PREFACE

In many fields of physiology, gender is considered to be a variable that should be "controlled for". Therefore, males and females are expected to respond differently to various interventions or conditions. Often, sample groups are restricted to the use of males, possibly because in a "neutral environment" (i.e. without intervention), male physiology is considered to remain relatively consistent from day to day. On the other hand, women are excluded due to the circamensal rhythm (known as the menstrual cycle) that most women between approximately 13 and 50 years old experience, where the ovarian hormones fluctuate predictably over (on average) 28 days. Therefore, these hormones are considered to be ever changing and although they primarily function to support reproduction, they have been reported to influence other physiological systems. So to include women in a subject group is like using a moving target. For this reason, gender studies have been conducted to compare established responses in men with the response in women. However, in these studies women are mostly studied only during the early stages of their menstrual cycle when the ovarian hormones are considered to be at their lowest, so as to avoid the moving target scenario.

Naturally, in reality, women in their own right function and compete in sporting events at all stages of the menstrual cycle. Therefore, some researchers have endeavoured to compare physiological responses in women between identified phases of the menstrual cycle, corresponding to accepted concentration ranges for the ovarian hormones. However, this field of research is plagued with many inconsistent findings. We speculate that most inconsistencies can be solved by a closer look at the hormone interactions, as the ovarian hormones are often reported to function antagonistically. Furthermore, the study of menstrual cycle physiology and response to exercise has not been exhaustive; that is, many virgin topics in this field are still open for investigation. In this thesis, we have ventured into some novel territories and results are presented from original menstrual phase comparative investigations.

BRIEF OUTLINE OF THE THESIS

Chapter 1 reviews the changes that the ovarian hormones can impose on the respiratory, thermoregulatory, cardiovascular, and metabolic systems, which is of particular relevance for female athletes as such changes may influence exercise performance. Such alterations to metabolism appear to have the most pronounced effect on exercise performance during endurance events. While a number of studies have addressed the effects of menstrual phase or changes in ovarian hormones on carbohydrate metabolism, studies investigating fat metabolism in eumenorrhoeic women is lacking. The advent of stable bio-tracer technology allows for a more comprehensive assessment of free fatty acid dynamics. Thus **Chapters 2 and 3** investigates the effects of menstrual phase and changing ovarian hormone concentrations on aspects of free fatty acid metabolism during exercise, with the use of stable tracers.

The use of stable (carbon) free fatty acid tracers for the purpose of measuring plasma free fatty acid oxidation rate is common practise, but its application in menstrual cycle comparative studies is limited. The validity of such measurements depends on the application of a metabolic correction factor (the acetate correction factor) that is separately derived and is influenced by various factors. While considering the parameters that influence the acetate correction factor, it became apparent that the menstrual cycle could be a further variable that may need to be controlled for when deriving the acetate correction factor. **Chapter 2** assess the effects of various menstrual phases on the acetate correction factor.

Chapter 3 is one of the first studies to measure plasma free fatty acid kinetics during exercise in eumenorrhoeic women and considers the relation between plasma free fatty acid kinetics during endurance exercise and oestrogen and progesterone concentration.

In the process of developing a protocol for preparing the free fatty acid infusate for Chapter 3, I explored the option of using an alternative carrier for the free fatty acid

tracer for the purpose of keeping the tracer in solution for parenteral delivery. This study was motivated due to the potential health risks associated with the routinely used carrier, human serum albumin. These results are discussed in **Chapter 4**.

Chapter 5 investigates the relation between the ovarian hormones and changes to ventilation during submaximal exercise and indirectly considers whether persistently higher ventilation (consequent to a change in the ovarian hormones) throughout endurance exercise will significantly increase the metabolic demand of exercise.

Chapter 6 investigates the influence of menstrual phase on exercise performance. Although previous studies have been conducted to assess the effects of menstrual phase or changing ovarian hormone concentration on exercise performance or capacity during endurance events (or exercise lasting more than 30 min), none have considered the menstrual phase that occurs just before ovulation (the late follicular phase). Thus we conducted a novel performance study, which included the late follicular menstrual phase in our comparison. This menstrual phase is characterised by the pre-ovulatory surge in oestrogen, where oestrogen transiently attains its highest concentration but progesterone concentration remains low.

In **Chapter 7**, the results from the studies presented in **Chapter 2 to 6** are summarised, conclusions drawn and future studies discussed.

As part of the **Declaration**, contributions of each author to each of the published and submitted studies are outlined below and other significant contributions that have not already been acknowledged are disclosed.

Chapter 2

Oosthuyse T, Bosch AN, Jackson S (2003). Effect of menstrual phase on the acetate correction factor used in metabolic tracer studies. **Canadian Journal of Applied Physiology** 28: 818-830.

The idea and design of the study was my own. I prepared the infusate solutions, ran all the experimental trials and analysed all samples besides the oestrogen and progesterone radioimmunoassay which was contracted out to SAIMR and pyrogen testing on infusate samples which was contracted out to Department of Infectious Control. Prof. Labross Sidossis advised me on infusion rates. I analysed the data and wrote up the manuscript, which my supervisors, Drs. Andrew Bosch and Sue Jackson reviewed.

Chapter 3

Oosthuyse T, Gray DA, Davidson B, Apps P, Deftereos DAJ and Bosch AN. Naturally cycling oestrogen and progesterone influences plasma free fatty acid kinetics during exercise. **Scandinavian Journal of Medicine and Science in Sports** (Submitted).

The idea and design of the study was my own. I prepared the infusate solutions, ran all the experimental trials and analysed all samples besides the oestrogen and progesterone radioimmunoassay which was contracted out to SAIMR, and plasma carbon-13 palmitate enrichment which was performed by Dr. Peter Apps of the CSIR. Dawn Deftereos operated the HPLC and performed the interpolation calculations on the extracted catecholamine samples. Prof. Labross Sidossis and Prof. Asker Jeukendrup advised me on infusion rates and mixing protocol. I analysed the data and wrote up the manuscript, which Drs. Andrew Bosch, Bruce Davidson and Dave Gray reviewed.

Chapter 4

Oosthuyse T, Jackson S, Bosch AN. Is 2-Hydroxypropyl-β-cyclodextrin a suitable alternative carrier to Human serum albumin for delivering FFA tracers by infusion in metabolic studies? **Scandinavian Journal of Clinical and Laboratory Investigation** (Submitted).

Prof. Duncan Mitchell suggested exploring the option of using cyclodextrins as a means of complexing free fatty acids (FFA) for intravenous infusion. Dr. Matthew Worthington from South African Druggists suggested the use of 2-Hydroxypropyl-β-cyclodextrin and supplied free samples and information. I ran all solubility tests, prepared infusates and ran experimental trials. John Lanham from the Light Isotope Laboratory at UCT analysed expired air samples for carbon-13 enrichment. I analysed the data and prepared the manuscript which was reviewed by Dr. Andrew Bosch.

Chapter 5

Oosthuyse T and Bosch AN. The influence of menstrual phase on ventilatory responses to submaximal exercise is dependent on the oestrogen and progesterone concentration. **South African Journal of Sports Medicine** (In press).

The idea and design of this study was my own. I ran all experimental trials, analysed all data and prepared the manuscript, which was reviewed by Dr. Andrew Bosch. Oestrogen and progesterone radioimmunoassay was contracted out to SAIMR.

Chapter 6

Oosthuyse T, Jackson S and Bosch AN (2005) Cycling time trial performance during different menstrual phases. **European Journal of Applied Physiology** 94: 268-276.

The idea and design of this study was my own. I ran all experimental trials and analysed all samples. I analysed the data and prepared the manuscript, which was reviewed by Drs. Andrew Bosch and Sue Jackson.

As **supervisor of the candidate**, I confirm that she has described the roles of her co-authors accurately, and that she has identified all other assistance received and that she functioned as principal investigator for all five studies.

Andrew N. Bosch:_____

Date:_____

Dave A. Gray:_____

Date:_____

Bruce Davidson:_____

Date:_____