heart rate method was similar to that found when using the doubly labelled water method. This has several implications for the heart rate monitoring method, not only is it more cost effective, but can provide TDEE data that are within acceptable levels of accuracy, for many applications.

As a consequence of this early work, and in response to some of the limitations highlighted, various studies have been conducted in which equations have been modelled (81;130) to manage the apparent inconsistencies, between resting heart rate and the disproportional increases in metabolic rate, at lower or resting activities. Schultz et al. (130) investigated four higher order type equations and compared these to measured energy expenditure, as determined using the doubly labelled water method, in six subjects. Estimated energy expenditure did not differ significantly (P<0.05) from energy expenditure measured using
the doubly labelled water method. The equation, which included a logistical order function to describe the relationship between heart rate and energy expenditure, was on average 12.7% lower than the doubly labelled water method, while the other equations produced values that were on average 9.8-17.2% higher than the doubly labelled water values. However, the logistical function equation provided the best fit for both the doubly labelled water reference value and the highest correlation coefficients for energy expenditure determined by doubly labelled water and estimated using the regression equations.

In another study by Li et al. (81), the reliability of minute-by-minute heart rate monitoring was investigated in a group of 40 women, participating in occupational work activities. In this study, a logistical function, based on a sigmoid curve was employed to fit the data obtained from the individual subject heart rate-energy expenditure calibration tests, as opposed to the flex heart rate method. Figure 1.3.4.2 provides a diagrammatical representation of the logistical function (sigmoidal curve) used by Li et al. (81) to describe the relationship between heart rate and energy expenditure. The hypothesis behind the use of the sigmoidal curve was the observation that heart rates recorded during sedentary or activities of low intensity do not have parallel increases in heart rate and oxygen consumption. Although Li et al. (81) originally used a total of 16 different activities and two BMR measurements, to establish the relationship between heart rate and energy expenditure, the final logistical curves, generated for each subject, were based on a series of 9 activities, which included one BMR measurement. In this study, Li accounted for 91% of the variance in measured energy expenditure, using the heart rate monitoring method and the logistical function. In addition, it was found that the accuracy of the estimation improved significantly when all 18 activities were used in the logistical function, as opposed to the selected nine activities (10.6% versus 20.4% error). Further, the accuracy of the final estimation was significantly reduced when the group calibration curve was applied to
the heart rate data, as opposed to the individual’s own curve. From this difference it was concluded that while heart rate monitoring provides useful data into the nature of energy expenditure, it should be used only in conjunction with individual subject calibration. Further, individual subject calibration should employ as many activities as feasibly possible, to establish the heart rate-energy expenditure relationship.

**Figure 1.3.4.2.** Example of the logistical sigmoidal function used to plot energy expenditure (kJ.min\(^{-1}\)) against heart rate (bpm). Adapted from Li et al. (81).

![Logistical sigmoidal function](image)

McCrory et al. (88) further investigated the between-day and within-day variability in estimation of TDEE from heart rate monitoring. Twelve healthy subjects completed a standardised calibration test on four different occasions. The calibration test consisted of 8 different activities, which included four “sedentary-type” activities, and four “active” stages. Two linear equations were generated for each subject, one used for sedentary activities and one for active periods. To determine which equation would be used during the energy expenditure estimation, the flex heart rate cut-off point was employed. Consequently, each subject completed an individual heart rate-energy expenditure calibration test, to determine his or her own flex heart rate point. The same calibration test was repeated on four
different occasions, two morning and two afternoon sessions, to determine how the time of
day affected the calibration procedure.

In this study by McCrory et al. (88) it was found that for the group, oxygen consumption (or
energy expenditure) in the afternoon was on average 9% higher for supine lying and
approximately 7% for sitting (P<0.003). However, for the rest of the calibration activity
data, there were no significant group differences for heart rate, oxygen consumption, or
carbon dioxide production, either within- or between-day. In addition, there were no
significant group differences for either the slopes or the intercepts for the sedentary or
active equations, between-day or within-day. The coefficient of variation for the group
average energy expenditure, using the four different calibration data sets was low, only
1.1%. The coefficient of variation for the individual differences ranged from 5-9%, and was
thought to compare favourably with doubly labelled water technique. The greatest source
of variability in the TDEE estimation was thought to be as a result of the intra-individual
variability in the establishment of the flex heart rate, and therefore with activities associated
with heart rates lower than the flex heart rate. McCrory et al. (88) therefore, concluded that
regression equations for group application were acceptable. As a result of the intra-
individual variability in identifying the flex heart rate, it was suggested, that using heart rate
regression equations might only be suitable for estimating energy expenditure during
physical activity. This work represented one of the first studies investigating the use of
group regression equations for the measurement of TDEE.

Researchers (62;113) have tried to identify the factors that impact upon the expression of
the relationship between physical activity heart rate and energy expenditure. Indeed,
individual variation may be ascribed to the factors such as gender, age and training status
(62;81;114;145). For example, individuals who participate in regular physical activity
appear to have lower resting heart rates than their sedentary counterparts (114;163). Wilmore et al. (163), found that following a 20-week endurance training program, exercise heart rates were significantly reduced at the same absolute workloads, and further that these changes were subject to differences in age, gender and race. Considering the impact of these factors may therefore be crucial to accurate energy expenditure estimation, particularly in the presence of physiological variation.

Hiilloskorpi et al. (62) was one of the first investigators specifically exploring the heart rate-energy expenditure relationship during physical activity that attempted to characterise the variables that significantly impact upon the energy expenditure estimation. Hiilloskorpi et al. (62) tested 87 male and female volunteers, each of whom completed two incremental tests, one on a cycle ergometer and one on a treadmill. Each incremental test was then followed by a 10-minute steady state test, performed on the same piece of apparatus. Using general linear models with random effects, significant variables included age, gender and body weight, two different models were investigated, one for cycling and one for walking. Using the final models, the equations accounted for 94% of the energy expenditure during a steady-state cycling activity and 82% during steady state walking. While energy expenditure estimated, using the final model, were overestimated during both the walking and cycling activities, Hiilloskorpi et al. concluded that the method was acceptable for the measurement of physical activity energy expenditure for purposes of health promotion.

Using statistical modelling, Hiilloskorpi et al. (62) therefore concluded that the prediction of physical activity energy expenditure was improved upon by the inclusion of factors such as gender and weight in the final model. Morphological differences between men and women play an important role in the estimation of energy expenditure during physical activity. Indeed, during the same absolute workloads, men have higher energy expenditure than
women, simply as a result of their increased muscle mass (87). Similarly, body weight significantly contributed to the final model, the higher the individual's body weight the higher the caloric use. In this particular work by Hiilloskorpi et al. (62), age did not appear to be a significant variable in the prediction equation. Finally, the impact of cardiorespiratory fitness on the model for predicting energy expenditure from heart rate was not explored.

Rennie et al. (114) were amongst the first investigators to include a measure of fitness in the prediction equation for physical activity energy expenditure, from heart rate monitoring. In a cohort of 789 subjects, heart rate and energy expenditure data were collected during a five-stage calibration test, for the purpose of developing a group-based equation for the estimation of physical activity energy expenditure from heart rate. The calibration test included data collected during both sedentary (1 supine measurement) and 4 cycling working workloads. The flex heart rate method was employed to distinguish between the lowest heart rate experienced during exercise and the highest during the supine resting and these data was used to anchor the linear regression line for the exercise data. Participants then proceeded to wear heart rate monitors for a period of 4 days. For minute-by-minute energy expenditure estimation, heart rates recorded above the flex heart rate, were entered into the linear prediction equation, while those below were assigned values equal to mean resting energy expenditure. Further, sleep energy expenditure was calculated as 95% of measured BMR. Using these data, daily energy expenditure was estimated for each subject, for the four days. A daily physical activity level was then computed from the energy expenditure data and averaged for the 4 days and finally, from these data, a regression model was developed for estimating physical activity levels.
Variables, which contributed significantly to the estimation of physical activity levels were weight, BMI, gender and sitting heart rate and these were evaluated with respect to the prediction of physical activity, the prediction of flex heart rate, the slope of the relationship and finally, the intercept. A model, which included only current heart rate, gender, weight and sitting heart rate, was then applied to an independent sample of 97 subjects for inner validation. The final model explained 67% of the variance in measured physical activity levels, in the independent sample. Further, when measured and estimated physical activity levels were broken down into quartiles, 59% of the scores were placed in the same quartiles, while 98% were placed in the same or adjacent quartiles.

The study by Rennie et al. (114) was significant as it recognised the importance of fitness, or physical training in the estimation of energy expenditure from heart rate. Indeed, numerous studies (27;53;133;162) have reported on the effects of exercise training and the various components of energy expenditure including RMR. In addition, studies (50;71;135;162) have reported significant heart rate adaptations to exercise. Therefore, the study by Rennie et al. (114) was novel, as it was also one of the first to report on physical activity energy expenditure results, calculated using models, which included a proxy for fitness. Further it included physical activity energy expenditure data estimated on an inner validation or independent sample, with good accuracy, illustrating the value of group based prediction equations, without the need for individual calibration.

Strath et al., (145) also recognised the importance of fitness in estimating energy expenditure. In a study of 61 subjects, representing a broad spectrum of age, individuals completed a series of 7 activities, each lasting about 15-minutes in length. The activities included both inside and outside tasks. For example, the inside activities included: vacuuming, sweeping, washing dishes, feeding and grooming animals, caring for children,
walking at 2 different speeds while carrying a load of 6.8 kg, stretching and light callisthenics. The outside activities included: lawn mowing, gardening, playing with children and animals, playing tennis, playing golf, slow walking and fast walking. During the 7 activities the subjects were continuously monitored for oxygen consumption and heart rate, and values from minutes 5 to 15 were used in the final analysis. Subjects did not perform a maximal oxygen consumption test; instead this was predicted, using the equation of Jackson et al. (64).

Initially, heart rate and oxygen consumption values, from the 7 activities, were entered into a linear regression model, heart rate accounted for 46% of the variance in measured oxygen consumption. Heart rate was then used to estimate energy expenditure, in METS, after adjusting for age and fitness level (using the calculated maximal oxygen consumption data) and accounted for $R^2=0.76$ of the measured energy expenditure, using the oxygen consumption data. While the energy expenditure estimation was not performed a different sample, on which the initial equation was based, for inner validation, accounting for the good correlation between estimated and measured energy expenditure, the importance of including a measure of fitness during energy expenditure estimation from heart rate was recognised.

Prior to the work of Strath et al. (145) and Rennie et al. (114), other studies (81;121) using heart rates entered into regression equations, to estimate energy expenditure, have cited poor agreement for measured and estimated energy expenditure. However, often these equations were either developed on small sample populations, or samples that did not reflect the population demographics used to generate the equation. For example Rutgers et al. (121) developed an equation, using the data from only 13 elderly subjects, to generate a group-based energy expenditure equation, be applied to the general population and
compared this to energy expenditure estimated from individual subject heart rate-energy expenditure calibration equations. Using the heart rate data from 3 days of continuous heart rate monitoring, it was found that the equation only accounted for 14% of the variance in measured energy expenditure, using the individual heart rate-energy expenditure calibration technique, in these 13 elderly subjects. As a result Rutgers et al. concluded that group based equations, were unsuitable for TDEE estimation in groups.

Another source of error may be as a result of the calibration procedure. Often, the regression equations are based on continuous, incremental, calibration tests (62;81;83;88;114;121). In addition, the heart rate response to intermittent activity (including heart rate recovery) is not taken into account. A study by Lothian and Farrally (84) found that oxygen consumption, estimated from either a heart rate-oxygen consumption regression equation or from indirect calorimetry, using the Douglas bag technique, was significantly different (P<0.01), during intermittent activity (running and walking) on a motorised treadmill. The heart rate method, significantly underestimated oxygen consumption by 4.3%, when compared to oxygen consumption measured using indirect calorimetry. It was also found that heart rate changes lagged behind changes in activity, i.e. when the subject went from running to walking or rest periods. Consequently it was suggested that using the heart rate monitoring method to estimate oxygen consumption, might result in error in the final estimation as a result of a delay in the evolution of the heart rate response, behind activity change. While the overall error in estimating the energy cost of the intermittent activity was only 4.3%, using heart rate to determine individual oxygen consumption might lead to errors in the estimation, particularly during low-intensity activities.
Similarly, a study by Bot et al. (20) demonstrated a temporal dissociation between heart rate and oxygen consumption during intermittent activities. In this study, participants completed simultaneous heart rate and oxygen consumption measurements, while performing either steady state or intermittent physical activity exercise. During the steady state incremental protocols using either small, large, or a combination of both muscle groups, a good association between heart rate and oxygen consumption was found, corroborating previous research. However, during the intermittent activity protocol, completed in an occupational nursing setting, there was a significant dissociation between the oxygen consumption and the heart rate measured, resulting in no overall relationship for heart rate and oxygen. Figure 1.3.4.3 is a diagrammatic representation of the heart rate and oxygen consumption relationship, observed during the intermittent activity protocol. The oxygen consumption data are represented by the squares numbered 1 to 9. Of importance are the oxygen consumption data points 2, 3, 5 and 8, which clearly indicate the dissociation between heart rate and oxygen consumption, during intermittent activities.
**Figure 1.3.4.3.** Data adapted from Bot et al. (20), diagrammatically illustrating the temporal dissociation between heart rate and oxygen consumption during intermittent activities, or activities of daily living.

The previous two studies (20;84) provide evidence for dissociation between heart rate and oxygen consumption, during intermittent activity. This may be problematic in the application of the heart rate monitoring technique for energy expenditure estimation, during intermittent activity, and further using equations derived from continuous incremental calibration protocols. Most of the previous investigations (29;62;81;88;114;121;130) using heart rate rely on calibration test protocols, that are firstly incremental in nature and secondly perhaps not as reflective of the real day-to-day nature of the activities of daily living. To our knowledge, there are no energy expenditure equations available for use during intermittent activity.

Finally, with respect to the heart rate monitoring technique for estimating energy expenditure it is important to mention that numerous authors (28;39;118;165) have found significant effects relating to the autonomic control of heart rate during exercise as well as a
result of exercise training. Most recently Yamamoto et al. (165) reported significant differences in resting and recovery heart rate, following a 6-week endurance training program. Following training, resting and recovery heart rate were significantly reduced by approximately 15 beats.min\(^{-1}\). Furthermore, this study was able to account for cardiac autonomic system modulation during both rest and recovery periods, and suggested that these were in part responsible for the heart deductions. This has important implications for the estimation of energy expenditure from heart rate monitoring, especially in situations where there is altered autonomic system control present. For example, a study by Darr et al. (39) showed significant differences in heart rate recovery time, irrespective of age, in 20 male subjects. Participants were categorised as either trained young, trained older, untrained young or untrained older. Following a maximal cycling ergometry test to volitional exhaustion, heart rate recovery was markedly increased for both the trained younger and older groups, compared to either untrained groups. The largest discrepancy between the trained and untrained individuals was apparent during the first 15-120 seconds of recovery, as opposed to the time between 120-240 seconds. This has significant implications when using heart rate to estimate energy expenditure as it highlights the need to take into account for the effects of the autonomic system as a result of training status or fitness.
Table 1.3.4.1. Summary of studies contributing to the validation of heart rate-energy expenditure estimation technique.

<table>
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<tr>
<th>Investigator</th>
<th>Subjects</th>
<th>Protocol</th>
<th>Result</th>
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<tbody>
<tr>
<td>Christensen et al. (31)</td>
<td>N=17 (5=healthy, 5=obese, 5=untreated thyrotoxicosis and 2=eating disorder.)</td>
<td>2 HR-VO₂ calibration sessions, using an incremental continuous protocol of 8 workloads, incorporating 2 resting workloads and 6 physical activity workloads, comprised 3 incremental intensity cycling and 3 incremental intensity walking, on consecutive days, to generate linear regression of HR and VO₂, followed by 24hr HR monitoring on 2 days.</td>
<td>HR correlated with VO₂, however high degree of variability between consecutive day calibrations, leading to poor agreement between duplicate measures of EE, only 7 out of 17 subjects had differences between duplicate measures that were less than 20%, no R² values given.</td>
</tr>
<tr>
<td>Spurr et al. (136)</td>
<td>N=16 men, N=6 women</td>
<td>Individual HR-EE calibration, using continuous incremental cycling protocol and flex HR determination (using 4 resting measurements) to generate linear regression (1 male and 1 female) between HR and VO₂. Equation validated during 22hrs in a respiration chamber, following fixed activity protocol for TDEE estimation.</td>
<td>Slopes for men and women significantly different during cycling (P&lt;0.001). Estimated TDEE (using individual HR-EE calibration) accounted for 76% measured TDEE. Maximum error for individuals was +20 and -15%.</td>
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<tr>
<td>Ceesay et al. (29)</td>
<td>N=11 men, N=9 women</td>
<td>Individual HR-EE calibration (6 workloads) and flex HR determination (3 resting measurements), incorporating 5-min breaks between activities (not used in individual linear regression generation). 21Hrs in respiration chamber.</td>
<td>Flex HR significantly different for men and women (P&lt;0.05). Individual HR-EE calibration method accounted for R²=89% of EE measured using respiration chamber.</td>
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Table 1.3.4.1. Continued.

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<th>Investigator</th>
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<tr>
<td>Schulz et al.</td>
<td>N=4 men</td>
<td>Individual HR-EE calibration, using continuous incremental protocol, and 4 models (both linear and non-linear) for estimating EE from HR, followed by 14 days DLW measurements during which 2 (random) days of 24hr HR monitoring</td>
<td>Different HR-EE models correlated differently with DLW method but were not statistically different (P&lt;0.05). A log function provided the best estimate of TDEE from HR monitoring when compared to DLW TDEE estimate, R=0.53.</td>
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<td>N=2 women</td>
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<tr>
<td>Livingstone et al.</td>
<td>N=9 men</td>
<td>Individual HR-EE calibration (2 activities-stepping and cycling, separated by 10-min rest) and flex HR determination (3 resting measurements), to generate linear regression equation, followed by 14 days DLW measurements and HR monitoring on 4-days, accumulating 16hrs of HR data.</td>
<td>HR derived EE values varied +52 to −22% from DLW measurements. 9 out of 15 measurements HR derived EE estimates were within 10% of DLW measurements.</td>
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<td></td>
<td>N=5 women</td>
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<tr>
<td>Hebestreit et al.</td>
<td>N=12 boys</td>
<td>Individual HR-VO₂ calibration, using linear regression equations, using 3 cycling workloads and during 4 different climatic conditions, of increasing temperature and humidity.</td>
<td>HR-VO₂ determined at 22°C provided zero value for comparison. At a given VO₂, HR increased linearly with temperature, approximately 1.05 beats.min⁻¹°C⁻¹.</td>
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<td></td>
<td>N=8 girls</td>
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9 out of 15 measurements HR derived EE estimates were within 10% of DLW measurements.
Table 1.3.4.1. Continued.

<table>
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<tr>
<td>Li et al. (81)</td>
<td>N=40 women</td>
<td>Individual HR-EE calibration, using a sigmoidal curve based on 16 workloads, during which subjects resting between activities to allow HR to return to base-line ~10-min for light activities and 30-min for intense activities. Analysis performed on a total of 9 and 18 workloads. Free-living HR accumulation during 1 day (~16hrs) was used to compare differences between- and within-subjects, with respect to EE estimation.</td>
<td>Relationship for HR and EE varied within and between subjects. Inter- and intra-individual variations were 14-18% and 11-20%, respectively.</td>
</tr>
<tr>
<td>Lothian and Farrally (84)</td>
<td>N=16 women</td>
<td>Laboratory- and field-based testing. Individual HR-VO₂ calibration (using linear regression equations), on a treadmill to estimate VO₂ during field-based activity, from HR monitoring.</td>
<td>HR overestimated VO₂ during field activity by approximately 5%.</td>
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<tr>
<td>McCrory et al. (88)</td>
<td>N=6 men N=6 women</td>
<td>4 Individual HR-VO₂ calibrations generated using 8 consecutive activities, each increasing HR by ~10bpm, during 2 morning and two afternoon sessions, incorporating the identification of flex HR (using 4 resting measurements) and linear regression equations, followed by 15hrs HR monitoring.</td>
<td>4 group mean HR-VO₂ EE values similar, regression equation did not differ for group, only individuals, with intra-individual variation &lt;10%. HR was more variable than VO₂ and variation ascribed to individual establishment of flex HR.</td>
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Table 1.3.4.1. Continued.

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<th>Investigator</th>
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<tr>
<td>Hiilloskorpi et al. (62)</td>
<td>N=42 women, N=45 men</td>
<td>Continuous HR and VO₂ measured during 2 incremental (cycling and walking) and two 10-min steady-state tests. Multivariate models developed for estimating EE from HR, using other variables.</td>
<td>HR, age, gender and body weight showed significant associations for measured EE. Final model overestimated EE by 18% during cycling, and 6% during walking.</td>
</tr>
<tr>
<td>Rennie et al. (114)</td>
<td>N=789 (model development), N=97 (inner validation)</td>
<td>HR-EE calibration and flex HR determination to develop regression model for EE from HR and other significant variables. Multivariate model applied during inner validation to estimate physical activity levels.</td>
<td>Age, weight, fitness level are all significantly associated with estimated EE using HR regression equations. Using the final model HR accounted for 66% of the variance in physical activity levels during the inner validation.</td>
</tr>
<tr>
<td>Strath et al. (145)</td>
<td>N=31 men, N=30 women</td>
<td>Continuous HR and VO₂ measured during both laboratory- and field-based activities, EE values averaged for 15min values and entered along with other variables into regression equation and compared to measured EE, using on-line gas analysis.</td>
<td>HR accounted for $R^2=0.46$ in measured VO₂. Using regression equation adjusting for age, fitness, HR accounted for 87% of variance in estimated EE.</td>
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<tr>
<td>Investigator</td>
<td>Subjects</td>
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<tr>
<td>Bot et al. (20)</td>
<td>4 subject populations: study 1 N=28, study 2 N=14, study 3 N=15 and study 4 N=5</td>
<td>Subjects performed both laboratory- and field-based activities, using steady- and non-steady state protocols.</td>
<td>During field- and laboratory-based interval test, HR accounted for $R^2=0.85$ measured $VO_2$ respectively. During non-steady state arm and leg activity, involving a field-test, contradictory results for HR and $VO_2$ found resulting in no relationship between HR and $VO_2$, $R^2=0.18$.</td>
</tr>
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In section 1.3.4, the heart rate monitoring technique was reviewed and while recent estimates in energy expenditure estimation have shown significantly increased accuracy (62;114), the heart rate monitoring technique is subject to both temporal dissociations during intermittent activities, reflective of the nature of activities of daily living as shown in a recent studies by Bot et al. (20) and Farrally and Lothian (84) as well as alterations in the autonomic nervous system, induced by exercise training (28;39;118;165).

A technique that may account for these irregularities as a result of immediate movement registration, are the electronic motion sensors. Briefly, electronic motion sensors, or accelerometers as they are commonly known, are another means by which free-living TDEE can be measured. However, as with the doubly labelled water method, it is necessary to include measurements of resting metabolic rate or sleep metabolic rate for accurate TDEE measurement. The first motion sensors were battery powered, uniaxial (single plane) and consisted of a piezoresistive ceramic plate, which registered both movement acceleration and deceleration (143). Following this initial model, a second model was introduced, which measured body accelerations in the 3 dimensional planes (the antero-posterior (x), mediolateral (y) and vertical (z) planes) and could measure accelerations and decelerations of up to three times as much as the first models (23). Three piezoresistive ceramic plates are mounted orthogonally in a resin block and each of the 3 axes is measured independently (79).

While motion sensors have been shown to have an excellent facility to measure energy expenditure during ambulatory physical activity, for measurement of TDEE, there is an underestimation in energy expenditure as a result of the monitors' inability to detect both
arm and external work (12), such as carrying heavy loads or heavy manual labour, running up inclines, or exercising on a stationary ergometer, for example stepping machines (i.e. elliptical rowers, and cycle ergometers', found in most fitness centres). The limitations to accelerometry are thought to be mostly biomechanical (24). As a result of these biomechanical limitations it has been suggested that a combination of motion sensing and heart rate monitoring would perhaps provide a more accurate estimation of energy expenditure. Brage et al. (24) has suggested that as a result of errors with either technique not be positively correlated or associated a combination of the two should yield more accurate results of energy expenditure estimations.

Accelerometry has been extensively validated for the measurement of physical activity energy expenditure (12;23;65;93). Bouten et al. (23) investigated the assessment of physical activity energy expenditure, using a triaxial accelerometer. In this study, 11 healthy men participated in a laboratory investigation, in which they performed a number of sedentary and walking activities, resembling the activities of daily living. Briefly, the protocol included sitting relaxed, sitting and writing, sitting with arm work, alternately sitting and standing for 10 seconds each and walking at five different speeds (3, 4, 5, 6, and 7 km.h\(^{-1}\)) on a motorised treadmill, while continuously measuring oxygen consumption and body accelerations with the triaxial accelerometer. Subjects spent an overnight stay in a human respiration chamber for the determination of sleep metabolic rate. In addition, relationships for the three-accelerometer planes were explored and compared to the total physical activity measured.

Initial results indicated that between 50 and 98% of the variance in physical activity energy expenditure could be explained by the accelerometer output. The relationship between measured and estimated energy expenditure was weakest during sedentary-type activities.
As the subjects progressed to the walking workloads, the variance, between measured and estimated energy expenditure, was significantly reduced, with the greatest accuracy being attained during the higher speed workloads. The variance for walking at a pace of 7 km.h⁻¹ was 8% while walking 3 km.h⁻¹ was 20%. This study was, however, not conducted in the free-living setting, and interpretations of these results may be limited as a result of the controlled laboratory setting. Further, this study did not investigate the effects of age, gender, weight and training status on the relationship between accelerations and energy expenditure, or investigate how these may change with individual physiological variation. Finally it was concluded that while the accelerometer provides a good measure of physical activity during activities that are weight bearing and ambulatory, care should be taken when interpreting data from static activity, where it was apparent that the accelerometer significantly underestimated the physical activity energy expenditure.

More recently, Jakicic et al. (65) compared energy expenditure during specific tasks measured using either indirect calorimetry or by triaxial accelerometer. In this study, 20 subjects performed a variety of different activities in the laboratory setting. Each activity was executed on a separate day and was randomised. The activities included treadmill walking, treadmill running, stationary cycling, stepping up and down steps and lateral sliding, performed on a slide board. Each exercise was performed for a total of between 20-30 minutes and cadence was graded and increased every 10-minutes, for walking, running, cycling, stepping, and side sliding. In addition treadmill grade was also increased every 10-minutes. Subjects were continuously monitored using indirect calorimetry, for the measurement of energy expenditure.

Initial results indicated that there were significant correlations between energy expenditure measured using on-line gas analysis and the accelerometers. The accelerometers accounted
for between 46 and 85% of the variance in measured energy expenditure. However, total energy expenditure was significantly underestimated, using accelerometry, during the walking activities (22%), with a similar trend for the stepping, lateral sliding and cycling activities. Interestingly, as the grade during the walking activities increased, the total error also increased to approximately 45%, highlighting the inability of the accelerometer to detect external work, such as walking up-hill, or as previously found by Bassett (12), carrying heavy loads. The error during the stepping and lateral sliding was approximately 40%. There were no significant differences between the energy expenditure measured using indirect calorimetry and accelerometers during the running workloads.

Finally, Bassett et al. (12) explored the validity of four motion sensors with respect to measuring moderate intensity physical activity energy expenditure. In this field-based study, 81 subjects completed a series of 28 activities, made up of six categories. The six categories included: 1) yard work, 2) occupation, 3) housework, 4) family care, 5) conditioning and 6) recreation. Each of the six categories was further comprised of several representative activities. Subjects performed each activity for 15-minutes, during which time oxygen consumption was continuously monitored using a portable indirect calorimetry system. Oxygen consumption data were converted into MET (metabolic equivalents) data. During the activities the subjects wore three uniaxial accelerometers and one electronic step counter. Initial data analysis indicated that all four of the motion sensors underestimated energy expenditure associated with the activities of daily living. Further analysis, however, indicated that data from the various units were significantly different and that, unlike the previous laboratory studies, the energy cost of walking was actually overestimated in two of the accelerometers.
This study demonstrates weaker relationships between energy expenditure during physical activity and motion sensors, compared to the previously mentioned laboratory-based studies. This may be, in part, as a result of the field-based study and that there was a wide range of moderate intensity physical activities, whereas, studies, such as Bouten et al. (23) and Jakicic et al. (65) were more tightly controlled, being executed in the laboratory setting. Despite these limitations, motion sensing is still thought to be a good indicator of physical activity energy expenditure. In addition, all the motion sensors had the ability to account for differences in the type of activity being performed, by individuals and therefore, may have the capacity to distinguish between persons completing different amounts of physical activity. As a result of these limitations, and the limitations to the heart rate monitoring technique there has been much interest in the technique of combined motion sensing and heart rate monitoring for physical activity energy expenditure measurement.

1.3.6. Combined motion sensors and heart rate monitors

It has been suggested that a combination of heart rate monitoring and motion sensing may provide a more accurate estimation of energy expenditure, as factors that affect heart rate such as emotion, environmental conditions, posture and fitness level (24;59) may either artificially elevate or decrease physical activity energy expenditure values, derived from heart rate monitoring. Therefore, by including a motion counter in the physical activity measurement from heart rate monitoring, only elevated heart rates, as a result of physical activity and where motion is triggered, will be considered for the final estimation (143).

Haskell et al. (55) performed one of the first studies evaluating this combined technique. In this study, 19 men were investigated during a laboratory study, using arm and leg activities. In addition to accelerometer and heart rate data, indirect calorimetry was used to estimate
oxygen consumption. Results showed an increased accuracy for oxygen consumption estimation when using a combination of the two techniques ($R^2=0.89$), compared to the energy expenditure, estimated from each technique separately. For example, adding accelerometry data to the heart rate data increased the $R^2$ for the prediction of oxygen consumption from 0.69 to 0.82.

In another study, by Moon et al. (94), this technique was evaluated in the field setting over 5 days of continuous heart rate-motion sensing in a sample of 20 adult men and women. On days 1 and 5 of the protocol, subjects spent 24hrs in a human respiration chamber, while days 2-4 were free-living, and included no restrictions to activity. Data were analysed in 3 different ways: 1) heart rate alone, where 4 non-linear models and 1 linear model were developed during a compulsory activity session the respiration chamber. 2) Combined heart rate-motion counting, where data during awake and sleep hours were separated and further entered into multiple regression equations to calculate an adjusted physical activity score. 3) 24Hr heart rate data from Day 1 in the respiration chamber were separated into either active or sedentary periods, using the motion data and identifying the flex heart rate point (83) and a physical activity score was then calculated from this.

Using data recorded during the first overnight stay in the respiration chamber, results for the heart rate monitoring method alone and individual non-linear equations, yielded estimates for energy expenditure that accounted for between 86-91% of the variance in measured energy expenditure. Using the second method, the combined heart rate-motion counting method accounted for a significant 92% of the variance in measured energy expenditure. While the third method, separating heart rate data into either sedentary or active periods, using the accelerometers to indicate the difference activity periods, produced the best predictors for oxygen consumption and carbon dioxide production ($R^2$ value not
given). The errors for the active periods were $-2 \pm 8\%$ for both the oxygen consumption and carbon dioxide production. While during the inactive periods, the errors in the estimation for oxygen consumption and carbon dioxide were $-4 \pm 6\%$ and $-6 \pm 5\%$ respectively. This study highlighted the fact that energy expenditure may be accurately predicted using a combination of heart rate monitoring and motion counting, and that this combination produced a more significant estimate for energy expenditure, than the heart rate method alone ($R^2=0.91$ versus $R^2=0.92$). This study provided further evidence for using the combined heart rate-motion sensing technique.

Rennie et al. (113) further explored this relationship in a group of 8 individuals. Initially, subjects completed an individual heart rate-energy expenditure calibration test, which consisted of both sedentary and active (submaximal) workloads, on a stationary cycle ergometer. Subjects then proceeded to spend 24hrs in a human respiration chamber, where they also completed a protocol consisting of periods of both physical active and inactive bouts. For the entire duration of the protocol, in addition to being continually monitored using indirect calorimetry, subjects wore a heart rate monitor and motion sensor. The heart rate data were then converted into energy expenditure, using the individual calibration curve and motion sensor to discriminate between periods of activity and inactivity. For the sake of comparison, energy expenditure was estimated using the flex heart rate method alone. The heart rate monitoring plus motion sensing method produced a highly significant zero percent error, while the heart rate method alone produced an error of approximately 16%. This study demonstrated the increased effectiveness of combining the two methods in a controlled setting.

Strath et al. (143) also explored this relationship in a study investigating 10 individuals initially completing a heart rate-motion sensing calibration test and then within a week,
performing 6 hours of free-living activity, while continuously monitored using a portable indirect calorimetry system. This study was novel, in that discrimination was made between upper (arm) and lower (leg) activity and, as a result, subjects completed two submaximal calibration protocols, separated by 30-45 minutes of supine lying. On arrival in the laboratory, subjects (2hrs post-prandial) were instructed to lie in a supine position for 10-minutes, they were then instructed to sit still for 5-minutes, followed by 5-minutes of standing. They then proceeded to complete the submaximal leg exercise test, consisting of walking on a motorised treadmill, every 3-minutes the speed and gradient were increased, until the subject reached 80-85% of the age predicted maximal heart rate. This was followed by the submaximal arm test, during which subjects were instructed to turn an arm ergometer at a specified cadence and resistance, which was increased by 25 kp every 3-minutes, until the subject reached 80-85% of age predicted maximal heart rate. Using the heart rate and oxygen consumption data, individualised regression equations were developed for the arm and leg activity. Within a week of these calibration tests, subjects were monitored in the free-living environment over a 6 hour period, engaging in activities such as television viewing, physical activity training, cooking, light housework, grocery shopping, yard work and general office work.

During the 6 hour free-living period, subjects were continuously monitored using a portable metabolic system, and proceeded to wear a heart rate monitor and two motion sensors, one positioned on the posterior aspect of the wrist, while the other was positioned on the mid-axillary line of the dominant thigh, orientated vertically to the femur. These data were then used to determine whether the activities performed in the field were either arm activity, leg activity or a combination of both and determined which individual heart rate-energy expenditure regression equation would be used for the estimation of energy expenditure. Further the flex heart rate method was used to determine the cut-off between sedentary
and "active" activities; all heart rates below flex were assigned values equalled to 1 MET. 82% Of the variance between predicted METS using portable metabolic unit was explained by the simultaneous heart rate-motion sensing method. Further, post hoc analysis indicated that scores obtained from the simultaneous heart rate-motion sensing were not statistically different from measured energy expenditure, using indirect calorimetry.

In addition, the simultaneous technique accurately predicted time spent in sedentary, moderate or hard activity. The flex heart rate method explained 63% of the variance in measured energy expenditure; post-hoc testing indicated that the method significantly overestimated energy expenditure, and that this effect was similar across genders. In the same study, energy expenditure estimated using the flex heart rate method was significantly different from that using the portable unit and the method underestimated time spent in the resting or light activities. This study firstly demonstrated the ability of the simultaneous heart rate-motion sensing to discriminate between upper and lower body activities. As a result of this energy expenditure estimation is improved as equations specific to those muscle groups are used to provide more accurate energy expenditure estimation. Further, this method discriminates between sedentary, moderate and vigorous activity, therefore providing important information regarding physical activity intensity. It has previously been shown that while other techniques, such as the doubly labelled water technique provide highly accurate estimates for TDEE; they cannot provide vital information as to the exact nature, i.e. intensity or duration, of the physical activity bout.

Finally, a recent study by Brage et al. (24) demonstrated the feasibility of developing group calibration equations for the simultaneous heart rate-motion sensing. Up until this time, most of the modelling for this technique had been based on individual calibration, as in the above studies, which while possibly more accurate, is time consuming. In this study, 12
men were investigated while spending 22 hrs in a direct respiration chamber. Prior to this, each subject was individually calibrated on a treadmill. The calibration was performed approximately 4 months prior to the chamber stay and consisted of 5-minute intervals of continuous walking, 2 workloads and running, 8 workloads. Equations developed for physical activity intensity were then calculated at both the individual and group levels, for conversion of motion sensing data, as well as, heart rate into physical activity intensity. Non-branched and branched equations were developed for the conversion of simultaneous heart rate-motion sensing. Data from the 22 hours recorded during the respiration chamber stay were converted into physical activity intensity using the derived equations and separated into sleep and “awake” periods, with only the awake periods contributing to physical activity energy expenditure estimation. Using the individual and group calibrations, results for physical activity energy expenditure estimation using the models were −4.4 and 3.5% respectively, when compared with calorimetry. Further, all branched models had significantly lower errors (P<0.035) than the single measures of energy expenditure estimated using either heart rate, where the error was ≤ 39% or motion sensing, where the error was approximately 45% or the non-branched combination (≤ 26%). The novelty of this study is in the introduction of accurate group calibration equations, as opposed to individual calibration equations, which may be more practical in the free-living setting, whilst conducting large epidemiological studies.

The above literature suggests that the inclusion of a motion sensor, used in combination with heart rate monitoring for energy expenditure estimation, may improve the final energy expenditure estimates. Further research and tool development and validation is, however, required, as to date, subjects are typically required to wear both a motion sensor (sometimes two: one on the upper body and one on the lower body) and a heart rate monitor, making the measurement process more cumbersome for the subject.
1.3.7. Physical activity questionnaires (PAQ)

In many instances, the only option for measuring physical activity levels or physical activity energy expenditure in large population-based studies is to administer physical activity questionnaires (PAQ). The techniques, previously described, have often been used to validate questionnaires designed to measure physical activity energy expenditure and provide some indication as to the limitations in interpreting data derived from their use. The advantages of the PAQ method include: 1) it can be administered over the telephone, 2) typically require between 15-20 minutes of the interviewee’s time, 3) may be self-administered, and 4) may be widely administered to large and diverse groups, and further translated into a home language.

For example, Ainsworth et al. (6) found that the Tecumesh Occupational Activity Questionnaire (TOQ) and the modified Stanford 7-day recall questionnaire (7-day recall) accounted for approximately 53% of the variance in light and moderate intensity occupational physical activity, when compared to activity recorded during motion sensing. In addition, total occupational activity energy expenditure using the TOQ and 7-day recall accounted for approximately 21% of the variance in total occupation activity energy expenditure measured using motion sensing. The 7-day recall requires about 15-minutes of an individual’s time to conduct the interview and information relating to their physical activity frequency and intensity during the previous 7 days is obtained (78). It includes information pertaining to occupational physical activity habits, moderate-intensity recreational activities, vigorous-intensity recreational activities and strength/toning activities (6). Subjects are required to estimate the number of hours spent in the different activities over a 7-day period. Accordingly MET values are assigned to each of the categories, and
used to estimate physical activity energy expenditure as kcal.day\(^{-1}\), which is averaged over a 7-day period to obtain the average daily physical activity energy expenditure (78).

Leenders et al. (78) compared the 7-day recall to the DLW, during 7 days of measurement, in 13 healthy women. During the 7 days of free-living activity measurements subjects also wore three motion sensors, a uniaxial and triaxial accelerometer as well as a step counter. Results showed that group estimates for physical activity energy expenditure estimated using the 7-day recall (2700 ± 147 kJ.day\(^{-1}\)) were not statistically different to the group estimates obtained from the doubly labelled water method (3400 ± 349 kJ.day\(^{-1}\), R\(^2\) values not given). These data were, however, subject to significant intra-subject variability, most apparent in the subjects who were classified as very active or sedentary. This variation was attributed to a possible under-reporting of moderate intensity physical activity, with the greatest variability seen in those individuals who either classified as being very active or very sedentary. Physical activity energy expenditure estimated using the three motion sensors significantly (P<0.05) underestimated that measured using the doubly labelled water technique (between 35-59%). This study highlights the potential accuracy of a 15-minute PAQ for estimating physical activity energy expenditure.

Another study, by Richardson et al. (117) investigated the utility of the 7-day recall to assess habitual physical activity over a one-month period. In this study, 77 participants completed two 7-day recall records, physical activity records, motion sensing, maximal oxygen uptake test and had their body fat percentage determined. The physical activity energy expenditure estimated using the 7-day recall yielded an R\(^2\) of 0.33 for men, and 0.10 for women, when regressed against total physical activity energy expenditure measured using the motion sensor. In addition, measurements for very hard activity were only significantly associated in the men (R\(^2\)=0.19). While physical activity energy expenditure
using the 7-day recall and measurements of body fat % were related only in the women. It was concluded that the 7-day recall was perhaps more suited to measuring vigorous than light physical activities. Also apparent inconsistencies between findings for the men and women, raise questions to the ultimate validity of the 7-day recall.

A study by Washburn et al. (149) was unable to account for physical activity energy expenditure using the 7-day recall method compared to the doubly labelled water technique and further showed inaccuracies in the measurement technique for individuals reporting higher levels of physical activity. A group of 46 men and women were measured using the doubly labelled water technique and the 7-day recall, over 14 days. The total daily energy expenditure estimated using the 7-day recall accounted for 34% of the variance of the energy expenditure measured using the doubly labelled water technique. However, the two estimates were not significantly correlated. An Altman and Bland plot (16) indicated that the magnitude of the underestimation increased as the subjects reported increased levels of activity.

A PAQ, which has been embraced by the international community, is the International Physical Activity Questionnaire (36). Indeed it is impossible to conduct large multi-cultural and multi-country studies, for the surveillance of physical inactivity on a global scale, without a single, validated instrument. The IPAQ was developed, with short and long versions of the questionnaire, which can be administered by telephone or can be self-administered. The short IPAQ assesses 9 items, and provides information as to the amount of time spent walking, in vigorous- and moderate-intensity activity and sedentary activity, while the long IPAQ is comprised of 31 items, collecting detailed information within the categories of household and yard work activities, occupational activity, transport, and leisure time physical activity as well as sedentary activities.
Craig et al. (36) reported on the reliability and validity of the IPAQ, during an investigation conducted in 14 centres, spanning 12 countries during 2000. The study was conducted over a 3- to 7-day period. Participants were contacted twice, and either completed the long or short version of the IPAQ. During the second contact the same version was repeated. During the validity part of the study, participants completed the same protocol, in addition to wearing a uniaxial motion sensor and recording basic subject characteristics (height and weight).

Results from this study indicated good test-retest reliability for the long version of the IPAQ, with an $R^2=0.64$. With regard to physical activity intensity, it was found that moderate intensity activity was not as well recalled as vigorous activity, however these differences did not impact upon the physical activity estimates. The reliability data from the short version of the IPAQ was not as high as the long version, but still thought to be acceptable ($R^2=0.47$). The short version also had good test-retest reliability ($R^2=0.49$) for sitting time, and physical activity energy expenditure compared favourably with that reported using the long version ($R^2=0.44$). For ease of administration, it is therefore preferable to administer the shorter version. This study is important, as it is one of the first attempting to provide a single standard for global health assessment, and provides an accurate tool for population-based surveillance. While the IPAQ is perhaps not as accurate as the indirect calorimetry method, or any of the other methods such as the doubly labelled water technique (102), heart rate monitoring (62,114,136) or accelerometry (23), it does provide a general overview of physical activity status of a population. And further is not subject to other environmental influences, which may effect the estimation of energy expenditure.
The above section therefore illustrates, that while there are many physical activity questionnaires available, careful selection should be made when choosing a physical activity questionnaire as a result of increased variability in the technique, when compared to other more sensitive measures of physical activity energy expenditure, such as the doubly labelled water. Further, the importance of validating PAQ's specifically for the population on which the PAQ is to be used, prior to measurement period. It important to acknowledge, that while PAQ's only provide an estimate of energy expenditure, that may not be as accurate as techniques such as the doubly labelled water technique, much of their usefulness lie in categorising large groups of the population, particularly in large population-based epidemiological research, investigating possible dose-response issues (161). For estimates of free-living energy expenditure that are more accurate, other indirect measures, such as the doubly labelled water technique, heart rate monitoring and motion sensing, should probably be used.
1.4. LITERATURE CONCLUSION

Free-living energy expenditure measurement has significantly progressed since the first studies conducted over 200 hundred years ago in Paris. The need for accurate energy expenditure measurement, in light of the now recognised dose-response relationship, for the health benefits of physical activity and the need to quantify the amount of exercise that is sufficient for the attainment and maintenance of good health is certainly fundamental.

The gold standard of energy expenditure measurement, the respiration chamber, is the technique of choice, however, it has little practical value for population-based epidemiological research, as a result of being demanding on both subject and investigator, and further removing the subject from their natural environment and placing them within the confines of a small room. As a result, numerous other techniques have been investigated, and include such techniques as the doubly labelled water technique, heart rate monitoring, motion sensing, physical activity questionnaires as well as various combinations of these.

The doubly labelled water technique provides accurate estimates of free-living energy expenditure, however with respect to the day-to-day variability and nature of physical activity energy expenditure, the interpretation of the results is limited and as a result, indirect tools such as the heart rate monitors and motion sensors have been evolved. Recent developments using the heart rate monitoring technique, for energy expenditure estimation, have moved away from individual subject calibration and introduced the concept of group-generated energy expenditure equations. These developments have, therefore, moved the subjects away from the laboratory and resulted in large studies being conducted in the subjects' own environment. However, the accuracy of these equations for total daily
energy expenditure measurement remains in question, as most of the studies have been conducted on subjects completing a single physical activity bout and do not include data with regard to the total daily energy expenditure.

Other techniques that also provide information as to the nature of the day-to-day variability include the motion sensing technique and physical activity questionnaire. Motion sensing, as with the heart rate monitoring technique, has undergone significant developments over the last several years, and from previously only being able to measure motion in an uniaxial plane, now provide information in the 3-dimensional planes, providing significantly improved estimates of physical activity energy expenditure. Similarly, physical activity questionnaires, while not providing as accurate an estimate of total energy expenditure as the previously mentioned methods, have also experienced recent improvements over the last couple of years, and while still reliant on a participant’s subjective perceptions, provide insight into the physical activity status of an individual.

All the above techniques provide estimates of total daily energy expenditure and physical activity energy expenditure, which vary not only in the accuracy of the estimates but also in the ease of administration of the technique. These energy expenditure estimates in turn provide pertinent information relating to the dose-response relationship and how much exercise is enough for good health.
1.5. AIMS AND OBJECTIVES

Accordingly, the aims and objectives of this thesis were to investigate indirect measures of free-living energy expenditure, with special reference to physical activity energy expenditure. Further, this thesis aimed to develop and improve upon existing techniques, in an attempt to make the measurement of free-living energy expenditure more accessible and cost-effective in population-based studies. This thesis also undertook to construct and put into operation a respiration chamber, in order to further validate and improve upon other existing energy expenditure measurement techniques. As a result, the specific objectives of this thesis were to:

1. Examine the current use of the heart rate monitoring technique, utilising individual subject calibration, and its usefulness in estimating total daily energy expenditure for both training and population-specific studies.

2. Explore the factors which impact upon the relationship between heart rate and energy expenditure, in an attempt to improve the estimation technique and further use these significant variables in developing group generated energy expenditure equations, for use during physical activity.

3. Explore the significant factors which impact upon the relationship between heart rate and energy expenditure during activities, which are reflective of the nature of the activities of daily living, and intermittent in nature. Further validate these equations in a newly constructed respiration chamber.
Finally, this thesis will explore the applicability of this equation in a setting, where cardiovascular function is altered and there assess the suitability and accuracy of these group generated equations in a unique setting.
CHAPTER TWO

FREE LIVING ENERGY EXPENDITURE, MEASURED USING HEART RATE MONITORING, IN RESPONSE TO TRAINING IN PREVIOUSLY SEDENTARY POSTMENOPAUSAL WOMEN

This chapter has been published in part:

INTRODUCTION

There is evidence to suggest that exercise training impacts on the maintenance of energy balance. This effect may be mediated through changes in fat-free mass or muscle mass and concomitant changes in resting metabolic rate. For example, Poehlman et al. (106) found that an 8-week exercise-training programme increased the resting metabolic rate (RMR) in a group of 18 elderly (mean age 66.1 years), healthy individuals by 7%. However, in other studies of older adults initiating exercise programmes, the effects on energy balance and total daily energy expenditure (TDEE) have been equivocal.

Meijer et al. (91) found that a 12-week moderate intensity physical activity programme failed to increase the non-training physical activity energy expenditure of 15 older adults (mean age 59 yrs), as measured using accelerometry. Subjects wore a tri-axial accelerometer for 14 days, pre and post the intervention period and physical fitness was measured during an incremental exercise test. The failure to increase total daily physical activity energy expenditure occurred, despite an overall increase in physical fitness and a subsequent lowering in the submaximal heart rate, at the same absolute workloads, pre and post the exercise intervention. Similarly, Goran et al. (54) reported that an eight week endurance training programme in older persons, consisting of three cycling sessions that progressively increased in intensity each week, was not associated with an increase in total energy expenditure, measured using the doubly labelled water method, as there was a compensatory decline in the energy expenditure for activities other than the exercise training. Conversely, Withers et al (164) found that aerobic training in a group of 24 women, aged 49-70 years old, resulted in an increase in the TDEE, as measured with doubly labelled water. This increase in energy expenditure was attributed to the physical activity itself and was not associated with a concomitant increase in resting energy expenditure. In
a review on older persons initiating exercise programmes, studies suggest that moderate exercise may be more effective in increasing TDEE than vigorous activity (104).

The aim of this study was, therefore, to determine the effect of an 8-week walking/running programme on 24hr free-living energy expenditure, estimated using heart rate monitoring, in previously sedentary postmenopausal women. This is particularly relevant as heart rate responses have been shown to be attenuated at the same workload, and training may therefore, impact on the individual heart rate-energy expenditure calibration.
METHODS

Experimental design

Postmenopausal women were selected for the study. Subjects were excluded if they had exercised regularly, more than twice a week, in the preceding 6 months. Subjects were included if they were on hormone replacement therapy, on condition that they continued to use the medication for the duration of the study. The Ethics and Research Committee of the University of Cape Town School of Health Sciences approved the study.

Nine subjects, who met the inclusion criteria, and who were new participants in a commercial walk/run exercise programme that met 3 times a week for 8 weeks (EX), volunteered for the study. Each group exercise session began at a designated time, with a warm-up, consisting of a 500m walk, followed by supervised stretching exercises. Participants initially began walking 3 km on a sports field and then progressed to walking or jogging between 3-6 km on the road. Subjects were encouraged to exercise at an intensity of 70-75% of their age-predicted maximum heart rate (220-age), during each session, and individuals could choose the length of their session once they progressed to the road route, i.e. between 3–6 km. Once the participants had progressed to walking or jogging on the road, they were no longer supervised directly by the exercise leader.

Ten subjects were selected for a control group (CON), from volunteers recruited by an advertisement in the local media and by the investigator. They were instructed not to undergo any major lifestyle changes for the duration of the study including any changes in physical activity. All subjects were tested before any intervention and again after eight weeks. They were offered access to the walk-run programme on completion of the study.
Body composition

Subcutaneous body fat was expressed as the sum of 7 skinfolds (biceps, triceps, subscapular, suprailiac, anterior thigh, abdominal and medial calf) and was also converted to body fat percentage using the equations of Durnin and Womersley (44).

Total daily energy expenditure estimated using heart rate monitoring technique

Total daily energy expenditure (TDEE) was estimated from individually determined heart rate-energy expenditure regression equations (81) and 24hr heart rate monitoring (Vantage XL, Polar Electro, Finland). Resting metabolic rate was measured early in the morning, in after an overnight fast. Subjects rested for a minimum of 30 minutes in the supine position, before resting oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured using an open-circuit ventilated hood technique as previously described (99). Briefly, subjects placed their head within a clear, dome shaped ventilated hood. A flap located at the opening of the hood, covering the neck and shoulders of the subject, ensured unidirectional airflow. Room air was drawn through the hood by a suction pump at a flow rate of approximately 38–40 l.min⁻¹. The flow rate was calibrated using a VMM Series ventilation measurement module prior to each test. Air expired by the subject became diluted in the ambient flow, and was directed via a drying cylinder containing “Drierite” to an Ametek S 3A/1 O₂ analyser (Ametek, Germany) and Ametek CD–3 CO₂ analyser (Ametek, Germany) for respective O₂ and CO₂ analysis. Both analysers were calibrated using analytical grade gasses (5% CO₂, 95% N₂). All subjects were familiarised with the ventilated hood before the start of the study. Mean VO₂, VCO₂ and respiratory exchange ratio (RER) were collected for 30 min, data were analysed from the last 10-minutes of data collection in
the resting state (99). The heart rate-energy expenditure (HR-EE) relationship was calculated for each subject. This relationship was determined by measuring heart rate, VO$_2$ and VCO$_2$ simultaneously for 7 to 8 workloads of increasing intensity, each lasting 4–5 minutes. Energy expenditure (kJ) was determined for each workload, using the equations of Weir (150). During the test the subjects wore a facemask attached to an Oxycon Alpha automated gas analyser (Jaeger, The Netherlands). Before each test the gas analyser was calibrated by using a Hans Rudolph 5530 3-litre syringe and a 5% CO$_2$–95% N$_2$ gas mixture. Analyser outputs were processed by a computer, which calculated breath-by-breath ventilation, oxygen consumption (VO$_2$), rates of carbon dioxide production (VCO$_2$) and respiratory exchange ratio (RER), using conventional equations (150). In the first 3 stages of the protocol, subjects were tested in the supine resting position, followed by quiet sitting and subsequently standing. Following these resting measurements, the subject performed 5 progressive stepping exercises, beginning at a step height of 30 cm, at 80 steps per minute, increasing to a step height of 50 cm at 100 steps per minute.

The heart rate-energy expenditure calibrations were carried out approximately 90-minutes after the ingestion of a light meal, typical of the subject’s self-selected breakfast, following the measurement of RMR. Individual heart rate-energy expenditure calibration curves were then calculated for each subject using a non-linear regression equation (81). This equation was as follows:

\[ Y = A + \left( \frac{B}{1 + \text{Exp}(C-(D \times \text{heart rate}))} \right) \]

Where: \( Y \) = energy expenditure (kJ. min$^{-1}$), \( A \) = actual resting metabolic rate (kJ. min$^{-1}$), entered as a constant for each individual while \( B \), \( C \) and \( D \) were derived from the regression equation.
Each subject wore a heart rate monitor that recorded heart rate every minute for 24hr under free-living conditions. The 24hr free-living TDEE was determined using the minute-by-minute heart rate values and predicting energy expenditure from the individual heat rate-energy expenditure (81).

**Treadmill test: time to fatigue and VO_{2}max**

Maximal oxygen consumption (VO_{2}max) using the Bruce Multistage Treadmill protocol (2) was determined after the HR-EE calibration test, on the same day, after a minimum of a 45-minute rest. During the treadmill test, respiratory exchange parameters were measured using the Oxycon Alpha automated gas analyser (Jaeger, The Netherlands) and analyser outputs were calculated as previously described in text. Peak treadmill running speed (PTRS) and the time to fatigue was determined from the workload at which the subject could no longer maintain the pace of the treadmill. Heart rate was recorded and oxygen consumption was measured at the end of each minute.

The day after 24hr heart rate was monitored, each subject completed a dietary recall for the previous 24hr. A registered dietician administered the food recall. Diets were analysed using the computer package Food Fundi professional (Penta Medical Systems). Total energy intake (TEI) (kJ), total carbohydrate (CHO), protein, fat and alcohol (g and % of energy) were calculated. At the start of the trial, each subject was given a daily activity diary (Appendix 10.1 and 10.2) in which she had to record the hours spent in 5 different levels of activity: sleep, sedentary activity (e.g. sitting at a desk and watching television), moderate (e.g. brisk level walking and housework), very active (e.g. brisk uphill walking and stair climbing) and extremely active (e.g. running and other aerobic exercises). The subject was
required to record the number of hours spent in each category of activity, each day. The
activity diaries were started once the subjects had completed the pre-intervention testing
and completed before the post-intervention testing. The activity diaries did not include the
days on which 24hr heart rate monitoring took place.

All the tests were repeated twice following the eight-week intervention period. The EX
subjects were tested on an exercise day and a non-exercise day within a 7-day period and
the control subjects were tested on two days within the same period.

Statistical analysis

Results are expressed as means ± standard deviations (SD). An analysis of variance
(ANOVA) with repeated measures was performed to determine significant differences
between the CON and EX with respect to the pre-test (pre), post-test 1 (exercise for EX) and
post-test 2 (non-exercise day for EX). A non-parametric Kruskall Wallis was used to analyse
differences in self-reported levels of physical activity. Statistical significance was accepted
at an alpha level of P<0.05.
RESULTS

Subject characteristics

Subject characteristics are shown in Table 2.1. The average age of the subjects in the EX group was 58 ± 7 years and for the CON group, 55 ± 5 years. Starting body mass ranged from 58.0 to 83.0 kg, (68.0 ± 9.1 kg), in the EX group and 54.0 to 90.0 kg, (72.0 ± 12.3 kg), in the CON group. Body fat percentages ranged from 28% to 37%, (33 ± 3%), in the EX and from 28% to 40%, (34 ± 4%) in the CON. There were no significant changes in body mass, body fat and sum of 7 skinfolds over the 8 weeks in either group (Table 2.1).

Maximal and submaximal exercise performances

Maximal oxygen consumption (VO$_2$max), maximum heart rate and time to fatigue during the treadmill test, before and after the 8 weeks are shown in Table 2.2. There were no significant differences for VO$_2$max or maximum heart rate, either between groups or between the pre and post measurements. The time to fatigue improved similarly in both the EX and CON groups (P<0.0005). The heart rate response at the end of each of the last 2 submaximal workloads during the heart rate-energy expenditure calibration test was significantly attenuated in the EX group after 8 weeks (Table 2.2). Heart rate decreased from 129 ± 16 beats.min$^{-1}$ to 115 ± 12 beats min$^{-1}$ before and after training in EX in submaximal workload 6 (30 cm step/100 steps.min$^{-1}$) and did not change significantly for the CON group (CON 112 ± 15 beats min$^{-1}$ versus 108 ± 11 beats min$^{-1}$) (P<0.001). During submaximal workload 7 (50 cm step/80 steps.min$^{-1}$), EX group significantly decreased their heart rate (140 ± 19 beats.min$^{-1}$ to 126 ± 16 beats.min$^{-1}$, (P<0.003) and the heart rate in the CON group did not change (122 ± 16 beats.min$^{-1}$ versus 119 ± 13 beats.min$^{-1}$).
Total daily energy expenditure and resting energy expenditure

The total daily energy expenditure, resting metabolic rate, resting respiratory exchange ratio and the sum of the 24hr heart rates for pre and post training are presented in Table 2.3. There were no significant differences in any of these measurements either between CON or EX or before or after the 8 weeks of exercise training.

Day-to-day variability in energy expenditure in non-exercising control subjects (N=10) was determined on the two non-exercising days. Total heart rate over 24hr correlated reasonably well between the two control days (r=0.71, P<0.02) and the coefficient of variation was 6.4%. However, when this was converted into TDEE using the individual regression equations, there was a great deal of variability between the two control days (r=0.31, P<0.383) and the coefficient of variation was 23.1%. We also calculated the limits of agreement for TDEE measured on two non-exercising control days, using the Altman and Bland technique (16). This is represented in figure 2.1, where the X-axis represents the average of the TDEE for the 2 CON days, and the Y-axis represents the deviation between the individual scores for the TDEE on the 2 CON days. The mean difference in estimated TDEE measured in sedentary women measured on two separate days was 11198 ± 3620 kJ.d⁻¹. The limits of agreement (mean ± 2 SD) were between 7966 and -6516 kJ.d⁻¹. These results suggest that daily energy expenditure estimated from heart rate is highly variable in these women.
24Hr heart rate monitoring heart beats

The heart rate data is shown in Table 2.3. There were no consistent increases in the amount of time subjects spent over 100 bpm or 120 bpm on the exercise day, compared to the non-exercise day in response to exercise training in the EX (164 ± 89 bpm versus 166 ± 243 bpm).

Reported food energy and nutrient intake

The food energy and nutrient intakes are shown in Table 2.4. Although there was a tendency for total energy intake to be lower on the non-exercising day compared to the exercise day after 8 week in the EX (8.3 ± 2.8 MJ versus 8.8 ± 1.4 MJ), this was not significant.

Activity diaries

Subjects in EX reported significantly greater levels of physical activity than CON for the categories of, "active" (P<0.000) and "very active" (P<0.0001) after exercise intervention. However, the EX subjects also reported greater levels of "sedentary" behaviour following the exercise intervention (P<0.02).
Table 2.1. Subject characteristics for body mass (kg), the sum of 7 skinfolds (mm) and the % body fat for the CON group (N=10) and the EX group (N=9) before and after the 8-wk exercise intervention.

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<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>72.0 ± 12.3</td>
<td>71.3 ± 12.6</td>
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<tr>
<td>Sum of 7 skinfolds (mm)</td>
<td>141.2 ± 38.6</td>
<td>137.7 ± 39.9</td>
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<tr>
<td>Body fat (%)</td>
<td>34.0 ± 4.0</td>
<td>33.9 ± 3.6</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
<td>26.6 ± 4.3</td>
<td>26.3 ± 4.2</td>
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Table 2.2. \( \text{VO}_2 \text{max}, \) maximum heart rate and time to fatigue (TTF) and submaximal heart rates for workloads 6 and 7 for CON group (N=10) and EX group (N=9) before and after the 8-week exercise intervention.

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<td>( \text{VO}_2 \text{max} \text{ (l.min}^{-1}) )</td>
<td>1.96 ± 0.23</td>
<td>1.99 ± 0.24</td>
<td>1.87 ± 0.42</td>
<td>1.94 ± 0.32</td>
</tr>
<tr>
<td>Max HR (bpm)</td>
<td>159 ± 17</td>
<td>154 ± 17</td>
<td>166 ± 14</td>
<td>162 ± 9</td>
</tr>
<tr>
<td>TTF (min)</td>
<td>7.87 ± 0.97</td>
<td>8.39 ± 1.29</td>
<td>7.30 ± 172</td>
<td>8.40 ± 1.62( ^Y )</td>
</tr>
<tr>
<td>Submaximal HR</td>
<td>112 ± 15</td>
<td>108 ± 11</td>
<td>129 ± 16</td>
<td>115 ± 13*</td>
</tr>
<tr>
<td>workload 6 (bpm)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Submaximal HR</td>
<td>122 ± 16</td>
<td>119 ± 13</td>
<td>140 ± 19</td>
<td>126 ± 15*</td>
</tr>
<tr>
<td>workload 7 (bpm)</td>
<td></td>
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</tbody>
</table>

\( ^Y \) Time to fatigue (TTF) significantly improved for pre- and post-tests for both CON and EX groups, (\( P<0.0005 \)).

* Submaximal heart rate significantly decreased in the EX group, pre- and post- exercise intervention, for Submaximal workload 6 (\( P<0.001 \)) and Submaximal workload 7 (\( P<0.003 \)).
Table 2.3. Total daily energy expenditure (TDEE), resting metabolic rate (RMR), resting respiratory exchange ratio (RER), the total 24hr heart beats (24hr HR) and actual time spent at a heart rate above 100 bpm and 120 bpm over 24 hours, for the CON group (N=10) and the EX group (N=9) before and after the 8 week exercise intervention. Post 1 represents an exercise day for EX group and post 2 represents a rest day. RMR decreased in the exercise group on the post non-exercise day, and increased in the control group on the post non-exercise day, (P<0.03).

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post 1</td>
<td>Post 2</td>
<td>Pre</td>
<td>Post 1</td>
<td>Post 2</td>
</tr>
<tr>
<td>TDEE (MJ.day⁻¹)</td>
<td>11.6 ± 2.0</td>
<td>10.7 ± 3.3</td>
<td>11.4 ± 3.0</td>
<td>11.0 ± 3.4</td>
<td>10.6 ± 3.0</td>
<td>11.2 ± 2.6</td>
</tr>
<tr>
<td>RMR (kJ.kgFFM⁻¹.day⁻¹)</td>
<td>134.2 ± 9.4</td>
<td>137.1 ± 12.9</td>
<td>136.9 ± 15.0</td>
<td>138.4 ± 6.4</td>
<td>142.1 ± 11.0</td>
<td>140.7 ± 14.2</td>
</tr>
<tr>
<td>Resting RER</td>
<td>0.91 ± 0.05</td>
<td>0.90 ± 0.03</td>
<td>0.90 ± 0.04</td>
<td>0.90 ± 0.07</td>
<td>0.89 ± 0.06</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>PAEE (TDEE-sleep EE)</td>
<td>9728 ± 1860</td>
<td>8942 ± 3436</td>
<td>9546 ± 2740</td>
<td>9217 ± 3321</td>
<td>8616 ± 2876</td>
<td>9409 ± 2637</td>
</tr>
<tr>
<td>24hr heart rate (beats)</td>
<td>110808 ± 12574</td>
<td>107880 ± 12231</td>
<td>107366 ± 12864</td>
<td>110188 ± 9219</td>
<td>114213 ± 11351</td>
<td>114590 ± 12750</td>
</tr>
<tr>
<td>Heart rate above 100bpm</td>
<td>123 ± 96</td>
<td>116 ± 119</td>
<td>83 ± 124</td>
<td>164 ± 122</td>
<td>164 ± 89</td>
<td>166 ± 243</td>
</tr>
<tr>
<td>HR above 120 bpm (min)</td>
<td>23 ± 26</td>
<td>21 ± 23</td>
<td>23 ± 47</td>
<td>31 ± 37</td>
<td>37 ± 28</td>
<td>20 ± 47</td>
</tr>
</tbody>
</table>
Table 2.4. Total energy intake (TEI) and the energy contributions of CHO, protein, fat and alcohol for the CON group (N=10) and EX group (N=9) before and after the 8 week exercise intervention. Post 1 represents an exercise day for the EX and post 2 represents a rest day.

<table>
<thead>
<tr>
<th>Variables</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post 1</td>
<td>Post 2</td>
<td>Pre</td>
<td>Post 1</td>
<td>Post 2</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TEI (MJ/d)</td>
<td>7.9 ± 2.2</td>
<td>8.2 ± 2.5</td>
<td>8.2 ± 2.2</td>
<td>9.4 ± 1.6</td>
<td>8.8 ± 1.4</td>
<td>8.3 ± 2.8</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% CHO</td>
<td>45 ± 11</td>
<td>46 ± 9</td>
<td>50 ± 14</td>
<td>49 ± 9</td>
<td>48 ± 5</td>
<td>47 ± 9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Protein</td>
<td>15 ± 3</td>
<td>18 ± 5</td>
<td>15 ± 3</td>
<td>15 ± 2</td>
<td>14 ± 3</td>
<td>17 ± 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Fat</td>
<td>39 ± 11</td>
<td>34 ± 7</td>
<td>35 ± 10</td>
<td>35 ± 11</td>
<td>38 ± 5</td>
<td>38 ± 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Alcohol</td>
<td>4 ± 5</td>
<td>5 ± 8</td>
<td>4 ± 4</td>
<td>7 ± 7</td>
<td>5 ± 3</td>
<td>5 ± 5</td>
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</tbody>
</table>
**Figure 2.1.** Represents the Altman and Bland technique for the limits of agreement between the TDEE, as measured on the two post days for the CON. The X-axis represents the average TDEE, between the two post days, while the Y-axis represents the deviation between the two post days.
DISCUSSION

The first finding from this study was that exercise training, 3 times per week, at low to moderate intensity, was sufficient to induce an increased efficiency during submaximal exercise in the absence of any improvements in maximal physical performance. This conclusion was based on the lower heart rate at the same absolute workloads after training of the exercising group. This increased efficiency was however, not linked to changes in body composition or fat free mass, nor to increases in maximal work capacity.

This finding has previously been reported by Dressendorfer et al. (43) where it was found that submaximal exercise efficiency improved following a moderate intensity exercise programme, but not overall maximal exercise performance. In the study by Dressendorfer et al. (43), the submaximal heart rate improvement is attributed to habituation or improved external work efficiency, and not aerobic conditioning where the heart rate becomes lower at the same oxygen consumption. On the other hand, a number of previous studies have found improvements in both submaximal and maximal exercise performance following endurance training (162;163;165). Most notably, Wilmore et al. (162), found that following a 20-week endurance training program, heart rates during exercise were significantly reduced at the same absolute workloads. These changes in heart rate were subject to differences in age, gender and race. In another study, Poehlman et al. (106) also found significant changes following endurance training in a group of elderly subjects. Following an 8-week endurance training programme, there were significant increases for resting metabolic rate and VO₂max (7 and 11%,
respectively) in the subjects. In addition, it was shown that fat oxidation was increased by a further 22% at rest, in the fasted state (105). In the current study, there were no measurable changes in the subjects' resting metabolic rate, or respiratory exchange ratio, as a consequence of exercise training.

Our study suggests that in previously-untrained, postmenopausal women who begin a programme of low to moderate intensity exercise training, there may be no changes in total daily energy expenditure, energy balance, resting metabolic rate or the energy expenditure associated with physical activity. When we further examined the amount of time (min) spent at heart rates above 100 bpm, or 120 bpm, during a 24hr period, responses were variable. There were no consistent increases in the amount of time subjects spent over 100 bpm on the exercise day, compared to the non-exercise day in response to exercise training. This lack of increase in heart rate may be attributed, in part to compensation, with an increased time spent in sedentary activities, or an increased energetic efficiency on the exercise day.

These results are similar to those reported by Goran et al. (54), who found that 24hr free-living energy expenditure is not affected or enhanced by a moderate 8-week exercise training programme, in healthy elderly individuals, measured using the doubly labelled water technique. However, Goran et al. (54) attributed this lack of increase in the TDEE to compensatory increases in the energy content of the diet and a lower level of physical activity, during the non-exercise part of the day. This assumption cannot be made with the present study as there were no significant differences between the pre-
and post- measures of diet intake, while there were statistically significant increases in the reported time spent in sedentary activity, as measured with the training diaries.

In another study of 13 elderly women (mean age 73 years), Rutgers et al. (121) found that energy expenditure estimated using 24hr heart rate monitoring and individual calibration curves was not significantly correlated with the total daily energy expenditure, as measured by indirect calorimetry and as calculated from a physical activity questionnaire. This suggests that in older individuals, there may be greater day-day individual variability, or alternatively, there are less measurable differences in heart rate during exercise activity compared to activities of daily living. However, Meijer et al. (91) also failed to show any increases in daily physical activity after an eight-week moderate intensity training programme, measured using tri-axial accelerometers.

In the present study, the lack of increase in total daily energy expenditure with training may be attributed to a number of factors. Firstly, there was a high degree of intra-subject and day-day variability between the two tests (CV=23%), and the limits of agreement on the 2 post days for TDEE for the controls vary between 7966 and -6516 kJ. These factors may have masked possible changes in energy expenditure associated with training.

For example the heart rate-energy expenditure method has previously demonstrated a day-day coefficient of variation of 15% for TDEE using the heart rate monitoring method in validating the protocol on eight fit, healthy men (Van den Oever, unpublished observations (148)). Ceesay et al. (29) validated the technique in a group of volunteers
(mean age 25 years), and found a significant correlation between the heart rate method and indirect calorimetry method. In that study, they found that the heart rate method provided individual estimates of energy expenditure that were within 10% of the calculated indirect calorimetry method. However, Ceesay did find that there was much inter-individual error in the estimation of energy expenditure when dealing with heart rates below the highest average heart rate during standing and the lowest heart when vigorously exercising (also called flex heart rate). This may be problematic for individuals who spent the majority of their day in heart rates below the flex heart rate.

It was for this reason that the current study used a logistic function to predict energy expenditure from heart rate, based on the actual measured resting energy expenditure. Li et al. (81) showed that the intra-individual coefficient of variation using this technique was between 11-20%. The coefficient of variation was significantly lower when 18 activities were included compared to the nine activities, 10.6% versus 20.4%. Therefore, the variability in energy expenditure in the present study may have been higher as a result of fewer workloads used in the calibration test used to calculate the heart rate-energy expenditure equations.

Other studies (31;88) have shown a significant day-to-day variability in the establishment of the individual heart rate-energy expenditure calibration equations. In a study by Christensen et al. (31), the individual heart rate-energy expenditure relationship was investigated on 2 consecutive days, followed by 2 days of 24hr heart rate monitoring, for energy expenditure estimation using the first and second days calibration models respectively, in 17 subjects. In this study it was found that duplicate
estimates (day 1 versus day 2) of energy expenditure were highly variable, with only 7 out of the 17 subjects obtaining duplicate measures for energy expenditure that were within 20%. The high variability was attributed to the large differences between the slopes and intercepts between the two calibration models and subsequent energy expenditure estimation using accumulated heart rate. In another study by McCrory et al. (88) calibration tests were performed on 2 consecutive days, during both the morning and afternoon, followed by 24hr heart rate monitoring, in 12 subjects. The heart rate derived energy expenditure estimates were nearly identical for the group, however as was found in Christensen et al. (31) there were also significant differences (P<0.005) for the slopes and intercepts, on different days, and further during the morning and afternoon sessions. It was concluded that heart rate monitoring derived estimates of energy expenditure were therefore only acceptable for group application.

Therefore, from the present study, we can conclude that exercise training in previously sedentary, postmenopausal women was not associated with changes in total daily energy expenditure, measured using the HR monitoring technique. These findings are supported by previous studies in older adults, demonstrating “compensation” for increased exercise energy expenditure by overall lower energy expenditure during non-exercise time. However, this failure to demonstrate an increase in total daily energy expenditure may also be due to the large degree of intra-individual variability in the 24hr method for estimating energy expenditure, as well as the self-selected nature of the exercise programme and intensity. These findings provide methodological insights into quantification of the effects of exercise training on energy expenditure, as well as insights into guidelines for exercise prescription in older adults. Exercise training
sufficient to improve submaximal exercise efficiency may not be sufficient to alter body composition or significantly impact on energy balance.

Finally, while we were unable to detect any changes in total daily energy expenditure or physical activity energy expenditure, using the heart rate monitoring technique, possibly as a result of "compensation". This is despite the indirect evidence for training adaptations with changes in submaximal heart rate. This study highlights the potential utility of the heart rate monitoring technique for measuring energy expenditure, but also the limitation of the technique, including a high degree of intra-subject variability, which may impact on the ability to distinguish change, for example, associated with training. Results from this study suggest that further improvements of the heart rate monitoring technique are needed, to enhance its usefulness for quantifying physical activity energy expenditure.
CHAPTER THREE

PHYSICAL ACTIVITY RECALL ON WORKING DAYS: VALIDATION USING 24HR HEART RATE MONITORING AND INDIVIDUAL HEART RATE-ENERGY EXPENDITURE CALIBRATION.
INTRODUCTION

In the previous chapter, we demonstrated that total daily energy expenditure (TDEE), measured using the heart rate monitoring technique, did not change with exercise training in older women. This is despite the fact that women who underwent training reported higher levels of both “active“ and “very active“ physical activity, as well as “sedentary activity“, using activity diaries. These data highlight the need for development and validation of accurate measures of physical activity, in particular, in light of the current international focus on physical activity dose-response and health (74).

Physical activity has been linked to reduced risk for non-communicable diseases, such as: cardiovascular disease, diabetes and hypertension (3;15;63;161). As such there are numerous epidemiological studies, attempting to characterise the amount of physical activity, or dose-response, which may be regarded as protective (63;66;123;161). These studies typically rely on validated measurement tools, which are easy to administer in large population-based studies.

A measurement tool that has received much attention is the physical activity questionnaire (PAQ) (33;103;149). For example, Hawkins et al. (56) examined physical activity in a large cohort of over 40 000 subjects, using 3 different physical activity questionnaires. Using the data from 3 different PAQ's, it was possible to obtain a broad overview of the physical activity of this particular population, and further categorise
physical activity according to intensity, moderate versus vigorous activity. As a result, public health recommendations could be put into place.

However, when compared to other methods for estimating physical activity energy expenditure (PAEE), such as the doubly labelled water technique, motion sensors or the heart rate monitoring technique, PAQ's only explain approximately 20-50% of the variance in measured energy expenditure (6;33;117;149).

For example, a study by Washburn et al. (149) compared the 7-day recall to the doubly labelled water technique (122), in a sample of 17 men and 29 women. Total daily energy expenditure (TDEE) including physical activity energy expenditure was estimated using the 7-day recall, while TDEE was measured using the doubly labelled water. The average TDEE estimated using the 7-day recall accounted for 34% of the variance ($R^2=0.34$, $P<0.01$) in TDEE measured using the doubly labelled water technique. The individual variability ranged from approximately $R^2=0.27-0.49$. Physical activity energy expenditure estimated using the 7-day recall did not differ statistically from the physical activity energy expenditure estimated using the doubly labelled water, however the two estimates did not correlate significantly to each other ($r=0.12$).

Most PAQ's are designed to primarily quantify leisure time physical activity, and while some, for example, the Minnesota Leisure Time PAQ (117) include questions related to household activities, other important physical activity domains, such as occupational and transport physical activity, are often ignored. Physical activity questionnaires which do not include any reference to either occupational or transport physical activity will not
accurately reflect physical activity prevalence, particularly in developing countries. For example, a study by Heini et al. (60) found that men working in a rural African community expended more than an estimated 16 MJ.day\(^{-1}\), and exhibited a physical activity index of more than 2.4 times the basal metabolic rate, when measured using the doubly labelled water technique. Physical activity is considered to be intense when the physical activity index is greater than 2.1. In another study, by Lambert et al. (73), it was found in our South African community that sugar cane workers expended between 12-14 MJ.day\(^{-1}\), using the individual heart rate-energy expenditure calibration technique. The average energy intake over the same period was 5MJ.day\(^{-1}\).

These studies highlight the importance of using accurate measures of physical activity, which encompass all the areas of the activities of daily living. In fact, the recent guidelines for physical activity and health (2), recommend that individuals accumulate 30-minutes of moderate physical activity on most, preferably all days of the week. However, a study by Sparling et al. (134) found that a group of South African men, reporting only low-to-moderate physical activity had the lowest overall coronary risk compared to those engaged in vigorous occupational activity. These results suggest that the questionnaire itself may not have been culturally sensitive, or that men from this community are not protected by vigorous occupational or leisure activity, which seems unlikely (134). This further highlights the need to accurately validate a physical activity questionnaire, if it is to be used in a community or population that is different than the one for which it was originally developed.
To date, studies (70;80) have already used various PAQ's in the South African population; however, their validity is unknown. For example, Levitt et al. (80) used the 7-day recall to investigate prevalence of diabetes and risk factors in a peri-urban community. Results from this work found that individuals who were physically inactive displayed a 70% increased risk, than persons who were active. While Kruger et al. (70) explored the associations between physical activity, along with other measures, and the impact on obesity, in a group of South African women, from a community undergoing transition. Physical activity was measured using a modified version of the Baecke (11) questionnaire. Results from this study indicated that physical activity correlated significantly (negatively) with BMI ($r=-0.135$) and waist circumference ($r=-0.147$), while those displaying the highest levels of physical inactivity, were the most likely to be obese ($P<0.001$).

Therefore, the primary aim of this study was to validate a PAQ, a physical activity recall questionnaire (7-day recall (122)) modified to 5 working days (5-day recall) (Appendix 10.3), using the heart rate-energy expenditure technique, in a group of South African adults. The heart rate monitoring technique currently relies on individual subject calibration, to generate a model for the relationship between heart rate and energy expenditure, as previously described by Li et al. (81) and used in Chapter Two of this thesis. The model is then applied to heart rate data, accumulated using minute-by-minute heart rate monitoring, over the entire measurement period. Using the heart rate-energy expenditure model, Li et al. accounted for 91% of the energy expenditure, measured in a whole room respirometry.
A second aim of this study was to identify the extent to which gender, age, occupation and education affect the error in the reporting of physical activity levels.
METHODS

Experimental design

Hospital employees from a local hospital were recruited to participate in the study. Participants had to be free from all known cardiac and metabolic disorders and not using any medication. The Ethics and Research committee of the University of Cape Town Medical School approved the study.

Fifty-nine hospital employees, ranging from unskilled labourers to medical professionals (women, N=37, men, N=22, age: 33±10 yrs) were interviewed using the modified 7-day recall (122) modified to 5 (working) days only, therefore for the purpose of this study will be called the 5-day recall. The subjects were ranked according to 2 levels of education (level 1= completed high school and level 2 = did not complete high school) and 2 levels of occupational status (level 1 = professional or clerical and level 2 = manual unskilled). The 7-day recall was originally developed for the Stanford Five Cities project (122) and is administered during a 15-20 minute structured interview, as previously described by Washburn et al. (149). Briefly, subjects are asked to recall the amount of time spent in sleep, moderate, hard and very hard activities, on working days (Appendix 10.4), during the previous week. The average amount of time spent in light activity is then estimated as the difference in time spent sleeping and in the previously mentioned categories. PAEE (kJ.day\(^{-1}\)) is then estimated, based on assigning of metabolic (MET) equivalent values to moderate, hard and very hard (3, 6, and 9 METS, respectively).
Body composition and resting metabolic rate estimation

Body fat was expressed as the sum of 7 skinfolds (biceps, triceps, subscapular, suprailliac, anterior thigh, abdominal and medial calf) (119), and also as a percentage (44).

Resting metabolic rate was estimated from fat free mass, using the equation of Ravussin and Bogardus (110).

Energy expenditure estimation using heart rate monitoring

Energy expenditure was predicted from individually determined heart rate-energy expenditure regression equations (81) and 24hr heart rate monitoring (Vantage XL, Polar Electro, Finland). Subjects were asked to avoid any physical activity in the 24hr prior to the measurement of energy expenditure. The heart rate-energy expenditure (HR-EE) relationship was calculated for each subject, as in the previous chapter. Briefly, the HR-EE relationship was determined by measuring heart rate, VO\textsubscript{2} and VCO\textsubscript{2} simultaneously for 7 to 8 workloads, lasting between 4-5 minutes in length, and increasing intensity. Energy expenditure (kJ.min\textsuperscript{-1}) was determined for each workload, using the equations of Weir (150), using the last 2-minutes (steady state) of each workload. Individual calibration curves were then calculated for each subject using a non-linear regression equation (81).
This equation was as follows:

$$Y = A + \frac{B}{(1 + \exp(C - (D \times \text{heart rate})))}$$

Where $Y =$ energy expenditure ($kJ\cdot min^{-1}$), $A =$ actual resting metabolic rate ($kJ\cdot min^{-1}$), entered as a constant for each individual.

Following the individual subject calibration, the subject was required to wear a portable telemetric heart rate monitor (Vantage XL, Polar Electro, Finland), which recorded heart rate and averaged it every minute, for a 24hr period. The subject was required to wear the heart rate monitor on one day during the normal working week. In addition, the subject was instructed to note both the time that they went to sleep and woke up. Further, subjects were briefed in full as to the workings of the heart rate monitor, in the event that it should stop recording, they would be able to restart it. Finally, subjects were issued with a contact telephone number of the investigator, should they require any instruction or have any questions, during the heart rate monitoring period.

The 24hr free-living TDEE was determined using the minute-by-minute HR values and predicting EE from the individual HR-EE calibration curves (81). PAEE was calculated as the delta between the subjects sleep time and total 1440 minutes (24hrs) of heart rate monitoring.
Physical activity energy expenditure estimation using 5-day recall

Following the individual subject calibration, each subject completed a modified 5-day recall questionnaire (Appendix 10.3), administered by an interviewer, for 5 working days only. Subjects were required to estimate to the nearest half hour the amount of time spent in activities of light, moderate, vigorous and very active activities (Appendix 10.4), on working days only, during work and leisure time. The time recorded was then assigned MET values, which represented the ratio of the activity metabolic rate to the sitting resting metabolic rate, which was further converted into kJ.day⁻¹.

Statistics

Results are expressed as means ± standard deviations (SD). Spearman’s rank order correlation coefficients for Non-Parametric data (data were censored, some individuals reported no activity) were used to determine if there were any significant relationships between the variables. Analyses of variance were used to determine differences in the reported levels of activity versus that measured with heart rate monitoring, between gender groups, or occupational groups, or by level of education. Statistical significance was accepted at an alpha level of P<0.05.
RESULTS

Subject characteristics

Subject characteristics are shown in Table 3.1. The 59 subjects (N=37 women, N=22 men) in this study represented a diverse group with body weights ranging from 49 to 127 kg and body fat percentages from 7.6% to 43%. The average age of the women was 33 ± 11 years and for the men was 33 ± 9 years.

The occupations were divided into either a professional or clerical- and manual unskilled-group. Within the women's group, there were 32 professionals or clerical workers and 5 manual unskilled workers, while in the men's group, there were 8 professionals or clerical workers and 14 manual unskilled workers. The educational level was divided into 2 levels, those who had completed high school and those who had not completed high school. Sixteen women had completed high school and 21 had not, and 14 of the men had completed high school, while 8 had not.

Direct validation of physical activity energy expenditure

Physical activity energy expenditure values measured either using the heart rate monitoring technique or the calculated from the 5-day recall are presented in Table 3.2 (means ± standard deviations) for men and women. In Table 3.3., these data are presented by level of education and occupation (professional clerical versus non professional or unskilled).
The average physical activity energy expenditure measured using the heart rate monitoring technique was 10167 ± 3524 kJ.day\(^{-1}\) and for the 5-day recall was 15726 ± 6184 kJ.day\(^{-1}\). The two estimates for physical activity energy expenditure correlated significantly with one another (r=0.36, P<0.05), however, the values for 5-day recall were almost uniformly over-reported. Figure 3.2 presents an Altman and Bland (17) plot of the average energy expenditure measured using the two methods, and the differences (delta) for the 5-day recall method and for the heart rate method physical activity energy expenditure (mean ± 2 standard deviations). The mean difference (heart rate monitoring technique subtract the 5-day recall) was −5559 ± 6276 kJ.day\(^{-1}\), while the limits of agreement were −18111 and 6993 kJ.day\(^{-1}\).

Physical activity energy expenditure measured using the heart rate monitoring technique correlated with moderate and vigorous physical activity energy expenditure (r=0.35, P<0.05), using the 5-day recall, and physical activity energy expenditure measured during non working hours (r=0.39, P<0.05) using the 5-day recall. Physical activity energy expenditure during working hours was not correlated to physical activity energy expenditure measured using the heart rate monitoring technique.

*Influence of gender, occupation or education on reporting of physical activity energy expenditure*

PAEE, using the heart rate monitoring and 5-day recall techniques, are presented for men and women, as well as occupation and education levels, in Tables 3.2 and 3.3.
Men had a significantly higher measured and reported levels of physical activity energy expenditure than women, $P<0.000007$ for PAEE using the heart rate monitoring technique, $P<0.01$ for PAEE using the 5-day recall, $P<0.0007$ for moderate and vigorous PAEE, using 5-day recall and $P<0.0001$ for non-working hours PAEE, using the 5-day recall. However, differences between the two techniques, in terms of measured versus reported PAEE were not significant between men and women, and both over-reported PAEE ($P<0.001$).

Similarly, although persons employed in manual labour had significantly higher levels of reported energy expenditure then those professionally or clerically trained ($P<0.0006$), both groups over-reported energy expenditure ($P<0.001$), and differences between groups were not significant. Results were similar for educational levels.

*Physical activity energy expenditure estimated during working and non-working hours*

The average physical activity energy expenditure measured during working hours, with the 5-day recall was $9063 \pm 4495$ kJ.day$^{-1}$, while the average total energy expenditure estimated during non-working hours was $6663 \pm 3511$ kJ.day$^{-1}$. There were no gender differences for reported physical activity energy expenditure during working hours, however men tended to report more physical activity than women. The average physical energy expenditure reported during working hours was significantly different ($P<0.002$) for occupation, those involved in manual unskilled ($7855 \pm 659$ kJ.day$^{-1}$) work reported a higher level of work time physical activity than those involved with either professional skilled or clerical work ($11606 \pm 956$ kJ.day$^{-1}$). Similarly, the average
physical activity energy expenditure reported during work time was significantly different (P<0.049) for those who had completed high school (8010 ± 775 kJ.day⁻¹) versus those who had not completed high school (10311 ± 843 kJ.day⁻¹).
**Table 3.1. Table of subject characteristics.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (N=37)</th>
<th>Men (N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>33 ± 11</td>
<td>33 ± 9</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>69.0 ± 14.0</td>
<td>78.1 ± 12.7</td>
</tr>
<tr>
<td><strong>Fat free mass (kg)</strong></td>
<td>46.7 ± 6.6</td>
<td>61.2 ± 9.3</td>
</tr>
<tr>
<td><strong>Body fat %</strong></td>
<td>31.0 ± 6.7</td>
<td>18.9 ± 6.0</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school completed</td>
<td>24 (36%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>High school not completed</td>
<td>13 (22%)</td>
<td>14 (24%)</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional skilled or clerical</td>
<td>32 (54%)</td>
<td>8 (14%)</td>
</tr>
<tr>
<td>Manual unskilled</td>
<td>5 (8%)</td>
<td>14 (24%)</td>
</tr>
</tbody>
</table>
Table 3.2. Physical activity energy expenditure estimated using either the heart rate monitoring or 5-day recall methods.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women (N=37)</th>
<th>Men (N=22)</th>
<th>Total (N=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAEE using heart rate monitoring kJ.day⁻¹</td>
<td>8553 ± 2282ᵃ</td>
<td>12881 ± 3615</td>
<td>10167 ± 3524</td>
</tr>
<tr>
<td>5-day recall PAEE kJ.day⁻¹</td>
<td>13709 ± 5328*</td>
<td>19119 ± 6140</td>
<td>15726 ± 6184</td>
</tr>
<tr>
<td>5-day recall moderate and vigorous PAEE</td>
<td>11068 ± 5306ᵇ</td>
<td>16391 ± 6219</td>
<td>13053 ± 6182</td>
</tr>
<tr>
<td>5-day recall total work PAEE kJ.day⁻¹</td>
<td>8369 ± 4194</td>
<td>10231 ± 4834</td>
<td>9063 ± 4495</td>
</tr>
<tr>
<td>5-day recall total non-work PAEE kJ.day⁻¹</td>
<td>5240 ± 2667ᶜ</td>
<td>8889 ± 3687</td>
<td>6663 ± 3512</td>
</tr>
</tbody>
</table>

ᵃ P<0.000001, gender difference
ᵇ P<0.01, gender difference
ᶜ P<0.0001, gender difference

* P<0.0007, gender difference
Table 3.3. Estimated physical activity energy expenditure, according to education and occupation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR monitoring method</th>
<th>5-day recall method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women completed school (N=24)</td>
<td>8463 ± 2501</td>
<td>13794 ± 5451</td>
</tr>
<tr>
<td>Women not completed school (N=13)</td>
<td>8719 ± 1892</td>
<td>13550 ± 5306</td>
</tr>
<tr>
<td>Men completed school (N=8)</td>
<td>13187 ± 3371</td>
<td>15538 ± 5190</td>
</tr>
<tr>
<td>Men not completed school (N=14)</td>
<td>12706 ± 3860</td>
<td>21166 ± 5831</td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women professional skilled or clerical (N=32)</td>
<td>8458 ± 3393</td>
<td>13485 ± 5341</td>
</tr>
<tr>
<td>Women manual unskilled (N=5)</td>
<td>9162 ± 2352</td>
<td>15134 ± 5604</td>
</tr>
<tr>
<td>Men professional skilled or clerical (N=8)</td>
<td>13098 ± 3404</td>
<td>16907 ± 6364</td>
</tr>
<tr>
<td>Men manual unskilled or clerical (N=14)</td>
<td>12757 ± 3850</td>
<td>20383 ± 5617</td>
</tr>
</tbody>
</table>
Figure 3.1. Physical activity energy expenditure estimated using either the 5-day recall or heart rate monitoring methods, $r=0.39$, $P<0.05$.

![Figure 3.1](image)

Figure 3.2. Altman and Bland plot of the mean differences between the two energy expenditure methods, and their limits of agreement. The mean difference was $-5559 \pm 6276 \text{ kJ.day}^{-1}$, the limits of agreement ($\text{mean} \pm 2 \text{ standard deviations}$ was $-18111$ and $6993 \text{ kJ.day}^{-1}$).

![Figure 3.2](image)
DISCUSSION

This study presents energy expenditure data, estimated either using a heart rate monitoring technique and individual subject calibration or a physical activity questionnaire (5-day recall), modified from the 7-day Stanford physical activity recall questionnaire (122). The first finding from our study is that in a group of people, both occupationally and educationally diverse, energy expenditure estimated, using the 5-day recall, consistently overestimated energy expenditure, compared to the heart rate monitoring technique, using individual calibration (P<0.05). This finding was consistent for both men and women. The two methods were still weakly, but significantly correlated (r=0.36, P<0.05).

Previously, Ainsworth et al. (6) compared the energy expenditure estimated using the 7-day recall with a 7-day occupational activity record and an accelerometer, during 7 consecutive days. Energy expenditure estimated using the 7-day recall and the occupational physical activity record was moderately correlated with energy expenditure, calculated as either MET.min⁻¹.week⁻¹ (r=0.45) or hours.week⁻¹ (r=0.78). However, energy expenditure estimated using the 7-day recall was not correlated with the energy expenditure estimated using the accelerometer. This dissociation was attributed to differences in measurement periods, i.e. the accelerometer accumulated 24hr data, while the 7-day recall accumulated occupational data. Similarly in our study, we found a weak relationship between the energy expenditure estimated using the 5-day recall and the energy expenditure estimated using the heart rate monitoring technique. The 5-day
recall only accounted for 13% of the variance in estimated energy expenditure using the heart rate monitoring technique.

Our study is one of the first to report a physical activity questionnaire validation using the individual heart rate monitoring technique. Other techniques more commonly used are accelerometry (6;78;117) and doubly labelled water (33;78;149). However, in our study, we could not discount the fact that energy expenditure was only estimated on 1 working day using the heart rate monitoring technique, and this single measurement was compared to energy expenditure averaged over 5 working days, using the 5-day recall method. In addition, our criterion measure or "gold standard" was the heart rate monitoring technique, using individual subject calibration, as previously described by Li et al. (81). While previous research has shown reasonable accuracy (81;88;136), when using this technique, a large amount of both inter- and intra-individuality has been reported. In Chapter Two of this thesis, we reported heart rate-derived energy expenditure data, using individual subject calibration, in a group of 9 exercising women and 10 control women, before and after an eight week exercise training program. The day-to-day variability in energy expenditure in the control group was as high as 23%.

A large part of the criticism in the heart rate monitoring method is related to energy expenditure estimates below what is termed as the "flex heart rate". Flex heart rate is the heart rate, calculated as the average of the highest heart rate recorded during sedentary activities and the average of the lowest heart rate recorded during physical activities. As result, energy expenditure estimates, below flex heart rate, are therefore related to sedentary activities, and energy expenditure data has only shown to be linear
above flex heart rate (136). Subsequently it has been suggested (83) that the heart rate monitoring method should only be used for activities above the flex heart rate cut-off point. In our study, we only found a weak relationship, 12% of the variance in moderate and vigorous physical activity energy expenditure estimated using the 5-day recall, could be accounted for by the heart rate monitoring technique.

In the present study, the physical activity energy expenditure, measured using the heart rate monitoring method correlated with total reported physical activity energy expenditure, reported moderate and vigorous total physical activity energy expenditure and physical activity energy expenditure during non-working hours, but not working hours. Discrepancies between physical activity recall during working and non-working hours have been found previously (5;69). For example Ainsworth et al. (5) reported differences between physical activity energy expenditure captured during the day, during working hours and habitual physical activity. It was therefore concluded that capturing physical activity energy expenditure during working hours was one of the most difficult components to assess.

Studies by Bonnefoy et al. (19) and Racette et al. (109) also report on an overestimation in energy expenditure estimated using the 7-day recall, when compared to the doubly labelled water method, thus corroborating our results. In addition, these studies all report a high degree of individual variability. Washburn et al. (149) attributed this high variability to the incorrect assigning of absolute MET values associated with physical activity, which would result in significant over or under estimations in energy expenditure estimates.
With respect to occupation and level of education, we found subjects involved with manual or unskilled occupations, or those with lower levels of education tended to over-report physical activity energy expenditure compared to subjects involved with skilled or clerical occupations, however, these differences were not statistically significant. This may be due, in part, to inadequate representation in groups, or small sample size, resulting in Type II error. With respect to level of education, we also found that subjects who had not completed high school tended to over-report physical activity energy expenditure, compared to subjects who had completed high school, but interpretation of results may be limited due to sample size.

In conclusion, this study provides an example for the use of the heart rate monitoring method for estimating physical activity energy expenditure and further, provides useful insights into the patterns of over- and under-reporting in physical activity recall. In the present study, subjects over-reported energy expenditure, irrespective of gender, levels of education or occupational status. However, we acknowledge that this study design would have been substantially improved had we measured heart rate on more than one working day. This is especially relevant, as we have previously demonstrated, along with others, that energy expenditure estimated using the heart rate monitoring method has a high degree of intra-individual variability. In addition, it remains somewhat impractical to consider individual heart rate-energy expenditure calibration, for larger groups of subjects, such as those used in epidemiological studies, and therefore, highlights the need to develop group-based heart rate-energy expenditure calibration equations.
CHAPTER FOUR

PREDICTION OF FREE-LIVING ENERGY EXPENDITURE FROM HEART RATE MONITORING DURING SUBMAXIMAL EXERCISE-
(Application Of Group-Based Equations).

This chapter has been accepted in part for publication:

INTRODUCTION

In Chapter Three, the heart rate monitoring technique, using individual subject calibration, was used to investigate potential errors in reporting physical activity energy expenditure and the possible confounders associated with the error in physical activity questionnaires. Reasonable correlation was found between the two measures of physical activity energy expenditure however; differences in two techniques for estimating energy expenditure could not solely be attributed to the physical activity recall questionnaire technique. In Chapter Two of this thesis, we found energy expenditure estimated using the heart rate monitoring technique and individual subject calibration, to have coefficient of variation for intra-individual differences of as much as 23%. Therefore, as a result of these differences we felt it necessary to further explore the heart rate monitoring technique and identify the factors which impact upon the relationship between heart rate and energy expenditure, particularly during physical activity.

During moderate physical activity there is a near linear relationship between heart rate and oxygen consumption. This heart rate-oxygen consumption relationship is subject to both intra- and inter-individual variability. Heart rate may be partially dissociated from energy expenditure by factors such as emotion, posture, and environmental conditions (58). Furthermore, the relationship between heart rate and energy expenditure is linear only within a relatively narrow range of approximately 90 to 150 beats per minute (the so-called flex heart rate), during physical activity (29;114;136). During light activity or inactivity, there is almost no slope to the relationship between heart rate and energy
expenditure and for the purpose of measuring energy expenditure from heart rate it is assumed, that energy expenditure is equal to resting energy expenditure (114). A non-linear, discontinuous function has been found to be more accurate than a linear relationship in predicting physical activity energy expenditure from heart rate (81).

Heart rate monitoring, for estimating free-living energy expenditure, has been extensively validated using indirect calorimetry, doubly labelled water technique, and whole-room respirometry and reported differences between measures range from −20 to +25% (85). In large groups of people, heart rate monitoring provides one of the most efficient and economical means of estimating energy expenditure. In addition, heart rate monitoring provides useful insights into the type of activity being undertaken over the measurement period. Other assessment methods, such as the doubly labelled water technique, can only convey the total amount of physical activity measured, whereas heart rate monitoring provides additional information about the type of activities being performed and describes the nature of day-to-day variability in energy expenditure (59;85). On the other hand, whole room respirometry and indirect calorimetry provide physiological information into the nature of the activity being performed, but these tools are not only costly to maintain and often take the subject out of their natural environment for the duration of the measurement period (85).

In the majority of previous studies in which heart rate has been used to predict energy expenditure, individual calibration was performed (29;81;136). Individual calibration requires that each subject complete a progressive exercise test, during which time heart rate is simultaneously measured, along with indirect calorimetry, to estimate energy
expenditure. However, in recent studies, efforts have been made to develop group-based, heart rate-energy expenditure regression equations (62;114). For example, Hiilloskorpi (62) developed a prediction equation for energy expenditure from heart rate, using multiple regression analysis, on a sample of 87 healthy, active men and women. Factors found to have a significant interaction with energy expenditure included: age, weight and gender. Mode of exercise (cycling versus running) did not contribute significantly to the model.

In a more recent study, Rennie (114) developed a prediction model using a sample of 789 individuals. In this large sample, factors found to have a significant effect on the relationship between heart rate and energy expenditure included: sitting heart rate, as well as, age, weight, gender. These variables were used to predict the slope and the intercept of the regression line between energy expenditure and heart rate. This energy expenditure equation was then further validated on an independent sample of 97 subjects, and found to have a correlation coefficient of r=0.73 (114). Rennie et al. (114) demonstrated the utility of developing equations for estimating physical activity energy expenditure, from heart rate-energy expenditure in large, representative samples of subjects, with reasonable accuracy and the potential for wide application in epidemiological studies.

The present study was therefore undertaken firstly, to further characterise the factors that influence the relationship between energy expenditure and heart rate during moderate to vigorous activity in regularly exercising persons. A second aim was to
develop a prediction equation for energy expenditure from heart rate, adjusting for these factors.
METHODS

Part 1: Developing energy expenditure prediction equation

Subjects

Subjects were recruited from a local fitness centre, group-based exercise programs, running clubs and cycle races. Regularly exercising men and women volunteered for the study (N=127, of which 115 had complete data). Subjects were familiarised either with a cycle ergometer or motor-driven treadmill, and ranged in age from 19 to 45 years. Subjects were free from any known cardiac or metabolic disorders and were not currently taking any chronic medication. Subject characteristics are presented in Table 4.1. Subjects were tested on two occasions, after self-selecting the mode of exercise (cycle ergometer N=69, or treadmill N=46). The Ethics and Research committee of the University of Cape Town, Faculty of Health Sciences, approved the study and informed consent was obtained from the subjects before the commencement of the trial.

A second sample of regularly exercising subjects (N=17) was subsequently recruited, independent of the first sample, to test the validity of the prediction model. The second sample was recruited from a local fitness centre, and represented a wide range of ages (21 to 53 years), body mass (51 to 105 kg) and fitness levels (34 to 74.3 ml.kg\(^{-1}\) min\(^{-1}\)).
Body composition

Body fatness was expressed as the sum of 7 skinfolds (mm, biceps, triceps, subscapular, suprailliac, anterior thigh, abdominal and medial calf) (119). Body fat % was estimated using the equations of Durnin and Womersley (44).

Maximal oxygen consumption ($\text{VO}_{2}\text{max}$)

During the first visit to the laboratory, maximal oxygen consumption ($\text{VO}_{2}\text{max}$), peak power output (PPO) or peak treadmill running speed (PTRS) and maximum heart rate were measured. $\text{VO}_{2}\text{max}$ was measured during either a progressive treadmill or cycle test to exhaustion. During the treadmill test, the starting treadmill velocity was 12 km.h$^{-1}$ for the men and 10 km.h$^{-1}$ for the women and treadmill speed was increased by 0.5 km.h$^{-1}$ every 30 s until volitional exhaustion, as described previously (100). In the cycle test to exhaustion, subjects were tested on an electronically braked cycle ergometer (Lode, Gronigen, The Netherlands). Each subject started cycling at an exercise intensity of 3.33 W.kg$^{-1}$ body weight for 150 s, after which the work rate was increased by 50 W for a further 150 s. The exercise intensity was then increased by 25 W every 150 s up to the point of exhaustion (57). Maximum heart rate was defined as the maximum heart rate achieved at the point of exhaustion.

During both the treadmill and cycle tests, subjects wore a facemask attached to an Oxycon Alpha automated gas analyser (Oxycon, Jaeger, The Netherlands). Before each test, the gas analyser was calibrated using a Hans Rudolph 5530 3-litre syringe and a
two point calibration technique, using 5% CO₂ – 95% N₂ gas mixture and fresh air. Rate of oxygen consumption (VO₂), rate of carbon dioxide production (VCO₂) and respiratory exchange ratio (RER), were calculated using conventional equations (150). PPO and PTRS were defined as the workload at which the subject could no longer maintain the pace of the treadmill or maintain an RPM of 70.

Submaximal testing and estimation of energy expenditure

Subjects returned to the laboratory within a week and performed a submaximal test. The cycle ergometer submaximal test protocol consisted of 3 consecutive workloads, each 15-minutes in duration, during which the subjects cycled at 25%, 55% and 70% of the previously determined PPO, corresponding to 41%, 63% and 80% of VO₂max respectively. The submaximal treadmill protocol consisted of 3 consecutive workloads, each lasting 10-minutes, at 35%, 50% and 70% (also corresponding to approximately 41%, 63% and 80% VO₂max respectively) of previously determined PTRS. Minute-by-minute heart rate was recorded using the Polar Vantage heart rate monitor (Polar Electro, Finland) and respiratory exchange measurements (VO₂ and VCO₂) were collected and used to estimate energy expenditure based on the equations of Weir (150), during the last 5-minutes of each of the stages. The submaximal heart rate data from the last 5-minutes of each stage were used to subsequently calculate predicted energy expenditure on the basis of individual regression equations. Factors that were significantly correlated with heart rate or VO₂ were used in the model to predict energy expenditure.
Part 2. Validation of prediction model on an independent sample

For the purpose of validation, the energy expenditure values from a twenty-minute self-selected, continuous exercise session, involving large muscle groups (cardiovascular session) were predicted on an independent sample of subjects, recruited from a local fitness centre. Subjects were instructed to choose either a single 20-minute cardiovascular workout, or two 10-minute exercise bouts.

Subjects

The 17 subjects (N=9 men, N=8 women) who participated in this study were free from known cardiovascular and metabolic disorders and participated in some form of cardiovascular physical activity at least 3 times a week. Subjects met the inclusion criteria of the original study and characteristics are presented in Table 4.2.

Body composition and maximal test to exhaustion

Subjects reported to the laboratory on two different occasions within one week. During the first visit, subjects had their body composition measured using the near infrared reactance technique (Futrex Inc., Gaithersburg, MD, USA). Subjects then performed a maximal test to exhaustion on an electronically braked cycle ergometer (Lode, Gronigen, The Netherlands) as previously described (57). During the test, oxygen consumption and carbon dioxide production were measured as described above.
Estimation of physical activity energy expenditure

During the second visit, subjects reported to the laboratory in a 2-hours post-prandial state. Subjects were instructed not to engage in any strenuous physical activity during the preceding 24 hours. All subjects completed a 20-minute cardiovascular exercise session, as part of an independent study in progress. The cardiovascular component was performed following a 5-minute warm-up, consisting of between 2-3 minutes of walking, and 2-3 minutes of light jogging. Subjects then chose to either complete one 20-minute continuous cardiovascular exercise session, or two 10-minute cardiovascular exercise sessions, on a self-selected piece of fitness centre equipment. For the duration of the exercise session, the subjects' HR, VO$_2$ and VCO$_2$ were continuously monitored using the K4b$^2$ portable gas analyser (Cosmed, Italy). Minute-by-minute energy expenditure (kJ.min$^{-1}$) was then determined using the non-protein caloric equivalents for oxygen consumption. Before each test the portable gas analyser was calibrated using a Hans Rudolph 5530 3-litre syringe and a two point calibration technique, using 5% CO$_2$ – 16% O$_2$ gas mixture and fresh air. Analyser outputs were processed, which calculated breath-by-breath ventilation, oxygen consumption (VO$_2$), rates of carbon dioxide production (VCO$_2$) and respiratory exchange ratio (RER), using conventional equations (150).

Statistical Analysis

Initially, 127 subjects volunteered to participate in the study. The final sample size was 115 subjects, due to incomplete heart rate and VO$_2$ max data collection on 12 subjects.
The initial exploratory data analyses to determine factors that may have significantly contributed to the relationship between heart rate and energy expenditure included Box plots and scatter plots for all variables (not shown). Univariate (means, standard deviations) and bivariate (correlation coefficients) summary statistics were then calculated for all variables.

Based on these analyses, we fitted a mixed model for predicting energy expenditure. Subject level factors gender, weight, age and VO₂max were modelled as fixed effects, and subjects as random effects with 3 repeated measurements of energy expenditure (and fixed heart rate) for each subject. In the model, the covariance matrix between the measurements for each subject was unstructured and compound symmetry was assumed for the covariances between subjects.

A second mixed model was fitted under the rationale that in certain settings, a test of maximal oxygen consumption might be impractical or not available. The second model included all the variables and assumptions in the original model, except VO₂max. For inner validation, both models were tested on an independent sample of subjects (N=17), completing a 20-minute cardiovascular bout of exercise.

The initial exploratory analyses were done with Statistica (Statsoft, Southern Africa Inc. (2002). Statistica data analysis software system, version 6.1). Statistical modelling was done with SAS® Proprietary Software Release 8.2 (USA).
RESULTS

Subject characteristics from sample used to develop prediction equation

Subject characteristics are presented in Table 4.1. Subjects represented a wide range of morphology and fitness with ages from 19 to 45 years of age, body weight ranging from 47 to 116 kg, % body fat from 4.8% to 37.8% and VO₂max from 27 to 81 ml.kg⁻¹.min⁻¹. There were no differences in mean age, weight, % body fat or VO₂max between subjects who underwent treadmill testing versus those that underwent cycle ergometer testing. There were significant gender differences in weight, % body fat and VO₂max (Table 4.1., P<0.0001).

Subject characteristics from sample used for inner validation

Subject characteristics are presented in Table 4.2. Subjects were comparable to those used in the original study and represented a broad range in body composition. Subjects in both samples were equally matched for age and weight. Subjects from the original study were slightly fitter than those used in the validation study (mean VO₂max of 54 ± 1 ml.kg⁻¹.min⁻¹ versus 48 ± 1 ml.kg⁻¹.min⁻¹ in Part 2), although the difference was not statistically significant.
Prediction equations of energy expenditure from heart rate: mixed model analysis

A mixed model was used to derive the following equation for predicting physical activity energy expenditure (EE):

\[ EE \text{ (kJ.min}^{-1}\text{)} = -59.3954 + \text{gender} \times (-36.3781 + 0.271 \times \text{age} + 0.394 \times \text{weight} + 0.404 \times \text{VO}_2\text{max} + 0.634 \times \text{heart rate}) + (1 - \text{gender}) \times (0.274 \times \text{age} + 0.103 \times \text{weight} + 0.380 \times \text{VO}_2\text{max} + 0.450 \times \text{heart rate}). \]

Where gender=1 for males and 0 for females. Table 4.3 represents the above model in a different format. The likelihood ratio test for goodness-of-fit statistic is \( \chi^2 \approx 262.73 \) on 5 degrees of freedom with \( P<0.0001 \). Results of type III tests for the fixed effects in the mixed model are given in Table 4.4. Degrees of freedom for the F-tests were calculated with Satterthwaite’s method.

In Figure 4.1, the measured energy expenditure is regressed against estimated energy expenditure. The coefficient of correlation was \( r=0.913 \), therefore 83.3% of the variance in measured energy expenditure, was explained by the model, in the original sample.
A second model, which contained no measure of fitness, was also fitted. The final prediction equation for energy expenditure using age, gender, weight and heart rate was:

\[
EE (\text{kJ.min}^{-1}) = \text{gender} \times (-55.0969 + 0.6309 \times \text{heart rate} + 0.1988 \times \text{weight} + 0.2017 \times \text{age}) + (1-\text{gender}) \times (-20.4022 + 0.4472 \times \text{heart rate} - 0.1263 \times \text{weight} + 0.074 \times \text{age}).
\]

Gender=1 for males and 0 for females. Table 4.5 represents the above model in a different format. The likelihood ratio test for goodness-of-fit statistic is \( \chi^2 = 360.68 \) on 5 degrees of freedom with \( P<0.0001 \). Results of type III tests for the fixed effects in the mixed model are given in Table 4.6. Degrees of freedom for the F-tests were calculated with Satterthwaite's method.

In Figure 4.3, using the second model, the measured energy expenditure is regressed against estimated energy expenditure. The coefficient of correlation was \( r=0.857 \), thus, only 73.4% of the variance in measured EE in the sample was explained by the second model.

*Independent sample analysis for inner validation*

Data from an independent sample of 17 subjects (8 women, 9 men) were used to validate both models. Predicted energy expenditure, using the first model, which included a measure of fitness (\( \text{VO}_2\text{max} \)), accounted for 70% of the variance in measured energy expenditure during self-selected cardiovascular fitness training (\( r=0.836 \),
P<0.0001, Figure 4.2). Using the second model which excluded a measure of VO\textsubscript{2}max, the correlation coefficient was r=0.77 (P<0.0001) and therefore, only accounted for 59% of the variance in measured energy expenditure (Figure 4.4.).

Because we have a mixed model (with random subject effects) we had to use maximum likelihood estimation and not least squares. The result is that even the estimates for the original sample are very slightly biased. The bias of the estimates and their random variation can be summarised as follows for the four sets of estimates: The bias is the difference between the predicted and the corresponding actual value of energy expenditure. The 95% limits of agreement were calculated as mean ± 1.96 x standard deviation (10) and the data are presented in Table 4.7.
Table 4.1. *Subject characteristics (means ± standard deviations) for equation development (N=115).*

<table>
<thead>
<tr>
<th></th>
<th>Treadmill</th>
<th>Cycle ergometer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>30 ± 7</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>76 ± 10</td>
<td>66 ± 11</td>
</tr>
<tr>
<td>% Body fat*</td>
<td>14.5 ± 4.8</td>
<td>26.8 ± 5.2</td>
</tr>
<tr>
<td>VO$_2$ max (ml.kg$^{-1}$.min$^{-1}$)*</td>
<td>65.0 ± 8.6</td>
<td>49.0 ± 9.7</td>
</tr>
<tr>
<td>Heart rate max (bpm)</td>
<td>189 ± 9</td>
<td>184 ± 9</td>
</tr>
</tbody>
</table>

(* P<0.0001, gender-differences)*

Table 4.2. *Subject characteristics (means ± standard deviations) for independent inner validation sample (N=17).*

<table>
<thead>
<tr>
<th></th>
<th>Men (N=9)</th>
<th>Women (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>29 ± 8</td>
<td>34 ± 10</td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td>81 ± 14</td>
<td>62 ± 9</td>
</tr>
<tr>
<td>% Body fat*</td>
<td>14.8 ± 5.1</td>
<td>26.0 ± 3.9</td>
</tr>
<tr>
<td>VO$_2$ max (ml.kg$^{-1}$.min$^{-1}$)*</td>
<td>54.3 ± 11.4</td>
<td>42.4 ± 5.4</td>
</tr>
<tr>
<td>Max HR (bpm)</td>
<td>190 ± 9</td>
<td>178 ± 15</td>
</tr>
</tbody>
</table>

(* P<0.00001, gender-differences)*
Table 4.3. The estimates and their standard errors for the fixed effects of the mixed model including fitness. Likelihood ratio test for goodness-of-fit $\chi^2 = 262.73$ on 5 degrees of freedom, $P < 0.0001$.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Male</th>
<th></th>
<th>Female</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Standard error</td>
<td>Estimate</td>
<td>Standard error</td>
</tr>
<tr>
<td>Intercept</td>
<td>-95.7735</td>
<td>9.5734</td>
<td>-59.3954</td>
<td>17.1314</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.6344</td>
<td>0.01370</td>
<td>0.4498</td>
<td>0.01648</td>
</tr>
<tr>
<td>Weight</td>
<td>0.3942</td>
<td>0.06423</td>
<td>0.1032</td>
<td>0.1166</td>
</tr>
<tr>
<td>VO$_2$max</td>
<td>0.4044</td>
<td>0.08374</td>
<td>0.3802</td>
<td>0.1575</td>
</tr>
<tr>
<td>Age</td>
<td>0.2713</td>
<td>0.1120</td>
<td>0.2735</td>
<td>0.2087</td>
</tr>
</tbody>
</table>

Table 4.4. Table with type III analysis for fixed effects of model including fitness.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Degrees of freedom</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>2</td>
<td>109</td>
<td>56.05</td>
</tr>
<tr>
<td>Heart rate x gender</td>
<td>2</td>
<td>125</td>
<td>1444.98</td>
</tr>
<tr>
<td>Weight x gender</td>
<td>2</td>
<td>100</td>
<td>19.23</td>
</tr>
<tr>
<td>VO$_2$max x gender</td>
<td>2</td>
<td>101</td>
<td>14.57</td>
</tr>
<tr>
<td>Age x gender</td>
<td>2</td>
<td>101</td>
<td>3.79</td>
</tr>
</tbody>
</table>
Table 4.5. The estimates and their standard errors for the fixed effects of the mixed model without fitness. Likelihood ratio test for goodness-of-fit $\chi^2 = 360.68$ on 5 degrees of freedom, $P<0.0001$.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Male Estimate</th>
<th>Male Standard error</th>
<th>Female Estimate</th>
<th>Female Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-55.0969</td>
<td>5.5780</td>
<td>-20.4022</td>
<td>7.2318</td>
</tr>
<tr>
<td>Heart rate</td>
<td>0.6309</td>
<td>0.0137</td>
<td>0.4472</td>
<td>0.0165</td>
</tr>
<tr>
<td>Weight</td>
<td>0.1988</td>
<td>0.0619</td>
<td>-0.1263</td>
<td>0.1061</td>
</tr>
<tr>
<td>Age</td>
<td>0.2017</td>
<td>0.1180</td>
<td>0.0740</td>
<td>0.1742</td>
</tr>
</tbody>
</table>

Table 4.6. Results of type III tests for fixed effects of model excluding fitness.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Degrees of freedom</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>2</td>
<td>109</td>
<td>14.43</td>
</tr>
<tr>
<td>Heart rate x gender</td>
<td>2</td>
<td>125</td>
<td>1428.63</td>
</tr>
<tr>
<td>Weight x gender</td>
<td>2</td>
<td>99.9</td>
<td>5.86</td>
</tr>
<tr>
<td>Age x gender</td>
<td>2</td>
<td>100</td>
<td>1.55</td>
</tr>
</tbody>
</table>
Table 4.7. Bias of the estimates and random variation for original and validation samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Model</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original sample</td>
<td>First</td>
<td>-1.06</td>
<td>7.83</td>
<td>-16.4</td>
</tr>
<tr>
<td></td>
<td>No fitness</td>
<td>-5.79</td>
<td>9.85</td>
<td>-25.1</td>
</tr>
<tr>
<td>Validation sample</td>
<td>First</td>
<td>8.19</td>
<td>9.19</td>
<td>-9.83</td>
</tr>
<tr>
<td></td>
<td>No fitness</td>
<td>6.27</td>
<td>9.65</td>
<td>-12.65</td>
</tr>
</tbody>
</table>
Figure 4.1. Graph representing estimated energy expenditure (kJ.min⁻¹) regressed against observed energy expenditure (kJ.min⁻¹) for first model and original sample (N=115), $r=0.913$.

Figure 4.2. Scatter plot of estimated energy expenditure versus observed energy expenditure (kJ.min⁻¹) for first model and independent validation sample (N=17), $r=0.836$. 

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Figure 4.3. Scatter plot of measured energy expenditure versus predicted energy expenditure for original sample (N=115) using the second model, without fitness, $r=0.857$.

Figure 4.4. Scatter plot of measured energy expenditure versus predicted energy expenditure for independent validation sample (N=17), using the second model, without fitness, $r=0.77$. 
DISCUSSION

In this study, we demonstrated that physical activity energy expenditure, during moderate-to-high intensity exercise, might be predicted with good accuracy in a group of individuals varying widely in age, fitness and morphology, without the need for individual calibration. This study represents an improvement over existing studies in the estimation of physical activity energy expenditure using heart rate monitoring. The proposed model (using heart rate, age, weight, gender and VO$_2$max) accounted for 70% of the variation in observed energy expenditure in an independent sample of subjects completing a self-selected 20-minute cardiovascular exercise session.

Previous studies (81;121) have cited poor agreement between energy expenditure estimated using heart rate monitoring and measured energy expenditure. These prediction equations were developed on small samples, not representative of the population to which the equation was to be applied. For example, Rutgers (121) developed a prediction equation based on the heart rate and energy expenditure data acquired from thirteen elderly subjects. It was concluded that the use of heart rate monitoring for energy expenditure measurement was inaccurate during 3 days of measurement, citing large discrepancies between energy expenditure estimation using the individual calibration curve and a group curve and therefore not accurate for application in large population-based studies. Li et al. (81) also found poor agreement for the estimation of energy expenditure using heart rate monitoring, between group and individually derived estimates. Once again, this sample was relatively small, consisting of only 40 subjects.
In a more recent study Rennie et al. (114) used the variables that significantly interacted with energy expenditure to predict the slope, intercept and the heart rate flex point for measured versus predicted physical activity energy expenditure. In that study, the variables of sitting heart rate, age weight and gender were found to have a significant impact on the slope, intercept and heart rate flex point. These investigators were then able to use the slope and intercept of the linear model to place 98% of the subjects in their sample in either the same or adjacent quartiles for the measured and estimated physical activity levels. Their model has implications for the classification of physical activity levels for epidemiological research. In the current study, we present linear equations, based on mixed model analyses. These equations yield predictions for a continuous estimation of physical activity energy expenditure, which correlate significantly with the test sample, as well as the independent validation sample.

Previously, Hiilloskorpi et al. (62) developed an equation to predict energy expenditure using the variables of heart rate, age, weight and gender. In that study it was shown that the mode of exercise, cycling versus running, did not significantly affect the final prediction of energy expenditure. We also found that the mode of exercise did not affect the estimation of energy expenditure, suggesting that the equation derived from these data may be suitable for both running and cycling activities. During our inner validation study, we even found good agreement with other modes of continuous activity such as stationary rowing ergometry and stationary stair climbing activities.
Hiilloskorpi et al. (62) did not include any measure of physical fitness or VO₂max in the prediction equation, citing a need to produce an equation for estimating energy expenditure independent of laboratory testing. We found that when a measure of the level of cardiorespiratory fitness, such as VO₂max, is included, the accuracy of the prediction improved, with an additional 10% of the variance in measured energy expenditure explained (R² = 0.83 versus R² = 0.73). In the inner validation sample, the model using VO₂max accounted for approximately 70% of the variance in measured energy expenditure, compared to the model without VO₂max, which accounted for only 59% of the variance in measured energy expenditure. It is well known that training results in adaptations in the heart rate response to increasing workloads (92; 162). Therefore, it is not surprising that an indirect measure of cardiorespiratory fitness improves the accuracy of the prediction of energy expenditure from heart rate. This finding corroborates that of Rennie et al. (62), in which sitting heart rate was found to play a significant role in the prediction of energy expenditure from heart rate monitoring. Rennie et al. (62) proposed that sitting, resting heart rate was a useful proxy measurement for fitness, since previous studies have found an inverse association between resting tachycardia and maximal exercise capacity (14), as well as a positive relationship between regular participation in physical activity and lower resting heart rate, independent of age (142). Strath et al. (145) also found that including a measure of fitness in the regression equation provided estimates of energy expenditure, which accounted for 76% of the variance in measured energy expenditure, using respiratory gas analysis, during the activities of daily living.
Hiilloskorpi et al. (62) found that including age in the regression model did not significantly improve the variance. This is different to the current study, in which we found that age did contribute significantly to the final mixed model. This difference may, in part, be explained by differences in sample characteristics. Hiilloskorpi et al. (62) acknowledges a relatively narrow age range, with few subjects older than 50 years or younger than 25 years. In our study, the mean age of the 72 men was 31 years (range 19 to 50 years of age), and for the 43 women was 30 years (range 22 to 44 years of age). In Hiilloskorpi et al. (62), the mean age was 40 years for the 45 men and 38 years for the 43 women. Their subjects were, notably older than those from the current study. In addition to the age discrepancies between the two studies, there were also discrepancies between the fitness of the two samples. In the current study, the mean VO₂ max for the men was 59.2 ml.min⁻¹.kg⁻¹ and for the women was 45.7 ml.min⁻¹.kg⁻¹ while in Hiilloskorpi et al. the average VO₂ max for the men was 48.5 ml.min⁻¹.kg⁻¹ and for the women was 39.5 ml.min⁻¹.kg⁻¹. These demographic differences may partially account for differences found between the two prediction models. Rennie et al. (114) also found that age impacted in the regression model of physical activity energy expenditure from heart rate.

It may be argued that the sample used to generate the prediction equation comprised a well-trained group of individuals, but we feel that they represented a typical, fitness centre population. VO₂ max values ranged from 27.0 to 64.1 ml.kg⁻¹.min⁻¹ in the women and from 38.0 to 81.4 ml.kg⁻¹.min⁻¹ in the men. It was the intention of the present study to apply this equation to the general exercising population, and as a result our subject recruitment was from a local fitness centre and amateur running clubs and cycling races.
While our average fitness levels were unlike those presented in both Hiiliskorpi et al. (62) and Rennie et al. (114), we have demonstrated that the inclusion of VO$_{2}$max, as a proxy for fitness, improves the predictability of our group-based equation.

While numerous other studies (62;81;114;145) have used similar approaches to develop prediction equations without individual calibration, not all of them (62;145) use an independent sample for inner validation of the developed model and in some cases, some studies actually report no inner validation of the developed model (145) or the use of the same sample, on which the original prediction equation was developed (62). This may lead to elevated levels of agreement between the prediction models and measured estimates, due to the homogeneous nature of samples. For example, Strath et al. (145) attempted to estimate physical activity during moderate intensity exercise, using heart rate monitoring and also reports good agreement ($r=0.87$) between measured and estimated energy expenditure. During this study, there was no inner validation reported on an independent sample of subjects. Conversely, Rennie et al. (114) validated a prediction equation for physical activity levels, developed on a sample of 789 individuals, on a smaller subset of 97 individuals. During this inner validation, 98% of the subset was placed in the same or adjacent quartiles during comparison of measured and estimated physical activity levels. In the current study, we found good agreement on an independent sample of subjects. The prediction equation explained 71% of the variance in estimated energy expenditure in an independent sample, during self-selected, cardiovascular exercise training.
Finally, for practical application the proposed equations represent an improvement in the estimation of energy expenditure from heart rate over existing equations. They may be used in large population-based studies for health purposes. Further research is needed in the area of simultaneous measurement of physical activity energy expenditure and heart rate, particularly during activities resembling the activities of daily living. Literature (153) suggests that not only is physical activity, in the form of exercise bouts, necessary for the promotion and maintenance of health, but also habitual physical activity. Therefore, predictive equations that estimate physical activity energy expenditure, for health-based research and promotion are required for a wider variety of activities, particularly for intermittent activity or activity conducted at lower intensities, reflective of the activities of daily living.
CHAPTER FIVE

A NOVEL PREDICTION EQUATION FOR ENERGY EXPENDITURE DURING INTERMITTENT ACTIVITY, BASED ON HEART RATE DURING THE CURRENT AND PRECEDING MINUTES.
INTRODUCTION

In the previous chapter, we demonstrated that it was possible to accurately predict physical activity energy expenditure from heart rate, along with other explanatory variables (e.g. age, gender, weight and level of fitness) using group-generated equations, thereby obviating the need for individual subject calibration. This is a consequence, in part, of a well-described relationship between heart rate and physical activity energy expenditure (29;81;85;136). As a result, it is possible to successfully predict free-living energy expenditure (62;85;136) using portable heart rate monitors and individual heart rate-energy expenditure regression equations. Typically this relationship has been determined for each subject by simultaneously monitoring heart rate and oxygen consumption (VO₂) during a heart rate-energy expenditure calibration test. This test usually consists of a series of at least eight continuous and incremental activities, which allow investigators to generate an individual heart rate and energy expenditure regression equation for each subject. In Chapter Two, we found individual differences using this technique of approximately 20%, while other studies (29;85) have validated the heart rate method against both whole body calorimetry and the doubly labelled water technique and found energy expenditure estimations using heart rate are within 10% of those derived using whole body calorimetry (29;81;83;130).

A number of significant methodological developments over the last several years have improved the accuracy of the prediction of energy expenditure from heart rate, and have improved the applicability of the technique to population-based studies. The first such development was the introduction of the concept of flex heart rate, which is the
heart rate above which the relationship between heart rate and workload or $\text{VO}_2$ becomes linear (136). The introduction of the flex heart rate concept into the modelling of energy expenditure from heart rate improved the accuracy of group estimates by $\pm 10\%$ (29;136).

The second development was the application of non-linear equations, for estimating energy expenditure from heart rate, and the inclusion of a greater number of steady state workloads during the individual heart rate and energy expenditure calibration testing (81). Indeed, numerous higher order regression equations were investigated (81;130) to manage the apparent non-linearity observed between heart rate and energy expenditure during lower intensity activities or periods of rest. It was further established that heart rate-energy expenditure calibration procedures using fewer workloads resulted in poor limits of agreement in the validation of energy expenditure prediction from heart rate (81).

As in our previous chapter, the third major development was the application of group data to derive population-based, multivariate prediction equations (62;114). Additional explanatory variables may be included in regression equations such as: age, weight and gender (62). One such population-based equation (114), derived from a continuous incremental physical activity heart rate-energy expenditure calibration test, yielded an $R^2=0.67$, ($P<0.01$), when compared to whole body indirect calorimetry measured in the laboratory setting, in 97 subjects who wore portable heart rate monitors continuously for 4 days. During this study, however, during minutes in which heart rate was lower than or equal to the flex heart rate, energy expenditure values equal to mean resting energy
expenditure were assigned, to account for the non-linearity between heart rate and energy expenditure during low intensity activity. In Chapter Four, we accounted for 70% ($r=0.836$) of the measured energy expenditure in an independent sample of subjects completing a self-selected cardiovascular training session, using an energy expenditure mixed model equation. This mixed model equation, therefore, further improves the estimation of energy expenditure from heart rate monitoring. However, we are, cautious to over interpret this data, as we acknowledge that energy expenditure estimation, during the inner validation, was only during 20-minutes of exercise, while Rennie et al. (114) estimated physical activity levels during a period of 4 days of heart rate monitoring.

Finally, in recent years there has been a trend to combine motion sensing, accelerometry and pedometry (130;143;144) with heart rate monitoring in subjects, in whom individual regression curves have been generated for the prediction of energy expenditure from heart rate monitoring. Combining these tools with the heart rate monitor technique compensates for weaknesses in the other method and improves the overall accuracy of the measurement of energy expenditure. Simultaneous heart rate monitoring and motion sensing yielded an $R^2$ of 0.81 for energy expenditure compared to VO$_2$ measurement during continuous activity monitoring (130). This may be compared to either the heart rate method, which yielded a $R^2$ of 0.75 for energy expenditure when compared to the whole body indirect calorimetry method (136) in 22 subjects, or the accelerometry method, which yielded an $R^2$ of 0.62, when used to measure habitual activity in 25 subjects (61). Accelerometry is limited in application to primarily those upper body activities involving locomotion and load-bearing activities.
The error in predicted energy expenditure has been as much as 60% for some activities based on indirect calorimetry (61).

The majority of these studies used incremental continuous physical activity heart rate–energy expenditure calibration tests to generate the prediction equations used to estimate energy expenditure from heart rate for each subject (31;62;88;130;136). Although the accuracy of the prediction of free-living energy expenditure has improved, a recent study by Bot (20), described a temporal dissociation between heart rate and oxygen consumption during intermittent activity, which is the typical characteristic of the activities of daily living. This rise in oxygen consumption lagged behind the heart rate values during each minute of recorded activity. Because of the real time lag in oxygen consumption, it is possible that the energy expenditure value may be more reflective of minute heart rate in the preceding minutes. On the basis of these findings, we hypothesised that it may be possible to further improve the accuracy of prediction of energy expenditure from heart rate monitoring, by considering the effects of heart rate in the preceding minute.

Thus, the initial aim of this study was to quantify the relative contributions of current heart rate as well as heart rate from the preceding minute, in the prediction of energy expenditure during physical activity and model a new group-based equation using a novel activity heart rate–energy expenditure protocol. Next we applied heart rate–energy expenditure data generated during intermittent activity to a prediction equation previously developed using the conventional continuous calibration procedure from Chapter 4. Therefore, the second aim of this study was to compare predicted versus
actual energy expenditure using the equations, derived from both the continuous heart rate–energy expenditure calibration protocol and the novel intermittent activity heart rate–energy expenditure calibration protocol generated during this investigation.
METHODS

Part 1: Development of a novel regression equation for predicting energy expenditure from heart rate during intermittent exercise

Subjects

Volunteers (N=65, 45 men, 20 women) were recruited from a local fitness centre. Subjects represented a heterogeneous sample of the population. All were free from known cardiovascular and metabolic disorders and regularly participated in some form of physical activity at least 3 times a week. The Research and Ethics Committees of the Faculty of Health Sciences of the University of Cape Town approved this study. All subjects were briefed in full, prior to the commencement of their participation in the trial. Subjects signed an informed consent form, after being given the opportunity to ask any questions.

Body composition and maximal test to exhaustion

Subjects reported to the laboratory on two separate occasions within one week. During visit one, subjects had their body composition measured using the near infrared reactance technique (Futrex Inc., Gaithersburg, MD, USA). Following this measurement, subjects performed a maximal test to exhaustion, using the Bruce multi-stage treadmill protocol (2). Briefly, subjects began walking at a grade of 10% and speed of 2.7km.h⁻¹ for three minutes, after which time both the treadmill speed and gradient were
increased every three minutes until the subject could no longer keep up with the pace of the treadmill. During the test the subject wore a facemask attached to an Oxycon Alpha automated gas analyser (Jaeger, The Netherlands). Before each test the gas analyser was calibrated with a Hans Rudolph 5530 3-litre syringe and a 5% CO₂ - 95% N₂ gas mixture. Analyser outputs were processed by a personal computer, which calculated breath-by-breath ventilation, oxygen consumption (VO₂), rates of carbon dioxide production (VCO₂) and respiratory exchange ratio (RER), using conventional equations (150).

*Novel energy expenditure calibration test*

This relationship was determined by measuring heart rate, VO₂ and VCO₂ simultaneously for 16 workloads, lasting 4–5 minutes each. Figure 5.1 presents the heart rate-energy expenditure calibration test in diagrammatical form. Energy expenditure (kJ.min⁻¹) was determined for each workload using the equations of Weir (150). During the test the subject wore a facemask attached to a K4b² portable gas analyser (Cosmed, Italy). Before each test the portable gas analyser was calibrated with a Hans Rudolph 5530 3-litre syringe and a three point calibration technique, using 5% CO₂ – 16% O₂ gas mixture and fresh air. Analyser outputs were processed, which calculated breath-by-breath ventilation, oxygen consumption (VO₂), rates of carbon dioxide production (VCO₂) and respiratory exchange ratio (RER), using conventional equations. During the first 2 stages of the protocol, subjects were tested while quietly sitting and standing. Following these resting measurements, the subject proceeded to a motorised treadmill, where they completed two workloads of walking at 4 and 6 km.hr⁻¹ respectively. Following the
walking measurements, subjects were required to rest, standing completely still for one minute. After the rest period, subjects completed two workloads on a stationary cycle ergometer (Lode, The Netherlands), at 30 and 60 W respectively. Immediately following these cycling measurements, subjects were required to rest completely still for one minute. During the next four activity stages, subjects performed progressive stepping exercises, beginning at a step height of 30 cm, and at a rate of 80 steps per minute, increasing to 100 steps per minute after 4-5 minutes. Following the initial two step workloads, on the 30 cm step, subjects rested for one minute, before completing the final two stepping workloads, on a step height of 50 cm, starting once again at a rate of 80 steps per minute and progressing to 100 steps per minute. Subjects completed their final one minute rest period, before completing a further two treadmill running stages at 9 and 11 km.hr⁻¹.

Statistical analysis for Part 1

Data from the 65 subjects were used in the final analysis. The initial exploratory data analyses, to determine factors that may have significantly contributed to the relationship between heart rate and energy expenditure included, Box plots and Scatter plots for all variables (not shown). Univariate (means, standard deviations) and bivariate (correlation coefficients) summary statistics were then calculated for all variables.

As in Chapter Four, we fitted a mixed model for predicting energy expenditure. Subject level factors gender, weight, age and VO₂max were modelled as fixed effects, and subjects as random effects with repeated measurements of energy expenditure (and
fixed heart rate) for each subject. In the model, the covariance matrix between the measurements for each subject was unstructured and compound symmetry was assumed for the covariances between subjects.

A second mixed model was again fitted under the rationale that in certain settings, a test of maximal oxygen consumption might be impractical or not available. The second model included all the variables and assumptions in the original model, except VO$_2$max.

Finally energy expenditure was also calculated using a conventional continuous model (Chapter Four) for estimated energy expenditure comparison.

The initial exploratory analyses were done with Statistica (Statsoft, Southern Africa Inc. (2002). Statistica data analysis software system, version 6.1). Statistical modelling was done with SAS® Proprietary Software Release 8.2 (USA).

**Part 2: Inner validation of the novel mixed model**

**Subjects**

The novel mixed model was applied to the same subject sample, used during the inner validation of the model developed in Chapter Four. Briefly, 17 volunteers were recruited from a local fitness centre (N=9 men, N=8 women).
Body composition and maximal test to exhaustion

As previously mentioned, subjects reported to the laboratory on two different occasions within one week, for body composition determination, using near infrared reactance (Futrex Inc, Gaithersburg, MD, USA) and a maximal test to exhaustion. The maximal test to exhaustion was performed on an electronically braked cycle ergometer (Lode, Gronigen, The Netherlands). Initially, the men began cycling at a workload of 150 W and the women at a workload of 100 W. Both workloads were increased by 50 W respectively after 150 seconds, and thereafter 25 W every 150 seconds until exhaustion or the subject could no longer maintain a RPM of greater than 70 RPM (57). During the test the subject wore a facemask attached to an Oxycon Alpha automated gas analyser (Jaeger, The Netherlands). Before each test the gas analyser was calibrated and analyser outputs were processed by a personal computer, as previously described in this text.

Estimation of physical activity using the novel mixed model

During the second visit for the determination of physical activity energy expenditure, using the novel mixed model, subjects reported to the laboratory to complete a 55-minute exercise session (unlike Chapter Four). This exercise session was considered to be representative of a normal physical activity training session and included a 5-minute warm-up, consisting of 2-minutes of walking and 3-minutes of slow jogging, a 20-minute toning circuit session, a 20-minute cardiovascular session, which could either comprise of one 20-minute continuous session, or two 10-minute sessions and a 5-minute cool-
down session. The toning circuit consists of both upper and lower body weight training exercises and require that participants complete as many repetitions of the respective exercise during a 45 second bout. At the end of the allotted time an auditory and visual signal is made, after which participants have 15-seconds to move to the next piece of equipment and prepare for the next 45-second exercise bout. Subjects were randomly assigned to either completing the cardiovascular or tone circuit first.

During the 55-minute exercise training session, individuals' heart rate, VO₂ and VCO₂ were continuously monitored using the K4b² portable gas analyser (Cosmed, Italy). Minute-by-minute energy expenditure (kJ.min⁻¹) was then determined for the exercise session using the non-protein caloric equivalents for oxygen (150). Before each test the portable gas analyser was calibrated as previously described. Analyser outputs were processed, which calculated breath-by-breath ventilation, oxygen consumption (VO₂), carbon dioxide production (VCO₂) and respiratory exchange ratio (RER), using conventional equations (150).

Statistical analysis for Part 2

The data from the 17 subjects was used for the validation of the novel model, developed during Part 1. We used both the initial sample (Part 1), as well as the validation sample (Part 2), to compare the novel model, developed in Part 1, to a previous conventional model (developed in Chapter Four), as well as to a model, which contained no measure of fitness. The association between the measured energy expenditure and estimated energy expenditure for each model (novel and conventional) and each sample (initial
(Part 1) and validation (Part 2) was measured using an interclass correlation coefficient. The validity of the estimated energy expenditure each model and each sample was examined by calculating 95% limits of agreement. The comparisons were done in Microsoft Excel XP.
RESULTS

Part 1

Subject characteristics

Subject characteristics are presented in Table 5.1a. Subjects represented a broad range in body composition, 4.2 to 25.1% body fat in men to 19.9 to 37.7% in women. Similarly, there was a wide range in the performance data (Table 5.2a) with the VO$_2$max values ranging from 39.4 to 80.4 ml.kg$^{-1}$.min$^{-1}$ in men and from 29.4 to 61.8 ml.kg$^{-1}$.min$^{-1}$ in women.

Prediction of energy expenditure from minute and preceding minute heart rate

Initially using simple correlation analysis, we identified variables that were significantly associated with measured energy expenditure. These included: age (years), VO$_2$max (ml.min$^{-1}$), current minute heart rate (bpm), preceding minute heart rate (bpm), weight (kg) and gender and an interaction variable between preceding minute heart rate and VO$_2$max. Additionally, we examined the relationships between explanatory variables to minimise the effects of co-linearity in the regression.
The final mixed model for predicting intermittent activity energy expenditure was:

$$EE (kJ.min^{-1}) = -59.2944 + (2 - \text{gender}) \times (-20.6364 + 0.5315 \times \text{age} + 0.1952 \times \text{VO}_2\text{max} + 0.5814 \times \text{current minute's heart rate} - 0.1564 \times \text{previous minute heart rate} + 0.002822 \times \text{VO}_2\text{max} \times \text{previous minute heart rate} + 0.2767 \times \text{weight}) + (\text{gender} - 1) \times (0.2234 \times \text{age} + 0.3854 \times \text{VO}_2\text{max} + 0.3798 \times \text{heart rate} - 0.04561 \times \text{previous minute heart rate} + 0.001747 \times \text{VO}_2\text{max} \times \text{previous minute heart rate} + 0.1486 \times \text{weight}).$$

Where gender = 1 for males and gender = 0 for females. Table 5.3 presents this model in a different format. The variance component for the repeated energy expenditure measurements for subjects was estimated as 18.22 and the variance between subjects as 26.65 for the novel model. Results of type III tests for the fixed effects in the novel model are given in Table 5.4. Degrees of freedom for the F-tests were calculated with Satterthwaite’s method.

In Figure 5.2, the measured energy expenditure is regressed against estimated energy expenditure, using the novel equation. The correlation coefficient is $r=0.90$ and the model explained 81.36% of the variation in measured energy expenditure in the original sample (N=65).

As in the previous chapter, a second model, which contained no measure of cardiovascular fitness, was also fitted. The final prediction equation for energy expenditure using age, gender, weight, heart rate and preceding heart rate accounted for 78.4% of the variance in estimated energy expenditure. Table 5.5 represents the
above model in a different format. Results of type III tests for the fixed effects in the second model are given in Table 5.6.

**Part 2: Inner validation of novel equation**

Energy expenditure was calculated using the heart rate and $V_O^2$ data from 17 subjects, completing a 55-minute exercise training bout, which included 20-minutes of cardiovascular training, and 20-minutes of intermittent weight training activity in a fitness centre, using both the novel and conventional models (developed during Chapter Four).

**Subject characteristics**

Subject characteristics are presented in Table 5.1b and Table 5.2b. This inner validation sample was not significantly different to the sample used to generate the equation, with respect to their height, weight, body fatness and maximal oxygen uptake. However, the males in this group were significantly older ($P<0.046$) than previous sample, while the women were comparable.

**Estimation of energy expenditure using novel activity equation**

Using the novel mixed model developed in Part 1 of this chapter, heart rate data was used to estimate the free-living physical activity energy expenditure from the 55-minute exercise training session. Measured energy expenditure was also calculated using $V_O^2$.
and respiratory exchange data. The average energy expenditure (kJ.min\(^{-1}\)) measured during the 55-minute exercise session was 30 ± 16 kJ.min\(^{-1}\). The mean total energy expenditure estimated using the novel mixed model was 35 ± 18 kJ.min\(^{-1}\), while the mean total energy expenditure estimated using the continuous or conventional equation was 39 ± 19 kJ.min\(^{-1}\). The two estimates for energy expenditure during the exercise session, using either the novel mixed or continuous models were both significantly different from the measured energy expenditure and from each other (P<0.00).

As in Chapter Four, and as a result of using mixed models (with random subject effects) we had to use maximum likelihood estimation and not least squares. The bias of the estimates and their random variation can be summarised as follows for the four sets of estimates and are presented in Table 5.7. The 95% limits of agreement were calculated as mean ± 1.96 x standard deviation (10).

In Figure 5.3, the measured energy expenditure is regressed against estimated energy expenditure, using the novel mixed model. The novel model explained 76.3% of the variance in measured energy expenditure in the sample. While in Figure 5.4, the estimated energy expenditure using the conventional equation is regressed against the measured energy expenditure. The models were comparable with 76% of the variance in predicted energy expenditure explained.

Figure 5.5 presents the estimated energy expenditure during the cardiovascular portion of the gym session, as calculated using either the novel model or the conventional equation. The novel model accounted for 71% of the variance in measured energy.
expenditure, while the conventional model explained 73% of the variance in measured energy expenditure, during the cardiovascular activity. Conversely the variance explained for the intermittent gym activity was 78.2% for the novel equation, while the conventional model produced an $R^2$ of 73.2% for the intermittent gym activity. The mean differences between predicted and measured energy expenditure using the 2 different models were significantly different for both the intermittent and cardiovascular activities ($P<0.000$). However, both models tended to over predict measured energy expenditure, using indirect calorimetry.
Table 5.1a. Subject characteristics (N= 65) for Part 1.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Body fat %</th>
<th>BMI (kg.m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (N=45)</td>
<td>35 ± 9</td>
<td>77.1 ± 8.4</td>
<td>16.2 ± 4.4</td>
</tr>
<tr>
<td>Women (N=20)</td>
<td>37 ± 9</td>
<td>62.8 ± 10.8</td>
<td>28.8 ± 5.0</td>
</tr>
</tbody>
</table>

Table 5.1b. Subject characteristics for inner validation sample (N=17), Part 2.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Body fat %</th>
<th>BMI (kg.m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (N=9)</td>
<td>28.6 ± 8.2</td>
<td>81.3 ± 14.4</td>
<td>13.7 ± 4.4</td>
</tr>
<tr>
<td>Women (N=8)</td>
<td>33.3 ± 10.4</td>
<td>61.0 ± 8.7</td>
<td>26.0 ± 3.9</td>
</tr>
</tbody>
</table>

Table 5.2a. Performance data (N=65) for Part 1.

<table>
<thead>
<tr>
<th>Max time</th>
<th>VO₂max</th>
<th>VO₂max</th>
<th>Max heart rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>(min)</td>
<td>(ml.min⁻¹)</td>
<td>(ml.kg⁻¹.min⁻¹)</td>
<td>(bpm)</td>
</tr>
<tr>
<td>Men (N=45)</td>
<td>14.4 ± 2.4</td>
<td>4527 ± 757</td>
<td>59.2 ± 10.4</td>
</tr>
<tr>
<td>Women (N=20)</td>
<td>10.6 ± 2.2</td>
<td>2288 ± 485</td>
<td>45.7 ± 7.6</td>
</tr>
</tbody>
</table>
### Table 5.2b. Performance data for inner validation sample (N=17), Part 2.

<table>
<thead>
<tr>
<th></th>
<th>Max time (min)</th>
<th>VO$_2$max (ml.min$^{-1}$)</th>
<th>VO$_2$max (ml.kg$^{-1}$.min$^{-1}$)</th>
<th>Max heart rate (bpm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men (N=9)</strong></td>
<td>13.3 ± 6.1</td>
<td>4360 ± 941</td>
<td>54.3 ± 11.3</td>
<td>190 ± 9</td>
</tr>
<tr>
<td><strong>Women (N=8)</strong></td>
<td>8.9 ± 2.8</td>
<td>2610 ± 420</td>
<td>42.3 ± 5.4</td>
<td>178 ± 16</td>
</tr>
</tbody>
</table>

### Table 5.3. The estimates and their standard errors for the fixed effects of the mixed novel model, including VO$_2$max.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Male Estimate</th>
<th>Male Std error</th>
<th>Female Estimate</th>
<th>Female Std error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-79.93</td>
<td>13.07</td>
<td>-59.29</td>
<td>24.20</td>
</tr>
<tr>
<td>Hear rate (HR)</td>
<td>0.5814</td>
<td>0.01725</td>
<td>0.3798</td>
<td>0.0272</td>
</tr>
<tr>
<td>VO$_2$max</td>
<td>0.1952</td>
<td>0.1248</td>
<td>0.3854</td>
<td>0.2809</td>
</tr>
<tr>
<td>Age</td>
<td>0.5315</td>
<td>0.1045</td>
<td>0.2234</td>
<td>0.1834</td>
</tr>
<tr>
<td>Previous min HR</td>
<td>-0.1564</td>
<td>0.0602</td>
<td>-0.0456</td>
<td>0.0856</td>
</tr>
<tr>
<td>VO$_2$max x previous min HR</td>
<td>0.002822</td>
<td>0.000956</td>
<td>0.001747</td>
<td>0.001769</td>
</tr>
<tr>
<td>Weight x gender</td>
<td>0.2767</td>
<td>0.1044</td>
<td>0.1486</td>
<td>0.1508</td>
</tr>
</tbody>
</table>
Table 5.4. Results of type III tests for fixed effects. Degrees of freedom for the F-tests were calculated with Satterthwaite’s method.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Degrees of freedom</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>2</td>
<td>73.6</td>
<td>21.69</td>
</tr>
<tr>
<td>Heart rate x gender</td>
<td>2</td>
<td>642</td>
<td>665.4</td>
</tr>
<tr>
<td>VO₂max x gender</td>
<td>2</td>
<td>154</td>
<td>2.17</td>
</tr>
<tr>
<td>Age x gender</td>
<td>2</td>
<td>56.5</td>
<td>13.69</td>
</tr>
<tr>
<td>Previous min HR x gender</td>
<td>2</td>
<td>657</td>
<td>3.52</td>
</tr>
<tr>
<td>VO₂max x previous min HR</td>
<td>2</td>
<td>658</td>
<td>4.84</td>
</tr>
<tr>
<td>Weight x gender</td>
<td>2</td>
<td>56.4</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Table 5.5. The estimates and their standard errors for the fixed effects for the model without VO₂max.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate</th>
<th>Std error</th>
<th>Estimate</th>
<th>Std error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>-16.067</td>
<td>11.579</td>
<td>-20.178</td>
<td>15.938</td>
</tr>
<tr>
<td>HR x gender</td>
<td>0.194</td>
<td>0.067</td>
<td>0.397</td>
<td>0.067</td>
</tr>
<tr>
<td>Previous min HR x gender</td>
<td>0.311</td>
<td>0.038</td>
<td>0.155</td>
<td>0.063</td>
</tr>
<tr>
<td>HR x previous min HR x gender</td>
<td>-0.002</td>
<td>0.000</td>
<td>-0.001</td>
<td>0.000</td>
</tr>
<tr>
<td>Weight x gender</td>
<td>-0.597</td>
<td>0.151</td>
<td>-0.174</td>
<td>0.191</td>
</tr>
<tr>
<td>Age x gender</td>
<td>0.353</td>
<td>0.118</td>
<td>-0.080</td>
<td>0.177</td>
</tr>
<tr>
<td>HR x weight x gender</td>
<td>0.007</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table 5.6. Results of type III tests for fixed effects, for the model not containing $VO_2^{\text{max}}$. Degrees of freedom for the F-tests were calculated with Satterthwaite’s method.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Degrees of freedom</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>2</td>
<td>115</td>
<td>1.76</td>
</tr>
<tr>
<td>HR x gender</td>
<td>2</td>
<td>641</td>
<td>21.6</td>
</tr>
<tr>
<td>Previous min HR x gender</td>
<td>2</td>
<td>643</td>
<td>36.74</td>
</tr>
<tr>
<td>Age x gender</td>
<td>2</td>
<td>58.3</td>
<td>4.6</td>
</tr>
<tr>
<td>Weight x gender</td>
<td>2</td>
<td>125</td>
<td>8.21</td>
</tr>
<tr>
<td>HR x previous min HR x gender</td>
<td>2</td>
<td>640</td>
<td>43.04</td>
</tr>
<tr>
<td>HR x weight x gender</td>
<td>2</td>
<td>641</td>
<td>36.72</td>
</tr>
</tbody>
</table>

Table 5.7. Bias statistics including limits of agreement for all models and samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Model</th>
<th>$R^2$</th>
<th>Mean deviation</th>
<th>Std</th>
<th>95% limits of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>Novel</td>
<td>0.8136</td>
<td>2.35</td>
<td>6.94</td>
<td>-11.26 15.97</td>
</tr>
<tr>
<td>Original</td>
<td>Novel No $VO_2^{\text{max}}$</td>
<td>0.7840</td>
<td>-0.05</td>
<td>7.36</td>
<td>-14.46 14.37</td>
</tr>
<tr>
<td>Original</td>
<td>Conventional</td>
<td>0.7888</td>
<td>1.58</td>
<td>7.45</td>
<td>-13.01 16.18</td>
</tr>
<tr>
<td>Validation</td>
<td>Novel</td>
<td>0.7555</td>
<td>4.71</td>
<td>8.76</td>
<td>-12.46 21.88</td>
</tr>
<tr>
<td>Validation</td>
<td>No $VO_2^{\text{max}}$</td>
<td>0.6468</td>
<td>7.39</td>
<td>9.80</td>
<td>-11.82 26.60</td>
</tr>
<tr>
<td>Validation</td>
<td>Conventional</td>
<td>0.6609</td>
<td>14.74</td>
<td>13.66</td>
<td>-12.04 41.52</td>
</tr>
</tbody>
</table>

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Figure 5.1. Diagrammatical representation of novel calibration protocol

Energy expenditure calibration test
Subjects fitted with $K_b^2$ portable gas analyser (Cosmed, Italy), and heart rate monitor (Polar, Finland)
Figure 5.2. Scatter plot reflecting estimated energy expenditure, using the novel mixed model equation, regressed against measured energy expenditure for the original prediction sample (N=65). The coefficient of correlation was \( r=0.90 \), and accounted for 81% of the variance in measured energy expenditure, \( P<0.000 \).

Figure 5.3. Scatter plot of estimated energy expenditure (kJ.min\(^{-1}\)), on an independent sample (N=17), using the novel mixed model regressed against the observed values, adjusted \( R^2=0.76, P<0.0000 \).
**Figure 5.4.** Scatter plot of estimated energy expenditure (kJ.min⁻¹), using conventional model (developed in Chapter Four), regressed against observed values, on an independent sample (N=17). Adjusted $R^2=0.76$, $P<0.0001$.

**Figure 5.5.** Estimated energy expenditure associated with all cardiovascular activity during the training session, calculated using novel mixed model and conventional model, regressed against measured energy expenditure. The novel model accounted for 71% of the variance in measured energy expenditure, while the conventional model accounted for 73% of the variance in measured energy expenditure.
Figure 5.6. Estimated energy expenditure estimated associated with all other activities not specifically cardiovascular during the gym training session, using either the novel mixed model or conventional equation, is regressed against measured energy expenditure. The novel model accounted for 78% of the variance in measured energy expenditure, while the conventional model accounted for 73% of the variance in measured energy expenditure.
DISCUSSION

The first finding from this study confirmed that heart rate in the preceding minute did contribute significantly to the model for predicting energy expenditure, during intermittent and continuous activity. Indeed, this finding corroborates previous work by Bot et al. (20) and Lothian and Farrally (84), in which a temporal dissociation between heart rate and VO$_2$, or energy expenditure during intermittent-type activity was demonstrated. To our knowledge, our study is one of the first studies to use this dissociation as a basis for incorporating the previous minute's heart rate into the regression equation for energy expenditure estimation.

Traditionally, energy expenditure estimation, using the heart rate method, has been calculated based either on an individual or group calibration test, which is usually continuous and incremental in nature (29;81;85;136). However, these calibration tests are perhaps not truly reflective of the nature of the activities of daily living, which are more stochastic or intermittent. Further, the effect of heart rate recovery, as a result of intermittent activity, is not typically incorporated into the energy expenditure estimation. It is documented (163;165), that exercise, particularly endurance training accelerates heart rate recovery. In a study by Yamatoto et al. (165), subjects completed a six-week endurance training program. Following training there were significant decreases in heart rate and parasympathetic control during both rest and post-exercise recovery periods, highlighting the effect that endurance training has on the post-exercise heart rate recovery and further the effect of the cardiac autonomic control in response to exercise training.

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As a result, the intermittent calibration test may be more appropriate for characterising the heart rate-energy expenditure relationship during intermittent or discontinuous activities more commonly associated with daily living. By incorporating scheduled stoppages into the calibration test, the heart rate in the preceding minute may either reflect increasing work rate or a recovery period. This is supported by the data from the inner validation component of the study. Using the technique described by Atkinson and Nevill (10), the differences between the observed energy expenditure and the estimated energy expenditure, were significantly greater for the conventional continuous model compared to novel model (Table 5.7.).

This provides evidence to support the inclusion of both the heart rate in the preceding minute, as well as the interaction variable of oxygen consumption and previous minute's heart rate, into the final regression model. It is well documented that heart rate recovery may be more rapid with increased levels of fitness and training (88;114;136;162;163).

Previously Strath (145) attributed the modest correlation found between heart rate and oxygen consumption during moderate intensity activities in the both the laboratory and field settings to the fact that trained individuals have a lower heart rate at any given oxygen consumption than non-trained individuals. While, Darr et al. (39) found a significant training effect on heart rate effect recovery, with heart rates in trained subjects falling more quickly in the first two minutes after stopping exercise, irrespective
of age (P<0.005). This therefore, provides good evidence for not only the use of a novel calibration test, but for including a measure of fitness in the regression equation.

In addition, during moderate intensity exercise of increasing length, a phenomenon known as cardiovascular drift is observed (35;45;46;49). Coyle et al. (35) describes cardiovascular drift as a progressive decline in stroke volume and systemic mean arterial pressure and parallel increase in heart rate during prolonged exercise. As a result of the increasing heart rate, energy expenditure may be overestimated in a model that does not consider the heart rate response in the preceding minute.

In our study, there was a greater attenuation of heart rate response with training at lower absolute workloads, while the heart rate response at higher workloads was more similar, irrespective of training or fitness levels. This is probably due to an increased sympathetic activation during higher intensity exercise. Yamamoto et al. (165) found greater than expected reductions in resting and post exercise heart rate, as a result of a relatively high training intensity. While, Arai et al. (8) demonstrated in a group of healthy subjects, compared to two groups of patients with cardiovascular disease, that heart rate sympathetic modulation was significantly increased during rest and post-exercise recovery. In contrast, during maximal exercise, this modulation was attenuated. This finding demonstrates the withdrawal of vagal activity during exercise experienced by healthy subjects, and the return during recovery.

To our knowledge, only two other studies (114;145) report a measure of fitness in the final model generated to predict energy expenditure from heart rate. In the study by
Rennie et al. (114), the prediction model included a variable that described sitting pulse rate and was correlated (P<0.001) with measured energy expenditure in both the men and women. In this particular study, sitting heart rate was chosen as a result of the above-mentioned heart rate alterations during both rest and post-exercise recovery, for those participating in regular physical activity training. While in the study by Strath et al. (145) subjects had their VO₂max predicted, using equations of Jackson et al. (64), to estimate VO₂ reserve. Using VO₂ and minute-by-minute heart rate data accumulated during 15-minute activities, resembling the activities of daily living, heart rate accounted for 47% of the variance in VO₂, and further accounted for 76% of the variance in measured energy expenditure, using on-line gas analysis.

While we acknowledge that it is not always practical to obtain an accurate measure of VO₂max, it is evident that some measure of fitness is important in the final energy expenditure estimation. Indeed, when a second model was generated (without a fitness measure), the goodness of fit was less than the model that included VO₂max (R²=0.81 versus R²=0.76). In the validation sample, the model without a measure of fitness accounted for only 64% of the variance in measured energy expenditure.

Finally, recent studies (85;143;144) have improved the prediction of energy expenditure from heart rate by including simultaneous heart rate-motion and other sensors to the final prediction models. In one such study (143), comparing simultaneous heart rate and activity motion sensing to the flex heart rate technique and indirect calorimetry, in 10 participants (mean age 26.6 years), the flex heart rate technique significantly overestimated energy expenditure (P<0.001), while the simultaneous heart rate-motion
sensing technique showed good agreement ($R^2=0.81$) with the indirect calorimetry method, during 6hrs of free-living activity. However, in this study, investigators discriminated between upper and lower body activities, developing two individual heart rate-energy expenditure calibrations for each subject, and placing two motion sensors on each subject, one for the upper body activities and one for the lower body activities. While it has been shown that motion sensing may improve energy expenditure prediction from heart rate alone, the method is a poor predictor when assigning metabolic cost to weight bearing, upper body activity and stair climbing (61). We have shown in this study that it is possible to improve the accuracy of predicting energy expenditure from heart rate, in particular for intermittent activity, at a level similar to or better than that achieved using both methods. This method may be more suitable for predicting during intermittent or stochastic activity energy expenditure representative of the activities of daily living, as well as exercise training.

In conclusion, in this study we provide evidence for the use of a mixed model regression equation, incorporating a measure of fitness and the preceding minute's heart rate to further increase the estimation of physical activity energy expenditure, particularly during intermittent or stochastic activity. This may be of particular benefit to estimate the energy expenditure associated with the activities of daily living. However, further work should be completed, exploring the accuracy of this equation, particularly with reference to total daily energy expenditure estimation during activities representative of habitual free-living.
CHAPTER SIX

PRACTICAL VALIDATION OF GROUP-BASED, HEART RATE-ENERGY EXPENDITURE EQUATIONS FOR PHYSICAL ACTIVITY AND ACCELEROMETRY, USING A RESPIRATION CHAMBER.
INTRODUCTION

In Chapter Three of this thesis, the need for accurate and reliable measures of free-living physical activity energy expenditure was discussed, particularly in light of recent focus on physical activity dose-response for the prevention of non-communicable disease. A technique which has been investigated thoroughly in both this thesis (Chapters Two through Five) and over the last 20 years is the heart rate monitoring technique for predicting free-living energy expenditure (29;62;81;82;85;136) (88;114). Based upon the assumption of a near-linear relationship that exists between heart rate and energy expenditure, heart rate monitoring is able to account for approximately 75% of the variance in energy expenditure measured using indirect calorimetry (29;136) and for between 87 to 97% of the energy expenditure measured using doubly labelled water method (130). In Chapters Four and Five we were able to account for 73% of the variance in measured energy expenditure, during a 55-minute gym training session, in an independent sample of 17 subjects, using the heart rate monitoring technique. These results represent a significant improvement compared to previous equations (62;114). However, the utility of this equation has yet to be determined over an extended period of time, and during free-living, daily activities.

In previous studies, heart rate-energy expenditure estimates have been derived largely from individual calibration, and validated against some measure of indirect calorimetry, during short-term exercise bouts (62;145) or over a more extended period of time, using techniques such as doubly labelled water, whole room respirometry, or even Douglas bags (29;136). Conversely, group-based predictions, derived by incorporating
various demographic or morphological characteristics into heart rate and energy expenditure regression equations have typically relied on either indirect calorimetry during short-term exercise bouts (145), or have not undergone validation by any means, on independent samples (62). In Chapters Four and Five of this thesis, we explored 2 novel, group-based equations (one continuous and one intermittent), for energy expenditure estimation, and validated them using indirect calorimetry, in an independent sample during a 55-min bout of physical activity. However, we have not yet studied the utility of such equations, during a more prolonged period of time, in which activities of daily living are incorporated.

Nearly all of the previous studies, which have attempted to predict energy expenditure using heart rate over a 24hr period have incorporated the use of a "flex heart rate". Spurr et al. (136) was one of the first investigators to explore this relationship between heart rate and energy expenditure during low intensity activity and introduced the concept of the flex heart rate. Briefly, flex heart rate is calculated as the average heart rate calculated from averaging heart rates associated with rest and heart rates associated with activity. Typically, heart rates below flex heart rate are assigned values equalled to measured resting metabolic rate. Using individual heart rate-energy expenditure subject calibration (as in Chapters Two and Three of this thesis) and the cut-off flex heart rate (substituting measured resting energy expenditure values) for each subject, Spurr et al. (136) was able to account for 76% of the variance in measured energy expenditure, in 11 subjects, during 22hours using a respiration chamber.
Another tool, which has received much attention, in the measurement of energy expenditure, during waking hours, is the accelerometer (12;22;23;72;155). Accelerometers are battery powered motion sensors, and more recently include measurements in the 3-dimensional planes, antero-posterior, medio-lateral, and vertical (22;23). Three piezoresistive ceramic plates are mounted orthogonally in a resin block and each of the 3 axes is measured independently (79). While motion sensors have been shown to have an excellent facility to measure energy expenditure during ambulatory physical activity, a limitation is the under-estimation of energy expenditure during arm external work (12), such as carrying heavy loads or heavy manual labour, running up inclines, or exercising on a stationary ergometer, for example stepping machines, rowers machines, and cycle ergometers', found in most fitness centres.

A study by Jakicic et al. (65) found that the energy expenditure measured using a 3-dimensional accelerometer correlated significantly (P<0.05) for various activities, accounting for between 46 to 85% of the variance in measured energy expenditure, using indirect calorimetry. In this particular study, the variance in estimated energy expenditure, increased with increasing intensity in the activity, i.e. as subjects had to walk or run faster, the difference between the measured and estimated energy expenditure increased. In spite of this limitation, accelerometers have gained worldwide acceptance as a tool for measuring free-living energy expenditure, in epidemiological research.

In another study by Powell et al. (107) the variability associated with a 3-dimensional accelerometer was explored. In this study two identical units were compared, during
two trials, 2-days apart, and during six different activities, including resting, walking and running activities. Results showed that the inter-individual variance, during activities involving locomotion to be less than 6%, however, during activities which involved sitting and standing, the variance was significantly increased, to as much as 25%. In addition, when data were analysed according the 3-axis', the vertical plane (x) showed the smallest amount of variance, compared to the anterior posterior and medio-lateral planes.

One of the objectives of this dissertation was to successfully construct and put into operation a respiration chamber. Further, one of the primary initial purposes of this chamber was to validate various other indirect techniques used for estimating total daily energy expenditure, and in particular physical activity energy expenditure. As shown above, studies, in which respiration chambers have been used to validate the heart rate monitoring and accelerometry techniques, have both accounted for approximately 85% of the variance in measured energy expenditure using these tools (72;136). However, to our knowledge, there has been no previous validation of a heart rate-energy expenditure equation that incorporates a measure of VO\textsubscript{2}max and considers heart rate in the preceding minute, nor one that was designed specifically for intermittent activity.

Therefore the aim in Part 1 of this study was to construct and validate a respiration chamber. On the successful completion of Part 1, our second aim (Part 2) was to validate the energy expenditure equation, developed in Chapter Five of this thesis, over a 24hr period, and thirdly compare energy expenditure estimated using this equation to energy expenditure estimated using a 3-dimensional accelerometer and energy
expenditure estimated using our conventional, continuous equation, developed in Chapter Four.
METHODS

Part 1

Construction and validation of a respiration chamber

The respiration chamber was based on the two existing models, currently in operation at the University of Maastricht, Netherlands, and is the only operational respiration chamber in Sub-Saharan Africa. Our chamber may be described as a push-type, open-circuit, indirect, respiration chamber. It is approximately 19.7 m$^3$ and furnished similarly to a normal household bedroom. The two windows and door allow the subject contact with the researcher, as well as views of outside. The windows are constructed of polycarbonate (900mm x 800mm, and 1200mm x 800mm), curtains ensure privacy and there is a two-way automated intercom for audible communication.

The chamber is fitted with a single bed, built-in sink and chest cabinet, dual colour television, video cassette player and a computer linked to a network portal. There is an electronically braked cycle ergometer (Lode, Gronigen, The Netherlands) as well as two stepping boxes (30 and 50 cm) and an electronic metronome for stepping exercises. The chamber is fitted with a freezer toilet (Scientific Technology Corporation, Cape Town, South Africa), for collecting faeces, while collection bottles are provided for urine collection. Two air locks provide entry and exit of food and collection of samples. The door to the chamber is operated using a quick release mechanism, operational from both sides.
**Ventilation**

The walls are constructed of 75mm chromodeck and the roof from 100mm chromodeck. Chromodeck can be described as polystyrene with waxed bonded 0.8mm hot dip galvanised epoxy sheet steel. The composite sheet end faces are formed into male-female interlocking ribs, which provide sealing during expansion and contraction, caused by internal pressure fluctuations. The heat leak factor is 61.6 watts per hour at an ambient temperature of 25°C. The two windows are manufactured from 3mm polycarbonate sheets. The windows are double glazed and triple sealed with a 75mm air gap in the window sandwich.

Air is forced into the chamber at a rate of 160 l.min⁻¹. Flow rate and barometric pressure are measured using a turbine transducer (Ventilation Measurement Module, Interface Associates, Aliso Viejo, California). Air is de-humidified and temperature-controlled (23–25 °C) using a single split wall mounted type air conditioner (LG Electronics, Spain). Humidity is detected via an interface to a Carel RUC Humidity Sensor, and temperature of sampled air will be measured using a modification of the Dallas DS1820 sensor. There is a louver fan (Sunbeam Products Inc, USA) to ensure good mixing of ambient air within the chamber. There is a fresh air inlet (30mm internal diameter), situated directly above the air conditioner, and an exhaust (30mm internal diameter) situated on the opposite side of the room, above the window.
Oxygen and carbon dioxide measurement systems

Continuous samples of chamber air, from the exhaust, and incoming fresh air, from the fresh air inlet, are sampled through 5mm sampling lines (Festo, Germany), connected to flow pumps (Ametek, Germany). From the pumps the sampling lines are directed into a solenoid manifold, which ensure continuous flushing of the sample, when not selected, therefore reducing dead time of the measurement sampling system. All sampling lines are fitted with membrane dryers (ME050-24-MFL, Perma Pure) as previously described (128), and which ensures enhanced drying of the sample gas. Oxygen is measured (0-22%) with a Xentra 4100 Gas Purity Oxygen Analyser (Servomex, United Kingdom) and carbon dioxide is measured (0-3%) with an Uras 4 NDIR Industrial Photometer Carbon Dioxide analyser (Hartmann and Braun, Germany).

All data are captured using a using a PC-based data acquisition system (Labview, National Instruments, USA). Continuous data is then further reduced to 3-minute intervals, and finally averaged to 1-hour values for the calculation of energy expenditure.

Chamber validation

Each month an independent check of the entire system is performed, by burning a known quantity of alcohol (99.9% ethanol) inside the chamber. The alcohol is placed inside a specially designed burner, and situated on a calibrated balance (Mettler-Toledo Spider 1-15, Mettler-Toledo, USA) (Figure 6.1). The reasoning behind the burn is that
when the alcohol is combusted, oxygen is consumed, while carbon dioxide is produced, thus simulating the normal measurement system. The final combusted mass is used to calculate the expected oxygen consumption and carbon dioxide production. Typically, the burns last between 12 to 18hrs in duration and the expected respiratory quotient for the combusted alcohol is 0.667 (90).

Part 2

Subjects

Five male volunteers were recruited from the University of Cape Town. All were free from known cardiovascular and metabolic disorders and regularly participated in some form of physical activity at least 3 times a week. The Research and Ethics Committees of the Faculty of Health Sciences of the University of Cape Town approved this study. All subjects were briefed in full, prior to the commencement of their participation in the trial. Subjects signed an informed consent form, after being given the opportunity to ask any questions.

Resting metabolic rate, body composition and maximal test to exhaustion

Subjects reported to the laboratory on two separate occasions within one week. During visit one, subjects arrived at laboratory early in the morning, following at least a 10hr fast. Subjects were instructed not to participate in any vigorous exercise training on the previous day. Subjects were then instructed to lie in the supine position, for a minimum
of a 10-minute period, before being placed under a ventilated hood (Vmax, Sensor medics, United Kingdom), for a minimum of a 30-minute period. Before each test the gas analyser was calibrated using the 2-point gas analysis technique and two gas mixtures; a 16% O\textsubscript{2}, 5% CO\textsubscript{2}, balance N\textsubscript{2} gas mix and a 25% O\textsubscript{2} gas mix. The flow was calibrated using a Hans Rudolph 3-litre syringe. Once the subject was placed under the hood, they were monitored closely to ensure the flow through the hood was at an optimal rate and that the CO\textsubscript{2} levels were acceptable.

On completion of the resting metabolic rate test, subjects proceeded to have their body composition measured using the near infrared reactance technique (Futrex Inc., Gaithersburg, MD, USA). Following this measurement, subjects then performed a maximal test to exhaustion, using an electronically braked cycle ergometer (Lode, Gronigen, The Netherlands). Briefly, after completing a 5-minute warm-up, each subject started cycling at an exercise intensity of 200 W for 150 s, after which time the work rate was increased by 50 W for a further 150 s. Thereafter, the exercise intensity was increased by 25 W every 150 s up to the point of exhaustion, defined either as volitional exhaustion or when the subject could no longer maintain a RPM of greater than 70 RPM (57).

During the test the subject wore a facemask attached to an Oxycon Alpha automated gas analyser (Jaeger, The Netherlands). Before each test the gas analyser was calibrated with a Hans Rudolph 5530 3-litre syringe and a 5% CO\textsubscript{2} – 95% N\textsubscript{2} gas mixture. Analyser outputs were processed by a personal computer, which calculated breath-by-breath ventilation, oxygen consumption (VO\textsubscript{2}), rates of carbon dioxide
production (VCO₂) and respiratory exchange ratio (RER), using conventional equations (150). Subjects were then familiarized with the respiration chamber.

**Accelerometry**

In addition to estimating energy expenditure using the heart rate monitoring technique, energy expenditure was also estimated using 3-dimensional accelerometry. Subjects were fitted with the RT3 triaxial accelerometer (Stay Healthy Inc., California, USA) for the duration of the 24hr measurement period. Data were downloaded using the manufacturer's software, and counts were converted to kJ.min⁻¹ (Stay Healthy Inc., California, USA).

*Energy expenditure determination using the respiration chamber*

On the second visit, the subject proceeded to spend 23hrs, in the respiration chamber, for the determination of total daily energy expenditure (TDEE). Initially subjects reported to the laboratory at 17h00. They were fitted with a telemetric heart rate monitor (Polar Electro, Oy, Finland) and three-dimensional accelerometer (RT3, Stay Healthy, California, USA). Both heart rate and body accelerations were measured during 1-minute intervals, for the entire measurement period. Subjects were then fully briefed with regards to the 23hr fixed activity chamber protocol. In addition, subjects received written instructions.
Briefly, the fixed activity protocol was as follows:

18h00 – 20h00 subjects enter the chamber
20h00 – dinner, 20h30-22h30 – video
10h30 – lights out
06h30 – subject woken
06h30-07h30 – RMR measurement, subject remain awake but lying
07h30-08h00 – ablutions
08h00 – breakfast
08h00-10h00 – free time
10h00-10h40 – cycling
10h40-12h30 – free time
12h30 – lunchtime
13h00-14h30 free time
14h30 – stepping exercises
15h00-17h00 – free time
17h00 – exit chamber

Resting metabolic rate measurement

The resting metabolic rate measurement was done immediately on waking, on the second day. Briefly, the subject was woken very early in the morning, and instructed to remain awake and lying down, for a period of one hour. The investigator monitored the
subject to ensure that they remained awake. Following this period subjects were then able to get up and carry out daily ablutions.

Meals

Subjects received 3 meals, dinner, breakfast and lunch, and 2 tea breaks, one mid-morning, and one mid-afternoon. Subjects were fed meals that comprised between 85-95% of their average habitual daily kJ intake, recorded in the preceding week to the overnight respiration chamber stay, using a 3-day dietary recall questionnaire (Appendix 10.5 and 10.6). Diets were not specifically matched with respect to habitual nutrient composition. Instead, the macronutrient composition of the diets was based on a standardised and prudent Western diet (146), which comprised approximately 60% carbohydrate, 15% protein and 25% fat.

Exercise protocol

There were two structured exercise sessions, performed during the day in the chamber, one mid-morning and a second one, mid-afternoon. The first exercise session was performed on a stationary cycle ergometer (Lode, Gronigen, The Netherlands) and comprised three 10-minute workloads, 25%, 50% and 70% of PPO obtained during the first visit. Initially, subjects performed a 5-minute warm-up at 75 W and proceeded into the first workload. Each cycling workload was pre programmed into the cycling ergometer, and this was automatically initiated once the subject reached a RPM of 25 W during the warm-up.
The second exercise session comprised 6 stepping workloads. All workloads were 5-minutes in length and conducted at a set cadence using an electronic metronome (Model SQ 50, Japan) and two different step heights. The step heights were 30- and 50cm, while the workloads were set at 80, 96 and 112bpm. Briefly, the subject began stepping at a cadence of 80bpm on the 30cm step, after 5 minutes they proceeded to the 50cm step and completed 5 minutes. They then rested for 1-minute before repeating the stepping but at 96bpm, completing 5-minutes on the 30cm step and then 5-minutes on the 50cm step. They rested again for 1-minute, and then proceeded to step at a cadence of 112bpm, again beginning on the 30cm step and then proceeding to the 50cm step.
Data preparation and statistical analysis

The data from the 23hr chamber stay; minute-by-minute heart rate monitoring and 3-dimensional accelerometer were averaged to hourly intervals. Incomplete heart rate and accelerometry data were excluded. For the purpose of data analysis, data were reduced and separated into awake, exercise and sleep periods. Accelerometer data and heart rate data were lost for two of the subjects, and therefore excluded. After the removal of sleep hours, data for the final analysis varied between 12 and 15 hours for each subject.

The energy expenditure equations, developed in Chapters Four (conventional continuous model) and Five (novel intermittent model) of this thesis, were modified for daily energy expenditure estimation. As a result of inconsistencies in energy expenditure estimates, during sedentary or low physical activity energy expenditure activities, a resting energy expenditure value was introduced as a constant into the models. As a result, heart rate derived energy expenditure estimates, falling below calculated resting metabolic rate, using the equations of Harris and Benedict (64), resting metabolic rate energy expenditure was inserted. Using the equations of Harris and Benedict, as opposed to measuring resting metabolic rate, upheld our aim of being able to apply generalised equations for energy expenditure estimation, without the need for individual subject calibration.

Data are presented as means ± standard deviations. An ANOVA, for repeated measures, was used to analyse the energy expenditure data, comparing the respiration chamber
measurements of 24hr energy expenditure to the estimates, using either heart rate monitoring or 3-dimensional accelerometry.
RESULTS

Part 1

Chamber Validation

Our respiration chamber was constructed in collaboration with the University of Maastricht, Netherlands, and now operates within the acceptable 5% error range for this equipment (128). Table 6.1 presents the chamber validation data, recorded during the independent entire system checks, from the last three alcohol burns. Figure 6.1. presents a diagrammatical representation of the burn and balance. The current analysis system produces a RQ of between 0.61-0.668 and both the O₂ and CO₂ recovery values are within the 5% error range.

Part 2

Subject characteristics

Subject characteristics and performance data are presented in Table 6.2. The subjects represented a fit, relatively young group of lean men. The mean age was 25.4 ± 3.5 years and mean body fat % was 11.6 ± 1.4%. The subjects' mean VO₂ max was 64.6 ± 8.8 ml.kg⁻¹.min⁻¹, while the average PPO was 332 ± 33W.
Food intake

The energy intake data is presented in Table 6.3. The average habitual intake recorded during 3 days, preceding the chamber visit, using a food diary, was 11644 ± 1001 kJ.day$^{-1}$. The average reported macronutrient intake (energy %) for the 5 subjects was approximately 3.5 ± 3.4% alcohol, 44.7 ± 9.0% carbohydrate, 18.7 ± 4.6% protein and 29.7 ± 7.3% fat. The average intake for the overnight chamber stay was 10714 ± 1251 kJ.day$^{-1}$, while the average macronutrient intake (energy %) for the overnight stay was 61.6 ± 1.8% carbohydrate, 15.2 ± 1.3% protein and 22.0 ± 1.9% fat. The diet energy content, during the chamber stay was on average 92 ± 5% of the subjects habitual energy intake.

Energy expenditure data

Resting metabolic rate

The average resting metabolic rate data, calculated using the ventilated hood technique was 4.70 ± 0.56 kJ.min$^{-1}$, while the average resting metabolic rate measured during the one hour before waking and calculated using the chamber technique was 5.5 ± 0.4 kJ.min$^{-1}$. This difference was statistically significant (P<0.48) and is presented in Figure 6.2.
Total daily energy expenditure for respiration chamber versus heart rate models and accelerometry techniques

The average total energy expenditure (including both non-exercise and exercise periods), for all subjects, is presented in Table 6.4 and Figure 6.3. The average total energy expenditure (including both non-exercise and exercise periods), measured in the respiration chamber was 11.3 ± 7.8 kJ.min⁻¹. Similarly, the total average energy expenditure estimated using the modified intermittent and conventional continuous heart rate models were 11.9 ± 9.5 kJ.min⁻¹ and 12.9 ± 10.4 kJ.min⁻¹, respectively, while the average energy expenditure measured using the accelerometer was 7.2 ± 2.8 kJ.min⁻¹. Energy expenditure estimated using the intermittent and continuous models accounted for 84% and 80%, of the variance in measured energy expenditure, respectively. Accelerometry accounted for only 62% of the variance in measured energy expenditure. Energy expenditure estimated using the two heart rate monitoring models was not statistically different from energy expenditure measured in the respiration chamber. However, energy expenditure estimated from the accelerometer was significantly lower (P<0.000) than that measured in the respiration chamber and versus the heart rate-energy expenditure equation estimation.

Table 6.5. presents the average bias and agreement limits, for individual estimates of total energy expenditure. The bias is the difference between the predicted and the corresponding actual value for energy expenditure, the closer the bias is to zero, the better the prediction. The 95% limits of agreement were calculated as mean ± 1.96 x standard deviation (10). The mean bias using the intermittent model, 0.6 ± 3.9 kJ.min⁻¹,
was lower when compared to the mean biases calculated for the conventional continuous model, 1.7 ± 4.8 kJ.min⁻¹ and accelerometry  -4.9 ± 6.2 kJ.min⁻¹. In addition, the intermittent model produced limits that were also less, -6.94 and +8.16 kJ.min⁻¹ versus -7.87 and +11.27 kJ.min⁻¹ and -17.1 and +7.4 kJ.min⁻¹, than both the continuous model and accelerometry, respectively.

The average energy expenditure (kJ.min⁻¹), for all awake time, but excluding both exercise sessions, measured using the respiration chamber was 8.8 ± 3.9 kJ.min⁻¹, while the average energy expenditure measured using the intermittent and continuous heart rate models were also 9.0 ± 6.0 kJ.min⁻¹ and 9.9 ± 7.0 kJ.min⁻¹, respectively. The average energy expenditure measured using the accelerometer was 6.1 ± 1.0 kJ.min⁻¹. Energy expenditure using the three methods was not significantly different.

Table 6.6. presents the average bias and limits of agreement data for all awake time, excluding exercise time. The intermittent model accounted for only 27% of the variance in measured energy expenditure, while the continuous model accounted for 30% of the variance in measured energy expenditure. The accelerometer method accounted for 0.32% of the variance in measured energy expenditure. The mean bias for the intermittent model and continuous models were -0.1 ± 2.5 kJ.min⁻¹ and 0.7 ± 2.9 kJ.min⁻¹, respectively. The mean bias for the accelerometer was -2.8 ± 1.6 kJ.min⁻¹. The limits of agreement for the intermittent model were between -5.1 and +4.9 kJ.min⁻¹, and for the continuous model, between -5.0 and +6.3 kJ.min⁻¹ and the limits of agreement for the accelerometer were -5.84 and +0.4 kJ.min⁻¹.
Physical activity energy expenditure

The physical activity energy expenditure data are presented in Table 6.4. and Figure 6.4. The average physical activity energy expenditure (kJ.min\(^{-1}\)) measured during the 30 minute cycling session using the respiration chamber method was 31.7 ± 11.3 kJ.min\(^{-1}\), while the average physical activity energy expenditure estimated using the either heart rate models were 34.9 ± 4.6 kJ.min\(^{-1}\) and 37.3 ± 3.4 kJ.min\(^{-1}\), intermittent and continuous respectively. The average cycling physical activity energy expenditure measured using the accelerometer was 12.8 ± 4.1 kJ.min\(^{-1}\) and was significantly different to energy expenditure estimated using the heart rate models or respiration chamber.

The average physical activity energy expenditure (kJ.min\(^{-1}\)) measured using the respiration chamber, during the stepping exercises was 17.2 ± 2.2 kJ.min\(^{-1}\), the average physical activity energy expenditure measured using either heart rate model were 20.6 ± 7.8 kJ.min\(^{-1}\) and 22.6 ± 9.8 kJ.min\(^{-1}\), intermittent and continuous respectively. The physical activity energy expenditure estimated from the accelerometry was 11.5 ± 2.6 kJ.min\(^{-1}\). Energy expenditure estimates were not different using the different tools.

Table 6.7. presents the average bias and limits of agreement for each method for the total physical activity energy expenditure. The intermittent model accounted for 74% of the variance in physical activity energy expenditure, during both exercise sessions, measured in the chamber. The mean bias for the intermittent model was 3.2 ± 5.9
kJ.min\(^{-1}\), and the limits of agreement were \(-8.3\) and \(+14.6\) kJ.min\(^{-1}\). Conversely, the continuous model accounted for only 64% of the measured energy expenditure. The average bias was \(5.5 \pm 7.8\) kJ.min\(^{-1}\), while the limits of agreement were between \(-9.8\) and \(+20.7\) kJ.min\(^{-1}\). Finally, the accelerometry accounted for only 7% of the total variance in physical activity energy expenditure measured in the chamber, the average bias was \(-14.6 \pm 9.9\) kJ.min\(^{-1}\) and the limits of agreement were between \(-34.0\) and \(+4.8\) kJ.min\(^{-1}\).

Figure 6.5. presents data from one subject, for all methods, including respiration chamber data, heart rate data and accelerometry.
Table 6.1. *Table with burn data from the last three burns conducted on the current system.*

<table>
<thead>
<tr>
<th></th>
<th>$O_2$ expected recovery (litres)</th>
<th>$O_2$ actual recovery (litres)</th>
<th>$CO_2$ expected recovery (litres)</th>
<th>$CO_2$ actual recovery (litres)</th>
<th>Measured RQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn 1</td>
<td>502.7</td>
<td>507.4</td>
<td>318.2</td>
<td>311.8</td>
<td>0.61</td>
</tr>
<tr>
<td>Burn 2</td>
<td>602.3</td>
<td>616.9</td>
<td>401.5</td>
<td>401.4</td>
<td>0.661</td>
</tr>
<tr>
<td>Burn 3</td>
<td>466.3</td>
<td>467.8</td>
<td>310.9</td>
<td>312.8</td>
<td>0.668</td>
</tr>
</tbody>
</table>

Table 6.2. *Subject characteristics (N=5).*

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Body fat (%)</th>
<th>VO$_2$max (ml.kg$^{-1}$.min$^{-1}$)</th>
<th>PPO (W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (N=5)</td>
<td>25.4 ± 3.5</td>
<td>71.7 ± 7.2</td>
<td>11.6 ± 1.4</td>
<td>64.7 ± 8.9</td>
</tr>
</tbody>
</table>
Table 6.3. Data of habitual energy intake, overnight chamber intake.

<table>
<thead>
<tr>
<th></th>
<th>Habitual intake (kJ)</th>
<th>Overnight intake (kJ)</th>
<th>% Of habitual intake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subjects (N=5)</strong></td>
<td>11644 ± 1001</td>
<td>10714 ± 1251</td>
<td>92 ± 5</td>
</tr>
<tr>
<td><strong>Carbohydrate %</strong></td>
<td>44.7 ± 9.0</td>
<td>61.6 ±</td>
<td></td>
</tr>
<tr>
<td><strong>Protein %</strong></td>
<td>18.9 ± 4.6</td>
<td>15.2 ± 1.3</td>
<td></td>
</tr>
<tr>
<td><strong>Fat %</strong></td>
<td>29.7 ± 7.3</td>
<td>18.0 ± 1.9</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol %</strong></td>
<td>3.4 ± 3.4</td>
<td>Not given</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.4. Final estimates for energy expenditure (kJ.min⁻¹) using the respiration chamber; the energy expenditure estimated from heart rate monitoring and the energy expenditure estimated using the 3-dimensional accelerometer.

<table>
<thead>
<tr>
<th></th>
<th>Chamber EE</th>
<th>Intermittent HR</th>
<th>Continuous HR</th>
<th>Accelerometry EE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>model EE</td>
<td>model EE</td>
<td></td>
</tr>
<tr>
<td>Resting metabolic rate (kJ.min⁻¹)</td>
<td>5.5 ± 0.38</td>
<td>Not measured</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>TDEE (kJ. min⁻¹)</td>
<td>11.3 ± 7.8</td>
<td>11.9 ± 9.5</td>
<td>12.9 ± 10.4</td>
<td>6.7 ± 2.2*</td>
</tr>
<tr>
<td>EE (kJ.min⁻¹) excluding exercise</td>
<td>8.8 ± 3.9</td>
<td>9.0 ± 6.0</td>
<td>9.9 ± 7.0</td>
<td>6.5 ± 1.9*</td>
</tr>
<tr>
<td>Overall exercise PAEE (kJ.min⁻¹)</td>
<td>24.4 ± 10.8</td>
<td>27.8 ± 9.7</td>
<td>30.0 ± 10.4</td>
<td>7.2 ± 8.2*</td>
</tr>
<tr>
<td>Cycling PAEE (kJ.min⁻¹)</td>
<td>31.7 ± 11.3</td>
<td>34.9 ± 4.6</td>
<td>37.3 ± 3.4</td>
<td>12.1 ± 3.4*</td>
</tr>
<tr>
<td>Stepping PAEE (kJ.min⁻¹)</td>
<td>17.2 ± 2.2</td>
<td>20.6 ± 7.8</td>
<td>22.6 ± 9.8</td>
<td>12.8 ± 4.1*</td>
</tr>
</tbody>
</table>

*P<0.000
### Table 6.5. Average bias and limits of agreement for total energy expenditure (kJ.min⁻¹) for each tool.

<table>
<thead>
<tr>
<th>Tool</th>
<th>R²</th>
<th>Mean</th>
<th>Std dev</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerometry</td>
<td>0.621</td>
<td>-4.851</td>
<td>6.243</td>
<td>-17.087</td>
<td>7.385</td>
</tr>
<tr>
<td>Continuous HR model</td>
<td>0.802</td>
<td>1.697</td>
<td>4.883</td>
<td>-7.874</td>
<td>11.269</td>
</tr>
<tr>
<td>Intermittent HR model</td>
<td>0.843</td>
<td>0.614</td>
<td>3.852</td>
<td>-6.936</td>
<td>8.164</td>
</tr>
</tbody>
</table>

### Table 6.6. Average bias and limits of agreement for physical activity energy expenditure (kJ.min⁻¹), during both physical activity bouts, for each tool.

<table>
<thead>
<tr>
<th>Tool</th>
<th>R²</th>
<th>Mean</th>
<th>Std dev</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerometry</td>
<td>0.071</td>
<td>-14.566</td>
<td>9.900</td>
<td>-33.970</td>
<td>4.837</td>
</tr>
<tr>
<td>Continuous HR model</td>
<td>0.644</td>
<td>5.466</td>
<td>7.785</td>
<td>-9.793</td>
<td>20.724</td>
</tr>
<tr>
<td>Intermittent HR model</td>
<td>0.743</td>
<td>3.151</td>
<td>5.851</td>
<td>-8.316</td>
<td>14.618</td>
</tr>
</tbody>
</table>

### Table 6.7. Average bias and limits of agreement for total energy expenditure (kJ.min⁻¹), excluding the physical activity bouts.

<table>
<thead>
<tr>
<th>Tool</th>
<th>R²</th>
<th>Mean</th>
<th>Std dev</th>
<th>Lower</th>
<th>Upper</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accelerometry</td>
<td>0.009</td>
<td>-2.739</td>
<td>1.581</td>
<td>5.838</td>
<td>0.360</td>
</tr>
<tr>
<td>Continuous HR model</td>
<td>0.297</td>
<td>0.668</td>
<td>2.874</td>
<td>-4.966</td>
<td>6.302</td>
</tr>
<tr>
<td>Intermittent HR model</td>
<td>0.266</td>
<td>-0.112</td>
<td>2.549</td>
<td>-5.108</td>
<td>4.884</td>
</tr>
</tbody>
</table>
Figure 6.1. *Diagram of the current alcohol burn set up, to perform the independent whole system checks, once a month.*

Figure 6.2. *Resting metabolic rate (kJ.min$^{-1}$), using either the respiration chamber or ventilated hood*

![Graph showing resting metabolic rate comparison between ventilated hood and metabolic chamber with p<0.048 significance]
Figure 6.3. Energy expenditure (kJ.min⁻¹) data for total awake time, either including or excluding physical activity energy expenditure data.

Figure 6.4. Cycling and stepping physical activity energy expenditure (kJ.min⁻¹) data for all subjects, during all exercise training activity.
Figure 6.5. Total energy expenditure, measured in the respiration chamber, and estimated from either the heart rate monitoring equation or accelerometer, for Subject 1.
DISCUSSION

Our initial objective was to construct and validate a respiration chamber. We successfully completed this objective, and have developed this technique, within the acceptable 5% error range. This respiration chamber represents the first of its kind in Sub-Saharan Africa. As a result, in this study, four different methods of measuring free-living energy expenditure were compared: indirect calorimetry, using the respiration chamber, the combined heart rate monitoring-resting metabolic rate technique, using the continuous equation developed in Chapter 4, and the novel energy expenditure equation, developed in Chapter five, and finally, accelerometry.

Energy expenditure data from both heart rate monitoring models accounted for approximately 80% of the variance in measured energy expenditure. These findings corroborate previous research conducted using heart rate monitoring techniques (136). Using a similar heart rate monitoring technique, Spurr et al. (136) accounted for 76% of the variance in measured energy expenditure, using minute-by-minute heart rate recordings, individual subject calibration and measured resting metabolic rate.

However, there was a difference in the physical activity energy expenditure estimates using either heart rate model. Using the novel intermittent model, we were able to account for 74% of the measured energy expenditure in the respiration chamber, while using the conventional continuous model; we were only able to account for 64% of the measured energy expenditure. Further the average bias using the intermittent model was smaller than the bias using the conventional model, 3.2 ± 5.9 versus 5.5 ± 7.8
kJ.min⁻¹, intermittent and continuous respectively. Similarly, the limits of agreement for the intermittent versus continuous models were also reduced, -8.3 and 14.6 kJ.min⁻¹ versus -9.8 and 20.7 kJ.min⁻¹, intermittent and continuous respectively. To our knowledge, our study is one of the first to validate a group-based equation for energy expenditure estimation, during the day in a respiration chamber.

Previously Rennie et al. (114) validated a group-based equation, to predict physical activity levels, in an independent sample of 97 individuals, during 4 days of heart rate monitoring, after developing a regression equation on a sample of 789 subjects. Estimates for physical activity levels, using the equation, and observed estimates of physical activity, from a heart rate-energy expenditure calibration test, Rennie et al. were able to account for 67% of the variance in measured physical activity level.

Strath et al. (145) explored the use of a group-based equation in a sample of 61 adults, performing a range of activities that included both inside and outside tasks, reflective of the activities of daily living. Using continuous heart rate monitoring and on-line gas analysis during 15-minute activity bouts, Strath et al. was able to account for 76% of the variance in measured energy expenditure using this technique. However, the sample on which the equation was generated was used for the final energy expenditure estimation, and activities were only performed during 15-minute bouts. Therefore we feel the current study represents a significant improvement in the validation of group-based energy expenditure equations.
In addition, we combined the use of resting metabolic rates, estimated using the equations of Harris and Benedict (64), and the heart rate monitoring techniques. It has been well recognised (29;81;82;85;88;114;136) that the relationship between heart rate and energy expenditure during activities that are sedentary is not linear, and therefore the heart rate monitoring technique used alone without the measurement or estimation of resting metabolic rate, is unlikely to provide meaningful estimates of total daily energy expenditure (29;81;83;114;136).

A study by Ceesay et al. (29) investigated 20 subjects spending 21 hours in a respiration chamber. Prior to the overnight chamber stay, subjects completed an individual heart rate-energy expenditure calibration test, during which time flex heart rate was identified. This cut-off heart rate was then used to substitute measured values for resting energy expenditure for heart rates falling below the flex heart rate. Ceesay et al. (29) was able to account for 89% of the variance in measured energy expenditure, using the respiration chamber in this sample. In another study, Livingstone et al. (83) used the doubly labelled water method to validate the heart rate monitoring technique, using individual subject heart rate-energy expenditure calibration. In a sample of 14 adults, energy expenditure was estimated during 4 days, after individual subject calibration and the identification of the flex heart rate. For heart rate values lower than flex heart rate, measured resting metabolic rate, using indirect calorimetry, was inserted, while during sleep hours, basal metabolic rate was used. Heart rate derived estimates for energy expenditure ranged from -22 to +52%, with the majority of the sample falling below 10% error ($R^2$ values not given).
The accelerometer data significantly underestimated energy expenditure measured, during the day, including both exercise sessions, using indirect calorimetry (P<0.000). Numerous investigations (12;23;65;72), have similarly reported under-estimation in total energy expenditure using the accelerometer technique. Kumahara et al. (72) most recently investigated the use of accelerometry to estimate total energy expenditure, placing 79 individuals, in a respiration chamber for a period of 24hrs. Subjects also completed two moderate exercise sessions and MET values were assigned to all levels of activity. As was found in our study, energy expenditure estimated using the accelerometry was significantly correlated with indirect calorimetry energy expenditure, however the accelerometer consistently underestimated both total and physical activity energy expenditure (P<0.001). During the exercise sessions, MET values were also strongly correlated with measured activity levels in the chamber (r=0.93). While estimates for accelerometry energy expenditure significantly underestimated energy expenditure, particularly during physical activity, significant correlations indicate that this tool may be useful for gaining insight into day-to-day variability in total energy expenditure.

Jakicic et al (65) examined energy expenditure in subjects performing five exercises (treadmill running, treadmill walking, stationary cycling, stepping and slide boarding) during workloads lasting 20-30 minutes. Initial results showed significant correlations between energy expenditure measured using the accelerometer and energy expenditure measured, using indirect calorimetry for walking, running, stepping and slide boarding, only. However, energy expenditure estimated during the cycling bout was not significantly correlated with energy expenditure measured using indirect calorimetry. In
addition, energy expenditure, measured using the accelerometer was significantly underestimated (P<0.001) during walking, stepping, slide boarding and cycling activities. Energy expenditure, during running was however, not significantly different. In the current study, the energy expenditure, measured using the accelerometer, during the submaximal cycling exhibited a greater discrepancy than the error in the stepping workload. This is not unexpected, as other studies (12;65) have shown that accelerometers have poor accuracy for predicting energy expenditure during non-weight bearing activities, such as cycling.

In summary, as part of the present study, we built, calibrated and put into operation a respiration chamber, in part, for the purpose of validating more indirect measures of daily energy expenditure. In this study, we showed that it is possible to estimate with good accuracy 24hr energy expenditure, using two new (a conventional continuous and novel intermittent) energy expenditure equations, and measured or predicted resting metabolic rate. Further we were able to show that there were significant differences for energy expenditure estimated using accelerometry. This finding corroborates previous research, and provides evidence for the combined heart rate monitoring resting metabolic rate technique use in total daily energy expenditure estimation. In addition, recent advances in the heart rate monitoring field, have made it possible for accurate energy expenditure estimation, without individual subject calibration, thus allowing for the use of the heart rate monitoring technique, in investigating the causal relationship between physical activity and mortality in large population-based studies.
CHAPTER SEVEN

SUMMARY AND CONCLUSIONS
The aims of this thesis were to explore the accuracy in measuring free-living total daily energy expenditure (TDEE), by examining existing indirect measures of energy expenditure (EE) measurement and further, developing new techniques, for improved accuracy and application, in population-based studies.

In the first part of this thesis, we explored the use of an existing free-living energy expenditure estimation technique, using 24hr heart rate monitoring, to identify changes in a group of previously sedentary postmenopausal women, before and after an eight week endurance training program. In this study, using the heart rate monitoring technique and individual subject heart rate-energy expenditure calibration to estimate energy expenditure, we could not account for any significant changes in total daily energy expenditure, following the endurance training, in spite of improvements in submaximal exercise capacity. The resultant lack of change in total daily energy expenditure could not conclusively be attributed to lack of physiological change, in spite of the training, as there was a significant inter-subject variability (23%), using the heart rate monitoring technique to estimate free-living energy expenditure, in our subjects.

During the second study, we again used the heart rate monitoring technique to estimate free-living energy expenditure, examining the validity of a modified physical activity questionnaire (PARQ). In a sample of 59 hospital employees, ranging in a wide variety of occupation and education, we administered the questionnaire on a week day, followed by an individual subject heart rate-energy expenditure calibration test and subsequent wearing of the heart rate monitor for a 24hr period, coinciding with a work day. Energy expenditure estimated using the PARQ and heart rate monitoring technique
correlated significantly \((r=0.39)\), however the PARQ significantly overestimated \((P<0.000)\) total energy expenditure compared to the heart rate monitoring technique. We also found significant gender differences. Overall the average energy expenditure estimated for the men, using either technique was consistently greater than the average energy expenditure estimated for the women. While the heart rate monitoring technique appeared to provide a reasonable method for validating the PARQ, we are unsure as to the level of accuracies of our criterion measure, especially in light of intra-subject variability, as seen in our first study. Therefore, the remainder of this dissertation focused on improving the prediction of energy expenditure using the heart rate monitoring technique. Furthermore, the real utility in these measures is the potential to apply them to research in which energy expenditure must be measured in large groups of subjects, and therefore, necessitated the investigation into group-generated equations.

Therefore, in our third study, we further investigated the use of the heart rate monitoring technique, this time, however, without individual subject calibration, during submaximal exercise. A second aim was to explore significant variables that impacted upon the relationship between heart rate and energy expenditure and included these in a mixed model regression analysis equation. A sample of regularly exercising individuals \((N=115)\) completed a submaximal exercise protocol, while continuously having their heart rate and energy expenditure measured. Significant variables that impacted upon the heart rate-energy expenditure relationship were: gender, age, weight, \(VO_2\) max and heart rate. The final mixed model accounted for 83% of the measured energy expenditure in the original sample, while in an independent validation sample, this
energy expenditure equation accounted for 73% of the measured energy expenditure, during a 20-minute self-selected cardiovascular training session. This study was novel as not only did it achieve a reasonable level of significance for energy expenditure estimation, but required that subjects no longer report to a laboratory for individual subject heart rate-energy expenditure calibration.

We further improved this model, by incorporating intermittent activity-rest calibration, which is more reflective of activities of daily living. We found that the following variables contributed significantly to the prediction of energy expenditure from heart rate: age, gender, weight, VO₂max, current minutes heart rate, preceding minutes heart rate and an interaction variable comprised of previous minutes heart rate and VO₂max. This new energy expenditure equation accounted for 81% of the measured energy expenditure on the original sample, but more significantly accounted for 76% of the measured energy expenditure in an independent validation sample (N=17) completing a 55-minute gym exercise training session, which included both continuous and intermittent training workloads. The validity of this equation, however, for total daily energy expenditure estimation had yet to be established.

We subsequently compared both equations and an additional technique of motion sensing or accelerometry on 5 subjects, using a respiration chamber, in which whole body energy expenditure is measured using indirect calorimetry. Total energy expenditure measured using the respiration chamber, was not significantly different to total energy expenditure estimated using either heart rate-energy expenditure equation, however, total energy expenditure estimated using the accelerometer was significantly
underestimated. The novel intermittent equation was more accurate than the continuous equation for both total and exercise energy expenditure. From this study we were able to conclude that energy expenditure estimation, using a group generated equation for heart rate monitoring could be achieved with a good level of accuracy, in the healthy population. Further, these results corroborated our previous results in Chapter 5, in which we found that the intermittent equation was more accurate for intermittent activity.

Together, these studies demonstrate that it is possible to estimate 24hr free-living energy expenditure with a good degree of accuracy, after adjusting for factors or variables, that impact upon the heart rate-energy expenditure relationship. Further, these studies provide evidence for the use of group-generated energy expenditure equations, and the need for modelling the effects of both fitness and the heart rate response in the preceding minute for potential broad-based application in predicting energy expenditure for use in epidemiological research.
CHAPTER EIGHT

ADDENDUM

A CASE STUDY INVESTIGATING TOTAL ENERGY EXPENDITURE IN SPACE, COMPARISON BETWEEN THE DOUBLY LABELLED WATER TECHNIQUE AND THE HEART RATE MONITORING TECHNIQUE.
INTRODUCTION

During the period of execution of this thesis, we were invited to participate in research conducted during a 10-day space mission, in a cosmonaut, part of a 3-man team aboard the Souyz-TM, space mission VC-3, a re supply mission to the International Space Station (ISS), part of the First African in Space project. The Souyz-TM crew joined the existing 3-man crew on-board the ISS for a 10-day period, during which time the Souyz-TM crew participated in the normal daily activities of living experienced on-board the ISS. Consequently we present data from a case study on one cosmonaut, during a 10-day space mission.

The overall aims of this thesis were to explore and validate indirect tools for the measurement of free-living energy expenditure. Accordingly, in Chapter Five, we presented evidence for the use of a novel energy expenditure equation, based on heart rate monitoring, while in Chapter Six, we explored the accuracy of this equation, during a 24hr free-living period and accounted for 85% of the measured energy expenditure, in a respiration chamber, using the novel energy expenditure equation. We now had the opportunity to investigate the accuracy of this equation, in an environment that presented known cardiovascular adaptations, microgravity.

The use of heart rate monitoring technique, for energy expenditure estimation, will always be subject to external, often uncontrolled, environment and physiological stresses (52;81;94). For example, Li et al. (81) reports that factors such as infection, lack of sleep, temperature, emotion (97), alcohol and caffeine consumption, smoking
(116) and physical activity (29;150) all affected the relationship between heart rate and energy expenditure. While Hebestreit et al. (59) provided evidence for a significantly altered relationship between heart rate and energy expenditure in temperatures above 22 degrees Celsius.

The microgravity environment provides another situation for altered heart rate-energy expenditure dynamics. Previous studies investigating microgravity (48;76;141) have shown that the cosmonauts heart rate is reduced by as much as 10 bpm in the microgravity environment, however in spite of these cardiovascular adaptations, studies have shown that energy expenditure in space remains similar to those levels achieved on Earth (76;140).

Most recently Lane et al. (76) used the doubly labelled water technique to measure the total energy expenditure in 13 male cosmonauts participating in 8–14 day Space Shuttle missions during 1992–1994. In this study, it was found that there was no difference in the total energy expenditure measured during the ground-based studies or in-flight-based studies. However, the total energy intake was significantly lower during the in-flight versus the ground-based studies. This significant difference was attributed to the motion sickness, which cosmonauts experience during the space mission. Body weight was also significantly different between the ground- and in-flight-based studies. This was as a result of the negative energy balance of the cosmonauts.

In another study, Stein et al. (140) compared the 16-day in-flight 1996 LMS mission with a 16-day 6° bed-tilt bed-rest study, using the doubly labelled water technique. In this
study it was found that microgravity resulted in significant difference in body weight, nitrogen retention and energy intake compared to best rest. During space flight, there were significant decreases in weight and nitrogen retention, in addition dietary intake was also reduced, while energy expenditure remained unchanged in-flight, resulting in a significant loss in overall body weight.

To date, studies investigating the impact of microgravity on total energy expenditure have relied on the doubly labelled water method. This stable isotope method requires that cosmonauts consume a dose of the stable isotope ($^2\text{H}_2^{18}\text{O}$) and collect daily urine and/or saliva samples for analysis, during their space mission and on their return to Earth. To our knowledge heart rate monitoring, for energy expenditure estimation has not been explored in the microgravity environment, despite cosmonauts being continuously monitored with ECG equipment during space flight, probably as a result of the above cardiovascular adaptations.

Therefore, the aim of this case study, was firstly to explore the use of the novel heart rate monitoring equation, developed in Chapter Five, in an environment which provided known cardiovascular adaptations and compare this to a commonly used method for energy expenditure estimation in space, the doubly labelled water technique. Secondly, compare energy expenditure estimated using the novel equation to the energy expenditure estimated using a conventional continuous energy expenditure estimation model.
METHODS

The study was conducted in two parts. Part 1 consisted of a 10-day Earth or ground-based study, conducted 2 months prior to a space mission, and Part 2; an in-flight or microgravity-based study was conducted over a 10-day space flight mission. The methodology used for Part 1 and Part 2 were identical, except for the microgravity environment experienced during Part 2. During Part 2, the subject participated as a working crewmember of a 3-man team aboard the Souyz-TM, space mission VC-3, a re-supply mission to the International Space Station (ISS). The Souyz-TM crew joined the existing 3-man crew on-board the ISS for a 10-day period, during which time the Souyz-TM crew participated in the normal daily activities of living experienced on-board the ISS. The normal working day on-board the ISS is eight to ten hours in length. Initially the Souyz-TM cosmonauts spent 36 hours aboard the Souyz capsule, before docking with the ISS where they proceeded to spend the next 8 days on board the ISS. Their return flight to Earth lasted approximately 10 hours.

The Medical Ethics committee of the University of Cape Town as well as the Rosaviakosmos* (*Russian space program) approved this study. The subject was briefed in full, prior to the commencement of this participation in the trial. The subject signed an informed consent form, after being given the opportunity to ask any questions.
Body composition

The subject had his body composition measured on three different occasions, using the skin fold callipers technique and the equations of Durnin and Womersley (44) for body fat assessment. The first measurement was completed during Part 1 of the study, while the second and third measurements were completed during Part 2, two months later. The second measurement was completed immediately prior to the 10-day space flight and the third measurement was taken immediately on the subjects return to Earth.

Resting metabolic rate measurement and individual heart rate-energy expenditure calibration

Resting metabolic rate was measured early in the morning, in the post-absorptive state. The subject rested for a minimum of 30-minutes in the supine position, before resting oxygen consumption (VO₂) and carbon dioxide production (VCO₂) were measured using the Cosmed K4b² portable gas analyser (Italy). Mean VO₂, VCO₂ and respiratory exchange ratio (RER) were collected for 30 min, data were analysed from the last 10-minutes of data collection in the resting state.

The subject's individual heart rate-energy expenditure relationship was determined during a heart rate-energy expenditure calibration test, for the estimation of total energy expenditure (81) from heart rate monitoring. This relationship were determined by measuring heart rate, VO₂ and VCO₂ simultaneously for 9 workloads of increasing intensity, each lasting between 4–5 minutes, to ensure the subject had achieved steady
state. Energy expenditure (kJ.min⁻¹) was determined for each workload, using the equations of Weir (150). During the test the subject wore a facemask attached to the Cosmed K4B² portable analyser system. Before each test the gas analyser was calibrated by using a Hans Rudolph 5530 3-litre syringe and a 5% CO₂ – 16% O₂ gas mixture. During the first 3 stages of the calibration test, the subject was tested in the supine resting position, followed by quiet sitting and subsequently standing. Following these resting measurements, the subject performed 6 progressive physical activity workloads, beginning at a walking pace of 4 km.hr⁻¹ and ending at a running pace of 12 km.hr⁻¹. The heart rate-energy expenditure calibration were carried out approximately 90 minutes after the ingestion of a light meal, typical of the subject’s self-selected breakfast. An Individual heart rate-energy expenditure calibration curve was then generated for the subject using a non-linear regression equation and the method described by Li et al. (81). This equation was as follows:

\[ Y = A + \left( B \right) / \left( 1 + \text{Exp} \left( C - (D \times \text{heart rate}) \right) \right) \]

Where: \( Y \)= energy expenditure (kJ. min⁻¹), \( A \)= actual resting metabolic rate (kJ. min⁻¹), entered as a constant for each individual while \( B \), \( C \) and \( D \) were derived from the regression equation.

Maximal treadmill test to exhaustion

On the same day that the subject performed the heart rate-energy expenditure calibration test, a maximal test to exhaustion was completed. This test followed the last
calibration workload, and begun at a treadmill speed of 12 km.hr\(^{-1}\), 0 % gradient. The treadmill speed was increased every 2 minutes, by 1 km.hr\(^{-1}\), until the subject could no longer maintain pace with the treadmill. Heart rate and treadmill speed were recorded throughout the duration of the test. Maximum heart rate and speed were recorded at the time of volitional fatigue.

24hr Heart rate collection

During Part 1 (completed on Earth), 24hr minute-by-minute heart rate data were collected on two separate days, using a heart rate monitor (Polar M series, Polar Electro, Oy, Finland), within the 10-day period. On both of these days, the subject performed a one-hour exercise session. During Part 2 (completed onboard the ISS, in Space), 24hr heart rate data were collected on four separate occasions. For heart rate collection, the subject was connected to a 6 lead portable ECG unit (FT 1000A Halter System, Spacelabs Medical, USA). On two of the four days, the subject performed prescribed exercise training in the weightless environment, while on the other two days the subject completed eccentric muscle contraction testing. The heart rate data were separated into waking and sleep hours. Heart rates recorded during sleep were assigned resting metabolic rate values, measured using indirect calorimetry.

The heart rate-energy expenditure calibration equation was then applied to the 24hr heart rate recordings, obtained during Part 1 and 2, of the study, for the estimation of total energy expenditure. In addition, the group-based equation, developed in Chapter Five, for intermittent activity, and incorporating heart rate in the preceding minute, was
also applied to the 24hr heart rate data. During Part 1, the subject performed 2 days of continuous 24hr heart rate monitoring, while during Part 2, the subject performed 4 days of continuous heart rate monitoring on board the ISS.

In Chapter Six, this equation was compared to 24hr energy expenditure in a small sample of men, measured in a respiration chamber. The equation, used in combination with measured resting metabolic rates, produced results which suggest that the energy expenditure estimated using the equation did not statistically differ from the energy expenditure, measured using indirect calorimetry. The combined heart rate monitoring-resting metabolic rate method accounted for 85% of the variance in measured energy expenditure, during the 24hr chamber stay.

**Doubly labelled water assessment**

Total energy expenditure was measured according the Maastricht protocol for doubly labelled water measurement (159). Briefly, doubly labelled water measurements covered the 10-days measurement period in both Part 1 (on Earth) and 2 (in Space). The subject was given a weighed dose of water, with a measured enrichment of approximately 5 atom% $^2$H (i.e. 5% of $^1$H atoms replaced by $^2$H) and 10 atom% $^{18}$O, so that isotope levels were increased 150 and 300 ppm above baseline for $^2$H and $^{18}$O, respectively. The doses were 103.97 g 10% $^{18}$O 5% $^2$H$_2$ and 103.57 g 10% $^{18}$O 5% $^2$H$_2$ for Part 1 and 2 respectively. The subject was administered a pre weighted dose of doubly labelled water on day 0 of the experiment, 10 hours prior to the collection of the second sample, to allow the doubly labelled water dose time to equilibrate with the body.
Saliva samples were collected using pre-dried dental swabs, for isotope determination on days 0, 1, 4, 7 and 10 of the experiment. Day 0 constituted the baseline sample. The subject was instructed to place the swab in the buccal cavity of the mouth, for a minimum 2-minute period. During Part 1 the saliva was immediately extracted from the dental swab and prepared for analysis. During Part 2, the subject placed the saturated dental swab in an airtight container, for analysis on return to Earth. On return to Earth, all samples were spun for 10-minutes at 3000 g, before being syringed into containers for sample analysis. A control subject collected samples, without doubly labelled water dosing, at identical times to the subject, during Part 1 and 2, to allow for corrections due to changes in background abundances. Isotope abundances in the saliva samples were measured with an isotope-ratio mass spectrometer (Aqua Sira, VG Isogas, Middlewich, UK). CO2 production was converted into energy expenditure by using an energy equivalent, calculated according to the average metabolic fuel quotient, estimated from the macronutrient composition of the diet and the use of body energy reserves. Background isotope levels in the experimental subject for the measurement of total energy expenditure was corrected according to the mean background changes noted in the control subjects.

Energy Intake

The subject completed 24hr energy intake records during Part 1 and Part 2. During Part 1 of the study, the investigator closely monitored and supervised the subject in an attempt to educate and prepare the subject for recording of energy intake during Part 2, while on board the ISS (Appendix 10.7 and 10.8), when the investigator was not be
present. Diets were analysed using Food Fundi 2 Professional, for Windows (PentaMedical Systems, South Africa).

Data preparation and statistical analysis

The data from all 24hr minute-by-minute heart rate monitoring periods were used to estimate energy expenditure. During Part 1, two heart rate days were selected, while in Part 2, 4 days of heart rate monitoring was recorded. Two energy expenditure equations (using the method of Li et al (81) and the equation developed in Chapter Five) were used for energy expenditure estimation. For estimation of energy expenditure from both heart rate methods, measured resting metabolic rate was used for heart rates recorded during sleep. In addition, for the novel energy expenditure equation method, energy expenditure estimates that were lower than measured resting metabolic rate were substituted with the resting metabolic rate measurement.
RESULTS

Body composition and maximal treadmill test to exhaustion

Table 8.1 represents the subject characteristics during the 2-month testing period. During Part 1 the subject weighed 78 kg. Immediately prior to the 10-day space mission, the subject weighed 75.5 kg and following his 10-day space mission he weighed 73.5 kg. His body fat percentage was 13.1 % during Part 1 and immediately prior to the 10-day space mission (Part 2) was 12.6%. On his return to Earth his body fat was 12.1 %. The maximum oxygen consumption reached during the treadmill test to fatigue was 4292 ml.min\(^{-1}\) O\(_2\) and the subjected exhausted after 8 minutes, at a peak treadmill speed of 15 km.hr\(^{-1}\).

Energy expenditure measurement

Table 8.2 represents the energy expenditure values derived using the different energy expenditure techniques, for Part 1 and Part 2. The average energy expenditure measured using the doubly labelled water technique was 13 400 kJ.day\(^{-1}\) for Part 1 and 13 400 kJ.day\(^{-1}\) for Part 2.
Energy expenditure data

Part 1

The average total energy expenditure calculated using the heart rate-energy expenditure calibration technique (81) was $21322 \pm 804 \text{ kJ.day}^{-1}$ for the first and second day of heart rate monitoring. Conversely, the average total energy expenditure measured using the novel equation (developed in Chapter Five) was $13781 \pm 347 \text{ kJ.day}^{-1}$ and ranged from 13536 to 14026 kJ.day$^{-1}$ for the first and second day of heart rate monitoring, respectively. This compares favourably with the measured energy expenditure using the doubly labelled water technique. The average energy expenditure estimated for waking hours, using the heart rate-energy expenditure calibration technique was $19462 \pm 1160 \text{ kJ.day}^{-1}$, and ranged from 18642 to 20283 kJ.day$^{-1}$, while the average energy expenditure estimated for the waking hours, using the novel equation was $11922 \pm 703 \text{ kJ.day}^{-1}$, and ranged from 11425 to 12419 kJ.day$^{-1}$.

The total accumulated waking heart rate, measured in beats, was $72090 \pm 4659$ beats and ranged from 68795 to 75384 beats.

Part 2

While on board the ISS, the average (for 4 days) total energy expenditure calculated using the heart rate-energy expenditure calibration technique was $18766 \pm 2266 \text{ kJ.day}^{-1}$ and ranged from 16057 to 21556 kJ.day$^{-1}$. Similarly, the average total energy
expenditure measured over the four days, using the novel equation was 9683± 1714 kJ.day\(^{-1}\) and ranged from 7635 to 11815 kJ.day\(^{-1}\).

The average energy expenditure estimated for waking hours, using the heart rate-energy expenditure calibration technique was 17494 ± 2520 kJ.day\(^{-1}\), and ranged from 14544 to 20560 kJ.day\(^{-1}\), while the average energy expenditure estimated for the waking hours, using the novel equation was 8411 ± 1922 kJ.day\(^{-1}\), and ranged from 6121 to 10819 kJ.day\(^{-1}\).

The total accumulated waking heart rate, measured in beats, in Part 2, was 75832 ± 7628 beats and ranged from 67518 to 84250 beats.

*Energy Intake*

The average energy intake during Part 1 (Earth) was 13 947 ± 2261 kJ.day\(^{-1}\) and ranged from 9266 to 16809 kJ.day\(^{-1}\), while during Part 2 the average intake on board the ISS was 9196 ± 5001 kJ.day\(^{-1}\) and ranged from 1886 to 15992 kJ.day\(^{-1}\).
<table>
<thead>
<tr>
<th></th>
<th>Part 1</th>
<th>Part 2 (Pre space flight)</th>
<th>Part 2 (Post space flight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
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<td>75.5</td>
<td>73.5</td>
</tr>
<tr>
<td>Sum of skinfolds (mm)</td>
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<td>58.2</td>
<td>53.4</td>
</tr>
<tr>
<td>Waist/hip ratio (arbitrary units)</td>
<td>1.006</td>
<td>0.932</td>
<td>0.92</td>
</tr>
<tr>
<td>Body fat %</td>
<td>13.1</td>
<td>12.6</td>
<td>12.1</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
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<td>9.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>67.8</td>
<td>66.0</td>
<td>64.6</td>
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Table 8.2. *Energy expenditure (kJ.day\(^{-1}\)) for Part 1 and Part 2.*

<table>
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<tr>
<th></th>
<th>DLW</th>
<th>HR-EE equation (kJ.day(^{-1}))</th>
<th>HR-EE equation (kJ.min(^{-1}))</th>
<th>Novel equation (kJ.min(^{-1}))</th>
<th>Novel equation (kJ.min(^{-1}))</th>
<th>Energy intake (kJ.day(^{-1}))</th>
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<tr>
<td></td>
<td></td>
<td>- awake</td>
<td>- total</td>
<td>- awake</td>
<td>- total</td>
<td></td>
</tr>
<tr>
<td>Part 1 (2 days of HR monitoring)</td>
<td>13 400</td>
<td>19462 ± 1609(^{\circ})</td>
<td>21322 ± 804</td>
<td>11922 ± 703</td>
<td>13781 ± 347</td>
<td>13947 ± 2262</td>
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<tr>
<td>Part 2 (4 days of HR monitoring)</td>
<td>13 400</td>
<td>17494 ± 2520</td>
<td>18766 ± 2266(^{\circ})</td>
<td>8411 ± 1922</td>
<td>9683 ± 1714</td>
<td>8968 ± 4543</td>
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</table>
Figure 8.1. Copy of heart rate tracing from 24hr heart rate monitoring, using heart rate monitor, recorded during Part 1.

Figure 8.2. Copy of heart rate tracing from 24hr heart rate monitoring, recorded during in-flight study in Part 2, using the portable halter ECG unit.
Figure 8.3. Energy expenditure (kJ.day\(^{-1}\)) and energy intake values (kJ.day\(^{-1}\)) during Part 1 (ground based) and Part 2 (In-flight) studies.
DISCUSSION

The first finding from this case study was that heart rate monitoring, using individual heart rate-energy expenditure calibration, overestimated 24hr energy expenditure, during both the Part 1, the ground based and Part 2, the microgravity environment, when compared to the doubly labelled water method. In Part 1, this overestimation in energy expenditure was as much as 64%, while in Part 2, the overestimation was 44%. This discrepancy may be due, in part, to methodological constraints. For example, McLaughlin et al. (89) found that the K4b2 portable unit slightly overestimated oxygen consumption, during cycling ergometry (50-200 W), when compared to oxygen consumption measured using the Douglas bag technique. This may have led to inaccuracies in the generation of the individual regression equation, during subject calibration. Furthermore, in previous studies, the heart rate monitoring method has been found to have large intra as well as inter subject variation (29;81;136). As a result of this intra subject variation the results obtained in this study are therefore within expected norms. In the above-mentioned studies, variations from underestimates of 20 % to overestimates of 25% were found for the heart rate monitoring, when compared to indirect calorimetry. Li et al. (81) found the inter-subject variation to be 14–18%, while the intra-subject variation was from 11–20 %.

In Part 2, the overestimation in energy expenditure, using the heart rate calibration method, was reduced (64% versus 44%, Part 1 and Part 2, respectively). This reduction may be as a result of the cardiovascular adaptations to space flight. Further, the total energy expenditure estimated, using the energy expenditure equation
(developed in Chapter Five), during the in-flight or microgravity component of the study was also markedly different to the energy expenditure measured using the doubly labeled water, 9683 versus 13000 kJ.day⁻¹. These results are almost certainly as a result of adaptations of the cardiovascular system to the microgravity environment. It is documented (68;108;132) that during space flight heart rate and arterial pressure are significantly reduced. As a result of this reduced heart rate, it is expected that energy expenditure, estimated from heart rate (involving individual calibration or group-generated regression equations) would be lower than values obtained during the ground based study. In addition, the majority of the methodological studies (62;81;83;114;136) using heart rate-energy expenditure regression equations typically are conducted in the healthy population. Inclusion criteria require that subjects be free from known cardiovascular and metabolic disorders and not smoking. This may explain differences in the energy expenditure estimate differences, using the novel equation between Part 1 and 2.

Li et al. (81), most comprehensively, reported on alterations to the heart rate-energy expenditure relationship, as a result of other external, or physiological factors which impact upon this relationship. These factors may include emotion, illness, alcohol and caffeine consumption (116), timing of the last meal (67), as well as environmental factors such as temperature (58;86). Hebestreit et al (58) reported on significant alterations to the heart rate-energy expenditure relationship for changes as small as 1.5 degree Celsius above 22 degrees Celsius. Therefore it should not be surprising that the energy expenditure estimated, using the novel equation, during Part 2, was significantly different to the energy expenditure estimated, using the novel equation, during Part 1,
simply as a result of the cardiovascular adaptations to the microgravity environment.

When the subject's heart rate data were used in a novel energy expenditure equation, the overall estimation of total energy expenditure more closely approximated energy expenditure measured with the doubly labelled water method, compared to the individual calibration test data. Total energy expenditure estimated during Part 1 was 13781 kJ.day\(^{-1}\) versus 21322 kJ.day\(^{-1}\) for novel equation and individual calibration method respectively, and similarly 9683 kJ.day\(^{-1}\) versus 18766 kJ.day\(^{-1}\) for Part 2. This finding was not surprising, given the accuracy of the estimation, using the novel equations, in Chapters' Five and Six. We were however; a little surprised at the difference between the energy expenditure estimated using the conventional continuous model and the doubly labelled water technique. Previously Li (81) has reported an increased accuracy with individual subject calibration. However, as shown in the previous chapters of this thesis, using a regression equation specifically developed for intermittent activity significantly increases the total daily energy expenditure estimation, compared to the conventional continuous energy expenditure equation.

It is important to note that total energy expenditure values obtained from the doubly labelled water method were identical during Part 1 and Part 2, over the 10 day measurement period, an average of 13 400 kJ.day\(^{-1}\) was found. Lane (76), who completed several doubly labelled water studies during short-term duration space flights, 8-14 days, in 13 male cosmonauts, have previously reported this finding. In these studies, there was no difference in the mean total energy expenditure recorded during either the ground or in-flight based studies.
When the subject's total awake heart beats were averaged during waking hours, it was found that during the microgravity part of the study (Part 2), it appeared that the subject's total waking hours heart beats (averaged over 4 days) exceeded that of the Earth based component (Part 1, averaged over 2 days), 75832 beats versus 72090 beats during Part 1. This would be in direct conflict with previous research, in which it has been shown that the microgravity environment decreases heart rate and arterial pressure in humans (48). However, on closer examination of the data, it was found that during the 10-day space flight mission, the subject slept on average approximately 87% less than during the pre-flight, the ground based study.

The second finding was that during the in-flight or microgravity part of the study, the subject was not in energy balance. This finding corroborates previous research by Lane et al. (76). The subject was in energy balance during Part 1, the ground based study. The energy deficit in Part 2, was reflected by the discrepancies between his average total energy expenditure as measured using the doubly labelled water method, and the 24hr food records. During Part 1 his energy intake was 13 497 kJ.day$^{-1}$ and his total energy expenditure was 13 400 kJ.day$^{-1}$. In Part 2, his average intake was 9197 kJ.day$^{-1}$ and total energy expenditure was also an average of 13 400 kJ.day$^{-1}$. The energy deficit during the in-flight study was approximately 3803 kJ.day$^{-1}$. Accordingly the subject lost a total of 2 kg during the 10 day or 1.5 kg of lean body mass. Previously Lane (76) and Stein (141) have reported similar findings. Stein reported an approximate 1 kg lean body mass loss during a 14-day space flight, with a reported average energy deficit of approximately 4500 kJ.day$^{-1}$ in four cosmonauts completing a 16-day space flight
mission. While Lane, reported and average energy deficit of approximately 4670 kJ.day\(^{-1}\) in 13 cosmonauts during short duration space flights (8–14 days).

Finally one cannot discount the fact that this is simply a case report. Previously much of the data collected during space flight has relied on the doubly labelled water technique, requiring that cosmonauts collect samples of either saliva or urine, or both, during the space mission. Sample collection is often time consuming and disruptive to the cosmonauts' activities of daily living, mostly as a result of the microgravity environment and as a result of the accurate records required for the technique. Heart rate monitoring provides investigators with a technique that is non-invasive, relatively inexpensive and easily administrable in the microgravity environment. Further, heart rate monitoring may provide information, as to the nature of the day-to-day routines experienced during space missions. For example, in Figure 8.2, the heart rate tracing provides information as to the nature of the cosmonaut's day and may help investigators account for some of the under-reporting experienced by cosmonauts.

However, this case study suggests that heart rate monitoring may not be suitable for interpreting energy expenditure during space flight, as a consequence of the cardiovascular adaptations that occur while in a microgravity environment. Finally caution should be made when interpreting energy expenditure estimated using the heart rate monitoring technique, especially under conditions when heart rate is likely to be dissociated from energy expenditure, such as in stressful or emotional situations, or where environments, both hot or cold, alter heart rate or where there are cardiovascular
adaptations as in cardiovascular or metabolic disease or as a result of smoking and other pharmaceutical agents.
1. Nutrition classics. A respiration calorimeter with appliances for the direct

2. American College of Sports Medicine Position Stand. The recommended quantity
and quality of exercise for developing and maintaining cardiorespiratory and
muscular fitness, and flexibility in healthy adults. Med.Sci.Sports Exerc. 30: 975-

3. Prevalence of no leisure-time physical activity--35 States and the District of

W. Thompson, D. A. Jones, C. A. Macera, and C. D. Kimsey. Comparison of three

5. Ainsworth, B. E., D. R. Jacobs, Jr., A. S. Leon, M. T. Richardson, and H. J.
Montoye. Assessment of the accuracy of physical activity questionnaire

6. Ainsworth, B. E., M. T. Richardson, D. R. Jacobs, Jr., A. S. Leon, and B.
Sternfeld. Accuracy of recall of occupational physical activity by questionnaire.


45. Ekelund, L. G. Circulatory and respiratory adaptation during prolonged exercise. 

46. Ekelund, L. G. Circulatory and respiratory adaptation during prolonged exercise 

47. Ekelund, U., M. Sjostrom, A. Yngve, and A. Nilsson. Total daily energy 
expenditure and pattern of physical activity measured by minute-by-minute heart 


decline during prolonged exercise is influenced by the increase in heart rate. 

50. Gallo, J. L., B. C. Maciel, J. A. Marin-Neto, and L. E. Martins. Sympathetic and 
parasympathetic changes in heart rate control during dynamic exercise induced 


10.1. PHYSICAL ACTIVITY DIARY, COVER PAGE.

PHYSICAL ACTIVITY DIARY

The physical activity diary needs to be completed on a daily basis. At the end of each day, calculate the number of hours spent on each MET level and record it in the physical activity diary.

MET LEVELS

<table>
<thead>
<tr>
<th>Intensity of activity</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep</td>
<td>Sleep</td>
</tr>
<tr>
<td>Sedentary</td>
<td>Leisurely walking, standing, driving, Light outdoor activity, playing a musical instrument, sitting at a desk, Watching TV, cooking, sewing</td>
</tr>
<tr>
<td>Active</td>
<td>Brisk level walking, gardening (lifting and digging), sweeping, mopping, cycling leisurely</td>
</tr>
<tr>
<td>Very active</td>
<td>Brisk uphill walking, climbing stairs, carrying a child, social dancing.</td>
</tr>
<tr>
<td>Extremely active</td>
<td>Running, aerobic exercise, carrying heavy loads.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sleep</th>
<th>Sedentary</th>
<th>Active</th>
<th>Very active</th>
<th>Extremely active</th>
<th>Total hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week1, day1</td>
<td>8</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td>Day 2</td>
<td>7.5</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>24</td>
</tr>
</tbody>
</table>

255
10.2. PHYSICAL ACTIVITY DIARY, DATA ENTRY PAGE.

Name: ____________________

<table>
<thead>
<tr>
<th></th>
<th>Sleep</th>
<th>Sedentary</th>
<th>Active</th>
<th>Very active</th>
<th>Extremely active</th>
<th>Total hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1, day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
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<td></td>
<td></td>
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<tr>
<td>Day 3</td>
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<tr>
<td>Day 4</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Day 5</td>
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<td></td>
<td></td>
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<tr>
<td>Day 6</td>
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<td></td>
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</tr>
<tr>
<td>Day 7</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Week 2, day 1</td>
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<td></td>
</tr>
<tr>
<td>Day 2</td>
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<td>Day 3</td>
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<td>Day 4</td>
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<td>Day 5</td>
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<td>Day 6</td>
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</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 3, day 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 2</td>
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<tr>
<td>Day 3</td>
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<tr>
<td>Day 4</td>
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<td></td>
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<tr>
<td>Day 5</td>
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<tr>
<td>Day 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
10.3. MODIFIED 5-DAY PHYSICAL ACTIVITY RECALL QUESTIONNAIRE

PHYSICAL ACTIVITY RECALL INTERVIEW

We would like to know about your physical activity levels during your work and non-work hours. The following questionnaire has been designed in order to get a more accurate estimate of your work activities.

1. How many days in the last week did you work? (This includes homekeeping, actual work etc.)

2. On the days which you worked, how many hours were spent working? (To the nearest 1/2 hours)

3. Please refer to the physical activity table in answering the following questions:
   a) Of the hours you worked, how many of those hours were spent in activities in which you had noticeable effort and resulted in slight sweating, similar to those in the MODERATELY ACTIVE CATEGORY.
   b) Of the hours you worked, how many of those hours were spent in activities in which there was sweating, increased breathing and increased effort, similar to those in the ACTIVE CATEGORY.
   c) Of the hours you worked, how many of those hours were spent in activities in which there was hard effort, heavy sweating and hard breathing, similar to those activities in the EXTREMELY ACTIVE CATEGORY.

4. On an average work day (Monday to Friday) how many hours do you spend in sleep each night? (to the nearest 1/2 hour)

5. On an average work day during your non-work time, how many hours do you spend in activities similar to those in the:
   MODERATELY ACTIVE CATEGORY
   ACTIVE CATEGORY
   EXTREMELY ACTIVE CATEGORY
10.4. **EXAMPLES OF MODERATELY, ACTIVE AND EXTREMELY ACTIVE CATEGORIES, USING THE 5-DAY RECALL.**

<table>
<thead>
<tr>
<th>MODERATELY ACTIVE:</th>
<th>brisk level walking</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gardening</td>
</tr>
<tr>
<td></td>
<td>sweeping</td>
</tr>
<tr>
<td></td>
<td>mopping</td>
</tr>
<tr>
<td></td>
<td>active child-minding</td>
</tr>
<tr>
<td></td>
<td>nursing</td>
</tr>
<tr>
<td></td>
<td>window cleaning</td>
</tr>
</tbody>
</table>

| ACTIVE:            | brisk uphill walk   |
|                   | climbing stairs     |
|                   | carrying a child    |
|                   | dancing             |
|                   | walking to and from work |

| EXTREMELY ACTIVE:  | running             |
|                   | aerobic exercise    |
|                   | swimming            |
|                   | carrying heavy loads |
10.5. 3-DAY DIETARY RECORD COVER PAGE.

BIOENERGETICS OF EXERCISE RESEARCH UNIT
OF THE MEDICAL RESEARCH COUNCIL (MRC)
AND THE UNIVERSITY OF CAPE TOWN

Sports Science Institute of South Africa Boundary Road Newlands 7700 South Africa
E2 115 Newlands 7725
Telephone (021) 6867330 International 27-21 6867330 Fax (021) 6867530

THREE DAY DIETARY RECORD FORM:

It is essential that you eat as you normally do, don't change your eating patterns or choices just because you are keeping a record.

Record your dietary intake on TWO weekdays and ONE weekend day.

When filling in the form:

- Record the approximate time that you ate/drank the meal/beverage.
- Record the food/beverage consumed and give a detailed description of the food:
  - use brand names if possible (e.g. Tussers cheese, Trim mayonnaise)
  - state how the food was cooked (steamed, boiled, roasted, fried etc.)
  - record if the meat was fatty or lean, was it crumbed?
  - was the chicken skin eaten?
  - record the type of margarine used and approximately how much (e.g. 1 tsp.)
  - record the type of cheese used (cheddar, feta, low fat cottage cheese, fat-free cottage cheese, brie etc.)
  - when eating canned food, state whether it is canned in oil or water or tomato?
  - record any additions to food such as cream, sugar etc.
  - when eating mixed foods such as stew or stir-fry, describe in as much detail the contents of the dish. If you prepared the food, record the quantities that you put in and divide by the proportion eaten.
- Record the AMOUNT of food consumed. Either record
  - the weight (in grams)
  - the volume (e.g. 250 ml)
  - use a household measure (1/2 cup, 1 tsp. etc.)
  - the dimension (e.g. 10 cm of boerewors, 20 cm diameter pizza)
  - draw the food on the back of the page (e.g. the size of a chop)
- Always report the COOKED weight
- When using household measures, record if it is a heaped or level spoon, what type of spoon (dessert, table or teaspoon)

Try and be as specific as possible, rather over-describe than under-describe as this will improve the reliability of the analysis. Use the back of the page if necessary. If you are not certain about anything, please contact me immediately.

Julia Goedecke RD (SA)
Tel: 666 - 7330 x 297 (w) or 481-1974 (h) or by Email: julieg@sports.uct.ac.za
### 10.6. 3-DAY DIETARY RECORD DATA INPUT PAGE

**DIETARY RECORD FORM:**

<table>
<thead>
<tr>
<th>NAME:</th>
<th>DAY:</th>
<th>DATE:</th>
</tr>
</thead>
</table>

**Is this representative of your USUAL daily intake?**

Yes  No  Explain:  

**Did you take any supplements? (vitamins or minerals or other supplements?)**

No  Yes  

If yes, what (brand name, dosage etc.)  

---

260
10.7. EXAMPLE OF 1-DAY FOOD DATA ENTRY ON BOARD THE
INTERNATIONAL SPACE STATION

<table>
<thead>
<tr>
<th>Time</th>
<th>Food type</th>
<th>Quantity</th>
<th>Fluid, mL</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>04:20</td>
<td>Listeroids (t-dopa)</td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04:20</td>
<td>Listeroids (t-dopa caps)</td>
<td>1/8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04:20</td>
<td>Extra (pseudoephedrine)</td>
<td>1/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>04:20</td>
<td>Listeroids (dextroamphetamine)</td>
<td>1/6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>06:30</td>
<td>Listeroids (t-dopa)</td>
<td>1</td>
<td>260 mL</td>
<td></td>
</tr>
</tbody>
</table>

**LUNCH**

<table>
<thead>
<tr>
<th>Time</th>
<th>Food type</th>
<th>Quantity</th>
<th>Fluid, mL</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:15</td>
<td>Extra (pseudoephedrine)</td>
<td>1</td>
<td>260 mL</td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td>Extra (pseudoephedrine caps)</td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td>Extra (pseudoephedrine cap)</td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td>Extra (pseudoephedrine)</td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td>Extra (pseudoephedrine cap)</td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12:15</td>
<td>Extra (pseudoephedrine)</td>
<td>1/2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DINNER**

<table>
<thead>
<tr>
<th>Time</th>
<th>Food type</th>
<th>Quantity</th>
<th>Fluid, mL</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:50</td>
<td>Pisco &amp; orange juice</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:50</td>
<td>Pisco</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:50</td>
<td>Orange</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:50</td>
<td>Orange</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:50</td>
<td>Orange</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: All entries are made in mL.
10.8. EXAMPLE OF 1-DAY FOOD DATA ENTRY ON BOARD THE
INTERNATIONAL SPACE STATION

---

**LOG SHEET (cont.)**

**Day 3 DATE: 2-7, 02**

### BREAKFAST

<table>
<thead>
<tr>
<th>Time</th>
<th>Food type</th>
<th>Quantity (grams or num. of units)</th>
<th>Fluid, mL</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>4:00</td>
<td>95% O.J.</td>
<td>100%</td>
<td>Water, 167 mL</td>
<td>Apples with past</td>
</tr>
<tr>
<td>5:00</td>
<td>Sausage</td>
<td>100</td>
<td>Water, 202 mL</td>
<td>Pork with sauce</td>
</tr>
<tr>
<td>6:00</td>
<td>Waffle</td>
<td>100</td>
<td>Water, 100 mL</td>
<td>Sugar and jam</td>
</tr>
<tr>
<td>7:00</td>
<td>Oatmeal</td>
<td>100</td>
<td>Water, 200 mL</td>
<td>Brown sugar</td>
</tr>
</tbody>
</table>

### LUNCH

<table>
<thead>
<tr>
<th>Time</th>
<th>Food type</th>
<th>Quantity (grams or num. of units)</th>
<th>Fluid, mL</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00</td>
<td>Juice box</td>
<td>100</td>
<td>250 mL</td>
<td>Salt and pepper</td>
</tr>
<tr>
<td></td>
<td>Cheese</td>
<td>50</td>
<td>50 mL</td>
<td>( \frac{1}{2} ) unit</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>20</td>
<td>20 mL</td>
<td>( \frac{1}{2} ) unit</td>
</tr>
</tbody>
</table>

### DINNER

<table>
<thead>
<tr>
<th>Time</th>
<th>Food type</th>
<th>Quantity (grams or num. of units)</th>
<th>Fluid, mL</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:00</td>
<td>Pizza</td>
<td>250 mL</td>
<td>250 mL</td>
<td>( \frac{1}{2} )</td>
</tr>
</tbody>
</table>